Final addendum to the

Renewal Assessment Report

- public version -

Risk assessment provided by the rapporteur Member State Germany and co-rapporteur Member State Slovakia for the active substance

GLYPHOSATE

according to the procedure for the renewal of the inclusion of a second group of active substances in Annex I to Council Directive 91/414/EEC laid down in Commission Regulation (EU) No. 1141/2010

October 2015

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Agrichem B.V.
AGRO TRADE GmbH
Albaugh UK Ltd.
Arysta LifeScience
Barclay Chemicals (R&D) Ltd.
Brokden S.L.
Bros Spolka Jawna B.P. Miranowscy
Cheminova
Dow Agro Sciences
Excel Crop Care (Europe) N.V.
Feinchemie Schwebda GmbH
Helm AG
Monsanto Europe S.A.
Nufarm GmbH & Co. KG
Rotam Agrochemical Europe Ltd.
Sabero Europe B.V.
SAPEC Agro S.A.
Société Financière de Pontarlier
Sinon Corporation
Syngenta
United Phosphorus Ltd. / Cerexagri s.a.s
Wynca UK Limited

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Renewal Assessment Report

18 December 2013

Revised
29 January 2015
31 March 2015

Glyphosate

Volume 1

Report and
Proposed Decision

RMS: Germany **Co-RMS: Slovakia**

Version history (Volume 1)

Date	**Reason for revision**
19 December 2013	Initial Renewal Assessment Report
29 January 2015	Updated following RAR peer review, taking into account the reporting table, the evaluation table and additional data
31 March 2015	Updated following discussions in Pesticides Peer Review Meetings 125 (25-27 February 2015) and 128 (03-05 March 2015)

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Level 1

Glyphosate

Statement of Subject Matter and
Purpose of Renewal Assessment Report

1 Statement of subject matter and purpose for which this report has been prepared and background information on the application

1.1 Context in which the renewal assessment report was prepared

1.1.1 Purpose for which the renewal assessment report was prepared

This renewal assessment report has been prepared in accordance with Commission Regulation (EC) No 1141/2010 and Guidance Document SANCO/10387/2010 rev. 8 in order to evaluate the application and the collective supplementary dossier submitted by Monsanto Europe N.V./S.A. on behalf of the European Glyphosate Task Force and to allow a decision on the renewal of the first approval of the active substance glyphosate.

According to Article 13 of Commission Regulation (EC) No 1141/2010, third parties could submit information to contribute to the assessment. Submissions from Agrar Koordination, PAN Germany and Umweltinstitut München containing either individual scientific papers or a list of published literature were received within the period prescribed and also considered in the evaluation.

1.1.2 Arrangements between rapporteur Member State and co-rapporteur Member State

According to Commission Regulation (EU) No 1141/2010 Germany was assigned rapporteur Member State (RMS) and Slovakia assigned Co-rapporteur Member State (Co-RMS).

A first draft of the fate section parts route and rate of degradation in soil, adsorption, desorption and mobility in soil, predicted environmental concentrations in soil and in groundwater as well as the review of the corresponding open literature were originally provided by the Co-RMS Slovakia. A review undertaken by the RMS led to the determination of different endpoints, particularly with regard to the kinetic evaluation of degradation rates in soil and with regard to adsorption in soil. As a consequence, these parts of the fate section and the calculations of the predicted environmental concentrations in soil and in groundwater were revised, based on the assessment of degradation rates and adsorption in soil.

1.1.3 EU Regulatory history for use in Plant Protection Products

Glyphosate was first evaluated as part of the 1st stage of the work-programme for existing active substances referred to in Article 8(2) of Council Directive 91/414/EEC with Germany being the designated rapporteur Member State.

In 1995 three task forces submitted each a joint dossier:

(1) The Tulip task force, comprising AgriChem, Aragonesas Agro SA, Industrias Afrasas, Calliope, Sundat and TKI Pinus Race,
(2) Monsanto and Cheminova and
(3) Barclay Chemicals and Portman Agrochemicals.

Further individual dossiers were submitted by Feinchemie Schwebda GmbH, Marubeni UK plc (Sinon EU Coorperation), Herbex Produtos, Quimicos Ltd, Luxan, I. Pi. Ci. Industrias Prodotti Chimici, Nufarm Limited, Alkaloida and Sanachem (subsequently Dow

AgroScience) all for glyphosate and Zeneca Agrochemicals (Syngenta) for glyphosate trimesium salt. Nufarm and Alkaloida officially withdrew their notifications prior to conclusion of the evaluation process.

The task force Monsanto/Cheminova as well as Feinchemie Schwebda GmbH were considered main data submitters for glyphosate. Zeneca Agrochemicals (Syngenta) was main data submitter for glyphosate trimesium.

Following a peer review organised by the European Commission glyphosate was included in Annex I of Council Directive 91/414/EEC with Commission Directive 2001/99/EC (OJ L 304/14, 21.11.2001), entering into force on 1 July 2002. According to Regulation (EU) No 540/2011 glyphosate is deemed to have been approved under Regulation (EC) No 1107/2009 as well.

The overall conclusions of the evaluation of glyphosate, as finalised by the Standing Committee on Plant Health on 29 June 2001, were provided in the Review Report (Glyphosate; SANCO/6511/VI/99-final, 21 January 2002).

The peer review concluded that only uses as herbicide may be authorised. These conclusions were reached within the framework of the following uses, which were supported by the main data submitters:

- herbicide against terrestrial annual weeds, perennial weeds and shrubs in fruit, vegetables, forestry, grassland, ornamentals and arable crops as well as non-crop uses

In agreement with Article 4 of Regulation (EC) No 1141/2010 Monsanto Europe N.V./S.A. on behalf of the European Glyphosate Task Force (www.glyphosatetaskforce.org) submitted an application to Germany as RMS and Slovakia as Co-RMS notifying the intention to renew the exsisting approval of glyphosate on 24 March 2011.

A collective supplementary dossier from the Glyphosate Task Force comprising 24 applicants was submitted on 25 May 2012.

In agreement with Article 4 of Regulation (EC) No 1141/2010 GAT Microencapsulation AG submitted an application to Germany as RMS and Slovakia as Co-RMS notifying the intention to renew the exsisting approval of glyphosate on 25 March 2011. This application contained several deficiencies. According to Article 6 (3) of (EC) No 1141/2010 the applicant was given a period of 14 days to render the application compliant. Thereupon the applicant withdrew the application on 20 April 2011.

1.1.4 Evaluations carried out under other regulatory contexts

Pesticide residues in food—2004 evaluations. Part II. Toxicological. WHO/PCS/06.1. World Health Organization, Geneva, 2006

1.2 Applicant(s) information

1.2.1 Name and address of applicant(s) for approval of the active substance

Applicant on behalf of the European Glyphosate Task Force:

Monsanto Europe N.V./S.A.
Haven 627
Scheldelaan 460
B-2040 Antwerp
Belgium

Contact:

[redacted]

Other Taskforce members (in alphabethical order):

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Pol. Ind. Fuente del Jarro
46988 Paterna (Valencia)
Spain

Agria S.A.
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Bulgaria

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Ireland

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Spain

Bros Spolka Jawna B.P. Miranowscy
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61 - 619 Poznan
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40126 Bologna Italy

[redacted]

Excel Crop Care (Europe) NV
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Belgium

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~~Edmund-Rumpler-Straße 6~~
~~D-51149 Köln~~
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Helm AG
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United Kingdom

United Phosphorus Ltd
The Centre,
Birchwood Park,
Warrington,
WA3 6YN
United Kingdom

Wynca UK Limited
32 Threadneedle Street,
London, EC2R 8AY
United Kingdom

[redacted]

1.2.2 Producer or producers of the active substance

Confidential information, see Annex C.

1.2.3 Information relating to the collective provision of dossiers

Confidential information, see Annex C.

1.3 Identity of the active substance

1.3.1 Common name proposed or ISO-accepted and synonyms

Active ingredient:	Glyphosate
Related salt-types:	Glyphosate- isopropyl-amine-salt
	Glyphosate-potassium-salt
	Glyphosate-ammonium-salt
	Glyphosate-dimethylammonium-salt

1.3.2 Chemical name (IUPAC and CA nomenclature)

Glphyosate:

IUPAC:	*N*-(phosphonomethyl)glycine
CAS:	*N*-(phosphonomethyl)glycine

Glyphosate-isopropyl-amine salt:

IUPAC:	*N*-(phosphonomethyl)glycine - isopropylamine (1:1) or isopropylammonium *N*-(phosphonomethyl)glycinate
CAS:	*N*-(phosphonomethyl)glycine compound with 2-propanamine (1:1)

Glyphosate-potassium salt:

IUPAC:	potassium *N*-[(hydroxyphosphinato)methyl]glycine
CAS:	*N*-(phosphonomethyl)glycine potassium salt (1:1)

Glyphosate-ammonium salt:

IUPAC:	ammonium *N*-[(hydroxyphosphinato)methyl]glycine
CAS:	*N*-(phosphonomethyl)glycine monoammonium salt

Glyphosate-dimethylammonium salt:

IUPAC:	*N*-(phosphonomethyl)glycine - dimethylamine (1:1) or dimethylammonium *N*-(phosphonomethyl)glycinate
CAS:	*N*-(phosphonomethyl)glycine compound with *N*-methylmethanamine (1:1)

1.3.3 Producer's development code numbers

With the submission of the "renewal"-dossier no explicite codes were given in Document M.

1.3.4 CAS, EC and CIPAC numbers

Glphyosate:

CAS	1071-83-6
EC (EEC)	213-997-4
CIPAC	284

Glyphosate-isopropyl-amine salt:

CAS	38641-94-0
EC (EEC)	254-056-8
CIPAC	284.105

Glyphosate-potassium salt:

CAS	70901-20-1
EC (EEC)	Not attributed
CIPAC	284.019

Glyphosate-ammonium salt:

CAS	40465-66-5
EC (EEC)	Not attributed
CIPAC	284.007

Glyphosate-dimethylammonium salt:

CAS	34494-04-7
EC (EEC)	Not attributed
CIPAC	284.102

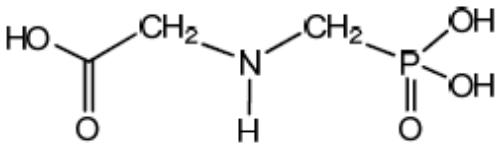
1.3.5 Molecular and structural formulae, molecular mass

Glyphosate:

Molecular formular: $C_3H_8NO_5P$

Molar mass: 169.1 g/mol

Structural formula:

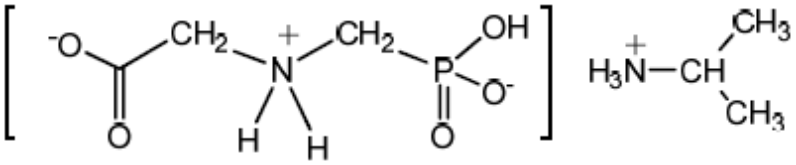


Glyphosate-isopropyl-amine salt:

Molecular formular: $C_6H_{17}N_2O_5P$

Molar mass: 228.18 g/mol

Structural formula:

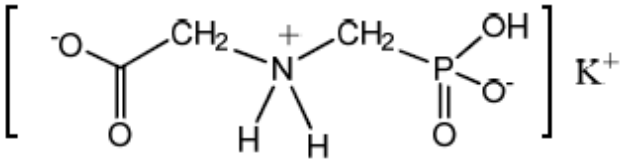


Glyphosate-potassium salt:

Molecular formular: $C_3H_7NO_5PK$

Molar mass: 207.18 g/mol

Structural formula:

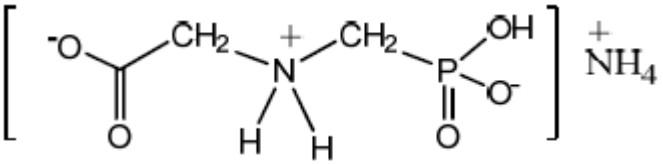


Glyphosate-ammonium salt:

Molecular formular: $C_3H_{11}N_2O_5P$

Molar mass: 186.1 g/mol

Structural formula:

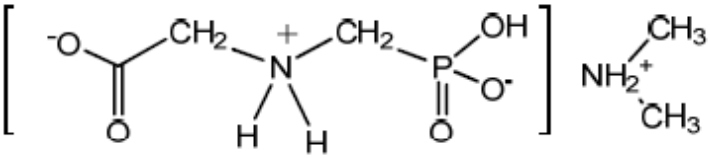


Glyphosate-dimethylammonium salt:

Molecular formular: $C_5H_{15}N_2O_5P$

Molar mass: 214.15 g/mol

Structural formula:



1.3.6 Method of manufacture (synthesis pathway) of the active substance

Confidential information, see Annex C.

1.3.7 Specification of purity of the active substance in g/kg

950 g/kg as stipulated in Regulation (EU) No 540/2011.

1.3.8 Identity and content of additives (such as stabilisers) and impurities

1.3.8.1 Additives

None.

1.3.8.2 Significant impurities

Confidential information, see Annex C.

1.3.8.3 Relevant impurities

Formaldehyde	maximum content < 1 g/kg
N-Nitroso-glyphosate	maximum content < 1 mg/kg

The impurity NN-IDA was proposed to be regarded as relevant impurity by the applicant. This was confirmed by the toxicological assessment. However, in none of the provided production batches the impurity was determined.

1.3.9 Analytical profile of batches

Confidential information, see Annex C.

1.4 Information on the plant protection product

1.4.1 Applicant

Applicant:
Monsanto Europe S.A.
Avenue de Tervuren 270-272
B-1150 Brussels
Belgium

Contact:
[redacted]

Monsanto applies on behalf of the Glyphosate Task Force (GTF). The representative formulation of the joint GTF submission (MON 52276) is a Monsanto product and some of the data presented in this Annex III dossier is proprietary and company confidential to Monsanto.

1.4.2 Producer of the plant protection product

Confidential information, see Annex C.

1.4.3 Trade name or proposed trade name and producer's development code number of the plant protection product

Trade name:	MON 52276 is registered and commercially available in most EU Member States under different trade names. An example of a trade name in Germany is 'Roundup Ultra'.
Code number:	MON 52276

1.4.4 Detailed quantitative and qualitative information on the composition of the plant protection product

1.4.4.1 Composition of the plant protection product

Confidential information, see Annex C.

1.4.4.2 Information on the active substances

Content of active substance:	Glyphosate, pure	360 g/L
Content as isopropylamine salt of glyphosate:		486 g/L

1.4.4.3 Information on safeners, synergists and co-formulants

Not applicable

1.4.5 Type and code of the plant protection product

Soluble concentrate (SL)

1.4.6 Function

Herbicide.

Glyphosate is a non-selective post-emergence, mono- and dicotyledonous herbicidal active substance.

1.4.7 Field of use envisaged

Herbicides containing glyphosate are commonly used for the control of annual and perennial mono- and dicotyledonous weeds and woody plants in agriculture, horticulture, viticulture, forestry, orchards, plantation crops, amenities, home gardening and greenhouses. Furthermore it is used for weed control on aquatic areas, on hard surfaces, on railways, along roads, and on non cultivated areas. Besides weed control, herbicides containing glyphosate are also used for the control of suckers, for the destruction of grassland before renewal and for harvest management (desiccation) in various crops.

1.4.8 Effects on harmful organisms

Glyphosate is a non-selective herbicidal active substance, belonging to the chemical class of glycines, with no or only low soil residual activity. Glyphosate is taken up by the leaves and other green parts of the plant and is translocated systemically (apoplastic and symplastic) in the whole plant, also in underground parts like roots, rhizomes or stolons. Glyphosate kills the plant by blocking the shikimic acid pathway. Glyphosate binds to and blocks the activity of its target enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), an enzyme of the aromatic amino acid biosynthetic pathway. The inhibition of the enzyme prevents the plant from synthesising the essential aromatic amino acids needed for protein biosynthesis. Action at the shikimic acid pathway is unique to glyphosate and the absence of this pathway in animals is an important factor of its low vertebrate toxicity.

1.5 Detailed uses of the plant protection product

1.5.1 Details of representative uses

Glyphosate containing products are used at rates as shown in the following table and control the most important dicot species like *Cirsium* spp., *Chenopodium album* and *Rubus* spp and monocot species such as *Elytrigia repens, Alopecurus myosuroides* and *Sorghum halepense* in stubble, cereals, peas, bean,oilseed rape, flax, mustard, orchards, pasture, forestry and industrial weed control.

There are various methods of application of glyphosate formulations including tractor-mounted hydraulic sprayers (Spray volumes ranging between 100 and 400 L for most uses. This is in line with the GAP supported for the renewal of glyphosate), hand held sprayers including rotary atomizers (ULV) and knapsacks, aerial (limited in the EU to Hungary for pre-harvest application in specific conditions in maize and sunflower only), weed wipers (rope wick) and cut stump treatments for trees. Specialised application techniques include shielded sprayers, spot applicators, brush applications to tree stumps, stem injections for trees and to persistent weeds such as Japanese knotweed and ready-to-use sprayers for the home and garden sector.

Applications are designed to avoid contact with the crop, with the exception of pre-harvest applications.

List of representative uses evaluated (Glyphosate)

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	L/ha product* min max	water L/ha min max	kg as/ha min max		
All crops** (all seeded or trans-planted crops)	EU		F	Emerged annual, peren-nial and bien-nial weeds	SL	360g/L	Spray	Pre planting of crop	1-2	21d (see remark)	1-6	100-400	0.36-2.16	F	Spring & autumn after harvest (incl. stubble and/or seedbed prep.) For all crops: Maximum applica-tion rate 4.32 kg/ha glyphosate in any 12 month period across use catego-ries, equivalent to the sum of pre-plant, pre-harvest and post harvest stubble applica-tions. The interval be-tween applications is dependent on new weed emer-gence after the first treatment, relative to the time of planting the crop.
All crops** (all seeded crops)	EU		F	Emerged annual, peren-nial and bien-nial weeds	SL	360g/L	Spray	Post planting/ pre emergence of crop	1		1-3	100-400	0.36-1.08	F	
Cereals (pre-harvest) wheat, rye, triticale,	EU		F	Emerged annual, peren-nial and bien-nial weeds	SL	360g/L	Spray	Crop maturity < 30 % grain moisture	1		2-6	100-400	0.72-2.16	7	Maximum applica-tion rate 4.32 kg/ha glyphosate in any 12 month period across use catego-ries, equivalent to the sum of pre-plant, pre-harvest and post harvest
Cereals (pre-harvest) barley and	EU		F	Emerged annual, peren-nial and bien-nial weeds	SL	360g/L	Spray	Crop maturity < 30 % grain	1		2-6	100-400	0.72-2.16	7	

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	L/ha product* min max	water L/ha min max	kg as/ha min max		
oats								moisture							stubble applications.
Oilseeds (pre-harvest) rapeseed, mustard seed, linseed	EU		F	Emerged annual, perennial and biennial weeds	SL	360g/L	Spray	Crop maturity < 30 % grain moisture	1		2-6	100-400	0.72-2.16	14	Pre-harvest uses in all crops include uses for weed control (higher doses) and harvest aid, sometimes referred to as desiccation (lower doses). The critical GAP is the high dose recommended used for weed control.

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	L/ha product* min max	water L/ha min max	kg as/ha min max		
Orchard crops, vines, including citrus & tree nuts	EU		F	Emerged annual, perennial and biennial weeds	SL	360g/L	Spray	Post emergence of weeds	1-3	28d	2-8	100-400	0.72-2.88	N/A	Stone & pome fruit, olives Applications to avoid contact with tree branches. Maximum cumulative application rate 4.32 kg/ha glyphosate in any 12 month period Note: Because applications are made to the intra-rows (inner strips between the trees within a row), application rates per ha are expressed per 'unit of treated surface area' the actual application rate per ha orchard or vineyard will roughly only be 33 %

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	L/ha product* min max	water L/ha min max	kg as/ha min max		
Orchard crops, vines, including citrus & tree nuts	EU		F	Emerged annual, perennial and biennial weeds	SL	360g/L	(ULV) Sprayer or Knapsack use (spot treatment)	Post emergence of weeds	1-3	28d	2-8	0-400	0.72-2.88	42 F 30 F	Stone & pome fruit Citrus & tree nuts Grapes Olives Applications made round base of trunk [0.0 L/ha water addresses ULV application of the undiluted product] Maximum cumulative application rate 4.32 kg/ha glyphosate in any 12 month period Note: Because applications are made round base of trunk and to the intra-rows, (inner strips between two trees within a row), application rates per ha are expressed per 'unit of treated surface area' the actual application rate per ha orchard or vineyard will roughly only be 33 %-50 %.

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	L/ha product* min max	water L/ha min max	kg as/ha min max		

Remarks:

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*eg.* fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) eg. biting and suckling insects, soil born insects, foliar fungi, weeds
(d) eg. wettable powder (WP), watersoluble granule (WG)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, eg. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, eg. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(i) g/kg or g/l
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) The minimum and maximum number of application possible under practical conditions of use must be provided
* former information on kg as/hL replaced by RMS
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions
** Crops including but not restricted to: root & tuber vegetables, bulb vegetables, stem vegetables, field vegetables (fruiting vegetables, brassica vegetables, leaf vegetables and fresh herbs, legume vegetables), pulses, oil seeds, potatoes, cereals, and sugar- & fodder beet; before planting fruit crops, ornamentals, trees, nursery plants etc.

1.5.2 Further information on representative uses

Representative uses for MON 52276 (360 g/L; SL) supported in the Annex I Renewal Dossier and their current corresponding authorisation status at national level are the following ones:

	USES AS LISTED IN THE REPRESENTATIVE GAP (detailed in Doc D-1)				CORRESPONDING REGISTERED MON 52276 USES AT NATIONAL LEVEL								
Crop	**Target**	**Situation of use (e.g. indoor)** **Growth stage & season**	**Application rate per treatment** **(kg as/ha)** **[Number of treatments]**	**Application method/**	**Country***	**Zone****	**Since**	**Reg. No.**	**Product**	**Product Application rate per treatment Min and Max l/ha**	**Active Substance Application rate per treatment Min and Max Kg as/ha**	**Number of treatments per Season Min and Max**	**Active Substance Max total dose/ ha Min and Max**
All crops**** (all seeded or transplanted crops)	Annual biennial and perennial weeds	Outdoor Pre-plant	0.36-2.16 [1-2]	Foliar spray	UK	C	1994	10320	Roundup Biactive	1.0-6.0	0.36-2.16	1-2	0.36-4.32
					SE	N	1992	3937	Roundup Bio	1.0-4.0	0.36-1.44	1-2	0.36-2.88
					FR	S	1992	9200293	Hockey Plus	n/a-6.0	n/a-2.16	1-2	n/a-4.32
All crops**** (all seeded crops)	Annual biennial and perennial weeds	Outdoor Post-plant pre-emergence of crop	0.36-1.08 [1]	Foliar spray	UK	C	1994	10320	Roundup Biactive	1.0-3.0	0.36-1.08	1	0.36-1.08
					SE	N	1992	3937	Roundup Bio	1.0-4.0	0.36-1.44	1	0.36-1.44
					FR	S	1992	9200293	Hockey Plus	n/a	n/a	n/a	n/a

	USES AS LISTED IN THE REPRESENTATIVE GAP (detailed in Doc D-1)				CORRESPONDING REGISTERED MON 52276 USES AT NATIONAL LEVEL								
Crop	**Target**	**Situation of use (e.g. indoor)** **Growth stage & season**	**Application rate per treatment** **(kg as/ha)** **[Number of treatments]**	**Application method/**	**Country***	**Zone****	**Since**	**Reg. No.**	**Product**	**Product Application rate per treatment Min and Max l/ha**	**Active Substance Application rate per treatment Min and Max Kg as/ha**	**Number of treatments per Season Min and Max**	**Active Substance Max total dose/ ha Min and Max**
Cereals (Pre-harvest) Wheat, rye, triticale,	Annual biennial and perennial weeds. Desiccation	Outdoor Pre-harvest	0.72-2.16 [1] Do not exceed 4.32kg/ha over a 12 month period	Foliar spray	UK SE FR	C N S	1994 1992 1992	10320 3937 9200293	Roundup Biactive Roundup Bio Hockey Plus	2.0-6.0 2.0-4.0 n/a-6.0	0.72-2.16 0.72-1.44 n/a-2.16	1 1 1	0.72-2.16 0.72-1.44 n/a-2.16
Cereals (Pre-harvest) Barley and oats	Annual biennial and perennial weeds. Desiccation	Outdoor Pre-harvest	0.72-2.16 [1] Do not exceed 4.32kg/ha over a 12 month period	Foliar spray	UK SE FR	C N S	1994 1992 1992	10320 3937 9200293	Roundup Biactive Roundup Bio Hockey Plus	2.0-6.0 2.0-4.0 n/a-6.0	0.72-2.16 0.72-1.44 n/a-2.16	1 1 1	0.72-2.16 0.72-1.44 n/a-2.16

	USES AS LISTED IN THE REPRESENTATIVE GAP (detailed in Doc D-1)				CORRESPONDING REGISTERED MON 52276 USES AT NATIONAL LEVEL								
Crop	**Target**	**Situation of use (e.g. indoor)** **Growth stage & season**	**Application rate per treatment** **(kg as/ha)** **[Number of treatments]**	**Application method/**	**Country***	**Zone****	**Since**	**Reg. No.**	**Product**	**Product Application rate per treatment Min and Max l/ha**	**Active Substance Application rate per treatment Min and Max Kg as/ha**	**Number of treatments per Season Min and Max**	**Active Substance Max total dose/ ha Min and Max**
Oilseeds (Pre-harvest) rapeseed, mustard seed and linseed	Annual biennial and perennial weeds. Desiccation	Outdoor Pre-harvest	0.72-2.16 [1] Do not exceed 4.32kg/ha over a 12 month period	Foliar spray	UK SE FR	C N S	1994 1992 1992	10320 3937 9200293	Roundup Biactive Roundup Bio Hockey Plus	2.0-6.0 2.0-4.0 n/a-6.0	0.72-2.16 0.72-1.44 n/a-2.16	1 1 1	0.72-2.16 0.72-1.44 n/a-2.16
Orchard crops, vines including citrus and nuts	Annual biennial and perennial weeds.	Outdoor	0.72-2.88 [1-3] Do not exceed 4.32kg/ha over a 12 month period	Foliar spray	UK SE FR	C N S	1994 1992 1992	10320 3937 9200293	Roundup Biactive Roundup Bio Hockey Plus	2.0-8.0 2.0-8.0 n/a-8.0	0.72-2.88 0.72-2.88 n/a-2.88	1-3 Do not exceed 4.32kg/ha over a 12 month period	0.72-4.32 0.72-4.32 n/a-4.32

	USES AS LISTED IN THE REPRESENTATIVE GAP (detailed in Doc D-1)				CORRESPONDING REGISTERED MON 52276 USES AT NATIONAL LEVEL								
Crop	**Target**	**Situation of use (e.g. indoor)** **Growth stage & season**	**Application rate per treatment** **(kg as/ha)** **[Number of treatments]**	**Application method/**	**Country***	**Zone****	**Since**	**Reg. No.**	**Product**	**Product Application rate per treatment Min and Max l/ha**	**Active Substance Application rate per treatment Min and Max Kg as/ha**	**Number of treatments per Season Min and Max**	**Active Substance Max total dose/ ha Min and Max**
Orchard crops, vines including citrus and nuts (Spot treatment)	Annual biennial and perennial weeds.	Outdoor	0.72-2.88 [1] 0.96-2.88 [1-3] Do not exceed 4.32kg/ha over a 12 month period	Foliar spray Knapsack	UK SE FR	C N S	1994 1992 1992	10320 3937 9200293	Roundup Biactive Roundup Bio Hockey Plus	2.0-8.0 (1%-4% sln)*** 2.0-8.0 (1%-4% sln)*** n/a-8.0 (<4% sln)***	0.72-2.88 0.72-2.88 n/a-2.88	1-3 Do not exceed 4.32kg/ha over a 12 month period	0.72-4.32 0.72-4.32 n/a-4.32

* Representative countries of the three regulatory zones and not a complete list of countries
** C-central; N-north; S-south (Reg 1107/2009)
*** Percent solution (spot treatment) based on a water volume of 200 l/ha
**** Crops including but not restricted to: root & tuber vegetables, bulb vegetables, stem vegetables, field vegetables (fruiting vegetables, brassica vegetables, leaf vegetables and fresh herbs, legume vegetables), pulses, oil seeds, potatoes, cereals, and sugar- & fodder beet; before planting fruit crops, ornamentals, trees, nursery plants etc.
n/a: Not applicable (no minimum rates for Hockey Plus)

1.5.3 Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

None.

1.5.4 Overview on authorisations in EU Member States

Some examples of glyphosate formulations currently registered in some countries of the EU are listed below:

Country	Trade Name	Approval number	Concentration	Salt of glyphosate	Formulation type
France	Roundup	7400057	360 g/L	IPA	SL
	Clinic	9800499	360 g/L	IPA	SL
	Roundup Max	2020293	450 g/L	IPA	SL
	Sting 2X	9400527	270 g/L	IPA	SL
BELGIUM	Clinic	9206/B	360 g/L	IPA	SL
	Roundup Turbo	9344/B	450 G/L	IPA	SL
GERMANY	ROUNDUP ULTRAMAX	005191-00	450 G/L	IPA	SL
	ROUNDUP TURBO	004960-00	680 G/KG	AMMONIUM	SG
HUNGARY	ROUNDUP BIAKTIV	02.2059/1/2010	360 G/L	IPA	SL
	ROUNDUP MEGA	02.5/10493-1/2010	450 G/L	POTASSIUM	SL
	DOMINATOR EXTRA	04.2/3239-2011	480 G/l	DMA	sl
ITALY	HOPPER 480	14969	480 G/l	DMA	sl
SPAIN	ROUNDUP ENERGY PRO	22959	450 G/L	POTASSIUM	SL
SWEDEN	cLINIC 360 SL	4378	360 G/L	IPA	SL
UK	ENVISION	MAPP 10569	450 G/L	IPA	SL
	ROUNDUP KLIK	MAPP 12866	450 G/L	POTASSIUM	sl
	ROUNDUP MAX	MAPP 12952	680 G/kG	AMMONIUM	SG
	GLYFOS 480	MAPP 10996	480 g/L	IPA	SL

Level 2

Glyphosate

Summary of
active substance hazard and
of product risk assessment

2 Summary of active substance hazard and of product risk assessment

2.1 Identity

2.1.1 Summary of identity

All data concerning the identity address sufficiently the requirements of Annex IIA and Annex IIIA.
However, it should be noted that based on the available studies which were submitted for the renewal of glyphosate, a conclusion on the equivalence for certain sources of the active substance cannot be made (For details see Volume 4)

2.2 Physical and chemical properties

2.2.1 Summary of physical and chemical properties of the active substance

Glyphosate and its salts (IPA, K, NH_4) are water soluble solids at room temperature without any explosive or oxidising properties.

2.2.2 Summary of physical and chemical properties of the plant protection product

MON52276 is a SL formulation without any explosive or oxidising properties that is stable for two years under the conditions applied (original container, at 20 °C).

2.3 Data on application and efficacy

2.3.1 Summary of effectiveness

Glyphosate controls the most important annual dicotyledonous species, for example *Chenopodium album*, biennial species such as *Cirsium* spp. and perennial dicotyledonous weeds such as *Rubus* spp. In addition glyphosate controls annual monocotyledonous species such as *Alopecurus myosuroides* and perennial monocotyledonous weeds including *Sorghum halepense* and *Elytrigia repens* in stubble, cereals, peas, beans, oilseed rape, flax, mustard, orchards, pastures, forestry and industrial weed control. For some species efficacy is not sufficient.

2.3.2 Summary of information on the development of resistance

Looking at the situation in Europe in more detail, currently 10 cases of resistance are known, encompassing five different weed species, the dicotyledonous *Conyza bonariensis*, *C. canadensis* and *C. sumatrensis*, as well as the monocotyledonous *Lolium multiflorum* and *L. rigidum*.
So far, resistant biotypes have been found exclusively in orchards, vineyards or olive groves (Italy, Spain and France) or on railways (Czech Republic). All cases have been reported from the last seven years, indicating a growing impact of glyphosate resistance. The most serious problem seems to be *L. rigidum* with reported cases from four different countries including a high number of affected sites. Apart from one case (*C. canadensis)* in the Czech Republic, Northern and Central Europe are not affected by glyphosate resistance up to now.

For all glyphosate-resistant weed species from Europe, research has shown that these particular biotypes may also be cross-resistant to other HRAC Group G herbicides. Multiple resistance seems to be a serious issue in the case of some glyphosate-resistant weeds outside Europe where resistance to up to four different herbicide groups (different modes of action) has been detected.
However, the design of the respective crop rotations and the associated frequency of glyphosate application may differ in the various Member States. A national assessment of the agronomic risk is therefore recommended. Compared to arable crops, there therefore seems to be a higher risk for resistance development in perennial systems, under non-crop situations or on railways, as glyphosate may be used as the only herbicide.

2.3.3 Summary of adverse effects on treated crops

In plants, glyphosate inhibits the shikimic acid pathway. Glyphosate binds to and blocks the activity of its target enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), an enzyme of the aromatic amino acid biosynthetic pathway. The inhibition of the enzyme prevents the plant from synthesizing the essential aromatic amino acids (e.g. phenylalanine, tyrosine, tryptophane) needed for protein biosynthesis. This reduces the production of protein in the plant, and inhibits plant growth. Because EPSPS is present in all plants, all crops, with the exception of genetically modified plants tolerant to glyphosate, are usually sensitive to glyphosate. In the normal crop rotation no effects are expected on any succeeding crops.

2.3.4 Summary of observations on other undesirable or unintended side-effects

In soils, glyphosate will be adsorbed quickly onto soil particles and inactivated. However, glyphosate can become unbound again in small amounts. The impact of this on plants and soil microorganisms has not been completely clarified. In soils (field), DT_{50} varies from 3 to 174 days depending on edaphic and climatic conditions. The major metabolite in soil is aminomethylphosphonic acid (AMPA).

Findings have shown that glyphosate can be transferred from the roots of target plants to the rhizosphere and non-target plants can also be influenced (e.g. reduced absorption of micronutrients – Mn and Fe deficiency). Glyphosate is a strong chelator to various divalent cations such as Ca, Fe, Cu and Mn. Glyphosate binds micronutrients in the soil and can cause micronutrient deficiencies in plants that increase their susceptibility to disease, especially on soils with pure nutrient content. However, the available scientific data suggest that the strong affinity of glyphosate and its metabolite AMPA to most soils prevents the uptake of these compounds by root systems of non-target plants.

There is a risk of crop damage using glyphosate in direct drilling systems. To avoid phytotoxic effects on crops in pre-emergence uses seeds must be totally covered with soil.

Glyphosate can reduce some beneficial organisms such as saprophytic fungi that decompose dead plant material and are important for soil fertility. Studies have shown that glyphosate stimulates the growth of a number of fungal pathogens that cause diseases in crops.

2.4 Further information

2.4.1 Summary of methods and precautions concerning handling, storage, transport or fire

Adequate information on methods and precautions concerning handling, storage, transport or fire is available.

2.4.2 Summary of procedures for destruction or decontamination

Adequate information on destruction or decontamination is available.

2.4.3 Summary of emergency measures in case of an accident

Adequate information on emergency measures in case of an accident is available.

2.5 Methods of analysis

2.5.1 Methods used for the generation of pre-authorisation data

Methods for the determination of the active substance and the impurities in the technical material are available.

2.5.2 Methods for post control and monitoring purposes

Methods to ensure the monitoring and enforcement of the respective limits are **not** completely available.

For the assessment of the analytical methods for the determination of glyphosate residues the following criteria were used:

- Mean recovery rates at each fortification level in the range of 70 to 110 % with a relative standard deviation of ≤ 20 %
- No interfering blanks (< 30 % of the LOQ)
- Methods must employ the simplest approach, involve the minimum cost, and require commonly available equipment.
- The enforcement method for food must be suitable for the determination of all compounds included in the residue definition and must be checked in an independent laboratory.
- The enforcement methods for environmental matrices must be able to analyse for all compounds of toxicological and/or ecotoxicological significance in soil, water and air.
- An additional confirmatory method for all matrices is supplied.

According to these criteria adequate analytical methods are listed in the Table 2.5-1.

Table 2.5-1: Methods for the determination of residues

Matrixtype	Matrix	Residue component	Method	Limit of quantification		Reference
Crop	Plums, limes, corn forage, corn grain, corn stover, corn oil, corn flour, corn grits, corn starch, corn meal, soybean forage, soybean seed, soybean hay, soybean oil, soybean meal, soybean hulls	Glyphosate and N-acetyl-glyphosate	LC-MS/MS of underivatised analytes with phenyl-hexyl column; m/z 170→88 (glyphosate), m/z 212→88 (N-acetyl-glyphosate)	0.05	mg/kg	Pentz and Bramble (2007a), DuPont-15444 (ASB2008-2635)
Crop	Grapes, soybean seed	Glyphosate and N-acetyl-glyphosate	LC-MS/MS of underivatised analytes with phenyl-hexyl column; m/z 170→88 (glyphosate), m/z 212→170 (N-acetyl-glyphosate)	0.05	mg/kg	Seal and Dillon (2007), DuPont-21313 (ASB2008-2637)
Crop	Flax, cabbage, melon, oat grain, rye straw, coffee	Glyphosate	GC-MS after derivaization with trifluoroacetic anhydride and hetafluorobutanol, BPX-5 column, m/z 612	0.05	mg/kg	Anderson and Ely, 2001; RAM 328/01 (ASB2012-12364)
Crop	Corn grain, soya forage, nutmeat	Glyphosate	GC-MS after derivaization with trifluoroacetic anhydride and hetafluorobutanol, DB-5 column, m/z 611.5	0.05	mg/kg	Alferness and Wiebe, 2001, Interlaboratory method validation study (ASB2012-12387)
Crop	Barley grain, barley straw maize green plant maize corn sugar beet root	Glyphosate	HPLC with post-column derivatization and fluorescence detection	0.05	mg/kg	Klimmek, 2007 DFG Method 405 (ASB2008-5606)
Crop	Oil seed rape, citrus fruit	Glyphosate	HPLC with post-column derivatization and fluorescence detection	0.05	mg/kg	Klimmek and Weber, 2008 DFG Method 405 (ASB2008-5607)
Crop	Potato, carrot, onion, cucumber, cabbage, cauliflower, lettuce, leek, tomato	Glyphosate	LC-MS/MS, Bio-Rad Fast Acid column, ESI-, m/z 168→68, m/z 168→79	0.05	mg/kg	Weber, 2012 (ASB2012-12489)

Ma-trixtype	Matrix	Residue component	Method	Limit of quantification		Reference
Animal matrices	Whole milk, skim milk, cream, whole egg, egg yolks, egg whites, muscle liver, kidney, fat,	Glyphosate and N-acetyl-glyphosate	LC-MS/MS of underivatised analytes with phenyl-hexyl column; m/z 170→88 (glyphosate), m/z 212→88 (N-acetyl-glyphosate)	0.025 0.05	mg/kg mg/kg	Pentz and Bramble (2007), DuPont-21372 (ASB2008-2636)
Animal matrices	milk, eggs, muscle liver	Glyphosate and N-acetyl-glyphosate	LC-MS/MS of underivatised analytes with phenyl-hexyl column; ESI+ m/z 170→88 (glyphosate), m/z 212→88 (N-acetyl-glyphosate) or ESI-, m/z 168→63 (glypho-sate)	0.025 0.05	mg/kg mg/kg	Karnik and Dillon (2007), DuPont-20009 (ASB2008-2634)
Animal matrices	Egg	Glyphosate	GC-MS after derivatization with trifluoroacetic anhydride and hepta-fluorobutanol, HP-5 MS column, m/z 612	0.01	mg/kg	Clarke and Robinson, 1998 (ASB2012-12398)
Animal matrices	Milk, egg, meat	Glyphosate	GC-MS after derivatization with trifluoroacetic anhydride and trifluoroethanol, CPSil 19 column, m/z 411	0.05	mg/kg	Schneider, 2001 a (MET2005-367)
Soil	Loamy sand	Glyphosate	GC-MS after derivatization with trifluoroacetic anhydride and trifluoroethanol, CPSil 19 column, m/z 411	0.05	mg/kg	Schneider, 2001 b (MET2005-371)
~~Soil~~	~~Szuter~~	~~Glyphosate~~	~~GC-MS after derivaization with trifluoroacetic anhydride and hetafluorobutanol, XTI-5 column, m/z 611~~	~~0.05~~	~~mg/kg~~	~~Szuter, 1996 (MET2000-699)~~

Matrixtype	Matrix	Residue component	Method	Limit of quantification		Reference
Water	Drinking water, ground water, surface water	Glyphosate	LC-MS/MS after derivatization with 9-fluorenylmethyl-chlorformate (FMOC), Synergi Fusion RP column, m/z 390→168, m/z 390→150	0.03	µg/L	Knoch, 2010 (ASB2012-12445)
Water	Drinking water	Glyphosate	LC-MS/MS after derivatization with 9-fluorenylmethyl-chlorformate (FMOC), Synergi Fusion RP column , m/z 390→168, m/z 390→150	0.03	µg/L	Geschke, 2011 (ASB2012-12426)
Air	Air, 35 °C / 80 % rel. humidity, 6 h	Glyphosate	GC-MS after derivatization with trifluoroacetic anhydride and trifluoroethanol, CPSil 19 column, m/z 411	5	µg/m^3	Schneider, 2001 c (MET2005-371)

This overview shows that an analytical method using HPLC-MS/MS is available for the determination of glyphosate and N-acetyl-glyphosate in all kinds of plant matrices with an LOQ of 0.05 mg/kg. This method is validated by a second laboratory, but does not allow the simultaneous confirmation of results. For confirmatory purposes a laborious GC-MS method is available, which does not allow the determination of N-acetyl-glyphosate.

A similar HPLC-MS/MS method was validated by two independent laboratories for the determination of residues of glyphosate and N-acetyl-glyphosate in all kinds of animal matrices. The LOQ of this method is 0.025 mg/kg in milk and egg and 0.050 mg/kg in liver, kidney and fat. Confirmatory methods are usually not available for animal matrices.

For the determination of residues of glyphosate in environmental matrices analytical methods are available except a confirmatory method for glyphosate and AMPA in soil.

The following methods for monitoring are missing:

- A confirmatory method for glyphosate in fat and kidney/liver.
- A confirmatory method for N-acetyl-glyphosate in dry plant materials and those with high water and high fat content.
- A confirmatory method for N-acetyl-glyphosate in all kinds of animal matrices.
- a confirmatory method for glyphosate and AMPA in soil.

It should be noted that currently the data requirements concerning analytical methods for the determination of residues in food stuff are covered only by studies of a company that is neither member of the Glyphosate Task Force (GTF) nor applicant in the context of the EU renewal procedure.

2.6 Effects on human and animal health

Methodological approach

This health evaluation of glyphosate is based on the following sources and approaches:

- More than 150 toxicological and ADME studies that had not been reviewed on EU level before were submitted by the Glyphosate Task Force (GTF) in 2012 and became subject to thorough evaluation.

- All toxicological and ADME studies that had been reported in the previous EU evaluation in the late 1990ies were re-evaluated by the RMS according to current quality standards (taking into account most recent OECD and EU test guidelines, if available).

- All toxicological studies were only used for risk assessment when regarded as acceptable or at least supplementary from a today's perspective. Justifications for disregarding now studies that had been formerly taken into consideration are given in the respective sections of Volume 3.

- A huge amount of more than 900 scientific publications (published since 2000 until 2014) and other relevant information was additionally considered.

- All these publication were assessed for relevance, quality and reliability and were used for risk assessment only on condition that the respective criteria had been met.

- After receiving comments from the Member States and EFSA, from the notifiers and from the public, the toxicological part of the RAR was revised. However, the main conclusions were not changed, apart from a correction of the dermal absorption estimate for the representative formulation. Most revisions and amendments (all marked in yellow) were due to inclusion of additional information that was either actually new or had not been known or available when the first draft was prepared. The main information sources for these amendments were as follows:
  - Some toxicological studies, a number of publications and additional data (*e.g.*, historical control data) that had been requested by the RMS and/or EFSA either in the first draft of the RAR or as a result of the commenting period and was submitted by the GTF in September, 2014.
  - All publications that were mentioned in the commenting phase but had not been included in the RAR before. (A major part of them simply became published after the first draft had been completed.)
  - Recent publications that became available in 2013 and 2014 which were identified by the RMS upon a repeated literature survey.

  In some cases, allocation of mainly published information to the different sections in Volumes 1 and 3 has been changed upon revision but this is clearly indicated.

A last amendment of the toxicological parts of the RAR (Vol. 1, 2.6; Vol. 3, B.6; List of endpoints) was made after the Pesticides Peer Review 125 expert meeting that has taken place in February, 2015. During that meeting, setting of an Acute Reference Dose (ARfD) was agreed, in contrast to the previous proposal of the RMS not to establish this additional reference value. This ARfD is now mentioned in the relevant parts of the RAR and its

experimental basis described. Furthermore, on request of the experts, the RMS collected and included additional data to substantiate the assumption of a high background incidence of malignant lymphoma in mice, especially in the Swiss mouse.

Introductory remarks to the evaluation in Volume 1

- If the mean daily dietary intake of the test substance was different for male and female animals in toxicological feeding studies, the lower value is given, except that the NOAELs/LOAELs were based on effects occurring in only one sex.

- Because of the large number of studies, summary tables for acute toxicity (with skin irritation and sensitisation) and some parts of genotoxicity testing are included only in Volume 3.

- Where possible, on the basis of all reliable studies and information, it was tried to establish "overall" NOAELs/LOAELs for a specific toxicological endpoint taking dose spacing into account.

- With regard to open literature, it should be noted that a major part of the publications is on plant protection products (PPP) or "formulations" which are different from the representative one.

- In many publications, the title or the conclusions are misleading because it is claimed that the active substance (a.s.) glyphosate had been tested but actually it was a specific formulation. Composition of the tested products, apart from glyphosate content, was mostly not reported. Therefore, this data is of limited value for this toxicological evaluation that is focussed on the active ingredient

- Despite the very high number of papers, only certain endpoints have been investigated and testing was performed often *in vitro* or sometimes *in vivo* using non-standard methods. Thus, the reference values were based on overall assessment of the regulatory studies that were performed with the active substance and submitted by the notifiers. Published articles were found unsuitable for this purpose.

- However, taken in the whole, the published data suggest a higher toxicity of certain formulations as compared to glyphosate itself.

2.6.1 Overall Summary

Following oral administration to rats, glyphosate is rapidly absorbed from the gut but only to a limited extent of approximately 20 %, *i.e.*, oral absorption is lower than 30 % as previously (DAR, 1998, ASB2010-10302) assumed. The absorbed portion is widely distributed with highest concentrations occurring in bone, kidneys and liver. Its elimination via urine is fast and complete, predominantly within 48 h. There is virtually no metabolism of absorbed glyphosate in rats. Unabsorbed substance is excreted in faeces, mostly unchanged, with only a small amount transformed to aminomethylphosphonic acid (AMPA). There is no evidence of accumulation of glyphosate. This pattern of toxicokinetics and metabolism is independent of sex, dose level, or repeated administration.

The acute toxicity of glyphosate was low *via* oral, dermal and inhalativ routes of exposure and in all species tested. The oral and dermal LD_{50} was above 2000 mg/kg bw and the inhalative LC_{50} was greater than 5 mg/mL air. Glyphosate was not irritating to rabbit skin and proved negative in all tests for skin sensitisation using different methods. However, glyphosate acid proved severely irritating to the eyes. Eye irritation properties of the glyphosate salts are much weaker.

In repeated dose studies in several species, it was noted that the high dose effects in the various studies may be quite different, depending on species, strain, laboratory or perhaps also the purity / impurities of the test substance. A part of these different effects may be due to acidic properties of glyphosate.
The short-term toxicity of glyphosate was rather low in all species under study and by all routes of administration. Oral subchronic studies revealed a maximum NOAEL of around 400 mg/kg bw/day in rats, of approximately 300 mg/kg bw/day in dogs and of approximately 500 mg/kg bw/day in mice. Toxic effects were confined to approximately 600 mg/kg bw/day and above. Severe toxicity resulting in mortality was observed in one dog study at the dose level of 1000 mg/kg bw/day when administered in capsules. Another dog study failed to confirm these effects. Dietary administration was generally tolerated better.
Target organs in the different species were the gastrointestinal tract (irritation with diarrhea or at least loose stool; in rats also distention, increased organ weight and mucosal atrophy of the caecum), the bladder (cystitis), and the parotid salivary glands (histological findings). In addition, effects on body weight gain, food consumption and efficiency or on red blood cell parameters were observed. Alterations in clinical chemistry parameters suggested weak toxic effects on the liver which were not supported by histopathological observations.
In subacute dermal studies on rats and rabbits, no systemic effects and only slight dermal irritation were observed up to the highest dose levels of 1000 or 5000 mg/kg bw/day.

The potential genotoxicity of glyphosate was tested in an adequate range of *in vitro* and *in vivo* studies providing no evidence of a genotoxic potential.

In long-term studies in rats, glyphosate was not carcinogenic. 100 mg/kg bw/day is considered to represent an overall NOAEL, based on combined assessment of four independent studies. The overall LOAEL was 350 mg/kg bw/day. Effects on body weight and body weight gain, an increase in liver and salivary glands weight, alterations in clinical chemistry parameters (*e.g.*, increase in AP activity and lower urine pH), histological salivary gland changes mainly of the parotis, cataracts and stomach mucosa irritation or caecum distention were observed but not consistently in all studies.

In the mouse, non-neoplastic effects resembled those in rats but were accompanied by liver pathology and epithelial hyperplasia of the bladder with an overall NOAEL of 150 mg/kg bw/day. All findings were confined to 800 mg/kg bw/day or above.
In one long-term study, an increase in the incidence of malignant lymphoma in male mice was observed at the highest dose level of 1460 mg/kg bw/day (10 000 ppm). This type of tumor is quite common in aging mice and was also found in a high incidence in the control group of this study. There was no statistically significant increase in the incidences of malignant lymphoma in 4 other studies even in the higher dose range of up to 40000 ppm (4348 mg/kg bw/day).
Therefore, glyphosate was considered unlikely to pose a carcinogenic risk in humans.

In a multitude of reproductive toxicity studies in rats, the overall NOAEL was approximately 300 mg/kg bw/day for both parental and offspring toxicity if all available studies are taken

into account. Parental effects in the different studies and generations occurred at a LOAEL of approximately 670 mg/kg bw/day and above and comprised increased food and water consumption, lower body weight gain and an increase in incidence and severity of cellular alteration of the parotid and submaxillary salivary glands. In offspring, body weight gain was reduced and, in one study, preputial separation was delayed at an exaggerated dose level. Regarding reproductive toxicity, the only finding was a decrease in homogenisation resistant spermatids in the *cauda epididymidis* in one study in F0 males receiving the limit dose level of 1000 mg/kg bw/day. Based on this effect, the NOAEL for reproductive toxicity was 351 mg/kg bw/day. Reproductive performance was not altered in any study.

The developmental toxicity studies in rats revealed an overall NOAEL for both maternal and developmental effects of 300 mg/kg bw/d based on clinical signs and reduced body weight gain in the dams and increased incidences of foetuses with delayed ossification and skeletal anomalies at 1000 mg/kg bw/day. In conclusion, the newly submitted studies confirmed the previous evaluation that there is no teratogenic potential of glyphosate in rats.
In rabbits, the overall NOAEL for maternal toxicity was 50 mg/kg bw/day, based on mortality and gastrointestinal disturbances from 100 mg/kg bw/day onwards. The lowest NOAEL for developmental effects was also established at 50 mg/kg bw/d, based on post-implantation loss at 200 mg/kg bw/day. Other developmental foetal effects comprised lower foetal weight and a delay in ossification.
In a developmental study in rabbits at a dose of 450 mg/kg bw/day, causing marked maternal toxicity, a slightly increased incidence of interventricular septal defects was observed. In all further acceptable developmental studies, this finding was not confirmed. However, excessive toxicity including maternal mortality at comparable dose levels was reported, making the evaluation difficult. It was concluded that there is no increased risk for foetal heart effects at levels of exposure below those that caused severe maternal toxicity.

Based on all reproductive and developmental toxicity studies, glyphosate was not considered teratogenic in rats or rabbits and is unlikely to cause reproductive or teratogenic effects in humans.

Glyphosate was not neurotoxic in rodents. As expected because of its chemical structure, the compound proved negative for delayed neuropathy in chicken. No evidence of immuno-toxicity was obtained in a dietary study in mice.

The major soil and plant metabolite aminomethyl phosphonic acid (AMPA) was extensively investigated in acute, short-term and developmental studies and mutagenicity assays. It was not genotoxic and was found to be of equal toxicity as the parent compound.

In epidemiological studies in humans, there was no evidence of carcinogencity and there were no effects on fertility, reproduction and development or of neurotoxicity that might be attributed to glyphosate. Poisoning incidents following accidental or suicidal ingestion of glyphosate formulations rather reflect the toxicity of the products (due to co-formulants) and cannot be directly assigned to the active substance itself. Transient irritation has been sometimes observed following direct eye contact or inhalative exposure.
Glyphosate has been found in the urine of operators and consumers, however, the measured values suggest a previously received systemic dose that is by orders of magnitude below the proposed reference values and, thus, is of no health concern.

In farm animals such as goat and cattle, the clinical picture of systemic intoxications after administration of high doses was mainly characterised by gastrointestinal and neurological

signs with mortality occurring at lower dose levels (minimum lethal dose of 790 mg Round-up/kg bw/day) than in rats.
Glyphosate was found in the urine of cows, mostly likely because of residues in their diet, but the systemic intake was very low with a wide margin of safety to doses that might cause clinical signs in cattle. Because the same enzyme (EPSPS) as in plants might be inhibited in many bacteria, there was concern about possible alterations in the composition of microbial communities and microbial metabolism in the rumen eventually resulting in disease. However, a new study in an artifical rumen system has shown that no adverse effects on microbial communities and animal health are to be anticipated.

The proposed ADI and AOEL for glyphosate are both based on the lowest NOAEL for maternal and developmental toxicity in the rabbit at 50 mg/kg bw/day since this was the most sensitive animal model. Applying the safety factor of 100, the resulting ADI is 0.5 mg/kg bw. The ADI for glyphosate is applicable also to AMPA if health evaluation of residues is needed.

For setting the AOEL, correction for 20 % oral absorption must be made. Accordingly, an AOEL of 0.1 mg/kg bw/day was established.

~~An ARfD is not warranted because glyphosate was of low acute toxicty and since there was no evidence that the effects seen in the studies with repeated administration might be caused by a single dose.~~
Clinical signs and mortality in pregnant rabbits indicated a need to establish an ARfD even though these were no acute effects occurring after single or very few administrations. Post-implantation losses in two developmental studies in rabbits were considered the most suitable experimental basis. Thus, based on an NOAEL of 50 mg/kg bw, an ARfD of 0.5 mg/kg bw was established that is also applicable to AMPA if health evaluation of residues is needed.

There is sufficient data available to prove that the toxicity of plant protections products containing glyphosate may be higher than that of the active ingredient. Most likely, this is due to certain co-formulants which may enhance the toxicity of the formulations. In particular, there is reliable evidence that polyethoxylated (POE) tallowamine surfactants may ~~be decisive~~ increase the toxicity of some formulations.
To facilitate a comprehensive risk assessment of products that contain both glyphosate and a POE-tallowamine and to ensure sufficient protection of operators, bystanders, workers, residents and consumers, reference values were established for the substance with CAS no. 61791-26-2 since this one was considered particularly toxic. A systemic AOEL, an ADI and an ARfD in the same magnitude of 0.1 mg/kg bw(/day) have been proposed. Furthermore, an inhalative AOEL of 0.0166 mg/kg bw/day was derived.

2.6.2 Summary of absorption, distribution, metabolism and excretion in mammals

The previous EU evaluation of glyphosate concluded that, following oral administration, glyphosate was rapidly absorbed from the gastrointestinal tract but only to a limited extent. This assumption was based on rather low elimination in the urine (DAR, 1998, ASB2010-10302). Meanwhile, data obtained in bile-cannulated rats ([redacted], 1996, ASB2012-11380; [redacted] 1996, TOX2000-1981) have demonstrated a negligible biliary excretion. Since the pulmonary route of elimination is also of no importance (< 0.2 %) as shown by [redacted] (1995, ASB2012-11379), [redacted] (1996, ASB2012-11380) and [redacted] (1996, TOX2000-1980), the systemically available glyphosate was confirmed to be excreted nearly exclusively in the urine. Thus, the extent of urinary excretion

may be considered the most appropriate measure for oral absorption. In the past, an oral absorption rate of around 30 % was estimated, based mainly on studies by [redacted] (1992, TOX9300343) and [redacted] (1988, TOX9552356). The study by Knowles and Mookherjee (1996, ASB2012-11380), provided confirmation of this estimate, at least for the low dose level that is usually considered for this parameter. However, a number of other experiments of a comparable high quality ([redacted] 1995, ASB2012-11379; Davies, 1996, TOX2000-1977; [redacted] 1996, TOX2000-1979) suggested a lower oral absorption. These findings are in line with data of [redacted] (1995, TOX9650071) that also included a comparison of excretion following oral and intravenous administration in the same study. Further confirmation came from a study in bile-cannulated rats receiving a high dose ([redacted] 1996, TOX2000-1981). The reasons for these apparent differences (see Table 2.6–1a and 2.6–1b) are not kown but the studies providing lower values must not be disregarded. Thus, 20 % is proposed as an appropriate estimate of oral absorption that is well in the middle of the percentage of urinary excretion in the various studies. This figure should be used for correction when the systemic AOEL has to be calculated, instead of 30 % as applied before (EU, 2001, ASB2009-4191).

Rapid absorption and distribution was confirmed by pharmacokinetic parameters. Peak plasma levels were observed within 4 – 6 h and elimination from blood and plasma was rapid with no evidence of accumulation in blood cells. A biphasic pattern of elimination from plasma has been suggested in several studies and terminal half lives of 8 – 10 hours have been estimated. Radiolabel in plasma was negligible after 24 h and not detectable any longer at 168 h ([redacted] 1995, ASB2012-11379; [redacted], 1996, ASB2012-11380; see also [redacted], 1988, TOX9552356; [redacted], 1992, TOX9552358; and, in the literature, [redacted]., 2009, ASB2012-11542).

Table 2.6-1a: Comparison of the distribution of radiolabelled glyphosate acid in excreta and tissues (in %) and its metabolism in rats in studies that were previously not evaluated in the EU

Reference, Study identification, Owner	Dosing regime and dose levels, length of post-observation period	Excretion / Distribution (mean % of applied dose)								Metabolism
		Urine		Faeces		Total organ / tissue / carcass residues		Bile		
		♂	♀	♂	♀	♂	♀	♂	♀	
[redacted] (1995), ASB2012-11379; Arysta	Single oral gavage, 168 h; satellite groups for plasma kinetics									Very limited, traces of AMPA in urine (< 0.3%) and of AMPA and another compound in faeces (<2 %)
	10 mg/kg bw	22.5	19.4	74.6	84.3	0.33	0.27	--	--	
	600 mg/kg bw	30.3	29.5	74.7	74.2	0.31	0.39	--	--	
[redacted] (1996), ASB2012-11380; Nufarm	Single oral gavage, 168 h; satellite groups for plasma kinetics and tissue residues (up to 72 h) and 48-h biliary excretion									Very limited with <1% transformed to a compound presumed as AMPA
	1 mg/kg bw	24.9	34.9	72.6	62.4	0.75	0.98	--	--	
	100 mg/kg bw	55.3	55.0	41.2	42.4	0.84	0.98	--	--	
	1 mg/kg bw	27.5	24.2	55.3	61.0	4.99	3.82	0.03	0.08	
[redacted] (1996), TOX2000-1981; Syngenta	Single oral gavage, 1000 mg/kg bw, 48 h	20.8	16.3	39.1	30.5	--	--	0.06	0.06	Very limited, <0.7% AMPA was found (based on examination of urinary and faecal samples obtained over 72 hours in other experiments from the same lab, i.e., Davies, 1996a-c)
[redacted] (1996), TOX2000-1977; Syngenta	Single oral gavage, 10 mg/kg bw, 72 h	13.3	11.1	88.5	88.7	0.54	0.46	--	--	Not investigated
[redacted] (1996), TOX2000-1978; Syngenta	Single oral gavage, 1000 mg/kg bw, 72 h	16.9	17.8	89.5	84.6	0.47	0.54	--	--	Not investigated
[redacted] (1996), TOX2000-1979; Syngenta	Single oral dose (gavage) after repeated (14x) dosing, 10 mg/kg bw, 72 h (after final dose)	10.6	10.7	86.8	90.7	0.47	0.41	--	--	Not investigated

Table 2.6-1b: Comparison of the distribution of radiolabelled glyphosate acid in excreta and tissues (in %) and its metabolism in valid ADME studies in the rat that were contained in the original DAR

Reference, Study identification, Owner	**Dosing regime and dose levels, length of post-observation period**	**Excretion / Distribution (mean % of applied dose)**								**Metabolism**
		Urine		Faeces		Total organ / tissue / carcass residues		Bile		
		♂	♀	♂	♀	♂	♀	♂	♀	
[redacted] (1995)[#], TOX9650071 / [redacted] (1995)[#], TOX9552251; ADAMAe	0.2 - 0.3 mg/kg bw, single oral dose, 168 h	12.3	9.6	82.9	83.3	--	--	--	--	No metabolites found in urine following oral high dose application
	200 mg/kg bw, single oral dose, 168 h	17.1	13.2	81.8	84.4	--	--	--	--	
	0.2 mg/kg bw, single i.v. dose, 168 h	90	88.6	5.6	7.2	< 0.1*	< 0.1*	--	--	
[redacted] (1992), TOX9300343; Cheminova	30 mg/kg bw, single oral dose, 168 h	29.0	30.7	58.8	56.5	0.62	0.64	--	--	No metabolites found in urine or faeces
	1000 mg/kg bw, single oral dose, 168 h	30.6	22.4	53.3	60.4	0.47	0.40	--	--	
	30 mg/kg bw, repeated (14x) oral application followed by a single radiolabelled dose, 72 h	34.3	34.6	49.6	46.7	0.96	0.83	--	--	
	30 mg/kg bw, single i.v. dose , 168 h	86.0	84.2	3.4	1.5	1.4	1.1	--	--	
[redacted] (1988), TOX9552356 / [redacted] (1988), TOX9552357; Monsanto	10 mg/kg bw, single oral dose, 168 h	28.6	22.5	62.4	69.4	0.48	0.36	--	--	Very limited, AMPA accounting for 0.2 – 0.4%
	1000 mg/kg bw, single oral dose, 168 h	17.8	14.3	68.9	69.4	<0.4	<0.4	--	--	
	10 mg/kg bw, repeated (14x) oral application followed by a single radiolabelled dose , 168 h	30.9	23.1	61.0	70.9	<0.7	<0.7	--	--	
	10 mg/kg bw, single i.v. dose , 168 h[$]	79.0	74.5	4.7	8.3	≈ 1.0	≈ 1.0	--	--	

[#] Study now considered supplementary by RMS. * Animals were different from those examined for excretion following i.v. application. Bone tissue not investigated. $ Total recovery was rather poor .

Distribution of the absorbed radioactivity into the organs and tissues was wide and rapid with generally low residues found in organs and tissues at termination. After a period of 3 to 7 days following oral administration, total body burden accounted for equal to or less than 1 % of the applied radioactivity ([redacted], 1988, TOX9552356; [redacted], 1992, TOX9300343; [redacted] 1996, TOX2000-1977, TOX2000-1978, TOX2000-1979; McEwen, 1995, ASB2012-11379, [redacted], 1996, ASB2012-11380). If measured, the highest residues were detected in bone, followed by kidney and liver. Of course, due to poor oral absorption, high amounts were also found in the gastro-intestinal tract (GIT). This pattern of distribution was confirmed by whole-body autoradiograms that showed the greatest intensity of radioactivity in bone and gastrointestinal tract at up to 24 hours after dosing which was reduced to negligible amounts within 48 hours ([redacted] 1992, TOX9552358; [redacted] 1996, TOX2000-1980). A certain affinity to bone tissue is not unexpected for a phosporous compound. Although elimination from bone is slower than from other tissues, the amount of radiolabel in bone after 168 h following a single oral dose was relatively low accounting for 0.02 – 0.03 % of the applied dose ([redacted] 1995, ASB2012-11379).
There was no evidence of a potential for accumulation in animals based on residue analysis in organs and tissues after 72 h -168 h after single or repeated doses.

Elimination of ingested glyphosate via faeces urine was rapid and virtually complete by 72 – 168 hours with the major part being excreted within the first 48 hours. Because biliary excretion was negligible, it can be concluded that faeces contained unabsorbed glyphosate and enterohepatic circulation was of no importance.

This pattern of absorption, distribution and elimination was not significantly changed by dose levels, by repeated administration of low doses or the sex of the test animals.

Most of the parent glyphosate is eliminated unchanged and only a small amount (less than 1% of the applied dose) is transformed to aminomethylphosphonic acid (AMPA). While AMPA is known to be the major metabolite of glyphosate in genetically modified plants and may occur also in soil and groundwater, its abundance in mammals has been shown to be very limited and is assumed to be due to gastrointestinal microflora activity rather than mammalian metabolic pathways ([redacted], 1991, TOX9551791). The metabolite AMPA has been tested in several toxicity studies which demonstrated that it is of lower toxicity than glyphosate acid (see 2.6.8).

In a rather old (supplementary) study with dietary administration of glyphosate over 14 days to rats ([redacted], 1973, TOX9552355), evidence of even a lower oral absorption than after gavage application was obtained. Total excretion was found equal to total intake.

A (supplementary) study in male rabbits ([redacted], 1973, TOX9552353) demonstrated a similar pattern of toxicokinetics and metabolism as in the rat.

Published information on toxicokinetics and metabolism of glyphosate in rats following oral administration is scarce and, so far available, does not contradict the results from the regulatory studies ([redacted], 1991, TOX9551791; [redacted], 1992, TOX9551954; [redacted] 2009, ASB2012-11542).

Following dermal exposure, glyphosate is poorly absorbed ([redacted] 2012, ASB2012-11459) but the actual extent of dermal absorption depends very much on the product in which the active ingredient is formulated. Therefore, it can be assessed only for the individual plant

protection products. ~~Data on dermal absorption of glyphosate from the representative formulation is presented under 2.6.14.~~ For the representative formulation MON52276, a dermal absorption of 1 % was estimated for all dilutions (see also 2.6.15).

As for most pesticides, reliable kinetic data for glyphosate obtained in man is hardly available. However, there are some studies in which glyphosate in urine was measured allowing at least a rough estimation of exposure either due to intake of residues or previous occupational use. The resulting estimates may be compared then to the toxicological reference values. This data is presented under 2.6.11 in a sub-section on human biomonitoring.

~~However, there is at least one publication that gives an idea of urinary excretion of glyphosate after systemic (agricultural) exposure. As part of a study in occupationally exposed farmers and there families in two U.S. states, glyphosate residues were analysed in 24-hr composite urine samples taken from 48 farmers, their spouses and 79 of their children (aged 4 to 18) before, on the same day and three days after application of herbicides containing glyphosate. The geometric mean of glyphosate in the urine samples was 3 µg/L (= 3 ppb since values were given in the original report in this unit) and the maximum value in a farmer was 233 µg/L. Examination of family members revealed that 4 % of the spouses and 12 % of the children had detectable urinary levels of up to 3 or 29 µg/L ([redacted]., 2004, ASB2012-11528). It may be concluded that urine is an important excretion route for glyphosate in humans following mainly dermal and/or inhalative exposure. Abundance of glyphosate in urine indicates systemic exposure and measured values might be used as a suitable basis for calculation of the magnitude of exposure. Based on the extraordinarily high level of 233 µg/L, the highest (individual) estimate for systemic intake in this study was 0.004 mg/kg body weight, *i.e.*, less than 1 % of the proposed ADI and approximately 4 % of the proposed AOEL. For most participants, the received dose can be assumed to be much smaller.~~

~~In a more recent study in 182 citizens from 18 European countries ([redacted] 2013, ASB2013-8037), glyphosate was found in urine samples of nearly one half of the participants at a mean concentration of 0.2 µg/L (0.2 ppb) with a maximum value of 1.82 µg/L and large interindividual differences. Although no details on recruitment of the participants, age, sex or profession were given, it might be presumed that people excreting glyphosate have been exposed via the dietary route. Based on these concentrations, a systemic dose may be calculated that is, for adults, always less than 0.1 % of the proposed ADI. Therefore, no health concern is anticipated.~~
~~In addition, AMPA was found in more than one third of the samples (mean: 0.18 µg/L, maximum: 2.63 µg/L) but the concentrations were not correlated with those of glyphosate. In facct, the source of these traces of AMPA is not known because there is virtually no metabolism of orally absorbed glyphosate. Since the ADI for glyphosate will also cover AMPA, there is no health risk due to residues of this substance.~~

2.6.3 Summary of acute toxicity

Glyphosate acid and its salts have been extensively tested for acute toxicity, skin and eye irritation, and skin sensitisation. An amount of 145 acute studies was submitted either for the previous EU evaluation or in the present GTF dossier.
In contrast to studies of other types, previously submitted acute studies were not re-evaluated so far no concern regarding the toxicological endpoint has been arisen.

2.6.3.1 Acute oral toxicity

For the previous EU evaluation, a large number of oral toxicity studies in rats and mice was submitted that had been conducted with either glyphosate acid or its salts. In the current GTF dossier, a variety of additional studies in rats with administration of glyphosate acid and two more studies in rats and mice with the IPA salt were provided and evaluated by the RMS (see Volume 3, B.6.2.1). These studies have not been reviewed before and confirm the low acute oral toxicity of glyphosate and its salts. General signs of oral intoxication were breathing difficulties, reduced activity, ataxia, piloerection, convulsions and hunched posture.

Overall, the studies on oral toxicity in rats and mice revealed LD_{50} values of at least > 2000 mg/kg bw and, therefore, classification according to criteria of DSD and CLP regulation is not required.

2.6.3.2 Acute dermal toxicity

Similarly, for the previous EU evaluation, a multitude of dermal toxicity studies in rats and rabbits were provided using glyphosate acid and its salts. Additional studies in rats with glyphosate acid were submitted for the current re-evaluation that confirmed the low toxicity after dermal exposure (see Volume 3, B.6.2.2).
Isolated signs of toxicity were body weight loss, diarrhea and slight local effects. The new studies revealed no mortality up to a dermal dose of 5000 mg/kg bw glyphosate acid in rats.

Overall, the studies on dermal toxicity in rats and rabbits submitted either for the previous evaluation or the current re-evaluation of glyphosate and its salts revealed LD_{50} values of at least > 2000 mg/kg bw/d. Therefore, classification according to criteria of DSD and CLP regulation is not required.

2.6.3.3 Acute inhalation toxicity

The former EU evaluation revealed the acute inhalation toxicity (LC_{50}) of glyphosate acid and its IPA salt in rats to be above the limit test dose of 5 mg/L air. For the current re-evaluation, eleven inhalation studies with glyphosate acid and two with glyphosate salts were submitted that confirmed the low acute inhalation toxicity of glyphosate and its salts (see Volume 3, B.6.2.3). Toxic signs after inhalative exposure were mainly irritation of the upper respiratory tract, hyperactivity, increased or decreased respiratory rate, piloerection, loss of hair, wet fur, slight body weight reduction, slight tremor and slight ataxia. It is noteworthy that these signs were not consistently observed throughout the studies.Based on this huge number of valid studies in rats, the inhalative toxicity of glyphosate and its salts is unequivocally low and therefore, classification according to criteria of DSD and CLP regulation is not required.

2.6.3.4 Acute intraperitoneal toxicity

Studies on acute intraperitoneal toxicity are not compulsory and no new studies for the current re-evaluation have been provided. For the previous EU evaluation, supplementary studies on intraperitoneal toxicity of glyphosate acid and salt (most probably IPA salt) in rats and mice had been submitted (DAR, 1998, ASB2010-10302). These studies revealed a higer toxicity compared to other exposure routes with LD_{50} values down to 134 mg/kg bw. The occurrence of late deaths was assumed to be caused by subsequent peritonitis instead of a direct toxic

drug effect. Because i.p. injection is an artificial exposure route with no relevance for operator exposure, these findings are not of concern. A few micronucleus assays with i.p. injection are reported in the genotoxicity sections of Volumes 1 (2.6.5) and Volume 3 (B.6.4) and confirmed that acute toxicity of glyphosate when adminstered via this route was much higher than in oral studies.

2.6.3.5 Skin Irritation

According to the previous EU evaluation, glyphosate acid and its salts were considered non-irritant to intact skin and only slightly irritant to abraded skin. For the current re-evaluation, 13 additional studies with glyphosate acid on rabbits were submitted (see Volume 3, B.6.2.4). Three of the new studies revealed mild or slight irritating effects, however, according to criteria of DSD and CLP regulation , classification for skin irritation is not required. Thus, the previous evaluation was confirmed.

2.6.3.6 Eye Irritation

Eye irritation was examined in numerous studies in rabbits that were performed either with the acid or its different salts.

Glyphosate acid

Due to the rapid occurrence (less than one hour), persistence (cornea opacity and conjunctival lesions not reversible within a period of up to 21 days) and severity (marked cornea opacity up to score 4, sloughing of the cornea, haemorrhage of the lower conjunctival membrane, blood stained discharge) of ocular effects, the previous EU evaluation already concluded glyphosate acid to be strongly irritating to rabbit eyes (for studies, see Table 2.6–2a). Therefore, in the sense of animal welfare considerations, it is impossible to understand why such a high number of additional studies on eye irritating potential in rabbits has been conducted afterwards (Table 2.6-2b).

Anyhow, most of the new submitted studies on eye irritating potential of glyphosate acid supported the previous evaluation. In contrast, the studies by [redacted] (2009, ASB2012-11429, ASB2012-11432, ASB2012-11433) failed to produce irritating effects. However, in those studies the instilled test substance was washed out after one hour instead of 24 hours according to the current OECD Guideline 405).
One of the newly submitted studies ([redacted] 2011, ASB2012-11438) was conducted in one male rabbit only. Due to severe effects observed up to 24 hours after instillation, the substance was considered to be corrosive and the study was therefore terminated.

Table 2.6-2a: Eye irritation tests with glyphosate acid in rabbits reviewed for previous EU evaluation

Reference; Study identification; Owner	Strain, number of animals	Purity	Amount applied	Results / Classification
[redacted] 1994; TOX9500249 Herbex	NZW, 1 female	95%	mg (~ 76 mg)	Due to the strong ocular effects (cornea opacity: score 4; iris: score 1; conjunctival redness: score 2; haemorrhage of the lower conjunctival membrane, blood stained discharge), the test was stopped for human reasons after 1 hour **Irritant**
[redacted] 1994; TOX9650146; Alkaloida	NZW, 4 females	97.2%	100 mg (pure)	Significant ocular lesions, especially chemosis **Irritant**
[redacted] 1991; TOX9551093; ADAMA	NZW, 2 males & 1 female	96.8%	100 mg	Mortality (1 female on day 2: severe enteritis and opacity); significant ocular lesions which were not reversible within 3 weeks **Irritant**
[redacted] 1991; Z101610; I.Pi.Ci.	NZW, 3 males	98 %	100 mg (pure)	Several ocular effects (score 1 and 2) in all animals, reversible within 3 days **Slightly irritant**
[redacted] 1990; TOX9500264; Agrichem	NZW, 3 females	98.1 %	100 mg	Cornea opacity, not reversible within 7 days (2 females); iris lesions, not reversible within 6 days (1 female); conjunctival redness, not reversible within 6 days (3 females) **Slightly irritant**
[redacted], 1989; TOX9552338; Monsanto	NZW, 1 male	98.6 %	100 mg (pure)	Due to strong ocular effects, the test was stopped for human reasons after the treatment of the first of six prepared animals **Irritant**

Table 2.6-2b: New eye irritation tests with glyphosate acid in rabbits reviewed for EU re-evaluation

Reference; Study identification; owner	Strain, number of animals	Purity	Amount applied	Effects / Result
[redacted] 1996; TOX1999-881; Cheminova	NZW, 6 male, 3 females	98.2 %	0.1 mL (65 mg)	Severely irritant in unwashed eyes: corneal opacity, conjunctival redness, chemosis, not reversible within 21 days (2 females); moderate irritation in washed eyes, reversible within 21 days **Irritant**
[redacted] 2007; ASB2012-11428; Nufarm	NZW, 1 male, 2 females	95.1 %	100 mg	Marked, early onset and transient ocular changes (Cornea opacity, conjunctival redness, chemosis), reversible within 10 days, no signs of corrosion or staining **Irritant**
[redacted] 2009; ASB2012-11429; Helm AG	Himalayan, 3 males	96.4 %	100 mg rinsed 1h post appl.	Slight signs of ocular changes, reversible within 7 days **Non-irritant**

Reference; Study identification; owner	**Strain, number of animals**	**Purity**	**Amount applied**	**Effects / Result**
[redacted] 1995; ASB2012-11430; Arysta LifeScience	NZW. 12 females	97.56 %	100 mg (pure)	6 females without eye irrigation: Cornea opacity: not reversible within 21 days (3/6 females); iris lesions: all females and reversible within 10 days; conjunctival redness & chemosis: all females and reversible within 16 days; 6 females with eye irrigation (30 sec. & 2 min. post application): reduced effects and faster recovery **Irritant**
[redacted] 2009; ASB2012-11431; Exxel	Expert statement		Test solution 1% purified	Test not performed because pH of the test solution was < 2 and corrosive properties were assumed **Corrosive**
[redacted] 2009; ASB2012-11432; Helm AG	Himalayan 3 males	98.8 %	100 mg rinsed 1h post appl.	**Non-irritant**
[redacted] 2009; ASB2012-11433; Helm AG	Himalayan 3 males	97.3 %	100 mg rinsed 1 h post appl.	**Non-irritant**
[redacted] 2009; ASB2012-11434; Helm AG	NZW 2 males 1 female	96.4 %	0.1 mL (93.2 mg)	Cornea opacity, iris lesions, conjunctival redness & chemosis reversible within 9 days **Irritant**
[redacted] 2005; ASB2012-11435; Helm AG	NZW 3 males	97.23 %	0.1 mL (60 mg)	All animals: corneal opacitiy, iris lesions, conjunctival redness & chemosis, reversible within 10 days Irritant
[redacted] 2008; ASB2012-11436; Helm AG	NZW 1 male 1 female	98.5 %	100 mg	Only 2 animals due to severe effects: Corneal opacity, iritis, conjunctival hyperemia, edema and secretion. Effects in female not reversible within 21 days **Irritant**
[redacted] 1988; Z35395; Monsanto	NZW 6 animals, likely 3/sex	97.76 %	100 mg	One rabbit died: considered not treatment related Corneal opacitiy, iritis, conjunctival redness, chemosis in 6/6 animals. Some effects not reversible within 21 days **Irritant**
[redacted] 1997; TOX2000-1986; Syngenta	NZW 6 females	95.6 %	100 mg	Corneal opacity, iritis,conjunctival redness and chemosis. All effects reversible within 8 days **Moderately Irritant** (according to Kay & Calandra)
[redacted] 2007; ASB2012-11437; Syngenta	NZW 1male 2 females	96.1 %	100 mg	Mild, early-onset and transient ocular changes (reversible within 7 days) **Irritant**
[redacted] 2011 ASB2012-11438; Syngenta	NZW 1 male	96.3 %	Glyphosate technical 100 mg	Based on results in one animal, study was terminated at 24 h: corneal opacity & erosion; conjunctiva: redness, chemosis, discharge, few black points; oedema of the eyelids; positive fluorescein staining at 24 h **Corrosive**

In conclusion, the former EU evaluation of glyphosate considered glyphosate acid to be severely irritant to rabbit eyes. Recently performed and submitted studies on the eye irritating potential in rabbits supported the previous findings and evaluation of glyphosate acid. Therefore, according to EU criteria, glyphosate acid has to be labelled and classified with Xi ('Irritant') and R41 ('Risk of serious damage to eyes') and corresponding to the GHS criteria with '~~Irreversible effects on the eye /~~ Causes serious damage to eyes (Category 1)', H318.

Glyphosate salts

In contrast to glyphosate acid, its isopropylamine, sodium, or ammonium salts caused only slight eye irritating effects, which were described in detail in the previous DAR and were reported in a summary table (Table B.6.2-49) in Volume 3 of the RAR. No new studies on the

eye irritating potential of glyphosate salts have been submitted for the current EU re-evaluation of glyphosate. Based on the results of available studies, classification according to criteria of DSD and CLP regulation is considered not needed for the salts.

2.6.3.7 Skin sensitisation

The previous EU evaluation revealed that neither glyphosate acid nor the salts exhibited skin-sensitising effects in guinea pigs. However, only the acid and the IPA had been studied at that time under the more stringent conditions of the Magnusson-Kligman Test (see Volume 3, B.6.2.6).

For the current re-evaluation, several additional studies for skin sensitising effects on glyphosate acid were provided: 8 Magnusson-Kligman Tests and 3 Buehler Tests in guinea pigs and two Local lymph node assays in mice. None of these studies (for detailed description, see Vol. 3, B.6.2.6) exhibited sensitising properties of glyphosate acid.
No further studies on glyphosate salts were provided.

In conclusion, all the available data in guinea pigs and mice confirm that glyphosate acid and its salts (IPA salt) do not possess a skin sensitising potential. Therefore, classification is not required.

Studies with formulations
For acute studies (including irritation and sensitisation) of the representative formulation, see sub-section 2.6.14. Data obtained with other formulations should be assessed on MS or zonal level for product authorisation and are beyond the scope of this RAR.

2.6.4 Summary of short-term toxicity

2.6.4.1 Subacute studies in different species and via different routes

Glyphosate was administered in few subacute studies (duration 14 or 28 days) by the oral route to rats and dogs and by the dermal route to rats and rabbits.

Toxicity upon dietary administration to rats was very low with only minor effects such as soft faeces or alterations in haematological and clinical chemistry parameters at high dose levels. The lowest NOAEL of 50 mg/kg bw/day ([redacted] 1989, TOX9552351) was mainly based on a higher incidence of nephrocalcinosis but this finding was not confirmed in a subsequent 90-day study in the same laboratory and rat strain ([redacted] 1991, TOX9552364) and, therefore, cannot be attributed to glyphosate administration. In dogs, there were no treatment-related findings observed up to 1000 mg/kg bw/day ([redacted] 1989, TOX9552352). The subacute oral studies did not indicate a need for setting an ARfD.

In both Sprague-Dawley ([redacted] 1993, TOX9552367) and Wistar-derived rats ([redacted] 1996, ASB2012-11461) as well as in NZW rabbits ([redacted] 1982, TOX9552366; [redacted] 1994, TOX9650151), no signs of systemic toxicity became evident following repeated application of glyphosate to the skin over a period of 3 or 4 weeks up to the highest tested dose levels of 1000 mg/kg bw/day in the rat and 5000 mg/kg bw/day in the rabbit. However, weak dermal irritation was observed at these high dose levels in both species ([redacted] 1982,

TOX9552366; [redacted] 1993, TOX9552367). Based on these results, there is no need to perform dermal toxicity studies of longer duration. Subchronic studies (90 days or longer) were conducted by the oral route only.

No valid short-term inhalative studies with the active ingredient is available. Since glyphosate is not volatile [VP = 1.31 x 10^{-5} Pa (25°C)], the conduct of 28- or 90-day inhalation studies, in principle, is not needed according to EU requirements. In addition, there was no concern about inhalation toxicity coming from acute studies. Therefore, the absence of valid subacute inhalation studies is not considered a data gap. A subacute inhalative study with a formulation is briefly mentioned below and reported in detail in the Appendix on tallowamines that may be found in Volume 3.

2.6.4.2 Oral subchronic (90 days and longer) studies

- Rat

All the studies that were used for current evaluation are summarised in Table 2.6–3a and Table 2.6–3b.

Table 2.6-3a: Valid oral subchronic studies in rats that were used for previous EU evaluation

Reference; Study identification; Batch, purity; Owner	Strain, duration, route	Dose levels	NO(A)EL	LO(A)EL	Main effects	Outcome of re-evaluation
[redacted], 1991; TOX9552364; Batch 206-JaK-25-1, 98.6%; Cheminova	Sprague-Dawley, 90 d, feeding	0-20-300-1000 mg/kg bw/d (dietary levels weekly adjusted)	300 mg/kg bw/d	1000 mg/kg bw/d	Bw gain↓ in m, urine pH↓ and some changes in clinical chemistry parameters in f ; m/f: cellular alterations in parotid salivary glands	Study acceptable; NOAEL confirmed
[redacted] 1993; TOX9650149; Lot 46540992, purity not given; Alkaloida#	Sprague-Dawley, 90 d, feeding	0, 2000, 6000, 20000 ppm	371 mg/kg bw/d (6000 ppm)	1262 mg/kg bw/d (20000 ppm)	Diarrhea in m/f; blood in urine; organ wt changes without pathological findings	Study supplementary, NOAEL increased from 2000 to 6000 ppm
[redacted] 1992; TOX9551096; Batch 60, 96.8%; ADAMA#	Wistar, 90 d (+28 d recovery, hig dose), feeding	0, 200, 2000, 20000 ppm (+20000 ppm for recovery) group)	147 mg/kg bw/d (2000 ppm)	1359 mg/kg bw/d (20000 ppm)	Bw gain↓ in f; AP activity↑ in m, glucose↑ in f	Study supplementary, NOAEL confirmed
[redacted] 1989; TOX9551821; Batch L16566, 97.1%; Barclay	Sprague-Dawley (CD), 90-92 d (+35 d recovery for additional control and top dose groups)	0, 2000, 3000, 5000, 7500 ppm (+ 7500 ppm for recovery)	7500 ppm (375 mg/kg bw/d assumed, mean dietary intake not caclculated)	> 7500 ppm	No effects up to highest dose	Study acceptable, NOAEL confirmed
[redacted] 1987; TOX9552362; Lot XLG 161, 95.2%; Monsanto	Sprague-Dawley, 90 d, feeding	0, 1000, 5000, 20000 ppm	1267 mg/kg bw/d (20000 ppm)	>1267 mg/kg bw/d (20000 ppm)	No effects up to highest dose	Study acceptable, NOAEL confirmed

supplementary study

Table 2.6-3b: More recent subchronic oral studies in rats that were first submitted for new EU evaluation

Reference; Study identification; Batch, purity; Owner	Strain, duration, route	Dose levels	NOAEL	LOAEL	Targets / Main effects
[redacted] 1996; TOX2000-1990; P15, 97.4%; Syngenta*	Wistar-derived (Alpk:APfSD), 90 d, feeding	0, 1000, 5000, 20000 ppm	414 mg/kg bw/d (5000 ppm)	1612 mg/kg bw/d (20000 ppm)	Bw gain↓ in m; alterations in some clinical chemistry parameters, in particular AP/ALAT activity↑, urine pH↓
[redacted] 1996; ASB2012-11451; H95D 161 A, 95.3%; Nufarm	Sprague-Dawley (CD), 90 d, feeding	0, 1000, 10000, 50000 ppm	79 mg/kg bw/d (1000 ppm)	730 mg/kg bw/d (10000 ppm)	Soft faeces, diarrhea; bw gain, food consumption, food efficiency↓ and hemoglobin in urine at top dose level, urine pH↓; alterations in some clinical chemistry parameters, in particular AP activity↑ and Ca↓ at mid and high dose levels; caecum: distention (top dose groups) and mucosal atrophy (at the two upper dose levels)
[redacted] 1995; ASB2012-11452; Batches: 940908, 95.7%; 941209, 95%; T-941209; 97.6%; Arysta	Sprague-Dawley (Crj: CD), 90 d, feeding	0, 3000, 10000, 30000 ppm	168 mg/kg bw/d (3000 ppm)	569 mg/kg bw/d (10000 ppm)	Bw gain↓ in m; alterations in some clinical chemistry parameters, in particular AP activity↑, urine pH↓; caecum: distention and wt (with contents)↑

* New study for EU evaluation but previously submitted for 2004 JMPR evaluation (ASB2008-6266)

Taken together, all these studies have demonstrated low toxicity of glyphosate in different rat strains upon repeated oral administration. It is not appropriate to select one of the fully acceptable studies as the most relevant. They all must be taken into account.

Soft stools and diarrhea, together with occasionally reduced body weight gain, might suggest some irritation of the gastrointestinal tract at high dose levels that is not unexpected for a compound of acidic properties and known irritancy at least to the eyes. In the same studies, blood ([redacted] 1993, TOX9650149) or hemoglobin ([redacted] 1996, ASB2012-11451) were observed in urine at high dose levels. A decrease in urine pH was rather frequently noted.
All these findings may be assumed to result from physico-chemical properties of glyphosate but this does not necessarily mean that they were not adverse. The same holds true for parotid salivary gland findings reported by [redacted] (1991, TOX9552364). Histological alterations comprised deep basophilic staining and enlargement of cytoplasm at all dose levels including very few control animals but were clearly more pronounced with regard to incidence and severity at the top dose level in males and females. They were not accompanied by organ weight changes neither of the parotid nor of the sublingual or submaxillary glands. In the latter two glands, no histopathological changes were noted. The absence of indications for such changes in other studies may be explained by the fact that different or no glands had been examined. [redacted] (1993, TOX9650149) reported swelling and reddening of sublingual salivary glands in a few animals but no dose response became apparent and histological examination did not reveal any remarkable findings. Salivary glands were not weighed. [redacted]

(1989, TOX9551821) and [redacted] (1992, TOX9551096) did not report pathological changes in the salivary glands (not further specified). [redacted] (1987, TOX9552362) examined the submaxillary gland only but did not detect any pathological changes. In the more recent studies by [redacted] (1996, TOX2000-1990) and [redacted] (1996, ASB2012-11451), salivary glands were reported to be taken but were apparently not weighed or examined histologically. [redacted] (1995, ASB2012-11452) performed histopathology of the sublingual and submaxillary glands without any remarkable findings but left the parotid gland aside. [redacted] (1992, TOX9551954), however, published a study in F344 rats in which they reported basophilic changes and hypertrophy of acinar cells in the submaxillary and, more pronounced, the parotid salivary glands at all dose levels (ranging from 3125 to 50000 ppm). Severity of these findings were clearly related to dose and, based on severity, the NOAEL was set at 6250 ppm, equal to about 400 mg/kg bw/day (JMPR, 2004, ASB2008-6266). These findings directly support the observations by [redacted] (1991, TOX9552364).

Alterations in clinical chemistry parameters in the majority of experiments, most often a higher activity of alkaline phosphatase, suggested a weak effect on the liver.

Two more recent studies ([redacted] 1995, ASB2012-11452; [redacted] 1996, ASB2012-11451) identified the caecum as an additional target organ because of certain findings (distention, elevated weight of this part of the intestines and its contents, mucosal atrophy) that had not been noticed before. Even if a specific vulnerability of Sprague-Dawley rats would be assumed, it is difficult to explain why such changes were not observed previously at higher dose levels by [redacted] (1987, TOX9552362), [redacted] (1991, TOX9552364) or [redacted] (1993, TOX9650149). One might expect that at least caecal distention would have been observed and reported if it had occurred.

It must be emphasised that toxic effects of glyphosate were confined to rather high doses. The large differences in the NOAELs/LOAELs are due to dose spacing. When the database is considered in the whole, it may be concluded that a robust "overall NOAEL" in the rat is in a magnitude ranging from 300 to slightly more than 400 mg/kg bw/day. For risk assessment purposes, where a "precise" figure may be needed, the ~~figure~~ NOAEL of 414 mg/kg bw/day ([redacted] 1996, TOX2000-1990) should be used, *i.e.*, the highest numeric value in this magnitude. This is well below the lowest LOAEL that had been ever established, *i.e.*, 569 mg/kg bw/day as reported by [redacted] (1995, ASB2012-11452). Effects at higher dose levels are relatively minor in nature but differ among the studies, depending on rat strain, laboratory and perhaps also test material (*e.g.*, impurities).

- <u>Mouse</u>

Toxicity of glyphosate to mice was investigated in a small number of subchronic studies. The NOAEL in the most recent valid 90-day study was 1221 mg/kg bw/day ([redacted] 1995, ASB2012-11453). A very high dose of *ca.* 6300 mg/kg bw/day caused a reduction in body weight gain, food consumption and efficiency and alterations in some hematological and clinical chemistry parameters with the latter findings pointing to liver toxicity. Gross necropsy revealed caecum distention that was supported by a higher organ weight but not accompanied by histological lesions. Cystitis of urinary bladder became histologically apparent in some high dose males. Urinary pH (most likely due to acidic properties of the test substance) was noted in all treated male groups. In a previous study ([redacted] 1991, TOX9552363), no effects were observed up to the highest dose level of 4500 mg/kg bw/day. While these two studies would suggest a lower toxicity in mice than in the rat, a published one from the U.S. NTP ([redacted], 1992, TOX9551954) provided a lower NOAEL of

about 500 mg/kg bw/day in another strain, based on histological changes in the parotid gland at about 1065 mg/kg bw/day and above. The findings comprised increased basophilia but also enlarged cells and acini with relativ redution in the number of acinar ducts. In the regulatory studies by [redacted] (1995, ASB2012-11453) or [redacted] 1991, TOX9552363), no effects on sublingual or submaxillary glands were noted but the parotid gland gland was not examined although it is obviously more sensitive to histological changes caused by glyphosate. Taking the salivary gland findings into account, toxicity of glyphosate acid in the mouse might be similar to that in the rat.

- Dog

In the dog, short-term toxicity (if compared to the life-expectancy of the species) of glyphosate was investigated in a number of (mostly quite recent) oral studies with oral administration, either via capsules or in the diet. The valid subchronic dog studies (90 days … 1 year) on which this evaluation is based are summarised in Table 2.6–4.

Table 2.6-4: Subchronic oral studies with glyphosate acid in dogs

Reference; Study identification; Batch, purity; Owner	Breed, duration, route	Dose levels	NOAEL	LOAEL	Targets / Main effects
[redacted] 2007; ASB2012-11454; H05H016A, 95.7%; Nufarm	Beagle, 13 wk, oral capsules	0, 30, 300, 1000 mg/kg bw/d	300 mg/kg bw/d	1000 mg/kg bw/d	Clinical signs (liquid/soft faeces, dehydration, vomiting) making termination of high dose groups after 11 wk necessary; bw/bw gain and food consumption↓; clinical chemistry and urine parameters altered; prostate aund uterus atrophy; histological lesions in many organs (such as kidney liver, bone marrow) related to moribund state
[redacted] 1999; ASB2012-11455; Lots 01/12/1997 and 01/06/1997, >95% both; ADAMA	Beagle, 90 d, dietary	0, 200, 2000, 10000 ppm (equal to 5.2/5.4; 54.2/52.8, 252.4/252.7 mg/kg bw/d in m/f)	252 mg/kg bw/d	> 252 mg/kg bw/d	No adverse effects up to highest dose level
[redacted] 1996; ASB2012-11456); T940308, 94.61%; Arysta	Beagle, 13 wk, dietary	0, 1600, 8000, 40000 ppm (*ca* 40, 198/201, 1014/1015 mg/kg bw/d in m/f)	1014 mg/kg bw/d	> 1014 mg/kg bw/d	Decrease in urine pH in high dose females not regarded as adverse; no further effects
[redacted] 1996*; TOX2000-1991; Lots D4490/1, P18, 99.1%; Syngenta	Beagle, 90 d, dietary	0, 2000, 10000, 50000 ppm (68/68, 323/334, 1680/1750 mg/kg bw/d in m/f)	323 mg/kg bw/d	1680 mg/kg bw/d	Bw gain↓; alterations in some clinical chemistry parameters (calcium, albumin↓ in m, AP↑ in f); liver wt↑
[redacted] 2008; ASB2012-11457; H05H016A, 95.7%; Nufarm	Beagle, 52 wk, capsules	0, 30, 125, 500 mg/kg bw/d	500 mg/kg bw/d	> 500 mg/kg bw/d	No adverse effects, calcium↓ in high dose m
[redacted] 1997; ASB2012-11458; T-950380, 94.61%; Arysta	Beagle, 12 mo, dietary	0, 1600, 8000, 50000 ppm (34/37, 182/184, 1203/1259 mg/kg bw/d in m/f)	182 mg/kg bw/d	1203 mg/kg bw/d	Bw gain↓, loose stool, alterations in some hematological and clinical chemistry parameters
[redacted] 1996*;	Beagle, at	0, 3000, 15000,	447 mg/kg	926 mg/kg	Bw gain↓ in f

Reference; Study identification; Batch, purity; Owner	Breed, duration, route	Dose levels	NOAEL	LOAEL	Targets / Main effects
TOX2000-1992; P24, 95.6%; Syngenta	least one year, dietary	30000 ppm (*ca* 91, 440/447, 907/926 mg/kg bw/d in m/f)	bw/d	bw/d	
[redacted] 1991[§]; TOX9552384; 206-JaK-25-1, 98.6%; 206-JaK-95-5, 99.5%; 229-JaK-5-1, 98.9%; Cheminova (/Monsanto)	Beagle, 52 wk, oral capsules	0, 30, 300, 1000 mg/kg bw/d	300 mg/kg bw/d	1000 mg/kg bw/d	Soft/loose/liquid stool, evidence of lower bw gain (not attending statistical significance)

* New study for EU evaluation but previously submitted for 2004 JMPR evaluation (ASB2008-6266)
[§] Study was available and reviewed during previous EU evaluation yet

In the whole, the results have shown that the dog is of similar sensitivity as the rat when the NOAELs/LOAELs are considered. However, high dose effects may be more severe than in rats or mice but appear somehow inconsistent among the studies.

In the most recent 90-day study by [redacted] (2007, ASB2012-11454), severe signs of toxicity were noted in the high dose groups receiving 1000 mg/kg bw/day. The test item administration induced marked clinical signs (liquid/soft faeces, dehydration, thin appearance, vomiting and pallor), caused lower body weight gain (males) and body weight loss (females) and reduced food consumption. This led to the early sacrifice of two moribund animals, and to the early termination of the entire group at week 11. Treatment-related histopathological changes in surviving animals consisted of an increased number of adipocytes in the sternal bone marrow in both sexes, as well as prostate and uterine atrophy and other, more infrequent changes in various organs. It is clear that the MTD was by far exceeded. Surprisingly, in the study by [redacted] (1991, TOX9552384), the same high dose of 1000 mg/kg bw/day was administered also in capsules for one year causing only minor effects. There is no explanation for this apparent differences although it is known from long-term studies in rats and mice that high-dose effects of glyphosate may differ considerably. A lower purity (and other source) of the test material applied by [redacted] (2007, ASB2012-11454) might be also relevant. In any case, it should be noticed that this dose level is by 2000 times above the proposed ADI and that no strong effects were seen in dogs at lower dose levels, independent from the application method.

In 90-day studies with dietary administration, very few findings were obtained. [redacted] (1999, ASB2012-11455) reported an initial decline in food consumption and body weight gain but normalisation to control levels was soonly achieved. The only clinical chemistry alteration that was likely related to treatment, *i.e.*, a higher bilirubin concentration, was not accompanied by any pathological change. Thus, these effects were not regarded as adverse.
[redacted] (1996, ASB2012-11456) did not find any effects (apart from a reduction in urine pH due to acidic properties of the test substance) in a study in which even higher dietary dose levels of up to 40000 ppm were employed. In the studies of [redacted] (1991, TOX9552384) and [redacted] (2007, ASB2012-11454), similarly high dose levels of 1000 mg/kg bw/day as by [redacted] (1996, ASB2012-11456) were included. A comparison of the three studies suggests that glyphosate is better tolerated when administered via the diet than in capsules.
In the study by [redacted] (1996, TOX2000-1991), weak toxic effects were noted at the exaggerated top dose of 50000 ppm, including a decrease in body weight gain and some

evidence of liver toxicity. The next lower dietary level of 10000 ppm (ca 320 mg/kg bw/day) was considered the NOAEL.

According to new EU data requirements for pesticides, and in contrast to the situation in other parts of the world, a one-year dog study is not mandatory any longer. However, if available, such data must be submitted and should be taken into consideration for risk assessment.

In these studies, effects were confined to high dietary doses of more than 500 mg/kg bw/day, even when the compound was administered in capsules. Compound–related findings comprised lower body weight gain, rather slight alterations in clinical chemistry and haematological parameters as well as a lower urine pH and clinical signs that indiate gastrointestinal irritation or disturbances.

In a 6-month study in which the IPA salt was administered to male and female Beagle dogs in gelatine capsules, [redacted] (1983, TOX9552361) did not find other effects than an increase in AP activity at the highest dose level of 300 mg/kg bw/day and an equivocal (not significant) decrease in body weight gain in males. In the absence of concomitant findings, this dose was considered the NOAEL suggesting that the toxicity of this salt to dogs was not higher than that of the acid. A higher dose of the IPA salt (MON 0139), from 625 mg/kg bw/day onwards, however, caused strong gastrointestinal irritation when given to Beagle dogs for five days ([redacted] 1982, TOX9552349). Clinical signs comprised diarrhea, vomiting and a decrease in body weight gain. A similar NOAEL of 312.5 mg/kg bw/day as in the 6-month study was established with a slightly lower food consumption being the only effect. It is likely that the gastrointestinal effects were not or only partly attributable to glyphosate because separate (single or repeated) administration of isopropylamine at a dose level of 72 mg/kg bw resulted in strong irritation of the gastric and oesophageal mucosa.

When all this data is taken into consideration, it seems reasonable to assume an "overall" NOAEL of 300 mg/kg bw/day for the short-term toxicity of glyphosate acid (and its IPA salt) in dogs with a LOAEL of more than 500 mg/kg bw/day.

Studies with formulations/Published data

A subacute inhalation study with the formulation MON 2139 in rats ([redacted] 1983, TOX2002-694) suggested a rather high inhalative toxicity of a glyphosate-containing product, most likely due to a tallowamine surfactant.. For description and discussion of this study, see the appendix on tallowamines in chapter B.6.13 of Volume 3.

In the open literature, no ~~further~~ short-term toxicity studies with glyphosate-based herbicides have been found.

2.6.5 Summary of genotoxicity

2.6.5.1 *In vitro*

The ability of glyphosate to cause gene/point mutations in bacteria was investigated in numerous studies by means of the Ames test. The available studies were all run with and without metabolic activation, using liver S9 mix to mimic *in vivo* liver metabolism. Because of quality problems, only two of the studies that had been previously evaluated (DAR, 1998, ASB2010-10302) could be still included in the current evaluation ~~following re-evaluation~~. However, many new studies have been submitted meanwhile. In sum, assessment for this genotoxicity endpoint is based on a total of ~~14~~ 16 valid studies with the active compound. Although test materials from various sources (manufacturers) and different purities were

applied, the result was consistently and unequivocally negative. All the valid studies are compiled in Table B.6.4-1 of Volume 3.

~~2.6.5.2 *In vitro*~~

Absence of genotoxicity *in vitro* was also confirmed in a small number of studies in mammalian cells, *i.e.*, in mouse lymphoma assays (Jensen, 1991, TOX9552372; Clay, 1996, TOX2000-1994) and in an HGPRT test (Li, 1983, TOX9552369). In an UDS assay in rat hepatocytes (Rossberger, 1994, TOX9400697) and in a Rec assay in *Bacillus subtilis* (Akanuma, 1995, ASB2012-11477), there was no impact on DNA damage and repair. No evidence of clastogenicity was obtained in the four available valid *in vitro* studies in human lymphocytes (Van de Waart, 1995, TOX9651525; Fox, 1998, TOX2000-1995) or Chinese hamster lung cells (Kyomu, 1995, ASB2012-11475; Wright, 1996, ASB2012-11476).

2.6.5.2 *In vivo*

Extensive testing of glyphosate for chromosome aberrations was performed *in vivo* by means of ~~9~~ 11 valid micronucleus assays or cytogenicity studies that all examined the bone marrow of either mice or rats. Five more ~~(negative)~~ studies (inclusing two that were submitted for the first time for the current re-evaluation) had to be excluded because of severe deficiencies in conduct or reporting or because dose levels were too low for meaningful analysis. A detailed explanation for rejecting the two new studies is given in Volume 3 (B.6.4.5).

General suitability of the bone marrow examinations is proven by the affinity of glyphosate to bone tissue as shown in the ADME studies, by the occasional observation of bone marrow toxicity in the tests themselves (*e.g.*, by [redacted], 1994, TOX9400323) and by the occurrence of hypoplasia in bone marrow in a long-term study in rats although at a very high dose ([redacted]., 2009; ASB2012-11490, see below). Thus, one can be convinced that the target tissue in these studies was actually exposed to the test compound.
As reported and discussed in the old DAR (1998, ASB2010-10302) already, a micronucleus test performed by [redacted] (1993, TOX9551100) indicated a weak increase in the incidence of micronuclei in females but not in males at the very high dose of 5000 mg/kg bw that was administered on two consecutive days (Table 2.6–5). This finding is somehow surprising since male mice are usually considered more sensitive with regard to micronucleus formation. Furthermore, a cytogenetic study conducted in the same laboratory and the same mouse strain under nearly identical conditions did not provide any evidence of chromosome aberrations. In this trial, test material of the same purity (although another batch) was applied at the same dose levels ([redacted] 1994, TOX9400323). Instead, a certain degree of cytoxicity to bone marrow cells at the highest dose level became apparent since the mitotic index was reduced. Although not measured in the preceding micronucleus test, such an effect could be expected for the this previous experiment, too, and cytotoxicity might have contributed to micronucleus formation. Last but not least, a statistical significance level of $p<0.1$ is not that convincing.

Table 2.6-5: Erythrocyte differentiation and incidence of micronuclei in bone marrow smears of Swiss albino mice at 24 h following two oral doses on two consecutive days ([redacted] 1993, TOX9551100)

Dose group	Sex	Ratio PCE:NCE	% PCE with micronuclei	% NCE with micronuclei	Total percentage of erythrocytes with micronuclei
Vehicle control	Males	1 : 1.1	0.69	0.62	0.65
Glyphosate, 50 mg/kg bw/d	Males	1 : 1.1	0.84	0.64	0.73
Glyphosate, 500 mg/kg bw/d	Males	1 : 1.2	0.73	0.22	0.45
Glyphosate, 5000 mg/kg bw/d	Males	1 : 1.3	0.89	0.47	0.65
Cyclophosphamide, 100 mg/kg bw/d	Males	1 : 1.9	2.33*	1.18*	1.58*
Vehicle control	Females	1 : 1.2	0.51	0.39	0.44
Glyphosate, 50 mg/kg bw/d	Females	1 : 1.3	0.28	0.15	0.21
Glyphosate, 500 mg/kg bw/d	Females	1 : 1.2	0.52	0.23	0.36
Glyphosate, 5000 mg/kg bw/d	Females	1 : 1.3	1.05*	0.46	0.72*
Cyclophosphamide, 100 mg/kg bw/d	Females	1 : 1.9	2.39*	1.65*	1.90*

* p<0.1, contingency test und t-test; PCE polychromatic erythrocytes, NCE normochromatic erythrocytes

[redacted] (1991, TOX9552374) as well as [redacted] (1996, TOX2000-1996) did not observe an increase in micronucleus frequency following administration of the same high oral dose of 5000 mg/kg bw. However, their studies were performed in different mouse strains and the test compound was given only once. All the other micronucleus assays cannot be used for direct comparison because dose levels were lower and/or the application route was different (i.p. versus oral). Nontheless, it should be noted that the tests by [redacted] (1999, ASB2012-11482) and by [redacted] (2008, ASB2012-11483) as well as the most recent by [redacted] (2012, ASB2014-9277) and [redacted] (2012, ASB2014-9333) were unequivocally negative. A statistically significant increase in PCEs was observed by [redacted] (2006, ASB2012-11478) after single i.p. injection of 600 mg/kg bw to CD-1 mice. However, this response was modest and within the historical range for vehicle control animals and, therefore, was not considered relevant. The two available studies in rats, *i.e.*, a cytogenetic study by [redacted] (1983, TOX9552375) and a micronucleus assay by [redacted] (2009, ASB2012-11479) were both negative, too. Thus, taking the weight of evidence approach, the RMS concludes that glyphosate is not clastogenic *in vivo.* An overview on the whole database for the genotoxicity endpoint ”chromosome aberrations *in vivo”* is given in Table 2.6–6.

Table 2.6-6: Summary of valid *in vivo* micronucleus assays or cytogenetic studies with glyphosate acid in mice and rats

	Reference; Study identification; Owner	Type of study	Test organism / test system, route/ treatment	Dose levels; batch/lot, purity; sampling	Results
Studies from the previous evaluation	[redacted] 1994; TOX9400323; ADAMA	Cytogenicity in bone marrow	Swiss albino mice; daily oral applications for 2 successive days	0, 50, 500, 5000 mg/kg bw/day; batch 046, 96.8%; sampling 24 h after second dose	Negative; mitotic index ↓ at 5000 mg/kg bw
	[redacted] 1993; TOX9551100; ADAMA	Micronucleus test in bone marrow	Swiss albino mice; daily oral applications for 2 successive days	0, 50, 500, 5000 mg/kg bw/day; batch 60, 96.8%; sampling 24 h after second dose	♂: negative ♀: weakly positive at highest dose
	[redacted] 1991; TOX9552374; Cheminova	Micronucleus test in bone marrow	NMRI mice, single oral application	0 – 5000 mg/kg bw; 206-JaK-25-1, 98.6%; sampling after 24, 48, 72 h	Negative
	[redacted] 1983; TOX9552375; Monsanto	Cytogenicity in bone marrow	Sprague-Dawley rats, single i.p. injection	0 – 1000 mg/kg bw; XHJ-64, 98.7%; sampling after 6, 12, 24 h	Negative
Studies not reviewed in the previous evaluation	[redacted] 2006; ASB2012-11478; Nufarm	Micronucleus test in bone marrow	CD-1 mice ♂; single i.p. dose	0, 150, 300, 600 mg/kg bw; H05H016A, 95.7%; sampling after 24 and 48 h	Stat. sign. ↑ in PCE at 600 mg/kg bw (24 h) but within historical control; overall: negative
	[redacted] 1999; ASB2012-11482; Nufarm	Micronucleus test	Swiss albino mice, ♂ + ♀, two i.p. injections (24 h interval)	0, 187.5, 375, 562.5 mg/kg bw; batch 037-919-113, 95.49 %; sampling 24 h after 2nd application	Negative
	[redacted], 1996; TOX2000-1996; Syngenta	Micronucleus test in bone marrow	CD-1 mice, 5 ♂+ 5 ♀/dose / sampling point; single oral dose	0, 5000 mg/kg bw; P24, 95.6%; sampling after 24 and 48 h	Negative
	[redacted] 2008; ASB2012-11483; Syngenta	Micronucleus test in bone marrow	NMRI mice 6 ♂/dose/sampling point; single oral dose	0, 2000 mg/kg, sampling after 24 and 48 h, 500 & 1000 mg/kg bw sampling after 24 h only; Batch 20070545, 99.1%	Negative
	[redacted] 2009b; ASB2012-11479; Helm	Micronucleus test in bone marrow	CD rat, single oral application	0, 500, 1000, 2000 mg/kg bw/day; batch 20080801, 98.8%; sampling after 24 and 48 h	Negative
	[redacted] 2012; ASB2014-9277; Dow	Micronucleus test in bone marrow	Swiss albino mice, ♂, two oral. injections (24 h interval)	0, 2000 mg/kg bw, sampling after 24 h; Lot 20061109, 98.9%	Negative
	[redacted] 2012; ASB2014-9333; Syngenta	Micronucleus test in bone marrow	NMRI mice 7 ♂/ sampling point; single oral dose	0, 2000 mg/kg bw, sampling after 24 and 48 h; Batch 56753, 96.3%	Negative

Studies in germ cells are usually not required if no genotoxic potential in somatic cells was observed. However, absence of such effects on male germ cells was additionally confirmed for glyphosate in two dominant-lethal tests in rats ([redacted] 1992, TOX9551102) and mice ([redacted] 1980, TOX9552377) that were both negative up to the highest dose levels of 5000 or 2000 mg/kg bw/day.

Based on the genotoxicity tests in the whole, and taking into consideration the outcome of long-term and developmental studies discussed below, the overall assesssment is that glyphosate is not a genotoxic carcinogen and that no teratogenicity/developmental toxicity due to genetic changes can be expected.

Studies with formulations/Published data

An earlier review of the toxicity of glyphosate and the original Roundup™ formulation concluded that neither glyphosate nor this formulation pose a risk for the production of heritable/somatic mutations in humans (Williams *et al.*, 2000, ASB2012-12053). An addendum of the RMS to the previous DAR (2000, ASB2013-2748, a slightly amended version may be found in Volume 3 under B.6.4.7) provided strong evidence that positive results obtained with different formulations in some test systems were likely due to cytotoxicity and perhaps even DNA reactivity of certain co-formulants but certainly not attributable to glyphosate itself.

A new review of more recent (since 2001) genotoxicity publications was submitted in this EU re-evaluation and included analysis of study methodology and incorporation of all the findings into a weight of evidence approach for genotoxicity (for details, see Volume 3, B.6.4.8).
As reviewed by Williams *et al.* (2000, ASB2012-12053), most gene mutation studies for glyphosate and glyphosate-based formulations were negative. Of fifteen gene mutation assays reported, there were only two positive observations. Subsequent to this review, only two new gene mutation studies have been published, both with negative or inconclusive results. These publications on gene mutation provided only limited additional information that was suitable for assessment of genotoxicity of glyphosate.
A large number of publications adressed chromosome aberrations. The weight of evidence from *in vitro* and *in vivo* mammalian studies supports the earlier conclusion that glyphosate and glyphosate-based formulationss are predominantly negative for this endpoint category. Exceptions were mostly observed in unusual test systems but there are also some unexplained discordant positive results in more widely used mammalian systems. However, these occasional findings are by far outweighed by the negative high quality studies reported above.

Likewise, several reports of positive results for DNA damage endpoint by means of the SCE (sister chromatid exchange), the alkaline SCGE (single cell gel electrophoresis) and the comet assay have been published for glyphosate and certain formulations. The data suggest that these effects were likely due to cytotoxic effects (*e.g.*, of surfactants) at high concentrations rather than to DNA reactivity.

A new comprehensive review on genotoxicity studies of glyphosate and glyphosate-based formulations was submitted by Kier and Kirkland (2013, ASB2014-9587). The authors concluded that an overwhelming preponderance of negative results in well-conducted bacterial reversion and *in vivo* mammalian micronucleus and chromosomal aberration assays indicates that glyphosate and its formulations were not genotoxic in these core assays. Negative results for *in vitro* gene mutation and a majority of negative results for chromosomal effect assays in mammalian cells add to the weight of evidence that glyphosate was not genotoxic. Mixed results were observed for micronucleus assays of formulations in non-mammalian systems. Reports of positive results for DNA damage endpoints indicate that some formulations tend to elicit DNA damage effects at high (toxic) dose levels but the data suggest that this is due to cytotoxicity rather than to DNA interaction, perhaps associated with the surfactants present in many products.
Taking a weight of evidence approach, it may be concluded that there is no *in vivo* genotoxicity and mutagenicity potential of glyphosate or its formulations to be expected under normal exposure scenarios, *i.e.*, below toxic dose levels.

2.6.6 Summary of long-term toxicity and carcinogenicity

Long-term toxicity and carcinogenicity of glyphosate were investigated in a large number of studies in rats and mice that were performed over the course of time on behalf of different notifiers. This situation, on one hand, may enhance reliance on the results but, on the other, will make evaluation difficult because all valid studies with partly different effects must be taken into account and differences due to dose spacing may play a role when overall NOAELs/LOAELs have to be set. For the previous EU evaluation, a total of 4 studies in rats and four in mice were available and reported in the DAR (1998, ASB2010-10302). All these studies were subject to rigorous re-evaluation for purposes of this RAR including an assessment of their quality and reliability according to current standards and it was checked if the NOAEL could be confirmed. The outcome of re-evaluation may be found in the summary Table 2.6–7a (rat) and Table 2.6–8a (mouse). For the new evaluation, five chronic or combined chronic toxicity/carcinogenicity studies in rats and three long-term studies in mice were additionally provided. These new studies are summarised in Table 2.6–7b (rat) and Table 2.6–8b (mouse). A few of them had been already reviewed by WHO/FAO for its 2004 evaluation of glyphosate (JMPR, 2004, ASB2008-6266).

2.6.6.1 Rat

Table 2.6-7a: Long-term studies in rats that were used for previous EU evaluation

Reference; Study identification; Batch, purity; Owner	Study type, strain, duration, route	Dose levels	NO(A)EL*	LO(A)EL*	Targets/ Main effects	Outcome of re-evaluation
[redacted] 1993; TOX9750499; 229-JaK-5-1, 98.9% and 229-JaK-142-6, 98.7%; Cheminova	Combined chronic toxicity/ carcinogenicity; 2 yr; Sprague-Dawley; feeding	0, 10, 100, 300, 1000 mg/kg bw/d (dietary levels regularly adjusted)	10 mg/kg bw/d (NOEL)	100 mg/kg bw/d (LOEL)	Bw gain↓, AP activity↑, urine pH↓, salivary glands: wt↑ and histo-logical fin-dings, liver wt↑	Study acceptable; NOAEL 100 mg/kg bw; LOAEL 300 mg/kg bw/d (assuming rele-vance at least of pronounced salivary gland findings and because of AP↑ and liver wt↑ from 300 mg/kg bw/d onwards)
[redacted] 1996; TOX9651587; 2 batches used, 96.8/96.0%; ADAMA	Combined chronic toxicity/ carcino-genicity; 2 yr; Wistar; feeding	0, 100, 1000, 10000 ppm (6.3/8.6, 59.4/88.5, 595.2/886 mg/kg bw/d in m/f)	6.3 mg/kg bw/d (100 ppm, NOEL) 60 mg/kg bw/d (NOAEL)	60 mg/kg bw/d (LOEL) 600 mg/kg bw/d (LOAEL)	AP activity↑ (f), slight in-crease in cataracts (m, no clear dose response in f)	Study acceptable; NOAEL 60 mg/kg bw/d (based on cataracts)

Reference; Study identification; Batch, purity; Owner	Study type, strain, duration, route	Dose levels	NO(A)EL*	LO(A)EL*	Targets/ Main effects	Outcome of re-evaluation
[redacted], 1990; TOX9300244; XLH-264, 96.5%; Monsanto	Combined chronic toxicity/ carcinoge-nicity; 2 yr; Sprague-Dawley; feeding	0, 2000, 8000, 20000 ppm (89/113, 362/457, 940/1183 mg/kg bw/d in m/f)	89 mg/kg bw/d (NOEL)	362 mg/kg bw/d (NOEL)	Bw and bw gain↓ in f, liver wt↑, stomach mu-cosal inflam-mation, cata-racts in m, urine pH↓, survival <50% in all groups incl. controls	Study acceptable; NOAEL 89 mg/kg bw/d (based on stomach mucosal irritation)
[redacted] 1981; TOX2000-595 and TOX2000-1997; XHJ-64, 98.7%; Monsanto	Combined chronic toxicity/ carcinoge-nicity; 26 months; Sprague-Dawley; feeding	0, 3/3.4, 10.3/11.2, 31.5/34 mg/kg bw/d in m/f (dietary levels adjusted according to values as measured in the 1st week)	31.5 mg/kg bw/d (NOEL)	Not estab-lished	No effects observed	Study not acceptable due to selection of too low dose levels and severe reporting deficiencies; study was not the sole basis for previous ADI setting

* according to the 1998 DAR and confirmed by subsequent EU evaluation

No evidence of carcinogenicity was obtained in any of these studies. Chronic toxicity was confined to rather minor effects occurring at high dose levels. The most uncommon findings were those in the salivary glands in at least one study ([redacted]., 1993, TOX9750499). They consisted of a higher organ weight of the parotid and, less pronounced, of the sublingual and mandibulary glands and histological alterations including hypertrophy and an increase in basophilic staining of acinar cells (described as ”weakly” for the mandibular to ”more deeply” for the parotid glands). [redacted] (1996, TOX9651587) did not find histological lesions but the salivary glands were apparently not weighed. Likewise, in the study by [redacted] (1990, TOX9300244), salivary glands were not weighed. No pathological changes were noted in the submandibulary glands whereas the parotid gland was not examined microscopically.

Table 2.6-7b: More recent long-term studies in rats that were submitted for EU re-evaluation

Reference; Study identifi-cation; Batch, purity; Owner	Study type, strain, duration, route	Dose levels	NOAEL	LOAEL	Targets / Main effects
[redacted]., 2009; ASB2012-11490; H05H016A, 95,7%; Nufarm	Combined chronic toxicity/ carcinoge-nicity; 2 yr; Wistar; feeding	0, 1500, 5000, 15000 ppm (progressively increased up to 24000 ppm), equal to 86/105, 285/349, and 1077/1382 mg/kg bw/d (m/f)	285 mg/kg bw/d	1077 mg/kg bw/d	Bw gain↓, transient increase in AP activity, changes in distribution of renal mineralisation, adipose infiltration of bone marrow (indicative of hypoplasia)↑, slight increase in cutaneous alterations

Reference; Study identification; Batch, purity; Owner	Study type, strain, duration, route	Dose levels	NOAEL	LOAEL	Targets / Main effects
[redacted] 2001*; ASB2012-11488; P30, 97.6%; Syngenta	Combined chronic toxicity/ carcinogenicity; 2 yr; Wistar-derived; feeding	0, 2000, 6000, 20000 ppm (121/145, 361/437, 1214/1498 mg/kg bw/d in m/f)	361 mg/kg bw/d	1214 mg/kg bw/d	Bw, food consumption and (initially) utilization↓, clinical chemistry findings (AP and ALAT activity↑, bilirubin↑, urine pH↓), kidney papillary necrosis, prostatis and periodontal inflammation↑ in high-dose males
[redacted] 1997; ASB2012-11484, 11485, 11486, 11487; T-941209, 97.56% and T-950308, 94.61%; Arysta Life Sciences	Combined chronic toxicity/ carcinogenicity; 2 yr; Sprague-Dawley; feeding	0, 3000, 10000, 30000 ppm (104/115, 354/393, 1127/1247 mg/kg bw/d in m/f)	104 mg/kg bw/d	354 mg/kg bw/d	Bw/bw gain, food consumption (initially) and utilization↓, loose stool↑, tail masses↑ due to follicular hyperkeratosis and abscesses, caecum: distention and wt↑, pH↓ and dark appearance of urine
[redacted] 1996*,#; TOX2000-1998; P24, 95.6%; Syngenta	Chronic toxicity; Wistar-derived; 12 months; feeding	0, 2000, 8000, 20000 ppm (141/167, 560/671, 1409/1664 mg/kg bw/d in m/f)	141 mg/kg bw/d	560 mg/kg bw/d	Bw, food consumption and utilization↓, AP activity↑, focal basophilia of acinar cells of parotid salivary gland (not weighed)

* New study for EU evaluation but referred to in 2004 JMPR evaluation (ASB2008-6266) # Supplementary study due to shorter duration than required for assessment of carcinogenicity

Again, there was no evidence of carcinogenicity of glyphosate in any of the studies. Chronic toxicity was confined to high dose levels in all the studies but remarkable differences became apparent in what was actually observed.

Effects on body weight (gain) and impairment of food consumption or food efficiency were quite common, as well as an increase in alkaline phosphatase activity. The latter findings might indicate some hepatotoxicity but liver weight was found elevated only by [redacted]. (1993, TOX9750499).

It is interesting to note that the previously known salivary gland findings were not reported in most of the new studies. In fact, [redacted] (1996, TOX2000-1998) described histological alterations which were not observed by [redacted] (2001, ASB2012-11488) although both studies were performed in the same laboratory and on the same Wistar-derived rat strain. An effect specific to one strain is not likely because of this striking difference and since [redacted] (1993, TOX9750499) had seen similar changes in Sprague-Dawley rats. In the study by [redacted] (1997, ASB2012-11484, ASB2012-11485, ASB2012-11486, ASB2012-11487), submaxillary (mandibular) and sublingual (but apparently not the parotid) salivary glands were taken and examined microscopically. No remarkable findings were reported. In the study by [redacted] (2009, ASB2012-11490), histological examination comprised the mandibulary and sublingual glands and was confined to control and top dose animals. No alterations were noted. Parotid gland was sampled only occasionally when gross lesions were apparent (as it was the case for one high dose male with acinar adenoma). Thus, at least in the latter two studies, there was obviously no real chance to confirm or contravene the histological findings in the parotid gland as reported by [redacted] (1996, TOX2000-1998). Salivary glands were not weighed in all these studies.

There were more findings that were reported in one or two of the studies only. [redacted] (1997, ASB2012-11484, ASB2012-11485, ASB2012-11486, ASB2012-11487) identified the caecum as an additional target organ. High dose effects (at 354/393 mg/kg bw/d and above) were characterised by distention and a marked increase in organ weight and may be related to the clinical sign of loose stool. However, there was no evidence of histological alterations in the mucosa. Similar clinical or necropsy findings were not noted in any other of the long-term studies in rats and, in line with usual experimental practice, the caecum was not weighed because this is required only if there are indications of an effect. So far examined microscopically ([redacted] 2009, ASB2012-11490), no remarkable lesions were reported for this organ.
Likewise, an increase in skin lesions became apparent by in-life observations and histology in the study by [redacted] (1997, ASB2012-11484, ASB2012-11485, ASB2012-11486, ASB2012-11487) but not in any further one with the possible exception of that by [redacted] (2009, ASB2012-11490). In this latter study, there were a slightly higher incidence of areas of necrosis or giant cell reaction to keratin and a non-significant increase in keratoacanthoma in high dose males (occurring in 6/51 animals as compared to 2/51 in the control group, 3/51 in the low and 0/51 in the mid dose groups; incidence in females always 0). In contrast to [redacted] (1997, ASB2012-11484, ASB2012-11485, ASB2012-11486, ASB2012-11487), the tail region was not particularly affected by skin lesions.
A slightly higher frequency of cataracts in animals receiving glyphosate at high dietary doses were reported by [redacted] (1990, TOX9300244) as well as by [redacted] (1996, TOX9651587) but were not noted in other studies. In other studies, neither ophthalmoscopy nor histological examination of the eyes confirmed this finding.
The bone marrow (increased fatty infiltration which might be indicative of lower cellularity. *i.e.*, hypoplasia) and kidney effects (less mineralisation of renal pelvis and papillary epithelium with a concomitant increase in cortical and/or medullary mineral deposition) in high dose rats in the most recent study of [redacted]. (2009, ASB2012-11490) were not seen in any other experiment although similarly high doses had been given. However, it should not be forgotten that other kidney effects (papillary necrosis) of high dose administration were also observed by [redacted] (2001, ASB2012-11488).

If all the valid long-term studies in rats are taken together, a robust "overall" NOAEL in the magnitude of 100 mg/kg bw/day may be assumed. A dose level of around 350 mg/kg bw/day s considered the LOAEL, based on the respective values obtained in the studies by [redacted] (1993, TOX9750499), Stout and Ruecker (1990, TOX9300244) and [redacted] (1997, ASB2012-11484, ASB2012-11485, ASB2012-11486, ASB2012-11487).

2.6.6.2 Mouse

Table 2.6-8a: Long-term studies in mice that were used for previous EU evaluation

Reference; Study identification; Batch, purity; Owner	Study type, strain, duration, route	Dose levels	NOAEL	LOAEL	Targets / Main effects	Outcome of re-evaluation
[redacted] 206-JaK-25-1, 98.6%; Cheminova	Carcinogenicity, 2 yr, CD-1, feeding	0, 100, 300, 1000 mg/kg bw/d (dietary levels regularly adjusted)	1000 mg/kg bw/d	> 1000 mg/kg bw/d	Equivocal evidence of enlarged/firm thymus and increase in mineral deposition in the brain	Study acceptable, NOAEL confirmed, mineral deposits are a common finding and thymus changes were not accompanied by histological lesions
[redacted] 1983; TOX9552381; NB 1782608/3 and 1782610/7, 99.7%; Monsanto	Carcinogenicity with chronic toxicity elements, 2 yr, CD-1, feeding	0, 1000, 5000, 30000 ppm 157/190; 814/955; 4841/5874 mg/kg bw/d in m/f)	157 mg/kg bw/d	814 mg/kg bw/d	Bw ↓ in high dose males, histological findings in liver (centrolobular hypertrophy) and bladder (epithelial hyperplasia) in males	Study acceptable, NOAEL confirmed

The previously known studies did not provide evidence of carcinogenicity up to the high dose levels tested. Effects on thymus and brain were equivocal and of doubtful toxicological relevance ([redacted], 1993, TOX9552382). In the elder study of [redacted] (1983, TOX9552381), hyperplasia of bladder epithelium was noted that might be due to acidic nature of the test substance. A possible effect on the liver was confined to an extremely high dose level of nearly 5000 mg/kg bw, as well as a decreae in body weight. These studies suggested that toxicity of glyphosate to mice was even less pronounced than to rats. Male mice appeared a bit more vulnerable than females.

The most recent 80-Week dietary mouse study was conducted by [redacted] (2009, ASB2012-11492) and can be considered very comprehensive with regard to histopathology. There were no adverse effects up to the the highest dose level of 5000 ppm, that was equivalent to 810 or 1081 mg/kg bw/day in males and females, respectively.
[It was noted that histological examination of salivary glands in this study covered submaxillary, sublingual and parotid glands, like in the study by [redacted] (1993, TOX9552382). However, no lesions similar to those found by [redacted] (1992, TOX9551954, see 2.6.3 above) in another mouse strain following administration of glyphosate ober 90 days at higher doses were observed in these long-term experiments. [redacted] (1997, ASB2012-11493), in contrast, examined only the sublingual and submaxillary glands while no precise information was given by [redacted] (2001, ASB2012-11491).]

Table 2.6-8b: More recent long-term studies in mice that were first submitted for EU re-evaluation

Reference; Study identification; Batch, purity; Owner	Study type, strain, duration, route	Dose levels	NOAEL	LOAEL	Targets / Main effects
[redacted] 2009, ASB2012-11492; Lot H05H016A, 95.7%; Nufarm	Carcinogenicity; 18 mo; CD-1 (ICR), feeding	0, 500, 1500, 5000 ppm (71/98; 234/299; 810/1081 mg/kg bw/d in m/f)	810 mg/kg bw/d	> 810 mg/kg bw/d	No effects observed
[redacted] 2001, ASB2012-11491; Lot 01/06/97, >95.14%; ADAMA	Carcinogenicity, 18 mo, Swiss albino, feeding	0, 100, 1000, 10000 ppm (15; 151; 1460 mg/kg bw/d, sexes combined since values were similar)	151 mg/kg bw/d	1460 mg/kg bw/d	Higher incidence of malignant lymphoma at top dose level (outside historical control range for males); cytic glands in stomach in m↑ (equivocal toxicological relevance)
[redacted] 1997, ASB2012-11493; T-941209, 97.56% and T-950308, 94.61%; Arysta	Carcinogenicity; 18 mo; CD-1 (ICR), feeding	0, 1600, 8000, 40000 ppm (165/153; 838/787; 4348/4116 mg/kg bw/d in m/f)	153 mg/kg bw/d	787 mg/kg bw/d	Bw gain, food consumption and efficiency↓, loose stool, caecum distended and organ wt↑, prolapse and ulceration of anus in m

The carcinogenicity study in Swiss albino mice by [redacted] (2001, ASB2012-11491) revealed an increase in malignant lymphoma incidence at the top dose level of around 1460 mg/kg bw/day. It must be emphasised that this tumor is quite common in ageing mice and that, at least in males, the number of affected animals in the control groups was actually higher in this strain than in the studies in CD-1 mice (see Table 2.6–8). In fact, malignant lymphoma accounted for 54.6 % of all tumours that were deteced in all animals in the study by [redacted] (2001, ASB2012-11491). However, it must not be disregarded that the malignant lymphoma incidence was significantly elevated as compared to the actual control groups in both sexes, was above the mean values of the (relatively small) historical control and, for males, outside the historical control range.

The high background incidence of malignant lymphoma in Swiss mice was confirmed in a literature search that was performed by the RMS on request of the Pesticides Peer Review 125 expert meeting. Its results are given in detail in Vol. 3 (B.6.5.2). According to older articles, control incidences in male mice of Swiss or Swiss-derived strains may reach 18–27.5 % and exceed 36 % in females (Sher, 1974, Z22020; Roe and Tucker, 1974, ASB2015-2534; Tucker, 1979, Z83266). Even though these historical rates were still lower than what was seen in the study by [redacted] (2001, ASB2012-11491) at least at the higher dose levels, they provide clear proof that Swiss mice are prone to developing lymphoreticular tumours. In a more recent publication, Tadesse-Heath *et al.* (2000, ASB2015-2535) even mentioned a nearly 50 % lymphoma (mostly of B cell orgin) incidence in a colony of CFW Swiss mice. The latter authors emphasised the contribution of widespread infections with murine oncogenic viruses to the high but remarkably variable incidence of tumours of the lymphoreticular system. No information is available on possible abundance of such viruses in the mouse colonies from which the animals used in the glyphosate studies were obtained.

To facilitate assessment of this finding, a comparison of malignant lymphoma incidence in all the available studies in the different mouse strains was made (Table 2.6–9). At first glance, the most recent study by [redacted] (2009, ASB2012-11492) seems not that useful for this purpose because its highest dose level was clearly below the critical one in the study by

[redacted] (2001, ASB2012-11491). On the other hand, a different strain was employed. Indeed, there was a higher incidence of the same tumour type in high dose males (5/51 vs. 0/51 ub tge control group) but the difference was not statistically significant. Likewise, in the study by [redacted] (1997, ASB2012-11493), there was a higher number of male mice affected at the exaggerated dose level of 40000 ppm (*ca* 4350 mg/kg bw/day) than in the control group (6/50 vs. 2/50) but, again, the difference did not gain statistical significance.

Table 2.6-9: Total incidence of malignant lymphoma in long-term studies with glyphosate in different mouse strains

Study, Strain		Males				Females			
[redacted], 2009, ASB2012-11492 Crl:CD-1 (ICR) BR	Dose (ppm)	0	500	1500	5000	0	500	1500	5000
	Affected	0/51	1/51	2/51	5/51	11/51	8/51	10/51	11/51
[redacted] 2001, ASB2012-11491 HsdOLA:MF1 (Swiss albino)	Dose (ppm)	0	100	1000	10000	0	100	1000	10000
	Affected	10/50	15/50	16/50	19/50*	18/50	20/50	19/50	25/50*
[redacted] 1997, ASB2012-11493 Crj:CD-1 (ICR)	Dose (ppm)	0	1600	8000	40000	0	1600	8000	40000
	Affected	2/50	2/50	0/50	6/50	6/50	4/50	8/50	7/50
[redacted], 1993, TOX9552382, CD-1 (not further specified)	Dose (mg/kg bw/d)	0	100	300	1000	0	100	300	1000
	Affected**	4/50	2/50	1/50	6/50	14/50	12/50	9/50	13/50

* increase statistically significant, for females based on percentage and not on total number of affected mice
** based on histological examination of lymph nodes with macroscopic changes

Historical control data might be of interest to put these findings into perspective. ~~but, unfortunately, were not submitted for these two studies in which a non-significant increase had been observed.~~ In fact, suitable historical control data was provided from the Japanese laboratory (Kitazawa, 2013, ASB2014-9146). In male mice, incidence of malignant lymphoma varied very much between 4 % and 19 % in the control groups from a number of studies and, thus, the 12 % incidence at the top dose level in the study by [redacted] (1997, ASB2012-11493) was well covered and at least this finding would not support the hyphothesis of an effect of glyphosate on the frequency of this tumour type in mice. ~~Unfortunately, no historical control data is available for the study by [redacted] (2009, ASB2012-11492).~~ For the study of [redacted] (2009, ASB2012-11492), historical control data from a total of nine studies were submitted on additional request ([redacted] 2015, ASB2015-2531) but were of not much use because incidences in male and female mice were not reported separately and since the data were apparently not from the same laboratory. However, the mentioned study incidences ranging from 0 % up to 32 % prove the large variability of malignant lymphoma frequency also in Crl:CD1 strains covering all male and female groups in the study under investigation. This view is supported by further historical control data for CD-1 mice collected from industry databases ([redacted], 2010, ASB2015-2529; Anonym, 2015, ASB2015-2532) or open literature (Son and Gopinath, 2004, ASB2015-2533). Lymphoma incidences in CD-1 strains are extremely variable and mostly

higher in females than in males but may reach even in males rates between 10 % and 20 %. In sum, neither the study by [redacted] (1997, ASB2012-11493) in the Crj:CD-1 strain nor that one by [redacted]. (2009, ASB2012-11492) in Crl:CD-1 mice in fact support the increase in malignant lymphoma that was observed by [redacted] (2001, ASB2012-11491) in Swiss albino mice. The slightly higher incidences in top dose males in the two studies in CD-1 mice were not statistically significant and fully covered by historical control data.

~~In contrast, n~~No increase in malignant lymphoma was seen by [redacted] (1993, TOX9552382) in CD-1 mice who applied a similar or even higher dose level as in the study by [redacted] (2001, ASB2012-11491) when for the (regularly adjusted) daily dose of 1000 mg/kg bw/day a dietary equivalent of at least 10000 ppm is assumed. In the study by [redacted] (1983, TOX9552381) in CD-1 mice, malignant lymphoma was not mentioned as a separate entity (therefore, it was not included in the table) but related tumours of the lymphoreticular system did not show an increase with dose even though a three times higher dietary dose had been fed than by [redacted] (2001).

Taking all this information together, a treatment-related effect in the study by [redacted] (2001, ASB2012-11491) in Swiss albino mice cannot be completely excluded. However, the weak increase in malignant lymphoma even over the historical control of the performing laboratory was clearly confined to this single study and strain since it was not reproducible in four other valid long-term studies. Thus, there is only very limited evidence of a carcinogenic potential of glyphosate as a high-dose phenomenon in mice of a susceptible strain. ~~Most likely,~~ Perhaps, age-related neoplastic changes might be exacerbated by long-lasting administration of high doses. Swiss albino mice with high background prevalence of malignant lymphoma could be more vulnerable than other strains.

Since the more frequent occurrence of malignant lymphoma was confined to a very high dose level that was administered over a long period, glyphosate ~~was~~ is considered unlikely to pose a carcinogenic risk in humans. Classification and labelling for carcinogenicity is not considered apppropriate by the RMS because of the following considerations:

(1) The presumed effect was observed ~~statistically significant~~ in only one of five long-term studies in mice in a strain with a rather high background incidence of malignant lymphoma. ~~Evidence coming from two other studies one more study is even more equivocal because a certain increase there did not gain statistical significance. In a third study, a (non-significant) increase in top dose incidence was explained and contravened by historical control data.~~ Taking into account the huge amount of information on historical control incidences, there was no evidence of a similar effect in any other study.
(2) Although the increase in lymphoma incidence in the study by [redacted] (2001, ASB2012-11491) was statistically significant in both sexes, it was still within the (small) historical control range of the performing laboratory for females. No evidence of a similar effect in female mice was obtained in any other study.
(3) No evidence of carcinogenicity was obtained in a total of six valid 2-yr studies in rats (see above) in which sufficiently high dose levels were employed.
(4) The dose with a significantly higher lymphoma incidence (1460 mg/kg bw/day) is by more 2900 times higher than the proposed ADI and the margin to the expected consumer exposure is even wider.
(5) There is no convincing evidence of carcinogenicity of glyphosate in humans coming from the epidemiological studies (see below).

In the Pesticides Peer Review 125 expert meeting (February 2015), it was agreed that there is no need to propose classification and labelling of glyphosate for carcinogenicity.

Another, non-neoplastic but presumably treatment-related effect found by [redacted] (2001, ASB2012-11491) was a more frequent occurrence of cystic glands of the stomach in male mice at all dose levels. However, there were no clear dose response and no evidence of an increase in severity of this lesion of which the clinical relevance is equivocal. Again, this finding was not reported in any other study in mice. Thus, based on the higher malignant lymphoma incidence, the mid dose level of 1000 ppm (*ca* 151 mg/kg bw/day) was considered the NOAEL. This figure was virtually the same as established by [redacted] (1983, TOX9552381) even though effects at higher dose levels were different.

In the third, previously not evaluated study in mice by [redacted] (1997, ASB2012-11493), the NOAEL was 153 mg/kg bw/day (1600 ppm), based on effects of glyphosate administration on body weight gain, food consumption and efficiency in female mice at the next higher dose level of 8000 ppm (equivalent to 787 mg/kg bw/day). At the extremely high dose of 40000 ppm (equivalent to 4348/4116 mg/kg bw/day in males and females, respectively) additional signs of toxicity included loose stools, caecum distention and increased absolute and relative caecum weight (without corollary histopathological findings), a higher incidence of anal prolapses and erosion/ulceration of the anus in male mice and some minor changes such as a decrease in urinary pH, lymphocytosis in females and few external signs (loss of tactile hair, pale-colored skin).

Based on the studies by [redacted] (1997, ASB2012-11493), [redacted] (2001, ASB2012-11491) and [redacted] (1983, TOX9552381), the overall NOAEL for long-term toxicity in the mouse can be set at 150 mg/kg bw/day. The overall LOAEL was around 800 mg/kg bw/day since first effects were observed at 787 mg/kg bw/day in females by Sugimoto (1997, ASB2012-11493) and at 814 mg/kg bw/day by [redacted] (1983, TOX9552381) in males. As in rats, the nature of high dose effects in mice was different in the various studies, depending on laboratory, strain, dose selection and, perhaps, purity/impurity profile of the test material.

Studies with formulations/Published data

Epidemiology

A number of epidemiology studies over the last decade have focused on pesticide exposure and associated health outcomes. Publications vary in the scope of their conclusions regarding either pesticides in general, certain classes of pesticides and in some cases individual insecticides, herbicides or fungicides. While some of these publications specifically mention glyphosate, few draw tenable associations with any specific cancer outcome. Publications suggesting glyphosate is associated with any cancer outcome are discussed below.

An essential consideration in both, risk assessment and interpreting the relevance of toxicology data, is exposure assessment. An inherent low level of confidence exists for epidemiological studies where tenuous links to exposure exist. Suggested associations between health outcomes and any possible causative agent are merely speculative if exposure cannot be confirmed and quantified.

The largest epidemiological study of pesticide exposure and health outcomes in the United States was the Agricultural Health Study (AHS) that also adressed and included glyphosate.

Dozens of publications have resulted from data generated in this study of approximately 57,000 enrolled farmers (applicators). Blair *et al.* (2009, ASB2012-11566) provided an overview of cancer endpoints associated with different agricultural chemicals reported in earlier AHS publications. Glyphosate was not reported to be associated with leukaemia, melanoma, or cancers of the prostate, lung, breast, colon or rectum. De Roos *et al.* (2005, ASB2012-11605) reported AHS data evaluating glyphosate use and multiple cancer endpoints. No association was noted for glyphosate with all cancers, including cancer of the lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate, melanoma, all lymphohematopoietic cancers, non-Hodgkin's lymphoma (NHL) and leukemia. In an earlier publication based on another data set, however, De Roos *et al.* (2003, ASB2012-11606) had reported an association between NHL and glyphosate use. Likewise, McDuffie *et al.* (2001, ASB2011-364) mentioned a non-significant positive association between self-reported glyphosate exposure and NHL in a Canadian study. Blair *et al.* (2009, ASB2012-11566), in contrast, did not report an association between glyphosate use and NHL in the AHS data but a "possible association" between glyphosate use and multiple myeloma was mentioned making reference to a "suggested association" between glyphosate use and multiple myeloma suggested by De Roos *et al.* (2005, ASB2012-11605). However, in this paper, no significant increase in relative risk for multiple myeloma was demonstrated. Both papers by De Roos *et al.* will be discussed in more detail below. Interestingly, a subsequent AHS review paper for the President's Cancer Panel (Freeman, 2009, ASB2012-11623) specifically referenced De Roos *et al.* (2005 ASB2012-11605) to provide no evidence of cancers of any type to be associated with glyphosate.

Lee *et al.* (2005, ASB2012-11882) reported a glyphosate association with gliomas, with the odds ratio differing between self-respondents (OR = 0.4) and proxy respondents (OR = 3.1). The authors expressed concern about higher positive associations observed for proxy respondents with glyphosate and several other pesticides. They suggested perhaps more accurate reporting of proxies for cases and underreporting by proxies for controls.

Monge *et al.* (2007, ASB2012-11909) investigated associations between parental pesticide exposures and childhood leukaemia in Costa Rica. Results are not interpretable for glyphosate as exposure was estimated with "other pesticides", including paraquat, chlorothalonil and "others". No association was noted for paternal exposures, but elevated incidence of leukaemias was associated with maternal exposures to "other pesticides" during pregnancy.
Some further epidemiological studies have focused on an association between pesticide exposure and Non-Hodgkin`s Lymphoma (NHL). Hardell and Eriksson (1999, ASB2012-11838) investigated in a case-control study the incidence of NHL in relation to pesticide exposure in Sweden. 404 cases and 741 controls have been included. The authors discussed an increased risk for NHL especially for phenoxyacetic acids. Glyphosate was included in the uni-variate and multi-variate analyses. However, only 7 of 1145 subjects in the study gave exposure histories to this agent. The authors reported a moderately elevated odds ratio (OR) of 2.3 for Glyphosate. This OR was not statistically significant and was based on only 4 "exposed" cases and 3 "exposed" controls. The major limitations of this study were: the reliance on reported pesticide use (not documented exposure) information, the small number of subjects who reported use of specific pesticides, the possibility of recall bias, the reliance on secondary sources (next-of-kin interviews) for approximately 43 % of the pesticide use information, and the dificulty in the controlling for potential confounding factors given the small number of exposed subjects.
A further study was submitted by Hardell *et al.* (2002, ASB2012-11839). This study pools data from the above mentioned publication by Hardell and Eriksson (1999, ASB2012-11838)

with data from a previously submitted publication from Nordström *et al.* (1998, TOX1999-687).
The authors found increased risks in an uni-variate analysis for subjects exposed to herbicides, insecticides, fungicides and impregnating agents. Among herbicides, significant associations were found for glyphosate and MCPA. However, in multi-variate analyses, the only significantly increased risk was found with a heterogenous category of "other herbicides" and not for glyphosate. No information is given about exposure duration, exposure concentration, as well as medical history, lifestyle factors (*e.g.*, smoking, use of prescribed drugs *etc.*). In all, the above mentioned limitations of the publication of Hardell and Eriksson (1999, ASB2012-11838) are also applicable to the publication by Hardell *et al.* (2002, ASB2012-11839).
Fritschi *et al.* (2005, ASB2012-11624) submitted a case-control study with 694 cases of NHL and 694 controls in Australia. Substantial exposure to any pesticide was associated with an increase of NHL. However, no association between NHL and glyphosate can be made on basis of this study. No information was given about exposure duration, used glyphosate products, and application rates. Therefore, the documentation is considered to be insufficient for assessment.
Eriksson *et al.* (2008, ASB2012-11614) reported a case-control study which included 910 cases of NHL and 1016 controls living in Sweden. The highest risk was calculated for MCPA. Glyphosate exposure was reported by 29 cases and 18 controls, and the corresponding odds ratio (OR) was 2.02. Results and reliability of the study are discussed below.
Alavanja *et al.* (2013, ASB2014-9174) reviewed studies on cancer burden among pesticide applicators and others due to pesticide exposure. In this article, the epidemiological, molecular biology, and toxicological evidence emerging from recent literature assessing the link between specific pesticides and several cancers including prostate cancer, NHL, leukemia, multiple myeloma, and breast cancer were integrated. Glyphosate was reported to be the most commonly used conventional pesticide active ingedient worldwide. However, the only association between the use of glyphosate and cancer burden mentioned in this review was the observation of Eriksson *et al.* (2008, ASB2012-11614, see above).

The following epidemiological studies did not reveal an association between glyphosate and specific cancer types.

- Alavanja *et al.* (2003, ASB2012-11535) reported on prostate cancer associations with specific pesticide exposures in the AHS; glyphosate did not demonstrate a significant exposure-response association with prostate cancer.
- Multigner *et al.* (2008, ASB2012-11917) also reported a lack of association between glyphosate use and prostate cancer. This data appears to have also been reported by Ndong *et al.* (2009, ASB2012-11922).
- The lack of association between glyphosate use and prostate cancer was also supported recently in an epidemiology study in farmers in British Columbia, Canada, by Band *et al.* (2011, ASB2012-11555).
- Lee *et al.* (2004, ASB2012-11883) reported a lack of association between glyphosate use and stomach and esophageal adenocarcinomas.
- Carreon *et al.* (2005, ASB2012-11585) reported epidemiological data on gliomas and farm pesticide exposure in women; glyphosate had no association with gliomas.
- Engel *et al.* (2005, ASB2012-11613) reported AHS data on breast cancer incidence among farmers' wives, with no association between breast cancer and glyphosate.

- Flower *et al.* (2004, ASB2012-11620) reported AHS data on parental use of specific pesticides and subsequent childhood cancer risk among 17,280 children, with no association between childhood cancer and glyphosate.
- Andreotti *et al.* (2009, ASB2012-11544) reported AHS data where glyphosate was not associated with pancreatic cancer.
- Landgren *et al.* (2009, ASB2012-11875) reported AHS data on monoclonal gammopathy of undetermined significance (MGUS), showing no association with glyphosate use.
- Karunanayake *et al.* (2011, ASB2012-11865) reported a lack of association between glyphosate and Hodgkin's lymphoma.
- Pahwa *et al.* (2011, ASB2012-11987) reported a lack of association between glyphosate and multiple myeloma.
- Schinasi and Leon (2014, ASB2014-4819) published the results of epidemiologic research on the relationship between non-Hodgkin lymphoma (NHL) and occupational exposure to pesticides. Phenoxy herbicides, carbamate insecticides, organophosphorus insecticides and lindane were positively associated with NHL. However, no association between NHL an glyphosate was reported.
- Kachuri *et al.* (2013, ASB2014-8030) investigated an association between lifetime use of multiple pesticides and multiple myeloma in Canadian men. Excess risks of multiple myeloma were observed among men reported using at least one carbamate pesticide, one phenoxy herbicide and ≥ organochlorines. However, no excess risk was observed for glyphosate.
- Cocco *et al.* (2014, ASB2014-7523) investigated the role of occupational exposure to agrochemicals in the aetiology of lymphoma overall, B cell lymphoma and its most prevalent subtypes. No increased CLL risk in relation to glyphosate was evidenced.
- Alavanja and Bonner (2012, ASB2014-9173) reviewed studies on occupational pesticide exposure and cancer risk. Twenty one pesticides identified subsequent to the last IARC review showed significant exposure-response associations in studies of specific cancers. No significant association was observed for glyphosate.
- El-Zaemey and Heyworth (2013, ASB2014-9473) reported a case control study on the association between pesticide spray drift from agricultural pesticide application areas and breast cancer in Western Australia. The findings support the hypothesis that woman who ever noticed spray drift or who first noticed spray drift at a younger age had increased risk of breast cancer. However, it was no possible to examine whether the observed associations are the result of a particular class of pesticides.
- Pahwa *et al.* (2011, ASB2014-9625) investigated the putative associaton of specific pesticides with soft-tissue sarcoma (STS). A Canadian population-based case-control study conducted in six provinces was used on this analysis. The incidence of STS was associated with insecticides aldrin and diazinon after adjustment for other independent predictors. However, no statistically significant association between STS and exposure to glyphosate or other herbicides was observed.
- Koutros *et al.* (2011, ASB2014-9594) studied associations between pesticide and prostate cancer. No statistically significant positive association between pesticides and prostate cancer were observed. There was suggestive evidence on an increased risk (OR>1.0) with an increasing number of days of use of petroleum oil/petroleum distillate used as herbicide, terbufos, fonofos, phorate and methyl bromide. However, no increased risk (OR>1.0) was observed for glyphosate.

In a comprehensive review of the AHS publications and data, Weichenthal *et al.* (2010, ASB2012-12048) noted that increased rates in the following cancers were not associated with

glyphosate use: overall cancer incidence, lung cancer, pancreatic cancer, colon or rectal cancer, lymphohematopoietic cancers, leukemia, NHL, multiple myeloma, bladder cancer, prostate cancer, melanoma, kidney cancer, childhood cancer, oral cavity cancers, stomach cancer, esophagus cancer and thyroid cancer.
Mink *et al.* (2012, ASB2014-9617) submitted a comprehensive review of epidemiologic studies of glyphosate and cancer. To examine potential cancer risks in humans they reviewed the epidemiologic literature to evaluate whether exposure to glyphosate is associated causally with cancer risk in humans. They also reviewed relevant methodological and biomonitoring studies of glyphosate. The review found non consistent pattern of positive assciations indicating a causal relationship between total cancer (in adults or in children) or any site-specific cancer and exposure to glyphosate.

Toxicological studies with formulations in laboratory animals

Chruscielska *et al.* (2000, ASB2013-9829) published the results of a combined long-term toxicity and carcinogenicity study in rats. The active substance glyphosate (apparently manufactured in Poland and fomulated as a 13.85 % solution of the ammonium salt in water) was used in the study that was performed mainly according to OECD guideline 453. The number of animals per dose group and sex (85 animals) was even higher than required. The highest dose level of the glyphosate salt was 2700 ppm. Study duration was 2 years. No carcinogenic effects have been found in the study. However, apart from tables with cancer incidences, no raw data has been reported and the whole report was very brief.

George *et al.* (2010, ASB2012-11829) used a 2-stage cancer model in mice to evaluate a glyphosate formulation for tumor promotion. A known tumor promoter, 12-o-tetradecanoyl-phorbol-13-acetate (TPA) was used as a positive control and for comparison with glyphosate effects after exposure to a tumor initiator, 7,12-dimethylbenz[a]anthracene. Proteomics were later applied to extrapolate a basis for glyphosate formulation tumor promotion. The results are considered by the authors to indicate a tumor promoting potential of glyphosate. However, the formulation Roundup was used in the study and not the active substance glyphosate. Furthermore, the up- and down-regulation of protein expression is not sufficient to prove a carcinogenic effect.

More recently, a two-year study in rats was published by Séralini *et al.* (2012, ASB2012-15514). Its main objective was to show a possible impact of long-term feeding of genetically modified (and glyphosate-treated) maize to rats but three of the test groups were administered a commercially available formulation (Roundup GT Plus, apparently authorised at least in Belgium) containing 450 g glyphosate/L at different concentrations ranging from 0.1 ppb (50 ng glpyphosate/L) to 0.5 % (2.25 g glyphosate/L) in drinking water. In these groups, the authors reported alterations in some clinical chemistry (blood and urine) parameters and hormone levels and histopathological lesions concerning the liver and the gastrointestinal tract but also a higher incidence of mammary tumours in females resulting in a shorter lifespan. This study was heavily discussed in the scientific community as well as in the general public where it gained remarkable attention due to massive promotion although it was clearly flawed by many serious deficiencies. A major point of concern was the small group size of only 10 males and 10 females per dose, *i.e.*, the test design was that one of a subchronic study. Such a small number of animals is not appropriate for a long-term study because age-related changes cannot be adequaetely taken into account. Following the receipt of contributions from many MS authorities, a comprehensive critical assessment was published by EFSA (2012, ASB2012-15513, EFSA Journal, 2012, 10 (11), 2986). The conclusion was that ”the currently

available evidence does not impact on the ongoing re-evaluation of glyphosate…". This opinion on the Séralini study is agreed with and supported by the RMS.
In reaction to this publication a large number of letters was send to the editor: Barale-Thomas (2012, ASB2013-10998), Berry (2012, ASB2013-10988), Grunewald (2012, ASB2013-11001), Hammond *et al.* (2012, ASB2013-10995), Heinemann (2012, ASB2013-10987), Langridge (2012, ASB2013-10986), Ollivier (2012, ASB2013-11000), Panchin (2013, ASB2013-10937), Pilu (2012, ASB2013-10992), Schorsch (2013, ASB2013-10996), Tester (2012, ASB2013-10994), Tien & Huy (2012, ASB2013-10984), Trewavas (2012, ASB2013-10989), Tribe (2012, ASB2013-10997), Wager (2012, ASB2013-10993), de Souza (2012, ASB2013-10999). In these letters, the study was mostly heavily critizised but sometimes also defended or certain methodical aspects were discussed in greater depth.

Mechanistic studies

Andreotti *et al.* (2012, ASB2014-9198) investigated the interaction between pesticide use and genetic variants that are involved in lipid metabolism and might increase a risk for prostate cancer. The authors examined the interactions between 39 pesticides and 220 single nucleotide polymorphisms (SNPs) in 59 genes. They found 17 interactions that displayed a significant monotonic increase in prostate cancer risk with pesticides exposure in one genotype but no significant assciations in the other genotypes under investigation. The most noteworthy association was for ALOXE3 rs 3027208 and terbufos. A higher risk was also reported with this method for glyphosate and other pesticides. However, the authors emphasize that glyphosate was not associated with a higher prostate cancer risk in the epidemiological studies, mainly the 'Agricultural Health Study' (AHS, see above).

Barry *et al.* (2011, ASB2014-9247) evaluated interactions between 39 pesticides and 394 tag single-nucleotide polymorphisms (SNPs) for 31 BER genes among 776 prostate cancer cases and 1444 male controls in a nested case-control study of pesticide applicators who had been involved in the AHS. The authors used likelihood ratio tests from logistic regression models to determine p-values for interactions between three-level pesticide variables and SNP (assuming a dominant model) and the false discovery rate multiple comparison adjustment approach. The authors observed notable interactions between several pesticides and BER gene variants with respect to prostate cancer. However, only fonofos x NEIL3 rs 1983132 showed an interaction fitting an expected biological pattern that remained significant after adjustment for multiple comparisons. No significant association was observed for glyphosate.

2.6.7 Summary of reproductive toxicity

2.6.7.1 Multi-generation studies

The reproductive toxicity of glyphosate was tested in a variety of multi-generation studies in rats. For the previous EU evaluation, a total of 8 studies in rats had been submitted of which four were now still considered acceptable or, in case of a single one-generation study, at least supplementary upon re-evaluation (Table 2.6–10a).

Table 2.6-10a: Valid reproductive toxicity studies in rats reviewed for previous EU evaluation

Reference; Study identification; Batch, purity; Owner	Study type, strain, duration, route	Dose levels	NO(A)EL	LO(A)EL	Targets/ Main effects	Outcome of re-evaluation
[redacted] 1993; TOX9300009; FSG 03090 H/05 March 90; 96.8%; ADAMA	Two-gen., Wistar rat, diet	0, 10, 100, 1000, 10000 ppm	Parental, offspring & reproductive 10000 ppm (700-800 mg/kg bw/d)	-	No treatment related effects	Study now considered supplementary (no effect dose), NOAELs confirmed
[redacted]., 1992; TOX9552389 ; 206-JaK-119-1, 99.2 %, Cheminova	Two-gen., Sprague-Dawley, diet	0, 1000, 3000, 10000 ppm	Parental, offspring: 3000 ppm (197 mg/kg bw/d); reproductive: 10000 ppm (668 mg/kg bw/d)	Parental, offspring: 10000 ppm (668 mg/kg bw/d)	Parental: bw↓, food & water ↑, cellular alterations of salivary glands in F0/F1 m/f	Study acceptable, previous NOAELs confirmed
[redacted] 1991; TOX9552388; 206-Jak-25-1, purity not stated; Cheminova	One-gen., range finding, Sprague-Dawley, diet	0, 3000, 10000, 30000 ppm	Not established	Maternal <3000 ppm (236 mg/kg bw/d) Offspring <3000 ppm (368 mg/kg bw/d)	Maternal salivary gland changes, GIT signs; Offspring: bw↓	Study supplementary (one gen, small number of animals), previous evaluation confirmed
[redacted] 1990; TOX9552387; XLI-203, 97.67%; Monsanto	Two-gen., Sprague – Dawley rat, diet	0, 2000, 10000, 30000 ppm	Parental, offspring & reproductive: 10000 ppm (720-760 mg/kg bw/d)	Parental, offspring & reproductive: 30000 ppm (~2000 mg/kg bw/d)	Parental: bw gain↓, soft stool, reproductive: litter size ↓ (equivocal), offspring: bw gain↓	Study acceptable, previous NOAELs confirmed

In these studies, parental toxicity was confined to minor effects at high dose levels only. The previously (see sections 2.6.3 and 2.6.5) described celluar alterations in salivary glands in F0 and F1 animals were reported in two studies from the same laboratory but were presumably not investigated in others.
No evidence of reproductive toxicity was observed. Equivocal effects suggesting a lower litter size were only seen far above the limit dose.
Weak effects on the offspring were indicated by a reduced pup weight but were confined to parentally toxic dose levels. Data from a range-finding study by [redacted] (1991, TOX9552388) suggested a decrease in mean pup weight at lower dose levels already but this was not confirmed in any of the more comprehensive studies with administration of the test substance for two generations in which much more animals were employed. The same holds true for the occurrence of salivary gland lesions in the dams at rather low dose levels in the same experiment.

Three new studies were provided in the GTF dossier and were considered acceptable by the RMS without restrictions. They are summarised in Table 2.6–10b and were submitted either

for the first time for this evaluation or had been subject to JMPR evaluation (JMPR, 2004, ASB2008-6266) yet.

Table 2.6-10b: New reproductive toxicity studies provided for EU evaluation 2012

Reference; Study identification; Batch, purity; Owner	Study type, strain, duration, route	Dose levels	NO(A)EL	LO(A)EL	Targets/ Main effects	Outcome of RMS evaluation
[redacted]., 2007; ASB2012-11494; H05H016A, 95.7%; Nufarm	Two-gen., Sprague-Dawley, diet	0, 1500, 5000, 15000 Ppm	Parental, reproductive, offspring: 5000 ppm (351 mg/kg bw/d)	Parental, reproductive, offspring: 15000 ppm (1000-1600 mg/kg bw/d)	Parental.: liver, kidney wt↑; Repro: homogenisation resistant spermatid count↓; Offspring: delay in preputial separation in F1 males	Study acceptable
[redacted] 2000; TOX2000-2000; Y04707/082, 97.6%; Syngenta	Two-gen., Wistar-derived AlpK, diet	0, 1000, 3000, 10000 Ppm	Parental, offspring: 3000 ppm (293 mg/kg bw/d) Reproductive: 10000 ppm (985 mg/kg bw/d)	Parental, offspring: 10000 ppm (985 mg/kg bw/d), reproductive: not established	Parental, offspring: bw↓ F1 pups & F1-adults	Study acceptable, JMPR evaluation confirmed
[redacted] 1997; ASB2012-11495; T-950308, 94,61%; Arysta	Two-gen., Sprague-Dawley, diet	0, 1200, 6000, 30000 Ppm	Parental, offspring: 6000 ppm (417 mg/kg bw/d), reproductive: 30000 ppm (> 2000 mg/kg bw/d)	Parental, offspring: 30000 ppm (> 2000 mg/kg bw/d), reproductive: not established	Parental: loose stool, bw↓, caecum distention, organ wt changes; offspring: bw↓, caecum distention	Study acceptable

In the first one of the new studies ([redacted] 2007, ASB2012-11494), increased absolute and relative organ weights of the liver (F0 & F1 females) and the kidneys (F0 females) were observed at the highest dose level of 15000 ppm (1000 – 16000 mg/kg bw/d). At the same high dose level, a significant decrease in homogenisation resistant spermatid (HRS) in the *cauda epididymidis* was noted in F0 males (Control: 399.9 million/gram; 15000 ppm: 309.0 million/gram). No similar effects were seen at lower dose levels or at any dose levels in F1 males. Furthermore, a significant delay in sexual maturation in male offsprings (F1) became apparent at the top dose level of 15000 ppm (~1000 mg/kg bw/d) because preputial separation was delayed (occurring after 45.9 days in the mean *versus* 43.0 days in the control group). Although this later onset of sexual maturation had no impact on subsequent reproductive performance, a treatment-related effect on the sexual development of male offspring cannot be excluded. However, it is important to note that this finding occurred at the limit dose at which parental toxicity was also apparent and, therefore, is not of concern. The overall NOAEL for parental, offspring and reproductive toxicity in this study was 5000 ppm (351 mg/kg bw/d).

In the study by [redacted] (2000, TOX2000-2000), there were no treatment-related mortalities or clinical findings in parents of either generation. The NOAEL for parental and offspring toxicity was considered to be 3000 ppm (293 mg/kg bw/day), based on a lower body weight

in F1 pups and a subsequent reduction also in body weight of F1 adult males at 10000 ppm corresponding to 985 mg/kg bw/day. No impact on sexual maturation was observed up to the highest dose level of 10000 ppm, however, a different rat strain was used than by [redacted]. (2007, ASB2012-11494). Administration of glyphosate at a dietary concentration of up to 10000 ppm for two generations had no effect on sexual function and fertility in males or females.

The third new two-generation study by [redacted] (1997, ASB2012-11495) revealed a number of parentally toxic effects but at an exaggerated dose of 30000 ppm (> 2000 mg/kg bw/day) only. Findings consisted of loose stool (F0/F1, m/f), reduced body weight (F0/F1, m) caecum distention (F0/F1, m/f), increased liver and kidney weights (F0/F1, m/f), and decreased prostate weight (F1). Histopathological alterations were not detected. Reproductive toxicity was not observed up to the highest dose level, despite lower gestation indices of F1 females at mid dose and high dose levels. This finding was considered not to be treatment related, because most of the F1 animals which failed to produce offspring showed normal reproductive performance after re-mating with untreated rats of either sex. Offspring toxicity was observed at the top dose level only and confined to reduced body weight and caecum distention in both sexes. Sexual maturation (preputial separation, vaginal opening) was not examined in this study. Thus, the NOAEL for parental and offspring toxicity was considered to be 6000 ppm (417 mg/kg bw/day) and that for reproductive toxicity was the highest dose of 30000 ppm (>2000 mg/kg bw/day).

In conclusion, based on both the previously and the more recently submitted reproductive toxicity studies, an 'overall' parental NOAEL might be in the magnitude of 300-400 mg/kg bw/day ([redacted] 1997, ASB2012-11495; Moxon, 2000, TOX2000-2000; [redacted] 2007, ASB2012-11494), and that is similar to what was observed in the subchronic studies. In fact, the lowest parental NOAEL of 197 mg/kg bw/day in a two-generation study and the lowest LOAEL of 668 mg/kg bw/day were established by [redacted] (1992, TOX9552389). High dose parental effects comprised gastrointestinal disturbance, decreased body weight and organ weight changes, furthermore also increased food and water consumption and an increased incidence of cellular alteration of the parotid (males and females) and submaxillary (females) salivary glands.
In offspring, body weight gain was reduced at parentally toxic dose levels and, in one study ([redacted] 2007, ASB2012-11494), preputial separation was delayed. The NOAELs/LOAELs were the same as for parental toxicity.
Regarding reproductive toxicity, equivocal effects on litter size was observed in one study ([redacted] 1990, TOX9552387) at the extremely high dose of 2000 mg/kg bw/day . In another study ([redacted] 2007, ASB2012-11494), a significant decrease in homogenisation resistant spermatid count in F0 males was observed at *ca* 1000 mg/kg bw/day. Based on the latter effect, the NOAEL for reproductive toxicity was 351 mg/kg bw/d.

2.6.7.2 Developmental toxicity and teratogenicity

The developmental toxicity and teratogenicity of glyphosate were tested in a variety of studies in rats and rabbits.

2.6.7.2.1 Rat

For the previous EU evaluation, a total of 5 studies in rats had been reported in the DAR of which four were still considered acceptable or at least supplementary and may be used for current evaluation (Table 2.6–11a). The previous assessment of the studies by [redacted] (1991, TOX9552393) and by [redacted] (1980, TOX9552392) and of the Alkaloida study (1981, TOX9650160, Author's name perhaps Antal but not verified) were confirmed by the RMS upon re-evaluation.
With regard to the single dose study by [redacted] (1991, TOX9551105), in contrast, the limit dose of 1000 mg/kg bw/day was confirmed to be the maternal NOAEL. However, it was acknowledged that a developmental NOAEL could not be established. At the same dose level, a higher incidence of delayed ossification (caudal vertebral arch, forelimb proximal & hindlimb distal phalanges) was observed and considered adverse, despite the fact that delayed ossification of other parts of the skeleton (skull) was more frequently seen in the control. However, these findings are not of concern because a robust NOAEL for developmental toxicity well below this high dose was established in the other studies.

Table 2.6-11a: Developmental toxicity studies in rats reviewed for EU evaluation 2001

Reference; Study identification; Batch, purity; Owner	Study type, strain, route, duration	Dose levels	NO(A)EL	LO(A)EL	Targets/ Main effects	Outcome of re-evaluation
[redacted] 1991, TOX9552393; 206-Jak-25-1, 98.6%; Cheminova	Developmental, CD, gavage, d 6-15 p.c.	0, 300, 1000, 3500 mg/kg bw/d	Maternal & developmental: 300 mg/kg bw/d	Maternal & dev.: 1000 mg/kg bw/d	Maternal: slight bw gain↓, noisy respiration (2/25); Dev.: ossification↓, skeletal anomalies	Study acceptable; previous evaluation confirmed
[redacted] 1991, TOX9551105; FSG 03090 H/05. March 90, 96.8%; ADAMA	Developmental, Wistar, gavage, d 6-15 p.c.	0, 1000 mg/kg bw/d	Maternal: 1000 mg/kg bw/d Dev. < 1000 mg/kg bw/d	Maternal: not established Dev.: 1000 mg/kg bw/d	Maternal: no effects; Dev.: ossification↓	Study acceptable as limit-test; maternal NOAEL confirmed, dev. NOAEL (previously 1000 mg/kg bw/d) not confirmed
[redacted] 1980; TOX9552392; Batch not stated, 98.7%; Monsanto	Developmental, Charles River, gavage, d 6-19 p.c.	0, 300, 1000, 3500 mg/kg bw/d	Maternal&dev.: 1000 mg/kg bw/d	Maternal & dev. 3500 mg/kg bw/d	Maternal:mortality, soft stool, diarrhea, Dev.: bw↓, post implantation losses	Study acceptable; previous evaluation confirmed

Reference; Study identification; Batch, purity; Owner	Study type, strain, route, duration	Dose levels	NO(A)EL	LO(A)EL	Targets/ Main effects	Outcome of re-evaluation
[redacted] 1981; TOX9650160; Batch not stated, purity 96.8%; Alkaloida	Developmental, CFY, diet, d 6-18 p.c.	Calculated to be 0, 22, 103, 544 mg/kg bw/d	Maternal & dev.: 544 mg/kg bw/d	Not established	No treatment related effects	Study supplementary; previuos evaluation confirmed

These previously submitted studies did not show any teratogenic potential in rats. The lowest NOAEL for both maternal and developmental effects was 300 mg/kg bw/day and the LOAEL was 1000 mg/kg bw/day, based on the study by [redacted] (1991, TOX9552393). In this most comprehensive rat study, evidence of delayed ossification and increased incidence of foetuses with skeletal anomalies was observed at 1000 mg/kg bw/day. At the very high dose level of 3500 mg/kg bw/day causing maternal toxicity and even mortality, post-implanation loss and the both skeletal variations and retardations were observed ([redacted] 1991, TOX9552393; [redacted], 1980, TOX9552392). No effects were seen neither in dams nor in foetuses, when the test substance was administered via the diet up to a daily dose of more than 500 mg/kg bw/day (*ca* 10 000 ppm).

In addition, two new studies in rats have been performed and were provided in the GTF dossier. They are summarised in Table 2.6–11b. These studies were submitted either for the first time for this evaluation or had been subject to JMPR evaluation in 2004 (ASB2008-6266) yet. They were considered acceptable by the RMS without restrictions.

Table 2.6-11b: New developmental toxicity studies in rats reviewed for EU evaluation 2012

Reference; Study identification; Batch, purity; Owner	Study type, strain, duration, route	Dose levels	NO(A)EL	LO(A)EL	Targets/ Main effects	Outcome of RMS evaluation
[redacted] 1996; TOX2000-2001 and 2002 ASB2012-10080; Y04707/034, 95.6%; Syngenta	Developmental, Alpk (Wistar derived), gavage, d 7-16 p.c.	0, 250, 500, 1000 mg/kg bw/d	Maternal & dev.: 1000 mg/kg bw/d	Not established	None	Study acceptable: JMPR (2004, ASB2008-6266) evaluation confirmed
[redacted] 1995 ASB2012-11497; 940908-1, 95.68%; Arysta	Developmental, CD (SD),, gavage, d 6-15 p.c.	0, 30, 300, 1000 mg/kg bw/d	Maternal&dev.: 300 mg/kg bw/d	Maternal& dev: 1000 mg/kg bw/d	Maternal: Loose stool Dev.: skeletal anomalies↑	Study acceptable

The study by [redacted] (1996, TOX2000-2001, 2002, ASB2012-10080) revealed a NOAEL for both maternal and developmental effects of 1000 mg/kg bw/day, *i.e.*, the highest dose tested. There was no evidence of developmental toxicity attributable to glyphosate as assessed by the number, growth or survival of the foetuses. Observations of the external appearance of the foetuses, examination of the viscera and assessment of the skeleton revealed no treatment-related findings.

The study by [redacted] (1995, ASB2012-11497) was conducted at doses of 30, 300 and 1000 mg/kg bw/day. At low and mid dose levels, no adverse effects were observed both in dams and foetuses. At the highest dose level, maternal toxicity was confined to loose stool and in the foetuses a slight increase in lumbar ribs were observed (11 out of 7 litters compared to 4 out of 2 litters in control animals). Therefore, the NOAEL for maternal and developmental toxicity is considered to be 300 mg/kg bw/d, despite absent statistical significance for the findings in foetuses.

In conclusion, the newly submitted studies confirmed the previous evaluation that there is no teratogenic potential of glyphosate in rats. At least the study of [redacted] (1995, ASB2012-11497) supported the previously established overall NOAEL of 300 mg/kg bw/day for both maternal and developmental effects.

2.6.7.2.2 Rabbit

Five developmental studies in rabbits had been submitted for the previous EU evaluation of which four may be still used following re-evaluation by the RMS. However, it has become necessary to revise the NOAELs and LOAELs in some cases and also to downgrade the reliability of individual studies (Table 2.6–12). In particular these rabbit studies have been recently discussed in scientific literature, mainly because of suspected occurrence of heart malformations and other findings that were interpreted as evidence of teratogenicity (*e.g.*, [redacted] 2012, ASB2012-15927**;** [redacted]., 2013, ASB2013-3462).

Table 2.6-12: Developmental toxicity studies in rabbits reviewed for previous EU evaluation that are still to be used for risk assessment

Reference; Study identification; Batch, purity; Owner	Study type, strain, duration, route	Dose levels	NO(A)EL	LO(A)EL	Targets/ Main effects	Outcome of re-evaluation
[redacted] 1993; TOX9551106; Batch 60, 96.8%; ADAMA	Developmental, NZW rabbit, d 6-18 p.c., gavage	0, 20, 100, 500 mg/kg bw/d	Maternal: 20 mg/kg bw/d. dev.: 100 mg/kg bw/d	Maternal: 100 mg/kg bw/d; dev.: not established due to low number of foetuses	Maternal: mortality, soft/liquid stool; dev.: no clear-cut effects up to 100 mg/kg bw/d, high dose group excluded due to low number of foetuses/ litters	Study supplementa-ry, previous NOAELs confirmed
[redacted], 1980; TOX9552390; Lot XHJ-64, 98.7%; Monsanto	Developmental, Dutch Belted rabbit, d 6-27 p.c., gavage	0, 75, 175, 350 mg/kg bw/d	Maternal: 75 mg/kg bw/d, dev.: 175 mg/kg bw/d	Maternal: 175 mg/kg bw/d, dev.: not established due to low number of foetuses	Maternal: mortality, soft stool, Diarrhea; dev.: none	Study supplementary, previous maternal NOAEL confirmed, dev. NOAEL revised
[redacted] 1991; TOX9552391;	Developmental,	0, 50, 150, 450 mg/kg	Maternal: 50 mg/kg bw/d,	Maternal: 150 mg/kg bw/d,	Maternal: GI-tract,	Study acceptable,

Reference; Study identification; Batch, purity; Owner	Study type, strain, duration, route	Dose levels	NO(A)EL	LO(A)EL	Targets/ Main effects	Outcome of re-evaluation
206-Jak-25-1, 98.6%; Cheminova	NZW rabbit, d 7-19 p.c., gavage	bw/d	dev.: 150 mg/kg bw/d	dev.: 450 mg/kg bw/d	food & bw gain ↓: dev.: late embryonic death, post implantation loss, cardiac malformations*	previous evaluation partly confirmed
[redacted], 1989; TOX9551960; Lot 38, 95%; Barclay, Luxan	Devleopmental, NZW rabbit, d 6-18 p.c., gavage	0, 125, 250, 500 mg/kg bw/d	Maternal& dev.: 250 mg/kg bw/d	Maternal&dev 500 mg/kg bw/d	Maternal: food, bw↓, abortion; Dev.: dead foetuses, malformations (external, visceral & skeletal)	Study supplementary due ot severe reporting deficiencies, previous NOAELs confirmed

* observed at excessive maternally toxic dose level

The study by [redacted] (1993, TOX9551106) must be considered supplementary, mainly because it was compromised by high maternal mortality. During treatment, 4 does of the mid and 5 females in the top dose group died. In addition, three more high dose females died after scheduled cessation of substance administration. In principle, the premature death of more than one half of the pregnant rabbits at the high dose level would have required immediate termination of this group. From the beginning of the experiment, there were less does in the treated groups than in the control (15 to 17 mated females *vs.* 26). Together with the animal losses and a case of complete litter resorption, this difference resulted in a very low number of litters and foetuses from the highest dose group that were available for teratological examination at scheduled sacrifice (Table 2.6–13).

Table 2.6-13: Foetal findings in the study by [redacted] (1993), copied from 1998 DAR

Dose group (mg/kg bw/d)	0	20	100	500
Percentage of foetuses with 'dilated heart'	0.0	5.1*	5.2*	17.9*
No. affected/total number of foetuses examined	-	4/78	4/77	5/28
Litters affected/no. of litters	-	3/13	2/12	2/6
Foetuses with major visceral malformations	4/133	6/78	6/77	8/28
Percentage of foetuses with extra 13th rib	0.0	1.3	2.6	3.6*

* statistically significant, p≤0.05

In fact, the percentage of foetuses with 'dilated heart' was significantly increased at all dose levels. However, because of the low number of foetuses and litters, it is hardly possible to interpret any of the results obtained in the top dose group. Furthermore, for all foetal effects at the mid and high dose levels, it must be taken into account that they occurred in the presence of overt maternal toxicity including lethality. If only the low and mid dose group would be considered, the absolute number of foetuses and litters with 'dilated heart' was quite small and did not show a difference between the two groups although the dose applied to mid dose females was by five times higher. Thus, there was no clear dose response what exactly one

would expect. Moreover, the diagnosis ‘dilated heart’ was not defined in the study report and neither criteria for this diagnosis nor any measurements of the heart and its size were provided.

At the highest dose, an increased percentage of foetuses with extra 13th rib was observed that gained statistical significance and was regarded in the 2001 DAR as treatment-related but here, again, the same reservations apply because of the low number of foetuses and litters and because of severe maternal toxicity.
In sum, the re-evaluation of the study by [redacted]. (1993, TOX9551106) by the RMS confirmed the previously established NOAEL for maternal toxicity at 20 mg/kg bw/day, based on probably treatment-related maternal deaths and gastroinstestinal signs. With regard to developmental effects, the study results do not allow meaningful assessment for highest dose level. If assessment is confined to the low and mid dose levels, there was no clear evidence of foetotoxicity or teratogenicity because the finding ‘dilated heart’ was not really substantiated in the study report and because of the lacking dose response. Thus, the NOAEL for developmental toxicity of 100 mg/kg bw/day from the previous evaluation may be also confirmed. There are strong reservations about the quality and validity of this study.

Re-evaluation of the study by [redacted] (1980, TOX2552390) revealed that the NOAEL for maternal toxicity may be confirmed at 75 mg/kg bw/day due to mortality and clinical signs at 175 mg/kg bw/day and above. No foetal effects were seen. However, the previously established NOAEL for developmental toxicity of 350 mg/kg bw/day was lowered to 175 mg/kg bw/day because of the low number of available foetuses in the high dose group avoiding meaningful evaluation. Like in the study by [redacted] (1993, TOX9551106), this low number of foetuses and litters was mainly due to the premature loss of more than one half of the does receiving the highest dose (10/17). This high mortality making assessment of high dose developmental effects impossible is the reason to consider the study now as supplementary only.

In contrast, the study by [redacted]. (1991, TOX9552391) is still considered acceptable and the NOAELs for maternal toxicity of 50 mg/kg bw/day as well as for developmental toxicity of 150 mg/kg bw/day were confirmed. The maternal NOAEL is based on clinical signs and decreased food consumption at 150 and 450 mg/kg bw/day. At the high dose level, one dam died following occurrence of clinical signs and abortion. The developmental NOAEL was established because of a higher frequency of late embryonic death at the highest dose level that was significantly elevated over the control value and was just at the upper edge of the historical control range. Furthermore, total embryonic losses were increased in all treated groups. However, this data is difficult to interpret since a comparison with historical control data from the performing laboratory proved a remarkably low percentage of post-implanation loss in the control group (5.7 %) that was below the historical control range (6.5 – 17.5 %). In contrast, the percentages for the low and high dose groups (19.5 and 21 %) were above its upper edge, but the 15.3 % in the mid dose group was well within and there was no clear dose response. In this study, there was also an increase in cardiac malformations, mainly interventricular septal defects, at 450 mg/kg bw/day. This finding was observed in four foetuses from 4 litters as compared to one foetus showing this defect in each the control, low and mid dose groups. It must be emphasised that these malformations are apparently different from what is presumably meant by [redacted] (1993, TOX9551106) as ‘dilated heart’.

The study by [redacted] (1989, TOX9551960) is now considered supplementary due to serious reporting deficiencies (*e.g.* no individual data, no results of maternal necropsy). However, the previous evaluation setting a maternal and developmental NOAEL of 250

mg/kg bw/day was confirmed. The previous DAR did not mention the external malformation in rabbits which are reported now in the present RAR (please refer to Volume 3). Total number of foetuses/litters with malformations was higher in the groups receiving mid and high doses of glyphosate without statistical significance, but it remains unclear, whether statistical analysis was performed. Ventricular septal defects as in the study by [redacted]. (1991, TOX9552391) were also noted but only in 2 out of 78 foetuses in the high dose group (control incidence 0/109). A higher number of further visceral malformations at the top dose level was due to absent kidneys or lung lobes.

In addition, three new studies in rabbits were provided in the GTF dossier and summarised in Table 2.6.-14. These studies were submitted either for the first time for this evaluation or had been subject to JMPR evaluation in 2004 yet and were all considered acceptable by the RMS.

Table 2.6-14: New developmental toxicity studies in rabbits reviewed for EU evaluation 2012

Reference; Study identification; Batch, purity; Owner	Study type, strain, duration, route	Dose levels	NO(A)EL	LO(A)EL	Targets/ Main effects	Outcome of RMS evaluation
[redacted] 1995, ASB2012-11498; T-041209, 97.56%; Arysta	Developmental, Japanese White rabbits (Kbl:JW), d 6-18 p.c., gavage	0, 10, 100, 300 mg/kg bw/d	Maternal: 100 mg/kg bw/d, Developmental: 300 mg/kg bw/d	Maternal: 300 mg/kg bw/d, Developmental: not Established	Maternal: Loose stool, abortion, 1 doe died; Dev.: none	Study acceptable
[redacted] 1996; ASB2012-11499; H95D161A, 95.3%; Nufarm	Developmental, NZW rabbit, d 7-19 p.c., gavage	0, 50, 200, 400 mg/kg bw/d	Maternal & dev.: 50 mg/kg bw/d	Maternal& dev.: 200 mg/kg bw/d	Maternal: bw gain ↓, Dev.: post-implantation loss	Study acceptable
[redacted] 1996; TOX2000-2002; Y04704/034, 95.6%; Syngenta	Developmental, NZW rabbit, d 8-20 p.c., gavage	0, 100, 175, 300 mg/kg bw/d	Maternal: 100 mg/kg bw/d, Dev.:175 mg/kg bw/d	Maternal: 175 mg/kg bw/d Dev.: 300 mg/kg bw/d	Maternal: food, bw gain ↓, clinical signs, dev.: foetal wt & ossification ↓	Study acceptable, 2004 JMPR evaluation confirmed

In the study by [redacted] (1995, ASB2012-11498), the NOAEL for maternal toxicity was 100 mg/kg bw/day, based on obviously gastrointestinal disturbances and a single death at 300 mg/kg bw/day. No effects on development were reported and, therefore, the highest dose of 300 mg/kg bw/day is considered the foetal NOAEL.

[redacted] (1996, ASB2012-11499) established a maternal NOAEL of 50 mg/kg bw/day, based on decreased body weight gain at 200 mg/kg bw/day and above. The NOAEL for developmental toxicity was the same because of increased post-implantation losses at the two upper dose levels. Due to some reporting deficiencies, it remains unclear whether the heart was part of visceral examination.

The study by [redacted] (1996, TOX2000-2002) was already evaluated by JMPR in 2004. The NOAEL for maternal toxicity of 100 mg/kg bw/day was based on clinical signs, reduced food consumption and body weight gain at the mid dose level of 175 mg/kg bw/day and above. Developmental toxicity comprised reduced foetal body weight and reduced ossification at 300 mg/kg bw/day, resulting in a foetal NOAEL of 175 mg/kg bw/day.

In conclusion, the previously known and newly submitted studies have clearly shown a particular vulnerability of pregnant females of this species.
The overall NOAEL for maternal toxicity was 50 mg/kg bw/d as found by [redacted] (1996, ASB2012-11499) and by [redacted] (1991, TOX9552391). It is further supported by the (supplementary) study of [redacted] (1980, TOX2552390) who had established 75 mg/kg bw/day and is well between the NOAEL of 20 mg/kg bw/day and the LOAEL in the (supplementary) study by [redacted] (1993, TOX9551106). However, in the latter study, mortality occurred already at the LOAEL of 100 mg/kg bw/day. This finding

might suggest a rather steep dose response curve and provided the lowest dose at which mortality was noted in any study and species following administration of glyphosate. In other developmental studies in rabbits, maternal deaths were seen at 175 mg/kg bw/day and above ([redacted]., 1980, TOX2552390; [redacted]., 1991, TOX9552391; [redacted] 1995, ASB2012-11498). Thus, the (pregnant) rabbit proved more sensitive than the other species which were employed in toxicological testing of glyphosate. Surprisingly, there were other studies in which the does tolerated similar or even higher amounts of glyphosate (300 to 500 mg/kg bw/day) much better, *i.e.*, without mortality at least ([redacted], 1989, TOX9551960; [redacted] 1996, ASB2012-11499; [redacted] 1996, TOX2000-2002). Apart from mortality in some studies, maternal toxicity was characterised by gastrointestinal signs, lower body weight (gains) and reduced food consumption and, occasionally, abortions. The lowest LOAEL was found in the study by [redacted] (1993, TOX9551106) whereas the respective values in the other experiments ranged from 150 to 500 mg/kg bw/day.

The lowest NOAEL for developmental effects was 50 mg/kg bw/day, based post-implantation loss at 200 mg/kg bw/day in the study by [redacted] (1996, ASB2012-11499). Due to dose spacing, the NOAELs in other studies were higher ([redacted] 1980, TOX2552390; [redacted]., 1991, TOX9552391; [redacted] 1996, TOX2000-2002) but consistently below 200 mg/kg bw/day. Beside post-implanation losses and late embryonic death ([redacted] 1989, TOX9551960; [redacted] 1991, TOX9552391), developmental findings at higher dose levels included a lower foetal weight and delayed ossification.

The (different) cardiac findings in foetuses in some studies had provoked much discussion. It seems clear that cardiac malformations (interventricular septal defects) occurred at very high dose levels of 450 mg/kg bw/day ([redacted]., 1991, TOX9552391) and of 500 mg/kg bw/day ([redacted], 1989, TOX9551960) although incidences were quite low. Similar effects were not reported in studies from the mid 90ies ([redacted] 1996, TOX2000-2002; Hojo, 1995, ASB2012-11498) but these researches did not administer that high dose levels which had proved clearly maternally toxic in other studies before [redacted] 1993, TOX9551106; [redacted], 1980, TOX2552390).

The study by [redacted] (1993, TOX9551106) must not be taken as supportive evidence because their findings (‘dilated heart’ that were claimed to occur at lower dose levels were of a completely different nature. Dose response for this ‘dilatation’ was questionable, descripton was poor and a similar effect was not described by others.
In any case, there seems to be no increased risk for foetal heart effects at the levels of exposure below those that caused severe maternal toxicity. Therefore, and because of the low incidence of the interventricular septal defects even at very higher dose levels, classification and labelling for developmental toxicity is not considered appropriate and necessary. This is in accordance to Guidance to regulation (EC) No 1272/2008 on CLP of substances and mixtures, 2012: Annex I: 3.7.2.4.4, *Maternal mortality: ‘an increased incidence of mortality among the treated dams over the controls shall be considered evidence of maternal toxicity if the increase occurs in a dose-related manner and can be attributed to the systemic toxicity of the test material. Maternal toxicity greater than 10 % is considered excessive and the data for that dose level shall not normally be considered for further evaluation’.*

Studies with formulations/Published data

Published studies on developmental and reproductive toxicity and on endocrine disrupting properties of glyphosate and glyphosate based formulations include *in vitro* and *in vivo* tests and epidemiological studies. Publications on endocrine disruption have been included in this

section because they are mainly related to developmental and reproductive toxicity. Different types of papers must be distinguished.

Reviews of existing regulatory studies

The developmental studies that had been submitted by the notifiers for the previous and current EU evaluations, have been, at least partly, subject to independent reviews:.

Kimmel *et al.* (2013, ASB2013-3462) analyzed the information from 7 unpublished developmental studies in rabbits and 6 developmental toxicity studies in rats to determine if glyphosate poses a risk for cardiovascular malformations. They concluded that there was no risk for increased cardiovascular defects as a result of glyphosate exposure during pregnancy.

In contrast, Antoniou *et al.* (2012, ASB2012-15927) blamed the regulatory authorities, in particular in Germany, for misinterpreting findings in the developmental studies that were reported in the old DAR (1998, ASB2010-10302). In addition, they reported a number of studies with adverse outcomes mainly from academic research to support their general view that the authorities would rely too much on industry-sponosored regulatory studies. In their review, they challenged also general principles of toxicology such as dose response, use of historical control data or weight of evidence. Very often, they ignored the differences in the test materials, *i.e.*, if the active substance glyhosate or certain formulations had been tested as well as the general problem to extrapolate from *in vitro* findings to the *in vivo* situation.

Experimental *in vivo* studies

Glyphosate was included into the U.S. EPA Endocrine Disruptor Screening Program's (EDSP) first list of 67 compounds that were foreseen to Tier 1 Screening. The compounds were selected on their potential for exposure rather than suspected interference with the endocrine system. The EPA will evaluate the full battery of Tier 1 screening assays for glyphosate's potential to interact with the oestrogen, androgen and thyroid endocrine pathways. ~~Accordingly, different tests will be performed. To date (March, 2013), their outcomes are not known. However, the~~ The first published data for glyphosate revealed no effects on androgenic or oestrogenic pathways in the Hershberger and Uterotrophic assays (Saltmiras *et al.*, 2012, ASB2012-12016). Levine *et al.* (2012, ASB2014-9609) published a short summary of the results. They concluded that, based on the Tier 1 assays that had been performed at different independent laboratories and taking into account the 'higher tier' regulatory safety studies glyphosate might not be considered an endocrine disrupter. Later on, Bailey *et al.* (2013, ASB2013-3464) summarized the first results of the male and female pubertal assays in which glyphosate did not exhibit evidence of endocrine disruption.

The following publications describe studies that were performed with commercially available formulations. Daruich *et al.* (2001, ASB2012-11601) studied the effects of a glyphosate herbicide formulation from Argentina on the activity of cytosolic enzymes in liver, heart and brain of pregnant rats and their foetuses and claimed to have found a variety of functional abnormalities. There was also a decrease in food and water intake and body weight gain and it cannot be excluded that the enzymatic changes were due to these findings. No consistent dose response was noted and unrealistically high doses were used.

Dallegrave *et al.* (2003, ASB2012-11600; 2007, ASB2012-2721) published results of two non-guidelines rat reproduction and developmental toxicity studies with a Brazilian Roundup formulation containing a polyoxethylated alkylamine, POEA, as surfactant. These studies did

not provide convincing evidence of reproductive toxicity or teratogenicity. However, the NOAELs were clearly below those in the regulatory studies with glyphosate pointing to higher toxicity of the formulation. They are described and discussed in detail in Volume 3 in chapter B.6.13 in the appendix 'Toxicological evaluation of the POE-tallowamine surfactant (CAS no. 61791-26-2)'.

Romano *et al.* (2010, ASB2012-12012) tested a glyphosate-based formulation in a male pubertal-like assay in rats and reported delayed preputial separation, reduced seminiferous epithelial height and increased luminal diameter of seminiferous tubules. Surprisingly, testosterone levels were decreased, despite a higher (relative) testis weight. More recently, Romano *et al.* (2012, ASB2012-12011) reported additional findings in male rats after supposed *in utero* and *post natal* exposures which included behavioural changes, histological lesions and endocrine effects. According to the authors, these changes were reflected by a 'hypersecretion of androgens and increased gonadal activity, sperm production and libido'. As in their first publication, Romano *et al.* (2012, ASB2012-12011) have based their hypothesis on rather selectively discussed literature implicating glyphosate as an endocrine disruptor, predominantly with citations to research from the Séralini laboratory (see below).

Paganelli *et al.* (2010, ASB2012-11986) exposed *Xaenopus laev*is frog embryos to a glyphosate formulation in the water where they dwelled or injected the test substance directly into frog embryos and, through a hole cut in the egg shell, into chicken embryos exposed directly to a glyphosate formulation. The authors claimed to have found evidence of teratogenicity, in particular neural crest lesions that might progress to craniofacial malformations. A mechanism similar to that of excess retinoic acid was suspected. However, the relevance of these findings must be questioned because of highly artifical routes of exposure as well as the application of excessive doses. Craniofacial malformations were not noted in developmental studies in rats or rabbits.

Chruscielska *et al.* (2000, ASB2013-9831) submitted a teratogenicity study in Wistar outbred rats. The used test guideline was not indicated. Doses of 0-750-1500-3000 mg/kg bw/day were administered from day 7-14 of pregnancy to 20 females per dose group. No embryotoxic and no teratogenic effects have been observed. Unfortunately, the test material was not properly described, apart from the information that it was glyphosate.

In vitro studies

There is a huge amount of *in vitro* research publications in which sometimes glyphosate but much more often glyphosate-based formulations were characterised as toxic and frequently also as 'endocrine disrupters'. Based on these findings, reproductive and developmental toxicity *in vivo*, also in humans, is often anticipated. Obviously, these assumptions are in contradiction to what was seen (or better, not seen) in the apical reproductive and developmental studies with glyphosate that are described above. They are also in contradiction to the absence of convincing evidence of adverse effects on fertility, reproduction and development in humans, despite worldwide and long-lasting use of herbicides containing this active substance (see 'Epidemiology' below). There may be at least three reasons for this discordance. At first, there is the general (and generally unsolved) problem of extrapolating from *in vitro* findings to the *in vivo* situation because of different behaviour and reactions of isolated cells as compared to cells, tissues, organs or regulation systems in an intact organism with a wide range of possibilities for feed back mechanisms, adaption and repair. Then, large differences in the exposure must be acknowledged. It is a clear difference between a steady concentration of a test substance in a cell culture (even if similar to what is applied, *e.g.*, on a field) and the variable and mostly shorter exposure of cells, perhaps of the same type, in the human or animal body, that is modulated, in case of glyphosate, by low oral or dermal absorption, wide distribution and fast elimination. The third reasons is more specific: glyphosate-based herbicides contain surfactants to allow or facilitate penetration into plant tissues. In general, surfactants are not suitable test substances to be used in *in vitro* cell models due to their physico-chemical nature. They alter the integrity of cellular membranes, including mitochondrial membranes, and thus may confound various endpoint measurements. (According to U.S. EPA Aromatase Inbibition Test Guideline, OECD 890.1200, 'Microsomes can be denatured by detergents [surfactants]. Therefore, it is important to ensure that all glassware and other equipment used for microsome preparations be free of detergent residue').
The French Agency for Food Safety (AFSSA, 2009, ASB2012-11532) mentioned that surfactant effects were known to increase membrane permeability, to cause cytotoxicity and to induce apoptosis.

In sum, the publications mentioned in the following are not suitable to amend toxicological evaluation of glyphosate. If this was the only available information, it would certainly suggest an endocrine, reproductive and developmental risk and higher tier animal studies would be needed for clarification. However, many of these studies are available for glyphosate and are reported and discussed in this RAR suggesting that there is not risk at least at exosure-relevant dose levels. Nontheless, the *in vitro* data suggest a higher toxicity of (certain) formulations as compared to the active ingredient.

From a laboratory at the University of Caen, France, a remarkable number of *in vitro* tests with glyphosate and glyphosate-based formulations was published (Richard *et al.*, 2005, ASB2009-9024; Benachour *et al.*, 2007, ASB2009-9018; Benachour and Séralini, 2009, ASB2012-11561; Gasnier *et al.*, 2009, ASB2012-11629; Gasnier *et al.*, 2010, ASB2012-11628; Gasnier *et al.*, 2011, ASB2012-11630; Clair *et al.*, 2012, ASB2012-11592; Mesnage *et al.*, 2012, ASB2012-11900). The investigated parameters and obtained findings were used to 'predict' *in vivo* effects including endocrine disruption, aromatase inhibition, oestrogen synthesis, placental toxicity, foetotoxicity, embryotoxicity and bioaccumulation. Nearly all findings were more pronounced *in vitro* when formulations of glyphosate were tested, as compared to the active ingredient. Thus, Richard *et al.* (2005, ASB2009-9024) compared effects of glyphosate and a Roundup formulation (that may be different from another

marketed under the same name) on human placental cells and aromatase and concluded that Roundup was more toxic than its active ingredient. Benachour *et al.* (2007, ASB2009-9018) studied time- and dose-dependent effects of Roundup on human embryonic and placental cells and came to the same conclusion that Roundup was more efficient than its active ingredient glyphosate. Benachour and Seralini (2009, ASB2012-11561) stated their 'work clearly confirms that the adjuvants in Roundup formulations are not inert'. In the most recent publication from this laboratory, the potential active principle for toxicity on human cells for 9 glyphosate-based formulations and the active ingredient was studied by Mesnage *et al.* (in press, ASB2012-13917). The authors have shown that 'ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity'.
Unfortunately, no surfactants alone, without glyphosate, were tested in these studies. However, there is some evidence coming from other fields of toxicological testing, that critical in vitro effects of formulations are mainly due to cytotoxicity of surfactants [see Addendum to the previous DAR (2000, ASB2013-2748) of which a slightly modified version may be found also in Volume 3, under B.6.4.7]. Levine *et al.* (2007, ASB2009-9030) presented convincing data to demonstrate a lack of *in vitro* synergism of glyphosate with the surfactant since the cytotoxic effects were completely independent of glyphosate. Identical dose-response curves were noted for a formulated product with and without the glyphosate active ingredient.

In line with that, Walsh *et al.* (2000, ASB2012-12046) reported that a glyphosate based formulation, but not glyphosate alone, affected the steroidogenesis pathway by inhibiting the progesterone production resulting in downstream reduction of mitochondrial levels of the StAR protein.

Glyphosate active ingredient was of no concern in other systems of potential relevance for reproduction when tested alone. Thus, Quassinti *et al.* (2009, ASB2012-12007) revealed clearly no effects on gonadal steroidogenesis in frog testis and ovaries.
Forgacs *et al.* (2012, ASB2012-11621) demonstrated no effect on testosterone levels in BLTK1 murine leydig cells *in vitro*. Furthermore, Hecker *et al.* (2010, ASB2012-11840) evaluated glyphosate within the OECD multi-laboratory validation of the Steroidogenesis Assay (that is also used for Tier 1 screening of the U.S. EPA EDSP, see above) and concluded that there was no impact on steroidogenesis.

Epidemiology

Several epidemiological studies on glyphosate exposure evaluated the following reproductive outcomes: miscarriage, fecundity, pre-term delivery, gestational diabetes mellitus, birth weights, congenital malformations, neural tube defects, attention-deficit disorder / attention-deficit hyperactive disorder (ADD/ADHD). In most instances, glyphosate and reproductive outcomes lack a statistically significant positive association (Mink *et al.*, 2011, ASB2012-11904).
For ADD/ADHD, however, a positive association with glyphosate use was reported by Garry *et al.* (2002, ASB2012-11626), but cases were parent reported with no clinical confirmation and the reported incidence rate of approximately 1% for the study population was well below the general population incidence rate of approximately 7%.

The absence of reproductive and developmental effects in humans is not surprising since human *in utero* exposures would be very limited. On one hand, the perfusion rate of glyphosate across the placenta is low (Mose *et al.*, 2008, ASB2012-11914). On the other hand, ~~at least with regard to general population (leaving people with occupational or~~

~~residential exposure aside), systemic intake is also quite limited~~ systemic intake of glyphosate in the general population is low. McQueen *et al.* (2012, ASB2012-11898) measured a very low dietary exposures ~~in Europe~~ of pregnant women in Australia ranging from 0.005% to 2% of the ~~current glyphosate~~ ADI of 0.3 mg/kg bw for glyphosate as established by the Australian authorities. In line with that, very low exposure of consumers and operators may be expected if calculated on the basis of glyphosate excretion in urine from Europe and the U.S. (see section 2.6.11, part on human biomonitoring, below). In combination, both facts will contribute to a nearly negligible *in utero* exposure.

One of the reasons for Paganelli *et al.* (2010, ASB2012-11986) to perform their research in *Xaenopus laevis* were reports on an increase in malformations in newborn children in rural areas in South America, *e.g.*, the Gran Chaco region, in which a large amount of agrochemicals is applied, frequently by aircraft. Reliable scientific information to confirm or exclude such an increase is scarce. In fact, Benitez-Leite *et al.* (2009, ASB2012-11563) reported the incidence of anomalies in newborn babies in a hospital in Paraguay but from this data it cannot be concluded if there was in fact an increase. Many of the reported anomalies were variations rather than malformations and, according to inquiries by the RMS, a similar incidence might be expected in an average German birth clinic. Furthermore, a ”single hospital-based” analysis is not sufficient to prove changes in the prevalence of malformations in a region. The authors themselves reported a (not specified) “high” exposure of the parents to agrochemicals and pesticides in general but glyphosate or glyphosate-containing herbicides were not explicitly mentioned.
Lopez *et al.* (2012, ASB2013-10534) reported an increase in malformations but also in cancer incidence from certain regions but these increases were more general without clear-cut evidence of a distinct anomaly or a certain cancer type. The general weaknesses of such data collected in so-called ”ecological” (“correlational”) studies are the unknown exposure level and the impossiblity to attribute a certain outcome to exposure to a single substance (Paumgartten and Cremonese *et al.*, 2012, ASB2013-10538). In everyday life, people in these rural areas were exposed to a great number of (agro)chemicals that, taken together, might result in a higher risk for adverse outcomes such as malformations or cancer, in particular if appropriate safety measures are not taken. However, such an information is of not much use for risk assessment that has to be performed for a single substance. Even if the claimed increases could be substantiated in future, it is unlikely that they were due to glyphosate, taking into account the extensive toxicological database and the long history of its worldwide safe use.

2.6.8 Summary of neurotoxicity

In the previous EU evaluation, it was concluded that glyphosate was devoid of a neurotoxic potential. However, this evaluation was only based on the absence of neurotoxicity in the acute, short-term and chronic studies because specific studies in rats were not available at that time. In addition, delayed neurotoxicity was not expected for this compound since it is not an organophosphate ester that will inhibit cholinesterases. According to the old DAR (1998, ASB2010-10302), data obtained in chicken had supported this assumption but these old studies must be regarded as not acceptable from a todays point of view.

Now, a more substantiated assessment can be made since three valid studies covering the endpoints acute and subchronic neurotoxicity (in rats) and delayed neuropathy (in chicken) have been performed. These studies (summarised in Table 2.6–15) confirmed that glyphosate was not neurotoxic.

Table 2.6-15: Neurotoxicity studies with glyphosate (all not available during previous EU evaluation)

Reference; Study identification; Batch, purity; Owner	Study type, species, strain, sex, number, duration, route	Dose levels	NOAEL	LOAEL	Findings
[redacted] 1996; ASB2012-11500; Y04707/034, 95.6%; Syngenta	Acute neuro-toxicity; rat, Alpk:AP_fSD (Wistar-derived); m/f, 10 per dose, oral gavage	0, 500, 1000, 2000 mg/kg bw/d	2000 / 1000 mg/kg bw (neuroto-xicity / systemic effects)	> 2000 / 2000 mg/kg bw	Clinical signs in females at top dose level including one death; no evidence of neurotoxicity (FOB, histology)
[redacted] 1996 ASB2012-11501; P24, 95.6%; Syngenta	Subchronic neurotoxicity; 90 d, rat, Alpk: AP_fSD (Wistar-derived); m/f, 12 per dose, feeding	0, 2000, 8000, 20000 ppm (156/166, 617/672, 1546/1631 mg/kg bw/d in m/f)	617 mg/kg bw/d (systemic) 1546 mg/kg bw/d (neuro-tox.)	1546 mg/kg bw/d (systemic) >1546 mg/kg bw/d (neuro-tox.)	Bw (gain) and food utilization↓ in high dose m; no evidence of neurotoxicity (FOB, histology)
[redacted] 1996; ASB2013-9828 95.6%; Syngenta	Delayed neurotoxicity, chicken (hybrid Lohmann brown)	0, 2000 mg/kg bw	2000 mg/kg bw	>2000 mg/kg bw	No evidence neither of systemic toxicity nor of delayed neuropathy; positive control substance triortho-cresyl-phosphate (1000 mg/kg bw) gave the expected response

Absence of neurotoxicity in rats was further supported by [redacted] (1996, ASB2012-12054) who performed *ex vivo* investigations with isolated rat gastrocnemius muscle as part of a screening study for pharmacological properties of glyphosate (see below). Evaluation of innervated muscle response showed that glyphosate technical, when injected at the maximum solubility concentration in physiological saline (12 mg/mL), did not cause any neuromuscular blocking activity after electrical stimulation of the sciatic nerve.
Because there were no indications for a neurotoxic potential of glyphosate in acute and subchronic neurotoxicity studies and no evidence of neurological disturbances in pups in the multi-generation studies in rats, a developmental neurotoxicity study (DNT) is not needed.

Data with formulations/Published information

Mechanistic studies *in vitro* and *in vivo*

The main focus of the available studies was on a possible link between an exposure to glyphosate and the development of Parkinson`s disease. ~~A possible link with Parkinson's disease~~ This hypothesis but also a link with other neurological diseases was examined in mechanistic studies in different systems such as *Caenorhabditis elegans* worms, in rats or cell cultures (Astiz *et al.*, 2009, ASB2012-11549; Negga *et al.*, 2011, ASB2012-11923; Gui *et al.*, 2012, ASB2012-11835). Sometimes, positive evidence was reported but these findings are not considered relevant when the extremely huge database in laboratory animals with no evidence of neurotoxicity and the absence of suggestive epidemiological data in humans is taken into

consideration. Chorfa *et al.* (2013, ASB2014-9328) studied the effects of four pesticides (paraquat, rotenone, maneb and glyphosate) on different molecular events in cell lines which are considered to be related to Parkinson`s disease. Three of the four pesticides triggered molecular events involved in Parkinson`s disease but glyphosate was the only one that did not exhibit such an effect.

A few more publications seem to support the lack of a neurotoxic potential of glyphosate. McConnell *et al.* (2012, ASB2014-9615) tested multi-well microelectrode arrays for neurotoxicity screening and found glyphosate negative with regard to its potential to cause neurotoxic effects. LeFew *et al.* (2013, ASB2014-9608) confirmed this finding when they evaluated microelectrode array data using Bayesian modeling as an approach for screening neurotoxicity and to facilitate prioritization for testing.

Even though glyphosate (N-phosphonomethyl glycine) is sometimes allocated to the organophosphates, it is well known not to inhibit the activity of the cholinesterases. In line with that, in poisoning incidents in humans, common symptoms of acute acetylcholinesterase inhibition such as salivation, lacrimation, urination and defecation have not occured.
Cole *et al.* (2004, ASB2012-11594) evaluated 15 different pesticides for neurotoxic endpoints in *C. elegans* with analytical grade active ingredients, mostly noting reduced cholinesterase activities for pesticides causing neurotoxicity but not for glyphosate. Interestingly, the authors reported a low pH effect resulting in reduced cholinesterase activity in the high dose of glyphosate. However, glyphosate formulations contain the salts instead of the technical acid and, thus, do not have a low pH.

Cattani *et al.* (2014, ASB2014-3919) studied neurotoxic effects of the formulation Roundup in the hippocampus of immature rats following acute (30 min) and chronic (during pregnancy and lactation) exposure. Results showed that acute exposure to Roundup increased the Ca^{2+} influx leading to oxidative stress and neuronal cell death. It was hypothesised that Roundup might lead to excessive extracellular glutamate levels and to glutamate excitotoxicity and oxidative stress in rat hippocampus. For re-evaluation of glyphosate, these findings obtained with a formulation are without relevance. Furthermore, they are not supported by the huge database of toxicological studies in rats and other species.

Epidemiology

Over the last decade, several published studies investigated an association of glyphosate with neurotoxicity endpoints. In three papers, two human cases of Parkinson's disease were reported that became manifest not long after glyphosate exposure. The first case followed acute exposure to a glyphosate formulation while spraying a garden (Barbosa *et al.*, 2001, ASB2012-11557; da Costa *et al.*, 2003, ASB2012-11598). The second one occurred following chronic exposure of a factory worker in China (Wang *et al.*, 2011, ASB2012-12047) in a facility where a variety of pesticides including glyphosate were produced. However, a causal relationship of these (not quantified) exposures to glyphosate with Parkinson's disease is not likely. Occupational health surveillance did not provide evidence of a higher frequency of Parkinson's disease in glyphosate production workers. If the widely used glyphosate was in fact a causative agent of this fairly common disease, one would expect a significant number of cases associated with either acute and/or chronic exposures. Furthermore, occurrence of Parkinson's disease in survivors of acute intoxications following ingestion of high amounts of glyphosate products has not been documented.

While some epidemiological studies have indeed suggested statistical associations of Parkinson's disease with general pesticide exposure or insecticide or herbicide exposure (Engel *et al.*, 2001, ASB2012-11612), there is no particular evidence ~~specifically~~ for glyphosate. In the largest study to date, *i.e.*, the U.S. Agricultural Health Study, no association with reported glyphosate use was found (Kamel *et al.*, 2007, ASB2012-11862). Freire and Koifman (2012, ASB2014-9479) conducted a review of the epidemologic literature over the past decade with regard to Parkinson`s disease risk. An increased risk has been associated with different pesticides but not with glyphosate.
Human non-cancer epidemiologic outcomes related to glyphosate have been recently reviewed by Mink *et al.* (2011, ASB2012-11904), and there was no convincing evidence for an increased incidence of Parkinson's disease or other neurological disorders in individuals reporting glyphosate exposure.

For a number of other neurological diseases, a possible association with pesticides in general or certain active substances was also reported but not for glyphosate.

Kim *et al.* (2013, ASB2014-9592) studied the relation between depressive symptoms and severity of acute occupational pesticide poisoning among male farmers in South Korea. Among the pesticides causing the poisonings, paraquat dichloride was found to be a significant predictor of depressive symptoms. Glyphosate did not cause significant effects.

Kamel *et al.* (2012, ASB2014-9586) summarized the literature on the association of amyotrophic lateral sclerosis (ALS) with pesticides. The meta-analysis suggested that ALS risk was associated with the use of pesticides. In particular, ALS was associated with aldrin, dieldrin, DDT and toxaphene. However, no relevant association was evidenced for glyphosate.

Faria *et al.* (2014, ASB2014-9477) analysed the association between occupational exposures to pesticides, nicotine and minor psychiatric disorders (MPD) among tobacco farmers in southern Brazil. The study reinforced the evidence of the association between pesticide poisoning and mental health disorders. However, in this study organophosphates were the only chemical group positively assciated with MPD. Glyphosate was not associated with MPD.

2.6.9 Summary of further toxicological studies

Mechanistic studies

Efforts were taken to elucidate the mechanism of salivary gland findings that were previously obtained in classical toxicological studies of different types in rats and occasionally in mice, *i.e.*, a higher organ weights and increased basophilic staining and enlargement of cytoplasm especially in the parotid salivary glands. By comparing the effects of high doses of citric acid and trisodium citrate dihydrate when given by gavage or in the diet, it became apparent that low pH conditions in oral cavity may result in similar effects mainly on the parotid salivary gland, perhaps due to local irritation ([redacted] 2010, ASB2012-11519). Acidic conditions may appear if a large amount of glyphosate is contained in the diet. Indeed, this effect should be considered rather adaptive than toxic but it will depend on its severity whether it should be regarded as potentially adverse.

[redacted] (1992, TOX9551954) found good evidence in F344 rats that an adrenergic mechanism may have also contributed to the salivary gland findings, at least at high dose

levels when they fed 50000 ppm of glyphosate alone or in combination with exposure to either the adrenergic agonist isoproterenol or the antagonist propranolol or both.

Leaving mechanistic considerations aside, [redacted] (1996, ASB2012-11520 and ASB2012-11537) identified clear differences among rat strains with regard to their sensitivity to this type of effects. Administration of diets containing 20000 ppm glyphosate acid to male rats for 4 weeks resulted in an increase in parotid salivary gland weights in the F344 and AP (Alpk:AP_fSD, Wistar-derived) strains but not in CD rats. This increase proved reversible in AP rats after a 4-week recovery period whereas in F334 rats, there was still a difference to the controls. However, after 13 weeks on untreated diet, parotis gland weights were not different any longer. Microscopic examination of the salivary glands showed the most pronounced effect, again, in the F344 strain where there was diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells. Similar but less pronounced effects occurred in the AP (Alpk:AP_fSD, Wistar-derived) and CD (Sprague-Dawley; Charles River) strains involving small foci of cells only. Complete recovery of histopathological changes was apparent in AP and CD strains following the 4-week recovery period. In the F344 strain, basophilia of parotid acinar cells was still to be seen in 5 out of 8 animals after a 13 week recovery period (control group: 1/8).

Although there is no evidence of necrosis, apoptosis or inflammation or that the cellular alterations would progress with time to preneoplastic or neoplastic lesions, the organ weight increase and histological alterations in salivary gland are considered clearly treatment-related. If exposure is sufficiently high, similar effects in man cannot be excluded. There is no reason to ignore these findings in risk assessment and at least if there is an increase in severity, they should be also taken into account for setting NOAELs/LOAELs in idividual studies.

Pharmacological activity of glyphosate was investigated *in vivo* in rats which received a single oral dose of 5000 mg/kg bw. One hour after dosing, no haematological, electrographic (ECG) or neurological (behavioural/functional) changes were observed when compared to control animals ([redacted] 1996, ASB2012-12054). When administered to isolated guinea pig ileum, glyphosate technical caused a contractile response similar to that seen with known parasympathomimetic agents. Results of exposure of isolated rat gastrocnemius muscle are reported above (see 2.6.7).

An immunotoxicity study in female mice did not provide evidence of suppression of humoral components of the immune system or effects on thymus and spleen weights after dietary administration of glyphosate at dose levels of up to 5000 ppm (*ca* 1450 mg/kg bw/day) over 28 days ([redacted] 2012, ASB2012-11521).

Further studies were aimed to elucidate the mechanism of (acute) toxicity and to investigate possible additive effects. Experiments with i.p./i.v. administration to mice or rabbits revealed an impact of glyphosate ammonium salt on heart and respiratory functions as well as a decrese of the lethal dose under anesthesia (Takahashi and Kakinuma, 1992, TOX9552421). Irritation of the intestinal and stomach mucosa that might contribute to toxicity was shown by Mizuyama (1987, TOX9552430) in dogs and was more pronounced with a complete Roundup formulation than with glyphosate, its IPA salt or the surfactant that was contained. Bhide and Naik (1987, TOX9551964) reported an additive effect of glyphosate and 2,4- D and possibly also dalapon on mortality of rats after simultaneous administration of high doses.

Toxicological studies in farm animals

Following single or repeated adminstration of glyphosate acid and its IPA salt (MON0139) at high dose levels of more than 1000 mg/kg bw(/day) to farm animals (goats and cattle), the clinical picture of systemic intoxication in these animals was mainly characterised by gastrointestinal signs but also by depression, ataxia, convulsions, sternal recumbancy or head tremors. However, in spite of these neurological signs, gross pathological examination did not provide evidence of lesions in the nervous system. Unfortunately, ~~and, therefore,~~ histopathology of nervous tissue was not performed. In fact, histopathological examination identified the kidney as a target organ in ruminants and revealed mucosal irritation in the GIT (Rowe *et al.*, 1987, TOX9552422; 1987, TOX9552423; 1987, TOX9552424). In a study with repeated administration of a Roundup formulation (MON2139, containing 41.1% of the glyphosate IPA salt) to cattle over 7 days (Rowe *et al.*, 1987, ASB2010-8131), a NOAEL for systemic toxicity of 400 mg/kg bw/day was obtained. The LOAEL was 500 mg/kg bw/day and mortality was seen at 790 mg/kg bw/day (due to treatment-related aspiration pneumonia) and above. However, gastrointestinal signs predominated and neurological signs did not occur.

In sum, it is not likely that there is a specific neurotoxic potential of glyphosate in ruminants but it seems that systemic poisoning in these species may result in neurological signs. Based mainly on mortality and severity of clinical signs, ruminants appear a bit more sensitive to glyphosate effects than monogastric animals. Higher toxicity of certain formulations as compared to the active ingredient was confirmed once more.

Urinary excretion of glyphosate by dairy cows

Recently, a study has been published (Krüger *et al.*, 2013, ASB2013-11599) in which a large number of cows from eight Danish dairy farms (30 per farm) was investigated for glyphosate residues in the urine by means of an ELISA. All cows excreted glyphosate, presumably because of residues in their feed (that was, however, not analysed for glyphosate), but the urinary concentrations in the individual dairy farms differed were very much with mean values ranging from 10 to more than 103 µg/L. Based on these figures, a maximum daily intake of up to 16 mg glyphosate was calculated that ~~may be compared to either the proposed ADI or to the NOAELs in toxicological studies in farm animals. Taking the first approach, calculated exposure would have been by 15 times lower than the ADI. A comparison to the NOAELs~~ would give a wide margin of safety (approximately 1:4200) to the NOAELs in toxicological studies in farm animals. Thus, an impact on animal health is very unlikely. The authors claimed that alterations in some clinical chemistry parameters would have been linked to glyphosate but, on one hand, there is no proof that these findings were in fact adverse since clinical signs or pathological changes were not reported and, on the other hand, the statistical correlation was very poor. Furthermore, the origin and basis of reference values were not given.

Very low serum levels of several trace elements such as cobalt or manganese were detected and, as a hypothesis, the authors ascribed this finding to a possible chelating mode of action of glyphosate. However, these considerations appear rather speculative, in particular against the background of the very low exposure. Even if glyphosate exhibited chelating properties, the ingested and absorbed amount is not expected to bind trace elements to such an extent that clinical signs might be expected to occur.
A main deficiency of the study is the absence of a control group of cows with no glyphosate in their urines and proven absence of glyphosate in the diet.

In a second study, Krüger *et al.* (2014, ASB2014-5024) reported a mean urinary concentration of slighly above 40 µg/L in Danish cows (n = 242). In German cows from conventional

husbandry (n = 343), a lower mean of 20 µg glyphosate/L urine was detected. In contrast, cows from "GM free" regions (n = 32) in which farmers try to avoid feed that is or might be produced abroad on the basis of genetically modified crops had hardly any glyphosate in their urines. On one hand, this finding might suggest that ingestion of glyphosate residues in imported feedstuffs will have contributed the most to the whole glyphosate intake. On the other hand, there is no information on possible differences in the application of glyphosate on farms in the "GM-free" regions as compared to other parts of Germany. Based on the calculation in the paragraph above, no health concern is anticipated but it is interesting (even though not surprising) to note that glyphosate concentrations in cattle urine in the mean are by about 10 times higher than in human urine samples (see 2.6.11) following dietary exposure. The measured values appear reliable since the correlation of the ELISA results with those from the more accurate GC-MS method was quiet good (R^2 of 0.96 for cattle urine, based on 21 samples that were measured in parallel).

Potential effects on intestinal bacteria

Moreover, there is some discussion about possible effects of glyphosate on gut microflora. Based on these speculations, Seneff *et al.* (2013, ASB2014-9729) even included glyphosate in theory on biochemical mechanisms that might underlie autism in humans.

The scientific background of a presumed effect on intestinal bacteria is the herbicidal mode of action of glyphosate. Its target enzyme, the 5-enolpyruvylshikimic acid-3-phosphate synthase, that is common in the plant kingdom does not occur in animals but in most bacteria and yeast and in many protozoan species. Thus, at least theoretically, microbial balance, *e.g.*, in the intestines, might be altered by ingestion of glyphosate ~~for which evidence is mainly claimed because of~~ and this idea was somehow supported by *in vitro* results (*e.g.*, Shehata *et al.*, 2012, ASB2013-8529). However, if such an effect would be of practical relevance, clinical signs or economic losses such as lower milk yield or slower body weight increase in fattening animals should have been frequently observed because of the extensive use of glyphosate-based herbicide worldwide. The gastro-intestinal signs that were observed after administration of very high doses in dogs (see 2.6.4), rats and rabbits (see 2.6.7) and also in goats and cattle (see above) were most likely due to the well established irritating properties of glyphosate acid and cannot ascribed to alterations of the intestinal microflora.

For more than 10 years, there are reports on ~~So far, a link of glyphosate residues in ruminants diet to~~ a new disease in cattle ~~that was~~ mainly occurring ~~reported from~~ in the Northern parts of Germany of which the etiology has not been elucidated so far. Clinical signs in cattle are predominately seen in the perinatal period and comprise indigestion with alternating constipation and diarrhea, apathy, ataxia, paralysis, retracted abdomen, breathing difficulties, a decrease in milk yield, and death. Involvement of *Clostridium botulinum* overgrowth and formation of *Clostridium* neurotoxins (Rodloff and Krüger, 2011, ASB2013-13311; Krüger *et al.*, 2012, ASB2013-13312) due to possibly glyphosate-caused microbial imbalance have been hypothesised (Krüger *et al.*, 2013, ASB2013-8527) but the evidence appears rather weak. There was no convincing proof for the etiological role of *C. botulinum* even though the disease is often (but perhaps erroneously) called "chronic" or "visceral botulism". Even if glyphosate would have an impact on intestinal bacteria, it is doubtful if the ingested amounts would be sufficient to cause any clinical effects. ~~hof has not been established and is not likely. there is no convincing proof~~

Preliminary results of a large case-control study (Seyboldt and Hoedemaker, 2014, ASB2014-10736) suggest that the observed clinical signs ~~that~~ were ~~indeed caused by~~ not related to the

abundance of *C. botulinum* or its toxins and there was also no correlation with the use of glyphosate on the included farms.

These findings are in line with the results of a research project that had been commissioned by the German Federal Institute for Risk Assessment (BfR) to check the hypothesis mentioned above and because of the public concern about the sick cattle. Its objective was to investigate (1) whether the quantitative composition of ruminal microflora or rumenal metabolism might be altered and (2) if there was evidence of *C. botulinum* overgrowth. Two different experiments were performed by means of the ”Rumen Simulation Technique” (RUSITEC). In the first one, the effects of a glyphosate-based herbicide (Plantaclean® XL; 360 g/l glyphosate, containing a tallowamine surfactant) on rumen fermentative parameters were studied. Total glyphosate doses per day were 0.26 or 2.31 mg per fermentation vessel. No major changes in physiological rumen parameters were detected except slight decreases in NH_3-N concentrations and increases in isovalerate production in response to the high dosage. There was an increase in (beneficial) *Bifidobacterium spp.* but, in general, the microbial communities were not affected. In the second trial, no effects of the herbicide on the growth of *C. sporogenes* was found that had been artificially added to serve as a surrogate for *C. botulinum*. Results have not been published so far but a preliminary research report (Riede *et al.*, 2013; ASB2013-14684) has been submitted. Even though also an *in vitro* system and technique were used, these experiments appear more comprehensive than those by, *e.g.*, Shehata *et al.* (2012, ASB2013-8529) or Krüger *et al.* (2013, ASB2013-8527) and might reflect real-world conditions better because an effect on microbial communities was investigated and not only on certain bacterial species in isolation.

~~However, because of the growing public concern about this disease especially in Germany and because an effect of glyphosate on micro-organisms due to inhibition of the enzyme EPSPS (that is common not only in plants but also in most bacteria and some other micro-organisms) cannot be excluded, the has commisioned a study by means of the ”Rumen Simulation Technique” (RUSITEC). Its objective was to investigate wheter (1) quantitative composition of ruminal microflora or rumenal metabolism might be altered and (2) there is evidence of *C. botulinum* overgrowth. Two different experiments were performed (Riede *et al.*, 2013; ASB2013-14684). In the first one, the effects of a glyphosate-based herbicide (Plantaclean® XL; 360 g/l glyphosate, containing a tallowamine surfactant) on rumen fermentative parameters were studied. Total glyphosate doses per day were 0.26 or 2.31 mg per fermentation vessel. No major changes in rumen parameters were detected except slight decreases in NH3-N-concentrations and increases in isovalerate production in response to the high dosage. There was an increase in (beneficial) *Bifidobacterium spp.* but the microbial community of *Clostridia* was not affected. In the second trial, no effects of the herbicide on growth of *C. sporogenes* (artificially added and used as a surrogate for *C. botulinum*) was found.~~

Teratogenicity in piglets

Malformations in newborn piglets on a Danish farm have been ascribed to glyphosate simply because residues of this active substance were found by means of an ELISA at extremely variable concentrations in different organs and tissues of these animals (Krüger *et al.*, 2014, ASB2014-8935). An increasing content of glyphosate in the diet that was fed to the sows was also claimed but not analytically verified. In the absence of any control group and taking into account the rather low dietary exposure of the sows and the results of a multitude of developmental studies (see 2.6.7), this assumption appears in no way substantiated.

2.6.10 Summary of toxicological data on impurities and metabolites

Aminomethyl phosphonic acid (AMPA)

Aminomethyl phosphonic acid (AMPA) is a major metabolite of glyphosate in soil and in genetically modified crops but was shown to occur only in traces in mammals (see 2.6.1). It is part of the residue definition for dietary risk assessment. AMPA was subject to comprehensive toxicological testing and can be currently regarded as one of the best known pesticide metabolites. It was of very low acute toxicity when adminstered to rats or mice via the oral and dermal routes and proved negative for skin sensitisation in Magnusson & Kligman tests.

Short-term toxicity was investigated in gavage/capsule and feeding studies in rats and dogs. Increased kidney weights in a subacute rat study (Heath *et al.*, 1993, TOX9300349) at 350 mg/kg bw/day and above were not confirmed in a 3-month study in the same laboratory under similar conditions up to the limit dose of 1000 mg/kg bw/day (Strutt *et al.*, 1993, TOX9300377). It is interesting to note that the specific histological lesions in salivary glands observed with glyphosate (see sub-sections above) were not confirmed by Heath *et al.* (1993, TOX9300349) for exposure to AMPA although these glands, including the parotis, were subject to careful examination. In a feeding study in rats (Estes *et al.*, 1979, TOX9552401), a decrease in body weight gain and food consumption, minor alterations in clinical chemisty parameters (increase in lactate dehydrogenase activity) and histological lesions (epithelial hyperplasia of bladder and renal pelvis) were observed at dose levels of 1200 mg/kg bw/day and above that, in the whole, resembled effects of exposure to high doses of glyphosate. At an exaggerated dose level of 4800 mg/kg bw/day, there was some mortality (25 %) in female rats which died shortly after interim or final blood collections with death preceeded by gastrointestinal signs and general morbidity. The NOAEL in this study was 400 mg/kg bw/day.
In dogs, not effects of AMPA administration were noted and the NOAEL in a 3-month study was at least 263 mg/kg bw/day, *i.e.*, the highest dose tested ([redacted] 1991, TOX9552406).

AMPA was extensively tested for mutagenicity and proved consistently negative *in vitro* in bacteria (Ames test), in the mouse lymphoma assay and in rat hepatocytes (DNA damage and repair). *In vivo*, it did not produce an increae in micronucleus frequence in mouse bone marrow in two strains using either the oral or the i.p. route ([redacted] 1993, TOX9300379; [redacted] 1993, TOX9552413).

Developmental toxicity of AMPA was investigated in two studies in rats. [redacted] (1992, TOX9300348) did not detect any evidence of toxicity neither in the dams nor in the foetuses up to the limit dose of 1000 mg/kg bw/day. [redacted] (1991, TOX9552414) established a maternal NOAEL of 150 mg/kg bw/day because of the occurrence of clinical signs and a reduction in bw gain and food consumption at the upper dose levels of 400 and 1000 mg/kg bw/day. There was no evidence of teratogenicity but the mean foetal weight was lowered at the top dose level. Thus, the developmental NOAEL in this study was 400 mg/kg bw/day.

Taking all this data into consideration, it appears that AMPA is of equal toxicity as its parent compound. There is no specific or additional concern with this metabolite and the same reference values as for glyphosate should apply also for AMPA. Setting of an ARfD is not needed. Further studies (long-term, multigeneration, developmental toxicity in a second species) are not considered necessary.

N-acetylglyphosate (NAG)

N-acetylglyphosate (NAG) is another important metabolite of glyphosate and is newly proposed to be part of the residue definition for monitoring and for dietary risk assessment. It will occur in certain genetically modified plants such as soy beans or maize following application of glyphosate and was evaluated by EFSA with regard to setting of import tolerances (EFSA, 2009, ASB2012-3480). For this purpose, a few toxicological studies had been submitted and were evaluated in 2008 by the RMS. This assessment resulted in the conclusion that NAG (and also N-acetyl AMPA that also may be formed) was of no higher toxicity than glyphosate and that, as for AMPA; the reference doses established for the parent compound, should also cover the. However, since this application scenario is beyond the scope of this re-evaluation of glyphosate in preparation of a decision on future approval in the EU, there is no need to report the studies with NAG in detail. Furthermore, the owner of these studies was not part of the GTF.

Impurities

Definitive conclusions can be drawn only after a specification has been agreed and comparison of the different test materials to this specification is possible. From a toxicological point of view, it cannot be excluded that some of the very different high dose effects in the various toxicological studies with glyphosate (see above) are rather due to impurities than to the active ingredient. It was noted that test materials of different purity were applied and it can be reasonably assumed that their impurity profiles will also differ. Toxicological evaluation of specification and of relevance of impurities is included in Vol. 4. It was noted that formaldehyde may occur as an impurity. Its content should be lower than 1 g/kg. Concentrations of 1 g/kg or higher in technical active ingredient would result in a classification as a 1B carcinogen.

2.6.11 Summary of medical data and information

Reports on medical surveillance on manufacturing plant personnel

Industrial hygiene air monitoring data for glyphosate from a Monsanto plant in Luling, Louisiana (U.S.A.) have been submitted for the years 1981-1998. Based on the measured low exposures to glyphosate in this manufacturing setting (well below the ADI) and because of low toxicological concern, glyphosate-specific medical monitoring was not considered necessary by Monsanto. No such data have been submitted from a Monsanto European manufacturing facility in Europe or by any of the other GTF member companies. Taking into account the large number of manufacturers and formulations, the RMS proposal is that such and perhaps more product-specific data on occupational exposures and occupational health surveillance of plant personnel should be requested on MS or zonal level for product authorisation.

Observations on exposure of the general population, human biomonitoring and epidemiological studies

~~Data on urinary excretion after occupational or presumably dietary exposure (Acquavella *et al.*, 2004, ASB2012-11528; Hoppe, 2013, ASB2013-8037) and the comparison of exposure estimates that may be calculated on this basis to the proposed reference values are reported under section 2.6.2 above.~~

When the revised version of this report was prepared in fall, 2014, a total of seven studies from Europe and the U.S. was available in which urine samples obtained from humans following either occupational or dietary exposure (sometimes perhaps overlapping) had been

analysed for glyphosate. These studies are described in detail in Volume 3 of this RAR in the sub-section B.6.9.3 and summarised in Table 2.6-16 below. A compilation and critical review of this data have been also prepared for a separate publication in a scientific journal (Niemann *et al.*, 2014, ASB2014-11029). The study quality and the analytical methods were as different as the number and origin of participants. Sometimes, important background information on these people and how they were recruited was missing. However, provided that the measured values can be considered reliable, this data may be used to roughly estimate previous human exposure to glyphosate. This approach can be taken because the systemically available dose, following oral, dermal or inhalative exposure, is rapidly and virtually completely excreted by the urinary route. Glyphosate does not undergo enterohepatic circulation and is eliminated chemically unchanged (see 2.6.1). The calculations of intake and exposure were made under the following (conservative) assumptions:

- daily urine volume of 2 L for an adult (might be an overestimation and would rather give a higher exposure estimate than the real one);
- body weight of 60 kg for an adult (at least most men in Europe will weigh more but, for risk assessment, this is well covered since the systemic dose in mg/kg bw would become lower with increasing body weight);
- oral absorption of only 20 % of the ingested dose (based on animal data, see section B.6.1).

Following "correction" for the low oral absorption rate, the resulting estimates for the amount of dietary intake, *i.e.*, the "external dose", can be compared to the proposed ADI if this is the most likely route of exposure.

If dermal and inhalative absorption during application will have contributed the most to total exposure, the resulting "systemic dose", *i.e.*, an "internal" one, it seems more appropriate to compare it with the AOEL.

In two studies (Acquavella *et al.*, 2004, ASB2012-11528; Mesnage *et al.*, 2012, ASB2014-3846), a predominantly occupational exposure has to be assumed. In male farmers (48 in total) from the U.S. Federal States of Minnesota and South Carolina who actually had applied herbicides containing glyphosate on the same day, a mean urinary concentration of 3.2 µg/L was determined. Similar values were measured in the few of their spouses in which glyphosate was found above the detection limit. However, individual urinary concentrations were as high as 233 µg/L even though this was an outstandingly high value. This concentration was obtained in one farmer whose son, in addition, had the maximum value for a child, *i.e.*, 29 µg/L (Acquavella *et al.*, 2004, ASB2012-11528). The resulting "systemic dose" was compared to the AOEL. If, as a worst-case calculation, only the application day is considered, the systemically available amount of glyphosate would be at least 466 µg (rounded for 500 µg in case that not all of it was excreted in urine the same day) giving, for a 60 kg weighing person, a systemic dose of 0.0083 mg/kg bw. This dose covers only 8.3 % of the proposed AOEL of 0.1 mg/kg bw/day on the first day and this percentage would become lower from day to day after application. Thus, even for this man who, in fact, had the highest ever measured amount of glyphoste in his urine, no health concern is anticipated. The same holds true for his teenage son even though a "precise" calculation cannot be made because his age is not known and body weight cannot be estimared. However, in any case, the received dose must have been much smaller since his urinary concentration was by 10 times below. For the geometric mean of 3.2 µg/L in the whole group of farmers, the systemic dose would hardly exceed 0.1 % of the AOEL.

Taking the same approach for evaluation of the case study published by Mesnage *et al.* (2012, ASB2014-3846), the urine concentration of 9.5 µg glyphosate/L as measured in a (presumably European) farmer at 3 hours after application would result in a systemic dose that is below 0.4 % of the AOEL.

A strong decline of urinary concentrations became apparent in both studies over two or three days following application.

In a study by Curwin *et al.* (2007, ASB2012-11597) that was performed in 2001 in Iowa with a total of 212 participants (children and adults), it remains open whether the mean urinary glyphosate concentrations of 1.1 to 2.7 µg/L in the different sub-groups (divided by sex, age, and place of residence) were rather due to occupational or residential use or dietary intake. This uncertainty is based on the surprising evidence that there was no statistically significant difference in urinary levels between study participants living on farms and those who had other places of residence, in contrast to what was seen with three other pesticides that were also subject to analysis.

The highest mean of 2.7 µg/L would account for not more than 0.1 % of the AOEL and even the maximum value of 18 µg/L as measured in a "farm" child would be not of concern. If dietary intake was more relevant, its likely amount must be calculated by adjusting for an oral absorption rate of only 20 % before it may be compared to the ADI. Thus, a mean urinary concentration of 2.7 µg/L (rounded to 3), *i.e.*, a daily excretion of 6 µg in urine, might indicate a maximum daily intake of 30 µg per person and day. The resulting (external) dose of 0.5 µg/kg bw as received by a 60 kg-adult would correspond to only 0.1 % of the proposed ADI of 0.5 mg/kg bw.

For the four remaining studies, comparison of calculated exposure solely to the ADI appears adequate since mainly dietary intake might be assumed. Unfortunately, important information on selection and background of the participants is missing.

Hoppe (2013, ASB2013-8037) found glyphosate concentrations above the limit of quantification (LOQ) of 0.15 µg/L in the urines of 80 among a total of 182 participants from 18 European countries. Overall, a mean of 0.21 µg/L was calculated with a maximum value of 1.82 µg/L in a sample from Latvia. Even this latter measurement would suggest a rather low total intake of less than 20 µg that would, for a 60 kg weighing adult, indicate a neglibible exposure in the magnitude of 0.0003 to 0.0004 mg/kg bw, *i.e.*, less than 0.1 % of the ADI. Surprisingly, the author had also found urine concentrations of AMPA in more than one third of the samples, even though without good correlation to glyphosate contents. Although the mean AMPA concentration of 0.18 µg/L was similar to that of glyphosate, it was remarkable that the maximum value of 2.63 µg/L (in a sample from Croatia) exceeded the highest glyphosate concentration by far. The source of AMPA in urine is not known. However, even if concentrations of glyphosate and AMPA would be summed up, the resulting intake was by far below (much less than 1 %) the ADI for glyphosate that is also applicable to AMPA because it is not more toxic (see 2.6.10 above).

Urinary concentrations in the same magnitude with maximum values of 0.65 µg/L for glyphosate and of 1.31 µg/L for AMPA were found in a so far unpublished study of the German Federal Environmental Agency (Markard, 2014; ASB2014-2057). A total of 40 urine samples were analysed that had been taken in the German city of Greifswald from 10 female and 10 male students in 1996 and from the same number, again, in 2012. All the fourty participants were 20 – 29 years old when the samples were taken. 22 out of 40 were tested

positive for glyphosate, *i.e.*, the LOQ of 0.15 µg/L was exceeded. Based on the maximum dose levels, again, exposure was lower than 0.1 % of the ADI. However, it was interesting to note that there was a trend of increasing glyphosate concentrations between 1996 and 2012, most probably reflecting a slightly higher dietary intake either due to a more freqeuent application of glyphosate to crops in Germany or to a higher residue level in imported foodstuffs. In contrast, AMPA concentrations appeared to decline if the 1996 and 2012 values were compared suggesting that there is no clear relationship between intake of glyphosate and this metabolite.

In a study that apparently had several hundred participants involved but was poorly reported, Krüger *et al.* (2014, ASB2014-5024) found mean urinary concentrations of slightly below 2 µg/L with maximum values in the magnitude of 5 µg/L. Even this latter amount in urine would result in a calculated daily intake of not more than 50 µg glyphosate covering less than 0.2 % of the ADI. People eating mostly products from "organic" agriculture, had lower urine concentrations as compared to those who preferred a more conventional diet.

Higher (individual) values were obtained by Honeycutt and Rowlands (2014, ASB2014-6793) who found glyphosate concentrations above the rather high LOQ of 7.5 µg/L in 13 out of 35 samples from the U.S. with a maximum value of 18.8 µg/L. If this concentration was due to dietary intake, a received systemic dose of 40 µg might be conservatively estimated. Based on the assumption of 20 % oral absorption, the total dietary intake could be then as high as 200 µg. The resulting dose of 0.0033 mg/kg bw would account for 0.66 % of the ADI. (If this urinary concentration would result mainly from occupational use, the systemic dose would be equivalent to 0.66 % of the AOEL.)

From this data, the following conclusions may be drawn:

- Current analytical techniques allow the detection and determination of much lower amounts of glyphosate in human urine than in the past. The results obtained with different methods are not that much different and, to some extent, confirm each other.
- Positive glyphosate findings in human urine are quite common and may result from occupational or residential exposure, from dietary intake or from both. The origin may often not be clearly distinguished and will probably overlap sometimes.
- Urinary concentrations in operators after application of plant protection products tend to be higher than those resulting from dietary intake of glyphosate by consumers.
- The by far highest concentrations were measured in the urine of one operator and his son and may indicate that the recommended protective measures were not properly taken.
- Even though the data is not representative, mean urine concentrations in consumers in the U.S. appear higher than those found in Europe. This result is likely to reflect differences in the agricultural use of glyphosate-based herbicides and the plantation of glyphosate-resistant, genetically modified crops in North America.
- There is a trend towards increasing glyphosate concentrations in measured urine samples also in Europe, probably reflecting more sensitive analytical techniques, more frequent use in agricultural practice in Europe or higher residues in imported foodstuffs.
- All measured values, even the highest, were of no health concern. The calculated human exposures were at least one order but mainly two or more orders of magnitude lower than the ADI and AOEL.

- The same holds true if urine concentrations of AMPA are taken into account. However, correlation between glyphosate and AMPA in urine is poor suggesting that other sources of AMPA than metabolism of glyphosate in plants should be considered.

Table 2.6-16: Urine concentrations of glyphosate in humans (mean and maximum values) and resulting estimates of previous exposure, compared to ADI or AOEL

Reference	Number and origin of samples	Analytical method, LOD/LOQ	Urine concentrations (µg/L)		Estimated exposure or systemic dose (µg/kg bw, mean/ maximum)	Percentage of ADI or AOEL (mean/ maximum)
			Mean	Maximum		
Acquavella *et al.*, 2004, ASB2012-11528	48 male farmers from Minnesota and South Carolina (USA), their spouses and 79 children	HPLC following ion exchange, LOD 1 µg/L	3.2	233 (adult) 29 (child)*	Systemic dose of 8.3 (maximum) or 0.11 (mean)	8.3 % / ca 0.1 % of AOEL
Curwin *et al.*, 2007, ASB2012-11597	48 women, 47 men, 117 children from "farm" and "non-farm" households in Iowa	Immunoassay (fluorescent microbeads), LOD 0.9 µg/L	2.7 (range of means 1.1 – 2.7 in different groups)	18 ("farm child")*	0.5 (dietary exposure; highest mean) Systemic dose: < 0.1 (highest mean)	0.1 % of ADI < 0.1 % of AOEL
Mesnage *et al.*, 2012, ASB2014-3846	One farmer, his wife and 3 children, presumably from Europe	HPLC-MS, LOD 1 µg/L, LOQ 2 µg/L	n.a. (only single values available)	9.5 (farmer) 2 (child)*	0.33 (systemic dose)	< 0.4 % of AOEL
Hoppe, 2013, ASB2013-8037	118 citizens from 18 European countries	GC-MS/MS following derivatisation, LOQ 0.15 µg/L	0.21	1.82	0.3 – 0.4 (dietary exposure, maximum)	Less than 0.1 % of ADI
Markard, 2014, ASB2014-2057	40 German students (20 female, 20 male)	GC-MS/MS (presumably), LOQ 0.15 µg/L	n.a. (22 above LOQ)	0.65	0.13 (dietary exposure, maximum)	Much less than 0.1 % of ADI
Krüger *et al.*, 2014, ASB2014-5024	> 300 from different groups	ELISA (partly validated against GC-MS), LOD/LOQ not given	≤ 2	5	0.83 (dietary exposure, maximum)	< 0.2 % of ADI
Honeycutt and Rowlands, 2014, ASB2014-6793	35 from women, men and children from U.S.A. (14 Federal states)	ELISA, LOQ 7.5 µg/L	n.a. (13 samples above LOQ)	18.8	3.3 (dietary exposure, maximum) 0.66 (systemic	< 0.7 % of ADI < 0.7 % of AOEL

Reference	Number and	Analytical	Urine concentrations (µg/L)		Estimated	Percentage
					dose, maximum)	

n.a. not applicable, * for children, calculations were not performed since age, body weight and assumptions of urine volume were not known

Recently, glyphosate findings in human breast milk had been reported by Honeycutt and Rowlands (2014, ASB2014-6793) but were considered not reliable (for discussion, see Vol. 3, B.6.9.3). Even if the measured values were true, no health concern would have resulted.

A number of epidemiological studies of different types, extent and quality have been published in which exposure to glyphosate-based formulations in the studied populations was postulated. These publications adress either carcinogenicity, neurotoxicity or reproductive endpoints (fertility, occurrence of malformations) and, accordingly, are discussed in the respective sections of Volumes 1 (2.6) and 3. Further reviews are reported in Vol. 3, chapter B.6.8.4.

Reports on clinical cases and poisoning incidents

For better understanding of the following, it must be emphasised that all the poisoning or irritation incidents resulted from exposure to glyphosate-containing plant protection products but not to the active ingredient. Thus, in principle, it is not possible to distinguish if they were due to the active substance or rather to co-formulants. From animal experiments, however, it is known that glyphosate acid was irritating to the eyes (*i.e.*, a sign that is frequently associated also with mucosal irritation) but of low toxicity *via* all relevant routes. Even eye irritation was less pronounced in studies with glyphosate salts as compared to the acid. These salts are used for formulation of commercial products. Accordingly, one might assume that (frequently irritant or even corrosive) co-formulants will have contributed the most to intoxications following systemic intake and perhaps also to the irritation in cases of eye contact with glyphosate-based herbicides.

The extensive and still increasing use of glyphosate as an active ingredient in herbicides worldwide, rather than the (low) toxicity of this compound, may explain the relatively large number of poisoning incidents that happened and was published. Extensive reviews of clinical cases were published by Bradberry *et al.* (2004, ASB2012-11576) and by Lee *et al.* (2008, ASB2012-11879).

In some countries, poisoning incidents due to plant protection products are reported to the regulatory agencies but this knowledge is often either not made public or not shared with authorities in other countries. Thus, apart from what was published in the open literature, the RMS was aware only of data collected in Germany and, to some extent, in Brazil.
Burger *et al.* (2009, ASB2013-11831) briefly summarised a total of 60 reports by physicians from Germany to the Federal Institute for Risk Assessment on cases of poisoning with glyphosate herbicides since 1990. In the vast majority of 52 cases, only slight health impairment was reported. In four cases, health disturbances were considered “moderate” whereas the only one actually life-threatening case was the result of ingestion of 200 mL of a herbicide containing glyphosate and a tallowamine surfactant with suicidal intent. In the three remaining cases, no symptoms were reported or their severity could not be evaluated.
More than 650 cases of intoxication/irritation ascribed to ingestion of/contact to glyphosate-based herbicides are mentioned in an overview on poisoning incidents from Brazil that was kindly provided to the RMS by the Brazilian National Health Surveillance Agency (ANVISA, 2012, ASB2013-13413). This data was collected between 2010 and 2012 in some federal

states but it is not clear if it is representative for the whole huge country in which the agricultural conditions in general and also those of pesticide use are extremely different. At the first glance, the exposure routes, ingested amounts, circumstances (accident, suicidal attempt), clinical signs and medical treatment are similar to what is known from Germany and from the literature. The much higher total number of cases seems to reflect the applied amount of glyphosate and its formulations that is by orders of magnitude higher in Brazil than in Europe. However, further analysis is considered necessary before these data can be used for risk assessment purposes.

What is known on the course of clinical cases, signs and symptoms is summarised below and presented in greater detail in Volume 3.

Clinical signs and symptoms of poisoning – skin and eye contact

The vast majority of reported clinical signs following exposures (apart from attempted suicides or rare accidents) comprise skin and/or eye irritation or irritation of the respiratory tract by inhalation of spray mist.

Contact with skin may produce a dermatitis similar to that caused by detergents (Bradberry *et al.*, 2004, ASB2012-11576) although the active ingredient was not irritating to the skin in laboratory animals.

Phototoxic reactions [sunlight or ultraviolet (UV) light induced skin reactions] have been reported. This was believed to be due to an antimicrobial additive (benzisothiazolone) which is present in certain residential use (*i.e.*, non-agricultural) products containing 10 % glyphosate or less (Bradberry *et al.*, 2004, ASB2012-11576).

Eye exposures have generally resulted in temporary conjunctival irritation, clearing either after irrigation or within 1-2 days. A review of ocular exposures to US glyphosate-surfactant formulations (1513 exposures over a 5-year period), showed no permanent eye injury (Acquavella *et al.*, 1999, TOX2002-699; Bradberry *et al.*, 2004, ASB2012-11576). Eye contact is not expected to cause systemic effects or serious ocular injury.

Clinical signs and symptoms of poisoning – Oral intake

Ingestions of more than approximately 50 mL ("one mouthful", if real amount unknown) of a product with >10 % glyphosate concentration may be clinically significant. In contrast, glyphosate concentrations of less than 10% have rarely if ever produced toxicity. Most serious illness was observed following ingestion of the 41% (glyphosate IPA salt) concentrate. In the absence of extensive clinical experience for the 11-40% concentration range, any ingestion of more than 50 ml of a preparation with greater than 10% glyphosate salts should be considered as a potential cause for the subsequently described symptoms.

Minor gastrointestinal exposures are likely to be asymptomatic but the patient may experience an unpleasant taste, tingling, mild self-limiting nausea and vomiting. Self-limiting diarrhoea may also occur.

After significant exposures, a burning sensation in the mouth and throat, salivation, oral erythema, sore throat, dysphonia, dysphagia, epigastric pain, nausea, spontaneous vomiting, abdominal pain and diarrhoea are common and may last up to a week.

Serum amylase may be elevated and isoenzyme analysis done in a few cases identified a salivary gland origin (Tominack *et al.*, 1989, TOX9552426).

Hypotension is common after ingestion of a mouthful or more of the concentrated product (not the diluted forms) and will usually favourably respond to intravenous administration of fluids and pressor amines. If not responsive to this treatment, however, hypovolemic shock may result in oliguria, anuria, organic failure and ultimately in death.

Severe or prolonged vomiting and diarrhoea may induce fluid and electrolyte imbalance.
Tachypnea, dyspnea, cough and bronchospasm including cyanosis have been seen in severe ingestions. Transient hypertension may also occur. In laboratory analysis, abrupt rises in BUN and serum creatinine may be seen. Hemoconcentration can result result from intravascular volume depletion and could possibly indicate severe capillary fluid leakage (Tominack *et al.*, 1989, TOX9552426; Bradberry *et al.*, 2004, ASB2012-11576).
Several case reports indicate clinically significant hyperkalemia following ingestion of large amounts of glyphosate-potassium salt concentrate solutions (Bando *et al.*, 2010, ASB2012-11556; Kamijo *et al.*, 2012, ASB2012-11863).

Metabolic acidosis is often seen in a severely poisoned patient (Bradberry *et al.*, 2004, ASB2012-11576) and may fail to respond to bicarbonate therapy. Although the exact etiology is unknown, a lactic acidosis is suspected.

There have been no reports of primary convulsions after ingestion and most patients are present with a clear sensorium unless another substance, such as alcohol, has been co-ingested or severe hypoxemia has occurred (Tominack *et al.*, 1989, TOX9552426). However, in other cases, "moderate disorders of consciousness" have been reported within 48 hours after ingestions of the concentrate with suicidal intention (Sawada and Nagai 1987, Z35531; Sawada *et al.*, 1988, Z35532).

Aspiration pneumonia, pulmonary oedema and respiratory failure have been seen although the exact role of aspiration has not been fully investigated.

Mild fever may occur even in the absence of infection. In addition, leukocytosis without evidence of bacterial infection has been noted in peripheral blood after ingestion of the concentrate (Bradberry *et al.*, 2004, ASB2012-11576).

No direct hepatotoxic effects have been noted; however, minor elevations in transaminases and bilirubin were reported (Tominack *et al.*, 1989, TOX9552426; Bradberry *et al.*, 2004, ASB2012-11576).

Respiratory distress requiring intubation, pulmonary oedema, shock (systolic BP < 90 mm Hg), altered consciousness, abnormal chest X-ray, ingestion of over 200 cc concentrate (41 %), or renal failure making dialysis necessary have been associated with a higher risk of poor clinical outcomes including mortality (Lee *et al.*, 2000, ASB2012-11512). These authors also developed a prognostic index based upon these factors. However, as onset of symptoms may be delayed, early use of such prognostic indicators and too much reliance on them may lead to an under-estimate of clinical severity.

<u>Clinical signs and symptoms of poisoning – Inhalation</u>
An isolated case report from Israel suggests the development of acute pneumonitis in a worker (smoker) shortly after he had repaird a spraying device (not in operation). From ”occupational history”, the occupational physicians concluded that he had been exposed to Roundup herbicde and suspected a polyoxyethylene amine surfactant in the product as the possibly responsible agent (Pushnoy *et al.*, 1998, ASB2012-11513). However, actual exposure and its extent could not be really substantiated in this case. Accordingly, the occurrence of pneumonitis in this individual is more likely to be coincidental by nature although a (different) occupational origin seems plausible (Goldstein *et al.*, 1999, ASB2012-11511).

However, Burger *et al.* (2009, ASB2013-11831) also reported severe acute dyspnoe, rise in body temperature and histological lung changes (acute alveolitis and bronchiolitis) in a 59 years old German farmer who had sprayed a herbicide containing glyphosate on a warm day for three hours without respiratory protection. First clinical symptoms occurred seven hours after spraying. The patient was given i.v. steroids at high doses and antibiotic cover. This therapy was successful but six months later, he still complained of moderate breathing difficulties under conditions of exercise. It was suspected that the combination of glyphosate with the tallowamine surfactant in the formulation might have caused this incident.
In addition, in the same reference, 20 cases of inhalative exposure among a total of 60 reports on confirmed or presumed poisoning incidents with glyphosate herbicides from Germany (since 1990) were mentioned with breathing difficulties occurring in 50 % of the affected people. No more details on clinical courses or outcomes were given but it was emphasised by the authors as ”striking” that the involved products nearly always contained tallowamines.

Thus, intoxications following inhalative exposure to glyphosate-based products may occur and it seems reasonable to assume that tallowamine surfactants might have played the crucial role in such incidents.

First aid measures and therapeutic regimes

First aid measures and therapeutic regimes have been proposed by the notifiers and may be found in Volume 3 but were not evaluated by RMS toxicologists.

Expected effects and duration of poisoning as a function of the route, extent and duration of exposure

The expected effects of acute exposures reflect the clinical experience as desribed above and may be summarised as follows:.

- Skin irritation following exposure to glyphosate-based herbicides is mostly due to surfactants and will be generally limited to topical irritation which will resolve within 3 days to 1 week following exposure. If exposure is aggravated by occluded conditions or physical abrasion, more severe skin injury with open skin injury may result and may take longer to fully resolve.

- Eye irritation will generally resolve within 3-7 days of exposure. Most irritation is minor but exposure to concentrate or the occurrence of a foreign body or of abrasions (from rubbing the eye) may result in corneal abrasion requiring topical antimicrobial therapy, often occurring in conjunction with topical corticosteroids and temporary eye patching to provide symptomatic relief. As noted above, a large study of ocular exposures to glyphosate-surfactant products in the U.S. demonstrated no long term eye injury.

- Following minor or incidental ingestions, or ingestion of fully diluted formulations, gastrointestinal upset with nausea, vomiting, and diarrhoea may occur. Nausea and vomiting usually resolve within a few hours of ingestion. Diarrhoea may last for several days but is generally not severe. Following ingestion of a larger amount, the onset of systemic symptoms may be delayed by several hours. For serious ingestions having major electrolyte disturbances or life threatening alterations of cardiovascular performance, medical intervention may be life saving. Fatalities due to cardiovascular failure are generally delayed by 12 – 36 hours. For serious but non-fatal cases, primary clinical injury generally is manifest within 72 hours but secondary complications such

as infection or respiratory distress syndrome may supervene. The majority of serious but surviving cases will fully recover within 7-10 days of ingestion. Individuals with complicate clinical courses can require a more extended and highly variable time to recover.

- Glyphosate products do not contain readily volatile ingredients and thus inhalation exposure will be limited to droplets, which will deposit primarily in the upper airways. Resulting irritant symptoms such as breathing difficulties, most likely due to surfactants, will generally resolve within hours to a few days following exposure. In rare cases, treatment for lung symptoms might become necessary.

Short- or long-term effects in consumers due to dietary exposure to glyphosate via residues are not to be expected when the whole toxicological profile of this active ingredient is taken into account and in particular when the wide margin between the exposure and the high dose levels causing adverse effects in laboratory animals is considered.

2.6.12 Toxicological end point for assessment of risk following long-term dietary exposure - ADI

In general, the ”Acceptable Daily Intake” (ADI) is based on the highest dose at which no adverse effect was observed in the most appropriate study in the most sensitive laboratory animal species. In case of glyphosate, this approach must be modified, simply because of the fact that most toxicological endpoints are covered by a number of studies from different notifiers but of the same quality. For most toxicological endpoints, it is not possible to rely mainly on one particular study and to leave the other studies either aside or to consider them as supportive or confirmative only. Instead, it is more appropriate to establish “overall” NOAELs/LOAELs for the areas of toxicological testing and for the different species, based on all valid studies that were submitted to adress this particular endpoint. It is acknowledged that separate assessment of the studies that were submitted by one or the other applicant might have resulted in different proposals for the reference doses but this approach cannot be taken and all available information must be taken together into consideration.

The same principle was followed in the previous EU evaluation resulting in the inclusion of glyphosate into Annex I of Directive 91/414/EEC (EU, 2001, ASB2009-4191). The ADI of 0.3 mg/kg bw was established on the basis of all long-term studies in rats which were available at that time.

In general, long-term studies are often the most suitable for deriving the ADI, in particular for a substance of that low acute and short-term toxicity as glyphosate. Based on the six combined chronic toxicity/carcinogenicity studies that were now considered valid by the RMS either upon first assessment or during re-evaluation (see Table 2.6–16), an ”overall NOAEL” in the magnitude of 100 mg/kg bw/day appears reasonable. This is a higher figure than established before (EU, 2001, ASB2009-4191) because for the previous evaluation, in line with former regulatory practice, the NOELs (see Table 2.6–7a) instead of the NOAELs had been used. In addition, an additional study by [redacted] (1981, TOX2000-595) was included that is now considered not acceptable any longer and in which no effects had occurred up to the highest dose level of 31 mg/kg bw/day.

Table 2.6-17: High quality combined chronic toxicity/carcinogenicity studies in rats forming the basis for EU evaluation of this endpoint

Study	Owner	NOAEL	LOAEL	Overall assessment
[redacted]., 2009 ASB2012-11490	Nufarm	285 mg/kg bw/d	1230 mg/kg bw/d	Not carcinogenic; high dose effects on bw gain, bone marrow, clinical chemistry, skin
[redacted] 2001 ASB2012-11488	Syngenta	361 mg/kg bw/d	1214 mg/kg bw/d	Not carcinogenic; high dose effects on bw and food consumption, clinical chemistry, kidney, prostate
[redacted] 1997 ASB2012-11484, ASB2012-11485 ASB2012-11486, ASB2012-11487	Arysta	104 mg/kg bw/d	354 mg/kg bw/d	Not carcinogenic; high dose effects on bw and food consumption, caecum, skin
[redacted] 1996 TOX9651587	ADAMA	60 mg/kg bw/d	595 mg/kg bw/d	Not carcinogenic; high dose effects on eyes (cataracts) and clinical chemistry
[redacted] 1993 TOX9750499	Cheminova	100 mg/kg bw/d	300 mg/kg bw/d	Not carcinogenic; high dose effects on bw gain, liver, salivary glands
[redacted] 1990 TOX9300244	Monsanto	89 mg/kg bw/d	362 mg/kg bw/d	Not carcinogenic; high dose effects on stomach mucosa (irritation), bw, liver, eyes (cataracts)

This "overall NOAEL" is further supported by the chronic (one-year) study in rats by [redacted] (1996, TOX2000-1998) in which the NOAEL was 141 mg/kg bw/day. It is below the NOAELs that were established for long-term toxicity in the mouse (150 mg/kg bw/day; based on [redacted] 2001 (ASB2012-11491), [redacted] 1997 (ASB2012-11493), and [redacted] 1983, TOX9552381), the multigeneration studies in the rat (lowest value for parental and offspring toxicity 197 mg/kg bw/day; [redacted] 1992, TOX9552389) or in the one-year studies in the dog (overall 300 mg/kg bw/day; [redacted] 1990 (TOX9552384) and [redacted] 1997, ASB2012-11458), *i.e.*, in the studies that are usually also taken into account for ADI setting.

When the usual assessment factor of 100 is used (and there is no apparent reason to select another one), an ADI of 1 mg/kg bw would result. This value is numerically the same as that one established by WHO/FAO (JMPR, 2004, ASB2008-6266), based on the 2-year rat study by [redacted] (1993, TOX9750499) for which the same NOAEL of 100 mg/kg bw/day was derived as by the RMS in the current EU re-evaluation.

However, since it is also a widely accepted requirement and general practice to look at the most sensitive species, the developmental studies in the rabbit must not be ignored.

At the same dose level of 100 mg/kg bw/day that caused no effects in long-term feeding studies in rats, there were several deaths in pregnant rabbits in the study by [redacted] (1993, TOX9551106) that must be considered treatment-related. In the studies by [redacted] (1980, TOX9552392), [redacted] (1991, TOX9552393 1), [redacted] (1996, ASB2012-11499) and [redacted] (1996, TOX2000-2001), the maternal LOAELs were in the magnitude of 150 to 200 mg/kg bw/day. Even though the effects were not that severe as observed by [redacted] (1993, TOX9551106) and mortality at these dose levels was only

occasionally seen ([redacted]., 1980, TOX9552392), these LOAELs were much lower than in any other type of studies with glyphosate. A particular vulnerability of the (pregnant) rabbit was further confirmed by the high mortality rate in the study by [redacted] (1980, TOX9552392) at 350 mg/kg bw/day as well as by abortion and one death reported at the top dose level of 300 mg/kg bw/day in the study of [redacted] (1995, ASB2012-11498).

In addition, first developmental effects in rabbits (mainly post-implantation losses) were observed at a dose level of 200 mg/kg bw/day ([redacted], 1996, ASB2012-11499).

Based on all the latter considerations, it seems most appropriate to derive the ADI from the NOAEL for both maternal and developmental toxicity of 50 mg/kg bw/day as established independently by [redacted] (1991, TOX9552393~~3~~1) and [redacted] (1996, ASB2012-11499). In fact, a lower maternal NOAEL of 20 mg/kg bw/day was found in the study by [redacted]. (1993, TOX9551106) but it is in no way mandatory and not usual to use the lowest available value if there is evidence that it results mainly from dose spacing. The proposed figure of 50 mg/kg bw/day is well below the LOAEL of 100 mg/kg bw/day in the study by [redacted] (1993, TOX9551106). In contrast, for the maternal NOAEL of 75 mg/kg bw/day in the study by [redacted](1980, TOX9552392), the margin to a dose causing maternal death in another experiment (100 mg/kg bw/d according to [redacted] 1993, TOX9551106) might be too small.

If 50 mg/kg bw/day is accepted as point of departure, the resulting **ADI** for glyphosate is **0.5 mg/kg bw.**

This reference dose as proposed by the RMS is slightly higher than the previously established value in the EU of 0.3 mg/kg bw and by 50 % lower than the ADI that was set by JMPR (2004, ASB2008-6266). However, it must be emphasised that the database that was available for the WHO/FAO evaluation did not contain many of the studies that have been considered for this EU review. Thus, only two of the developmental studies in rabbits ([redacted] 1991, TOX9552393~~3~~1 and [redacted] 1996, TOX2000-2001) were evaluated by JMPR in 2004.

The notifiers, *i.e.*, the GTF, had proposed a markedly higher ADI of 3 mg/kg bw, derived from the NOAEL in the long-term rat study by [redacted] (1993, TOX9750499) which had been set at 300 mg/kg bw/day, in contrast to evaluation by the RMS.

The ADI of 0.5 mg/kg bw and the approach taken for its setting as proposed by the RMS were confirmed on the Pesticides Peer Review 125 expert meeting (25 – 27 February 2015).

2.6.13 Toxicological end point for assessment of risk following acute dietary exposure - ARfD (acute reference dose)

~~A low acute oral toxicity of glyphosate was proven in a huge number of studies. In an acute neurotoxicity study, the NOAEL for systemic effects was 1000 mg/kg bw (*i.e.*, the limit dose that might justify a need for an ARfD) and there was no evidence of neurotoxicity. There is no evidence that adverse effects in repeated dose studies, including developmental studies in rats or rabbits, might result from a single oral exposure or would occur within the first days of treatment.. Accordingly, **no ARfD** is needed.~~
~~The notifiers have also not suggested an ARfD. The same approach has been taken by the WHO/FAO in its 2004 evaluation (JMPR, 2004, ASB2008-6266).~~

In the Pesticides Peer Review 125 expert meeting (February, 2015), the original proposal of the RMS not to establish an ARfD was not confirmed, in contrast to what was decided on the ADI and the AOEL.

Even though acute toxicity of glyphosate, as well as acute neurotoxicity, is low and no evidence of acute effects was obtained in studies with repeated dietary administration, MS experts were concerned about the maternal toxicity, including mortality, in the developmental toxicity studies in the rabbit. Usually, treatment-related deaths at dose levels below 1000 or even 500 mg/kg bw may trigger a need for setting an ARfD. In pregnant does, excessive mortality occurred at dose levels from 175 mg/kg bw/day onwards in the study by [redacted] (1980, TOX9552392) and even at 100 mg/kg bw/day and above in the study by [redacted] (1993, TOX9551106). Single maternal deaths were observed in the studies by Hojo (1995, ASB2012-11498) and [redacted] (1991, TOX9552391) at 300 or 450 mg/kg bw/day, respectively. Based on these fatalities, an ARfD might be indeed warranted. On the other hand, pregnant does died not in the beginning of the studies but after having received a number of daily gavage administrations. Thus, strictly spoken, it is questionable whether an ARfD should be established on this basis (alone). In additional, developmental toxicity was taken into account. Although it was confirmed in the Pesticides Peer Review meeting 125 that glyphosate was not teratogenic, post-implantation losses that might be due to a single or very few administrations should be taken into account as a possible basis for deriving the ARfD. Post-implantation losses were observed in two of the 7 studies in rabbits, *i.e.*, in the studies by [redacted]. (1991, TOX9552391) and that one of [redacted] (1996, ASB2012-11499). In the latter one, an increase in post-implantation losses was seen at the mid and high dose levels of 200 and 400 mg/kg bw/day with an NOAEL of 50 mg/kg bw/day. This dose had been used to establish an overall NOAEL for developmental toxicity in the rabbit (see sub-section 2.6.7.2.2 above) and was considered in the Pesticides Peer Review meeting the most appropriate basis to derive the ARfD. Using the safety factor of 100, an **ARfD of 0.5 mg/kg bw** was established.

2.6.14 Toxicological end point for assessment of occupational and bystander risks – AOEL

For AOEL setting, usually a suitable NOAEL from one of the so-called "mid-term" studies is chosen to reflect the expected length of operator exposure. This selection of studies comprises the 90-day studies in rodents and dogs and, if available, the one-year dog study, subchronic neurotoxicity, the reproduction (one- or two-generation) and developmental studies. In case of glyphosate, this is a huge database that is summarised in Table 2.6–18.

Table 2.6–18: Studies to be taken into account for deriving a systemic AOEL for glyphosate

Study type/endpoint	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	References
90 d, subchronic, rat	300 … 414 (overall)	569 (lowest)	[redacted] (1991, TOX9552364), [redacted] (1993, TOX9650149) and [redacted] (1996, TOX2000-1990) for NOAEL; [redacted] (1995, ASB2012-11452) for LOAEL
90-d, subchronic, mouse	500 (lowest)	1065 (lowest)	[redacted] (1992, TOX9551954)

Study type/endpoint	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	References
90 d, subchronic, dog	300 (overall)	1000 (lowest)	[redacted] (1996, TOX2000-1991), [redacted] (1999, ASB2012-11455) and [redacted] (2007, ASB2012-11454)
1-yr, subchronic, dog	300 – 500 (overall)	926 (lowest)	[redacted] (1990, TOX9552384), [redacted] (1996, TOX2000-1992), [redacted] (2007, ASB2012-11457)
Subchronic neurotoxi-city, rat	617 (systemic effects)	1546 (systemic effects)	[redacted] (1996, ASB2012-11501)
Reproduction, rat	Parental: 300-400 (overall) Reproductive: 351 (lowest) Offspring: 300-400 (overall)	Parental: 985 (lowest) Reproductive: about 1000 (lowest) Offspring: 985 (lowest)	[redacted] (1997, ASB2012-11495), [redacted] (2000, TOX2000-2000), [redacted] (2007, ASB2012-11494)
Developmental toxicity, rat	Maternal: 300 (lowest) Developmental: 300 (lowest)	Maternal: 1000 (lowest) Developmental: 1000 (lowest)	[redacted]. (1991, TOX9552393), [redacted] (1995, ASB2012-11497)
Developmental toxicity, rabbit	Maternal: 50 (overall) Developmental: 50 (lowest)	Maternal: 100 (lowest) Developmental: 200 (lowest)	[redacted] (1991, TOX9552393), [redacted] (1993, TOX9551106), [redacted] (1996, ASB2012-11499)

The rabbit appeared the most sensitive species providing the lowest NOAELs and LOAELs with the most serious effects among all species at relatively low dose levels.
Based on the same considerations as for the ADI, the AOEL should be derived from the NOAEL of 50 mg/kg bw/day for maternal and developmental toxicity in the rabbit ([redacted] 1991 (TOX9552393~~1~~); [redacted], 1996, ASB2012-11499), supported by [redacted]. (1993, TOX9551106) and [redacted]. (1980, TOX9552390). The maternal NOAEL of 75 mg/kg bw/day in the latter study had been used as the basis for AOEL setting during the previous EU evaluation (DAR, 1998, ASB2010-10302). Using the safety factor of 100 and the previous assumption of 30 % oral absorption, a numeric value of 0.2 mg/kg bw/day was calculated.

The safety factor of 100 should be maintained but oral absorption is now assumed to be as low as 20 % (see 2.6.1). If 50 mg/kg bw/day is used as the suitable point of departure [because the NOAEL in the study by [redacted] (1980, TOX9552390), was to close to a dose causing maternal deaths in another study in rabbits] the resulting **AOEL** is **0.1 mg/kg bw/day**.

The GTF had proposed a different systemic AOEL that was by 12 times higher. The suggested numeric value of 1.2 mg/kg bw/day was based on the 90-day rat study by [redacted] (1996, TOX2000-1990) in which the NOAEL was 414 mg/kg bw/day. This NOAEL was agreed with by the RMS but it is higher than the overall NOAELs for both maternal and developmental toxicity in the rat and, in particular, does not take into consideration the developmental studies in rabbits. The latter studies were not used because it was argued that the effects were due to gavage administration of a low pH organic acid causing mucosal irritation in the gut to which the rabbit is particularly sensitive. However, it was not proven

that the effects were local by nature and also the low pH is an inherent property of glyphosate that might produce adverse effects.
Numerically, the proposed NOAEL was also slightly higher than the (overall) NOAEL for subchronic toxicity in the dog and the NOAELs for parental, offspring and reproductive toxicity as obtained in two-generation studies in rats.
Furthermore, the GTF used the previous assumption of 30% oral absorption for correction of the systemic AOEL instead of 20% as it is proposed now by the RMS.

Apparently, there is no reason to derive separate dermal or inhalative AOELs.

The systemic AOEL of 0.1 mg/kg bw/day and the approach taken to derive it were confirmed by the Pesticides Peer Review 125 expert Meeting (25 – 27 February 2015).

2.6.15 Summary of product exposure and risk assessment

MON 52276 exhibits low acute oral, dermal and inhalation toxicity, is slightly irritant to skin, slightly to moderately irritant to eyes and is not a skin sensitiser. No additional classificaction has to be adopted for MON 52276 due to known toxicological properties of the actice substance or any of the co-formulants.

For a short summary see Table 2.6–19 and Table 2.6–20 below.

Table 2.6-19: Summary of evaluation of the studies on acute toxicity including irritancy and skin sensitisation for MON 52276 by the RMS

Type of test, model system (Guideline)	Result	Acceptability	Classification (acc. to the criteria in Dir. 67/548/EEC)	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
LD_{50} oral, rat (OECD 401)	> 5000 mg/kg bw	Yes	None	None	[redacted] 1991 TOX9552438
LD_{50} dermal, rat (OECD 402)	> 5000 mg/kg bw	Yes	None	None	[redacted] 1991 TOX9552439
LC_{50} inhalation, rat	Not submitted, not necessary. Justification presented in Vol. 3, B.6.11)				
Skin irritation, rabbit (OECD 404)	Non-irritant	Yes	None	None	[redacted] 1991 TOX9552440
Eye irritation, rabbit (OECD 405)	Non-irritant	Yes	None	None	[redacted] 1992 TOX9552441
Skin sensitisation, guinea pig (OECD 406, Buehler (9 applications)	Non-sensitising	Yes	None	None	[redacted] 2001 TOX2005-1135

Type of test, model system (Guideline)	Result	Acceptability	Classification (acc. to the criteria in Dir. 67/548/EEC)	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
Supplementary studies for combinations of plant protection products	No data – not required				

Table 2.6-18: Additional toxicological information relevant for classification/ labelling of MON 52276

	Substance (Concentration in product, % w/w)	Classification of the substance (acc. to the criteria in Dir. 67/548/EEC and/or in Reg. 1272/2008)	Reference	Classification of product (acc. to the criteria in Dir. 67/548/EEC, in Dir. 1999/45/EC and/or in Reg. 1272/2008)
Toxicological properties of active substance (relevant for classification of product)	None			
Toxicological properties of non-active substances (relevant for classification of product)	None			
Further toxicological information	No data – not required			

Dermal absorption of glyphosate in the representative formulation MON 52276 was very low. ~~In the high, mid and low concentrations, absorption rates of 0.1, 0.2, and 0.3 % were obtained~~ For all three concentrations levels, a dermal absorption rate of 1 % was established that is based on in an *in vitro* experiment with 24-hour exposure on human epidermis (Ward, 2010, ASB2012-5383). ~~The suitable figures from above were used for exposure calculations and risk assessment.~~

MON 52276 is a herbicide used for foliar spray application in various crops outdoors. The maximum recommended application rate is 3 x max. 2.88 kg a.s./ha (maximum dose per season not to be exceeded 4.32 kg a.s./ha) in orchard crops, vine etc. or 2 x 2.16 kg a.s./ha in crops treated with tractor-mounted ground boom sprayers such as cereals etc. The estimated operator exposure according to the German model does not exceed the AOEL of 0.1 mg/kg bw/day if prescribed PPE is worn by operators in the case of applications using knapsack sprayers. In contrast, no PPE is necessary for tractor-mounted applications using the German model for exposure estimation. ~~without PPE.~~
In the case of using tractor-mounted spray equipment, the estimated operator exposure according to the UK POEM will be below the AOEL only if gloves are worn during mixing/loading and application (without PPE: 261 %, with PPE: 49 % of AOEL). ~~estimated~~ If knapsack sprayers in orchard crops are used, the AOEL will be exceeded by the operator exposure estimated ~~etc. estimated operator exposure~~ according to the UK POEM irrespective

of whether PPE are worn or not (without PPE: 568 %, with PPE: 149 % of AOEL). ~~accounts for 207 % of the AOEL without PPE, so that gloves during mixing/loading and application as well as an impermeable coverall during application are necessary (85 % of AOEL). On the other hand, no PPE is needed according to the UK POEM for crops treated using tractor-mounted ground boom sprayers (76 % of AOEL).~~

Predicted worker exposure does not exceed the AOEL ~~either~~. Even for prolonged inspection or maintenance tasks of 8 hours the AOEL is exploited with 28.8~~8.6~~ % without PPE.

Estimated bystander and resident exposure is below the AOEL even if direct applications on pasture or lawn are considered for residents (bystanders: adults 4.05~~1.35~~ %, children 3.43~~1.33~~ % of AOEL; residents: adults 5.53~~1.85~~ %, children: 20.84~~16~~ % of AOEL).

Remarks on surfactants included into glyphosate-containing plant protection products

All glyphosate-containing plant protection products contain surfactants or - if not present as an integral component – are to be mixed with surfactants as a compulsory additive to produce the ready-to-use dilution. As has already been discussed during the first Annex I inclusion procedure for glyphosate it became apparent that glyphosate-containing products were more toxic than glyphosate alone. This phenomenon was attributed predominantly to the presence of particular surfactants, namely the POE-tallowamines.
Some MS may wish to allow for this in the context of the national risk assessment for POE-tallowamine containing glyphosate formulations. Therefore, a toxicological evaluation of POE-tallowamines (including reference values) is provided in a separate paragraph within Vol. 3 (B.6.13.3) of this RAR.

MON 52276 which is the representative formulation here **does not** contain any POE-tallowamines.
Instead, a different type of surfactant, i.e. a quarternary ammonium compound, is used for MON 52276.
Since studies on MON 52276 concerning acute toxicity, skin and eye irritation as well as skin sensitisation were performed with the original preparation of MON 52276 the results for these toxicological short-term endpoints also reflect possible effects provoked by the surfactant. No further studies are needed according to the data requirements for plant protection products. Therefore, no toxicological long-term studies were submitted using the formulated product or the surfactant alone. Moreover, up to now no reference values have been considered necessary for the surfactant used, thus, no respective risk assessment was required.
According to the material safety data sheet for the surfactant provided by the applicants this co-formulant was not mutagenic in an Ames-test. No further information on toxicological long-term endpoints was given in this material safety data sheet.

In addition, MON 52276 has been authorised within the EU for many years. There are no medical data which have been collected by occupational physicians or poisoning emergency centres describing long-term adverse health effects for operators provoked by this plant protection product until today.

2.7 Residues

2.7.1 Summary of storage stability of residues

The storage stability of glyphosate and AMPA was investigated in all matrix groups. For N-acetyl-glyphosate and N-acetly-AMPA only high water content, high oil content, high starch content and other plant matrices were investigated. The following intervals were identified without a significant decline of the residue (>70 % remaining):

Glyphosate

High acid content matrices (oranges; tomatoes)	>14 to >31 months
High water content matrices (clover; maize forage, green plant and stover; soya bean forage; sorghum stover; sugar beet roots and leaves)	>9 to 31 months
High oil content matrices (linseeed; rape seed; soya beans)	>18 to >24 months
High starch content matrices (barley, maize, rye, sorghum and wheat grain)	18 to >48 months
High protein content matrices (beans, dry)	>18 months
Other plant matrices (barley, rye, soya bean and wheat straw; soya bean hay)	18 to >45 months
Animal commodities (fat, muscle, liver and kidney from swine, cattle and poultry; milk; eggs)	14 to >26 months

AMPA

High acid content matrices (oranges; tomatoes)	>14 to >31 months
High water content matrices (clover; maize forage, green plant and stover; soya bean forage; sorghum stover; sugar beet roots and leaves)	6 to 24 months
High oil content matrices (soya beans)	>24 months
High starch content matrices (barley, maize, rye, sorghum and wheat grain)	10 to >31 months
High protein content matrices	not investigated
Other plant matrices (barley, rye, soya bean and wheat straw; soya bean hay)	6 to >24 months
Animal commodities (fat, muscle, liver and kidney from swine, cattle and poultry; milk; eggs)	14 to >26 months

N-acetyl-glyphosate

High acid content matrices	not investigated
High water content matrices	6 to >12 months
(maize forage, green plant and stover; soya bean forage)	
High oil content matrices	>12 months
(soya beans)	
High starch content matrices	>12 months
(maize grain)	
High protein content matrices	not investigated
Other plant matrices	>12 months
(soya bean hay)	
Animal commodities	not investigated

N-acetyl-AMPA

High acid content matrices	not investigated
High water content matrices	>1 to >12 months
(maize forage, green plant and stover; soya bean forage)	
High oil content matrices	>1 months
(soya beans)	
High starch content matrices	>12 months
(maize grain)	
High protein content matrices	not investigated
Other plant matrices	>1 months
(soya bean hay)	
Animal commodities	not investigated

2.7.2 Summary of metabolism, distribution and expression of residues in plants, poultry, lactating ruminants, pigs and fish

The metabolism of glyphosate in non-tolerant plants was investigated in numerous crops, covering all crop groups. The active substance was applied via soil treatment, hydroponic application, stem or trunk treatment and foliar treatment. Following direct treatment via foliar, trunk, stem or hydroponic treatment unchanged glyphosate was the only significant residue. In presence of soil as a substrate the active substance is quickly degraded, leaving AMPA at rates comparable or even higher than parent glyphosate. However, the uptake via the roots and the translocation in the plants was very low, not resulting in significant residue levels as confirmed by plant metabolism and confined rotational crop studies. A major part of the glyphosate was degraded into CO_2.

In glyphosate tolerant plants the metabolism may differ significantly. Depending on the kind of modification AMPA (GOX modification), N-acetyl-glyphosate (GAT modification) and N-acetyl-AMPA (GAT modification) become major metabolites in plant commodities, often being present at higher amounts than the unchanged parent. While CP4-EPSPS, an enzyme much lower susceptible to glyphosate, does not affect the metabolic pattern, the ratio between glyphosate and AMPA was approximately 1:1 in GOX modified plants. For GAT modified plants some commodities showed only little glyphosate remaining above the LOQ. N-acetyl-AMPA was the major residue in most commodities while parent glyphosate was only present at low level or even undetected.

The metabolism of glyphosate in rotational crops was investigated in several confined studies involving application rates to bare soil equivalent to 3.87 - 6.5 kg as/ha. The investigation of soil samples in these studies demonstrated the quick degradation of the parent substance, showing AMPA as major residue with levels up to 10 times higher than the glyphosate remaining. TRR levels in samples obtained from rotational crops contained substancial residues, equivalent to concentrations of up to 4.4 mg eq/kg. However, as demonstrated in soil treatment metabolism studies, most of the radioactivity remained unextracted due to incorperation of $^{14}CO_2$ from the degradation of glyphosate in the soil. In the extracts glyphosate levels depended on the interval between treatment and sampling. After short intervals up to 14 weeks glyphosate levels were higher than AMPA (glyphosate: 19.6 - 62 % of the TRR, AMPA: 2.3 - 15.6 % of the TRR). In samples collected after longer intervals glyphosate was only present in minor amounts of 10 % of the TRR or less (absolute levels <0.001 mg eq/kg to 0.026 mg eq/kg) while AMPA was the dominant residue with up to 20 % of the TRR (up to 0.05 mg eq/kg). Further metabolites were not identified.

In livestock animals the metabolism of glyphosate, AMPA and N-acetyl-glyphosate was investigated in lactating goats and laying hens. All three analytes are slowly degraded. Most of the residues was recovered unchanged as administered. The major part of the administered dose was excreted via the faeces. For bioavailable residues the excretion was observed mainly via urine, resulting in highest residue levels found in the kidney. Muscle, fat and milk gave very low residues, normally being present below the LOQ. In liver some metabolisation of glyphosate into AMPA was observed. However, the levels of both analytes were much lower than in kidney. In eggs the residue increase during the whole dosing period of up to eight days. A plateau was observed in livestock feeding studies on laying hens after 14 days. Again, most of the residue was identified as unchanged substance administered.

2.7.3 Definition of the residue

Definition of the residue for plant commodities
Residue definition for enforcement purposes in sweet corn, oilseeds rape, soya beans and maize (non-tolerant and tolerant, all modifications):
sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate

Residue definition for enforcement purposes in other plant commodities:
glyphosate

Residue definition for dietary intake purposes in plant commodities:
sum of glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA, all expressed as glyphosate equivalents

(For the future generation of residue data N-acetly-glyphosate and N-acetyl-AMPA are only mandatory analytes in GAT-modified crops.)
Definition of the residue for animal commodities
Residue definition for enforcement purposes in animal commodities:
sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate

Residue definition for dietary intake purposes in animal commodities:
sum of glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA, all expressed as glyphosate equivalents

(For the calculation of the maximum dietary burden for the purpose of MRL setting, only glyphosate and N-acetyl-glyphosate need to considered, since the reformation of both analytes from AMPA or N-acetly-AMPA is unlikely).

2.7.4 Summary of residue trials in plants and identification of critical GAP

For glyphosate several GAPs were reported:

The application of glyphosate before planting/sowing is reported for all crops involving one to two treatment at 2.16 kg as/ha each. The definition of a PHI was not necessary. A corresponding dataset of supervised field trials covering all crop groups was submitted. In these trials no residues of glyphosate or AMPA above the LOQs of 0.02 mg/kg to 0.05 mg/kg were found except for single detects for cereal straw.

In orchards and vineyards glyphosate is sprayed for weed control onto the ground with up to three applications per year with 2.88 kg as/ha each (maximum of 4.32 kg as/ha and year). Corrresponding supervised field trial data on tree nuts, pome fruit and stone fruit showed no detectable residues above the LOQ of 0.05 mg/kg in the fruits. Special circumstances need to be taken into account for grapes and olives.
For grapes low hanging fruits may be exposed to the spray solution, resulting in residuse up to 0.3 mg/kg in the grapes. For olives ground-picking is a common agricultural practice. Therefore only supervised field trials were taken into account involving ground-picking. The corresponding residues in the olives were between <0.05 mg/kg to 0.93 mg/kg for glyphosate and <0.05 mg/kg to 1.0 mg/kg for the sum of glyphosate and AMPA, expressed as glyphosate.

In oilseeds (rapeseed, linseed and mustard seed) the desiccation two weeks before harvest in conducted with application rates of 2.16 kg as/ha. However no supervised field trial data was submitted matching the reported application rate within the accepted interval of ± 25 %.

Cereal grain (barley, oats, rye and wheat) are also sprayed for desiccation with application rates of 2.16 kg as/ha and a PHI of 7 days. Supervised field trial data matching the GAP are numerous. For barley and oats grain glyphosate residues were between 1.2 mg/kg and 21.4 mg/kg and for the sum of glyphosate and AMPA, expressed as glyphosate, residues of 1.3 mg/kg to 21.6 mg/kg were found. In wheat and rye grain the parent substance was present at levels between 0.05 mg/kg to 17.5 mg/kg. The total residues (sum of glyphosate and AMPA, expressed as glyphosate) were 0.125 mg/kg up to 18.1 mg/kg.

2.7.5 Summary of feeding studies in poultry, ruminants, pigs and fish

Livestock animal feeding studies were provided for glyphosate, AMPA, glyphosate-trimesium and N-acetly-glyphosate in lactating cows, laying hens and swine.
The residues found in all species were in line with the metabolism studies submitted and covered the calculated mean and maximum dietary burden.

2.7.6 Summary of effects of processing

Under simulated processing conditions glyphosate, AMPA and N-acetyl-AMPA were stable.

The effect of processing was investigated in citrus, potatoes, olives, linseed, rapeseed, soya bean, maize, barley, rye, oats and wheat. In summary glyphosate and AMPA are polar components mainly present on the surface of the commodities analysed. In fatty compartiments (oil) normally no residues above the LOQ were found. An increase in the residue concentration was observed in dried commodities or bran. In view of the high amount of studies please refer to the list of end points or Volume 3 for an detailed overview of processing factors derived.

2.7.7 Summary of residues in rotational crops

In soil glyphosate is quickly degraded into AMPA and finally into CO_2. In experiments conducted as confined rotational crop studies or plants metabolism studies involving soil or hydroponic treatment only a minor uptake via the roots was observed. Both analytes are not further metabolised in the plants, however in rotational crops a higher relative amount of AMPA has to be expected compared to foliar treatment due to its formation in the soil. In confined rotational crop metabolism studies a high degree of incorperation of radioactivity into natural products (carbohydrates, lipids and protein) was observed. The absolute levels of glyphosate rarely exceeded 0.01 mg/kg after treatment at higher application rates (1.07 to 23.2 kg as/ha) than the representative GAPs (up to 4.3 kg as/ha and year). In summary it can be concluded that neither glyphosate nor AMPA show a potential uptake into rotational crops.

Filed studies on the behaviour of glyphosate and AMPA in rotational crops are not required. In supervised field trials involving pre-emergence/pre-sowing application no residues above the LOQ were found except single detect for cereal straw.

2.7.8 Estimation of the potential and actual exposure through diet and other sources

The chronic intake of glyphosate based on the representative uses resulted in a maximum utilisation of 2.5 % of the ADI (0.5 mg/kg bw) for children from Denmark (EFSA PRIMo Rev. 2). The German NVS II model gave a utilisation of 1.5 % of the ADI for the general population aged 14-80 years.
~~Due to the low acute toxicological properties of glyphosate and its metabolites the allocation of an ARfD was not necessary.~~
The acute intake based on the EFSA PRIMo as well as the German NVS II model resulted in a maximum IESTI of 4.7 % of the ARfD (0.5 mg/kg bw) for the consumption of oats from children from Germany aged 2-4 years. For adults the EFSA PRIMo resulted in a maximum utilisation of the ARfD of 8.5 % for barley, based on the consumption data from the

Netherlands. The German NVS II model indictated the highest intake for the General population aged 14-80 years with 5.7 % of the ARfD, also based on barley. All other commodities under consideration in this report gave lower utilisations of the ARfD.

In summary it can be concluded In summary it can be concluded that the chronic and acute dietary intkae is unlikely to present a public health concern.

2.7.9 Proposed MRLs and compliance with existing MRLs

Based on the representative uses the following MRLs were calculated:

Citrus fruits	0.05* mg/kg
Tree nuts	0.05* mg/kg
Pome fruit	0.05* mg/kg
Stone fruit	0.05* mg/kg
Grapes	0.5 mg/kg
Strawberries	0.05* mg/kg
Table olives	2 mg/kg
Root and tuber vegetables	0.05* mg/kg
Bulb vegetables	0.05* mg/kg
Fruiting vegetables, except sweet corn	0.05* mg/kg
Sweet corn	0.1* mg/kg (potential tolerant crop, higher LOQ)
Brassica vegetables	0.05* mg/kg
Leaf vegetables & fresh herbs	0.05* mg/kg
Legume vegetables	0.05* mg/kg
Stem vegetables	0.05* mg/kg
Pulses, except lentils	0.05* mg/kg
Oilseeds, except rape seed and soya beans	0.05* mg/kg
Maize, rape seed, lentils, soya beans	0.1* mg/kg (potential tolerant crop, higher LOQ)
~~Olives~~	~~2 mg/kg~~
Barley, oats	30 mg/kg
Rye, triticale, wheat	20 mg/kg
Buckwheat, millet, rice, sorghum, others	0.05* mg/kg
Herbal infusions	0.05* mg/kg
Sugar plants	0.05* mg/kg
Swine, muscle~~, fat and liver~~	0.05* mg/kg
Swine, fat and liver	0.1* mg/kg
Swine, kidney	0.2 mg/kg
Bovine, goat and sheep, muscle	0.05* mg/kg
Bovine, goat and sheep, fat	0.1 mg/kg
Bovine, goat and sheep, liver	0.1 mg/kg
Bovine, goat and sheep, kidney	2 mg/kg
Milk	0.05* mg/kg
Poultry, muscle~~, fat and liver~~	0.05* mg/kg
Poultry, fat and liver	0.1* mg/kg
Poultry, kidney	0.1 mg/kg
Eggs	0.05* mg/kg

In comparision to established MRLs for glyphosate (according to Reg. (EC) 396/2005) the MRLs for barley and oats (20 mg/kg), wheat and rye (10 mg/kg), table olives (1 mg/kg) and bovine kidney (2 mg/kg) are lower than the values estimated in this document. Higher MRL proposals for plant commodities result from the implementation of the harmonized OECD MRL Calculator procedure.

2.7.10 Proposed import tolerances and compliance with existing import tolerances

No import tolerances were reported for glyphosate.

The residues definitions for enforcement purposes in plant and animal commodities are proposed to be amended for compliance with the residue definitions for glyphosate defined by the Codex Alimentarius.

2.8 Fate and behaviour in the environment

2.8.1 Summary of fate and behaviour in soil

2.8.1.1 Aerobic soil degradation

Route and rate of degradation

The degradation route and rate of glyphosate in soil under aerobic conditions was investigated in various soils with different soil characteristics at incubation temperatures of 10 °C, 20 °C and 25 °C and different soil moisture contents. Under aerobic conditions at 10, 20 and 25 °C, glyphosate degrades in soil forming only one soil metabolite AMPA in significant concentrations. The maximum amount of AMPA formed in soil ranged from 13.3 to 50.1 % AR after 8 -91 days. Several other minor components were detected; however, none of these metabolites were formed in amounts greater than 2.3 % AR. At the end of the soil studies (after 60 – ~~180~~ 366 days) at 20 °C and 25 °C, 16.9 to 79.6 % AR was mineralized to CO_2 and 2.5 to 43.2 % bound residues were formed. At the end of the study (after 60 days) at 10 °C, 48.2 % AR CO_2 and 2.4 % AR bound residues were formed. Volatiles other than CO_2 remained ≤0.3 % of AR.

Under aerobic conditions, degradation of glyphosate in soil mostly follows mostly biphasic kinetic with a few occasions of SFO kinetic. DT_{50} and DT_{90} values of glyphosate under different soil moisture conditions and temperatures of 20 or 25 °C to be used for persistence calculations range from 1.0 - ~~67.7~~ 37.75 d and from 9.3 - ~~471.4~~ 1661 d, respectively. At 10 °C, the degradation of glyphosate also followed biphasic kinetic with a DT_{50} value of 8.1 d and a DT_{90} value of 50.8 d. Normalised SFO or back-calculated SFO DT_{50} at 20 °C and pF2 derived from best fit kinetics to be used for evaluation of the P-criterion of potential PBT, vPvB or POP substances range from 3.6 d to ~~133.8~~ 427.8 d with a geometric mean of ~~18.7~~ 19.7 d. The geometric mean of normalised SFO or back-calculated SFO DT_{50} of glyphosate at 20 °C and pF2 to be used for modelling purposes is ~~21.0~~ 20.5 d.

The metabolite AMPA was formed during aerobic degradation of glyphosate at 20 °C and 25 °C with formation fractions between 0.1817 - 0.6076 and mean formation fraction of ~~0.3680~~ 0.3595. AMPA subsequently degraded following SFO kinetics with DT_{50} and DT_{90} values under different soil moisture conditions and temperatures of 20 or 25 °C to be used for persistence calculations ranging from 39.0 - 300.7 d and 129.5 - 998.9 d, respectively. The geometric mean of normalised SFO DT_{50} of AMPA at 20 °C and pF2 is 88.8 d.

2.8.1.2 Anaerobic soil degradation

The anaerobic degradation of glyphosate and glyphosate trimesium was investigated for the 2001 EU evaluation of glyphosate and in several newly submitted studies. The results of the new studies demonstrate that glyphosate degrades under anaerobic conditions although at a slower rate than the aerobic conditions when applying more realistic anaerobic conditions found in the arable cropping environment, which are held under an aerobic/anaerobic gradient. Under complete anoxic conditions, as demonstrated in a study submitted for 2001 EU evaluation of glyphosate, glyphosate degradation was negligible.

2.8.1.3 Soil photolysis

In the 2001 EU evaluation of glyphosate several soil photolysis studies were reviewed and considered acceptable by the RMS. In a summary of the study results, it was concluded that

the photolytic degradation of glyphosate on soil surfaces to AMPA is a slow process and is, at most, a very minor pathway for the degradation of glyphosate in soil. Therefore, no new soil photolysis studies were submitted by the applicant in the renewal dossier.

2.8.1.4 Field dissipation

The dissipation of glyphosate under field conditions has been investigated at ten sites in Europe (Germany, Switzerland). Glyphosate shows similar degradation rates under field conditions compared with half lives under laboratory conditions. The metabolite AMPA was observed with a maximal occurrence between 19.7 and 53.8 % AR (glyphosate equivalents (molar based)).

A re-calculation of trigger endpoints following FOCUS Kinetics guidance (2006) was provided by the applicant. The un-normalised DT_{50} and DT_{90} values of glyphosate derived from field dissipation studies by best fit-kinetics and used as persistence endpoints and triggers for higher-tier experiments range between 5.7 and 40.9 days and between 66.9 and 386.6 days, respectively. The appropriate DT_{50} and DT_{90} values of the metabolite AMPA range between 283.6 and 633.1 days and between 942.39 and >1000 days, respectively and follow first order kinetics.

For the use in PEC_{Soil} calculation the worst case of the half life values of un-normalised field dissipation studies should be used. In the case of glyphosate in six of eight cases the degradation doesn´t follow first order kinetics, but biphasic models (DFOP and FOMC) were considered best fit kinetics. As input parameters for PEC_{Soil} calculation of glyphosate the kinetic parameters of the DFOP kinetic (k_1, k_2, g) of the trial Kleinzecher, Germany, should be used in the program ESCAPE 2. The overall DT_{50} and DT_{90} values for this trial are 38.3 and 386.6 days for glyphosate.

According to the Draft Guidance on “Evidence needed to identify POP, PBT and vPvB properties for pesticides” of EFSA expert group from 25.09.2012-rev.3 the maximal non-SFO-DT_{90} value divided by 3.32 is relevant with regard to P-criterion. The recalculated SFO-DT_{50} of 116.4 days from trial Kleinzecher, Germany is lower than the trigger of 120 days for PBT substances and therefore, the P-criterion is not fulfilled.

As input parameters for PEC_{Soil} calculation of AMPA the kinetic parameters of the SFO kinetic of the trial Unzhorst, Germany, should be used in the program ESCAPE 2. The DT_{50} value for this trial is 633 days for AMPA.

Normalised field degradation data are not available. Field studies are not triggered by the results from laboratory degradation studies and therefore, not necessary.

2.8.1.5 Adsorption and desorption

The adsorption and desorption behaviour of glyphosate in soil was evaluated during the 2001 EU evaluation of glyphosate. One study (Livingston et al., 1986, BVL no 2325589) was evaluated as acceptable based on the evaluation criteria and guidance in force at that time. The adsorption/desorption characteristics of glyphosate resulting from this study have been reported in the Glyphosate Monograph and ranged from 3800 to 60000 mL/g (K_{foc} values). The

study of Waring (1992, BVL no 1932008) was also evaluated as acceptable. The K_{doc} values reported in the Glyphosate Monograph ranged from 884 to 50660 mL/g.

There are three additional studies of the adsorption and desorption behaviour of glyphosate in soil available from GTF members but not considered during the 2001 evaluation (Thomas and Lane, 1996, BVL no 2310260; van Noorloos and Slangen, 2001, BVL no 2310257; Kolk, 1996, BVL no 2310258), one of which has been evaluated at Member State level already (Thomas and Lane, 1996 BVL no 2310260). Furthermore, there is one more study available (Schneider, 1993, BVL no 1027844) submitted during national authorisation in Germany.

Considering all compliant adsorption/desorption studies of glyphosate in (including already EU evaluated and additional studies), the K_{foc}/K_{doc} values for glyphosate range from 884 to 60000 mL/g (arithmetic mean: ~~15844~~ 15388 mL/g). The RMS proposes to use 1/n default values of ~~0.9 in cases where no reliable 1/n value could be derived in the study and~~ 1.0 in cases where no investigations of the relationship between soil solution concentration and adsorption behaviour were conducted in the study.

The adsorption and desorption behaviour of AMPA in soil was evaluated during the 2001 EU evaluation of glyphosate. The adsorption/desorption characteristics of AMPA derived from one acceptable study (Weeden, 1993, BVL no 2325586) have been summarized in the Glyphosate Monograph. K_f and K_{foc} values for AMPA from this study ranged from 15 to 1554 and 1160 to 24800 mL/g, respectively. One additional AMPA adsorption and desorption study (Muller and Lane, 1996, BVL no 2310266), conducted by a GTF member to support its own registrations, has also been evaluated at the EU-Member State level.

Additionally, two studies (Knoch, 2003, BVL no 2310262; Wittig and Bockholt, 2002, BVL no 2310266) with a wide range of soil characteristics were available from GTF members. Neither of these studies was evaluated during the glyphosate 2001 EU evaluation or at EU Member State level.

Considering the results of all compliant adsorption/desorption studies available for AMPA the K_{foc} values for AMPA based on multiple concentration tests range from 1119 to 45900 mL/g (arithmetic mean: 9749 mL/g). ~~The RMS proposes to use 1/n default values of 0.9 in cases where no reliable 1/n value could be derived in the study.~~ The results of all studies show that AMPA has a high adsorption potential.

Table B.2.8-1: Adsorption values for the glyphosate and its metabolite AMPA metabolites

Substance	number of soils (n)	K_{foc}/K_{doc} values (mL/g)		1/n values		Method
		arithmetic mean	range	arithmetic mean	range	
Glyphosate	24	~~15844~~15388	884 - 60000	~~0.914~~ 0.93	0.72 - 1.16	OECD 106
AMPA	16	9749	1119 - 45900	~~0.853~~ 0.81	0.75 - 0.98	OECD 106

2.8.1.6 Predicted environmental concentrations in soil (PEC_{Soil})

The PEC_{Soil} calculations were performed with ESCAPE 2.0 based on the recommendations of the FOCUS workgroup on degradation kinetics (2006). A soil bulk density of 1.5 g/cm^3, a soil depth of 5 cm and a tillage depth of 20 cm (arable crop)/5 cm (permanent crops) were assumed. Initial concentrations, maximum and minimum plateau concentrations, and actual and

time weighted average concentrations of glyphosate and AMPA in soil were calculated for a single maximum application rate of 4320 g glyphosate acid/ha as worst case approach and additional for all seven intended uses. It is important to mention that single application rate of 4320 g glyphosate acid/ha is not supported in the representative GAP, but rather represents the recommended maximum total annual application rate for all crops and therefore, a conservative worst-case approach.

For PEC_{Soil} calculations the worst case of the half life values of un-normalised field dissipation studies should be used. As input parameters for PEC_{Soil} calculation of glyphosate the kinetic parameters of the DFOP kinetic (k_1, k_2, g) of the trial Kleinzecher, Germany, should be used in the program ESCAPE 2. The overall DT_{50} and DT_{90} values for this trial are 38.3 and 386.6 days for glyphosate. As input parameters for PEC_{Soil} calculation of AMPA the maximum DT_{50} value of the trial Unzhorst, Germany, of 633 days should be used.

The summary of PEC_{Soil} values for glyphosate and its metabolite AMPA after maximal application of active substance to all crops and for all intended uses are provided in Table B.2.8-2.

Table B.2.8-2: Maximum PEC_{Soil} values for glyphosate and its metabolite AMPA after maximum field applications of 4320 g as/ha to all crops (PEC_{Soil})

Indication	Intended use	Application rate (g/ha) interception (%)	PECact (mg/kg)	PECaccu * (mg/kg)
Glyphosate				
worst case	all crops annual	1 x 4320 (0%)	5.7600	5.974
	all crops permanent	1 x 4320 (0%)	5.7600	6.6162
001	all crops annual	2 x 2160 (0%)	4.7514	4.9572
	all crops permanent	2 x 2160 (0%)	4.7514	5.5746
002	all crop annual	1 x 1080 (0%)	1.4400	1.4935
	all crop permanent	1 x 1080 (0%)	1.4400	1.6538
003, 004	cereals	1 x 2160 (90%)	0.2880	0.2987
005	oil seed rape	1 x 2160 (80%)	0.5760	0.5974
006	orchard crop, vines, citrus&tree nuts	3 x 2880 (0%) only 33% of area treated	2.5490	3.0648
007	orchard crop, vines, citrus&tree nuts	3 x 2880 (0%) only 50% of area treated	3.8235	4.5973
AMPA				
worst case	all crops annual	1 x 4320 (0%)	2.036	3.0719
	all crops permanent	1 x 4320 (0%)	2.036	6.1797

* a tillage depth of 20/5 cm was considered for calculating the background concentration for annual/permanent crops

2.8.2 Summary of fate and behaviour in water and sediment

2.8.2.1 Hydrolysis

Several hydrolysis studies on glyphosate were assessed as acceptable during the EU review of glyphosate (2001) and the results were summarized in the Monograph. Additional studies that were not previously reviewed are available from GTF members (see IIA 2.9.1). In these studies glyphosate was found to be stable to hydrolysis. No significant degradation products have been found in these studies. Therefore, no hydrolysis study for AMPA was conducted. However, because of chemical structure similarity of glyphosate and AMPA and the general observation of the prolonged stability of AMPA in highly alkaline (*e.g.* 0.1 N NH_4OH solvent commonly used to extract glyphosate and AMPA from soil) and acidic aqueous solutions (*e.g.* 6 N HCl elution solvent in AMPA crop method), AMPA also could also be characterized as stable toward hydrolysis.

2.8.2.2 Photolysis

The metabolite aminomethylphosphonic acid (AMPA) does not absorb light significantly at wavelengths longer than 230 nm. Thus, in highly purified sterile water, in which direct photolysis is the only mechanism for photo-transformation, AMPA is expected to be photo-stable. In addition, in accordance with the discussion presented in Annex Point IIA 2.9.2, in regard to the photolysis of [^{14}C]glyphosate in aqueous buffers under the influence of simulated artificial sunlight, it can be concluded that both glyphosate and AMPA should be stable to direct photo-transformation in the purified sterile water.

2.8.2.3 Ready biodegradability

In the 2001 EU evaluation of glyphosate, several studies assessing glyphosate's ready biodegradability have been reviewed (Henshal et al., 1972, BVL no 1934355; Brightwell et al., 1978, BVL no 1932009; Wüthrich, 1990, BVL no 1934369; Carrick, 1991, BVL no 2325628; Anonymus, 1990, BVL no 1934372; Neven, 1990a; Neven, 1990b). Two out of these reviewed studies were conducted according to the OECD guideline 302 for test on inherent biodegradability (Wüthrich, 1990, BVL no 1934369; Carrick, 1991, BVL no 2325628). One addition study according to OECD guideline 301 F (Mamometric Respirometry Test) was prepared by a Glyphosate Task Force (GTF) member (Feil, 2009).

In all studies, glyphosate did not show mineralisation of more than 60 % within 28 days. Therefore, the active substance is classified as not ready biodegradable.

2.8.2.4 Water-sediment system

The fate of glyphosate in several different water/sediment systems was evaluated during the 2001 EU evaluation of glyphosate (Möllerfeld and Römbke, 1993, BVL no 1934113; Muttzall, 1993, BVL no 1982136; Steginsky and Powell, 1995, BVL no 1934389; Henshall and Brightwell, 1972, BVL no 1934355; Kesterson and Jackson, 1990; Honegger, 1992, BVL no 2325652; Brightwell, 1978, BVL no 1932052). There is one additional wa-

ter/sediment study (Bowler and Johnson, 1999, BVL no 2154357) conducted with ^{14}C-glyphosate trimesium. In the initial Annex I submission, an AMPA water/sediment study was not provided by any Notifier; however, one study (Knoch and Spirlet, 1999, BVL no 1934122) was provided later. In addition to the above studies one other glyphosate and three other ^{14}C-AMPA water/sediment studies are available which were not reviewed during the 2001 EU glyphosate evaluation (Heintze, 1996, BVL no 1939626; Feser-Zügner, 2002, BVL no 2310270; McEwen, 2004, BVL no 2310275 and Knoch, 2003, BVL no 2310273).

In summary, the results of the plausible and valid water/sediment studies show that, in addition to microbial degradation, a major contributor to the aquatic dissipation of glyphosate is adsorption to the sediment. They also demonstrated that from approximately 6% to 48% of the applied glyphosate is mineralized to carbon dioxide during 91 or 100 days of incubations. Radioactivity associated with non-extractable residue was between 8% and 35% of the applied glyphosate during 97 or 91 days of incubation. The principal metabolite of glyphosate in water/sediment system is AMPA. The maximum amounts of AMPA detected were 16% (water phase), 19% (sediment) and up to 27 % (total system) of the total glyphosate applied. These studies that were independently conducted with ^{14}C-AMPA as a test substance also established that AMPA quickly dissipates from the water phase by both adsorption to the sediment and by degradation by the sediment micro-flora. Studies demonstrated that from 8% to 40% of the applied AMPA is mineralized to carbon dioxide. Several other minor components were also detected in these studies.

DT_{50} values from all relevant water/sediment studies have been re-calculated according to the recommendations of FOCUS kinetics guidance (FOCUS, 2006, 2011). In most cases, the degradation behaviour of glyphosate in the water/sediment systems does not follow first order kinetics. The recalculated half-life values of glyphosate for the total system ranged between 13.8 and 329.9 days leading to a geometric mean of 67.7 days (n = 6). Water phase DT_{50} values of glyphosate varied between 6.8 and 21.8 days. A geometric mean value of 9.6 days resulted for the water phase. Sediment DT_{50} values of glyphosate ranged between 34.1 and 75.6 days.

The recalculated half-life values of AMPA for the total systems ranged between 69.3 and 102.9 days leading to a geometric mean value of 86.1 days (n = 4). Water phase DT_{50} values of AMPA varied between 2.1 and 15.5 days. A geometric mean value of 5.5 days resulted for the water phase. Sediment DT_{50} values of AMPA could not be derived.

2.8.2.5 Predicted environmental concentrations in surface water, sediment and groundwater (PEC_{SW}, PEC_{Sed} and PEC_{GW}) (IIIA 9.2.1, 9.2.3)

Surface water and Sediment

Predicted environmental concentrations of the active substance glyphosate in surface water (PEC_{SW}) and sediment (PEC_{Sed}) were estimated using the programs FOCUS Steps 1-2 for FOCUS surface water Step 1 and 2 modelling. As worst case covering all intended uses, PEC_{SW} and PEC_{Sed} were derived for pre-emergence application of glyphosate to various field crops and for post-weed emergence use of glyphosate to the soil and trunks of pome/ stone fruit trees representing the intended use in orchard crops, vines including citrus & nut trees (= perennial crops). Additionally, FOCUS surface water Step 1 and Step 2 PEC_{SW} and PEC_{Sed} values were estimated for the metabolites AMPA and HMPA.

For glyphosate, maximum PEC_{SW} and PEC_{Sed} at Step 1 were 104.8 µg/L and 10300 µg/kg, both for the pre-emergence use in field crops and post-weed emergence use in perennial crops. Maximum PEC_{SW} and PEC_{Sed} of glyphosate at Step 2 for the intended pre-emergence use in field crops ranged from 18.49 to ~~23.58~~ 23.38 µg/L and from ~~1570~~ 1560 to ~~3600~~ 3570 µg/kg, respectively. For the intended post-weed emergence use in perennial crops, maximum PEC_{SW} of glyphosate at Step 2 were 39.73 µg/L with maximum PEC_{Sed} ranging from 2070 to ~~4780~~ 4770 µg/kg.

For AMPA, maximum PEC_{SW} and PEC_{Sed} (Step 1) were 40.90 µg/l and 3300 µg/kg, both for the pre-emergence use in field crops and the post-weed emergence use in perennial crops. Maximum PEC_{SW} and PEC_{Sed} at Step 2 for the intended pre-emergence use in field crops ranged from 6.67 to 15.76 µg/l and 628.4 to 1520 µg/kg. For the intended post-weed emergence use in perennial crops, maximum PEC_{SW} and PEC_{Sed} at Step 2 ranged from 7.32 to 17.16 µg/l and 685.1 to 1640 µg/kg.

For HMPA, maximum PEC_{SW} and PEC_{Sed} at Step 1 were 6.71 µg/l and 696 µg/kg, both for the pre-emergence use in field crops and post-weed emergence use in perennial crops. Maximum PEC_{SW} at Step 2 for the intended pre-emergence use in field crops was 1.22 µg/l and maximum PEC_{Sed} ranged from 86.8 to 196 µg/kg. For the intended post-weed emergence use in perennial crops, maximum PEC_{SW} at Step 2 was 2.63 µg/l and maximum PEC_{Sed} ranged from 128 to 294 µg/kg.

Groundwater

Predicted environmental concentrations of the active substance glyphosate and its metabolite AMPA in groundwater (PEC_{GW}) were estimated using the program FOCUS PELMO 4.4.3.

The exposure assessment was based on a representative use pattern derived from the representative GAP, i.e. PEC_{GW} was derived for pre-emergence application of glyphosate to winter cereals and to potatoes, for pre-emergence plus post-harvest application of glyphosate to spring cereals and for post-weed emergence use of glyphosate to pome fruits. A worst-case zero interception was assumed for all applications.

As input parameters for the PEC_{GW} calculations of glyphosate and AMPA the geometric mean of all DT_{50} values (~~21.03~~ 20.51 and 88.84 days, respectively) as well as the arithmetic mean of all K_{oc} values (15844 and 9749 ml/g, respectively) were used.

In all simulations the 80th percentile PEC_{GW} values of glyphosate acid and AMPA at 1 m soil depth were below the groundwater threshold value of 0.1 µg/L indicating that the use of glyphosate as intended is not likely to pose an unacceptable risk to groundwater via direct leaching.

2.8.3 Summary of fate and behaviour in air

Glyphosate has low vapour pressure (1.31×10^{-5} Pa at 25 °C) and therefore significant concentrations are not expected to be found in air through volatilisation following the use of the compound according to the proposed GAP. The 2001 EU glyphosate evaluation concludes that glyphosate can be classified as not volatile based on its Henry's law constant and on volatilization experiments from soil and plants with no significant rates. Due to no significant

UV-absorption, direct photolysis in air will not occur. Once in the atmosphere rapid photochemical oxidative degradation of glyphosate will occur.

An atmospheric oxidation rate estimation for the active substance glyphosate based on a calculation procedure by means of quantitative structure reactivity relations (QSAR) developed by Atkinson (AOPWIN (version 1.92) shows a half life of 1.6 hours assuming a OH-radical concentration of 1.5 x 10^6 cm^{-3} and a time window of 12 hours. Thus long range transport via air can be excluded.

2.8.4 Summary of monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products

Surface water

Maximum glyphosate and AMPA concentrations in European surface waters as measured in comprehensive monitoring campaigns (Horth, 2012, BVL no 2310291) range between 1.3 - 370 µg/L and 0.22 - > 200 µg/L for glyphosate and AMPA, respectively. It has to be noted that glyphosate and AMPA monitored in this study exceed the predicted environmental concentrations for glyphosate acid and AMPA in surface water (PEC_{SW}) calculated using the FOCUS (2000) surface water models, even though worst case applications was assumed. Nevertheless, the calculated TER values referring to the monitored concentrations in the study by Horth (2012) with the respective acceptability criteria show that the risk for aquatic organisms is acceptable. Compared to the findings published by Horth (2012), the maximum concentrations which were published in open literature are rather low, namely in the range of 0.4 - 1.37 µg/L and 0.2 - 13 µg/L for glyphosate and AMPA, respectively. Therefore, information published in open literature does not really modify the already existing assessment of glyphosate and AMPA occurrence in surface water.

In addition, control and inspection programs at local authority level in Germany show high rates (35 % in 2009 and 36 % in 2010 during controls that were not event-related) of non-compliance regarding the application of plant protection products on walks and places in housing areas, foot-walks, traffic islands and paved surfaces in private properties (Anonymous, 2011, BVL no 2537364 and Anonymous, 2012, BVL no 2537365). This indicates that misuses of plant protection products on paved surfaces, which often contain glyphosate as active ingredient, by non-professional users occur to a relatively large extent. Therefore, current discussions in Germany are focusing on whether the findings of glyphosate and its metabolite AMPA in surface waters originate to some extent from these misuses of plant protection products containing glyphosate on paved surfaces by non-professional users. However, a monitoring study performed in UK (Ramwell et al., 2014, see point B.8.11.6.2) indicates otherwise: Glyphosate losses from urban areas that arise solely from amateur usage have been quantified. Thereby, it was shown that in spite of overdosing occurring, glyphosate concentrations in drain flow were lower than concentrations reported elsewhere from professional use in urban areas. Due to the fact that the representativeness of the study for other EU Member States conditions is called into question and as a precaution, the other EU Member States should look at this issue and, if necessary, take appropriate risk management measures at the national level. ~~In any event, the other EU Member States should be aware of this problem and, if necessary, take appropriate risk management measures at the national level.~~

Next to the use of glyphosate based products by non-professional users, we point to the environmental risks associated with the intended uses of glyphosate as desiccant. An herbicide application to time harvest poses additional ecological risks for surface waters and for non-target species (please refer also to chapter 2.9.6). Member States might consider the

appropriateness of additional herbicide uses especially when addressing sustainable use of plant protection products according to Directive 2009/128/EC.

Groundwater
Regarding the groundwater monitoring data, it has to be pointed out that glyphosate has been detected in Europe with 0.64% above the limit concentration 0.1 µg/L; and AMPA with 0.77 % above 0.1 µg/L (Horth, 2012, BVL no 2310291). Detailed groundwater monitoring studies demonstrating that glyphosate (at least partly) exceeded 0.1 µg/l are available from Italy (Calliera et al., 2011, BVL no 2310280), Germany (Schmidt and Reichert, 2006, BVL no 2310282), The Netherlands (Franke et al., 2010, BVL no 2310284), Sweden (Carter and Pepper, 2005, BVL no 2310285), France (Anonymous, 2012, BVL no 2310289) and Spain (Sanchís et al., 2012, BVL no 2537361). In some cases, clarification could be presented by the authors; e.g. causes for glyphosate findings in groundwater aquifers > 0.1 µg/L were point source contamination, affection by waste deposit, deficient analysis, no fully protected wells, potential for direct hydrological connectivity between surface water and shallow groundwater via artificial drainage systems and short-term contamination of shallow groundwater or spring water. However, it remains often unclear if findings above the authorisation limit originate from a technically correct and regu-lation compliant use of the respective plant protection products in agricultural areas, or misuses or if construction defects on the groundwater abstraction points are reasonable for the limit exceedances etc. Another emerging issue is that other sources of glyphosate than agricultural applications, e.g. the control of weeds on streams and drains, around railways, roads, sports fields and industrial areas have to be considered as well. Regarding the pathways of glyphosate into groundwater when used for agricultural purposes as intended, RMS considers that groundwater contamination > 0.1 µg/L via direct leaching is generally not expected as the substance is strongly adsorbed to soil particles. Exceptions may be made, e.g. for preferential flow. Within a study from Spain (Sanchís et al., 2012, BVL no 2537361), it is described that surface waters exist in 10 out of 11 sampling sites where glyphosate was (at least partly) detected. Due to this fact, surface run-off and/or drainage into these waters with subsequent bank filtra-tion into groundwater cannot be excluded as pathway. During the EU evaluation of the active substances the pathway surface run-off and drainage into an adjacent ditch with subsequent bank filtration into groundwater is not considered until now.
Within the open literature review, groundwater monitoring data were obtained for 4 EU-countries (Spain, Denmark, Norway and Germany), whereas one citation was found for a non-EU site (Canada). However, information is rather heterogeneous. Maximum glyphosate and AMPA concentrations in either groundwater or drinking water well are in the range of 0.02 - 2.56 µg/L and 0.02 - 0.45 µg/L, respectively, whereas maximum glyphosate and AMPA con-centrations as obtained from comprehensive monitoring programs (Horth, 2012, BVL no 2310291) are in the range of 0.01 -24 µg/L and 0.02 - 19 µg/L, respectively. Therefore, in-formation published in open literature does not really modify the already existing assessment of glyphosate occurrence in groundwater.

2.8.5 Definition of the residues relevant to the environment

In the following the residues are defined considering their occurrence in the representative environmental fate studies and for groundwater, in addition, on the basis of PEC_{GW} simula-tions and the results of lysimeter studies.

<u>Soil</u>
The major residues in soil from the environmental fate point of view are glyphosate and its metabolite aminomethylphosphonic acid (AMPA) (max. occurrence in soil 53.8 % AR).

Ground water
The major residues in soil from representative environmental fate studies are glyphosate and its metabolite aminomethylphosphonic acid (AMPA) (max. occurrence in soil 53.8 % AR).

In all simulations the 80th percentile PEC_{GW} values of glyphosate acid and AMPA at 1 m soil depth are below the groundwater threshold value of 0.1 µg/L indicating that the use of glyphosate as intended is not likely to pose an unacceptable risk to groundwater via direct leaching.

Lysimeter studies usually show that the overall risk for the leaching of glyphosate to groundwater was assessed to be low. Glyphosate was either not detected in the leachate or the mean annual concentrations were significantly below 0.1 µg/l. A similar pattern was observed for its metabolite AMPA. Exceptions may be made, e.g. for soils with low content of organic matter and clays, recharge generated by irrigation and heavy rain, and possible preferential solute transport and/ or colloidal mediated transport.

For these reasons stated above, the definitive relevant residue for further groundwater assessment is defined as glyphosate (parent compound).

Surface water
The major residues in surface water, relevant for further risk assessment from the environmental fate point of view are glyphosate and its metabolites aminomethylphosphonic acid (AMPA) (max. occurrence in the water phase 15.7 % AR and max. occurence in the sediment phase 18.7 %) and hydroxymethylphosphonic acid (HMPA) (max. occurrence in water up to 10.0 % AR).

2.8.6 Summary of exposure calculations and product assessment

Predicted environmental concentrations (PEC's) in soil, surface water, sediment and groundwater were calculated for the active substance glyphosate and its metabolite AMPA. The estimated PEC values for the environmental compartments named above will be used for further risk assessment. From the PEC_{GW} value, it can be concluded that glyphosate and its metabolite AMPA pose no risk to groundwater via direct leaching in Europe.

2.9 Effects on non-target species

2.9.1 Summary of effects on birds and other terrestrial vertebrates

Birds
A number of different avian acute oral, short-term dietary and long-term studies have been carried out with glyphosate acid and are already evaluated during the initial EU assessment of glyphosate acid. New studies not evaluated during the first evaluation of glyphosate in the EU peer review of 2001 have been included ([redacted] 1997, BVL no 2310906; [redacted] 1996, BVL no 2310909; [redacted] 1996, BVL no 2310912; [redacted] 1999, BVL no 2310910; [redacted] 2003, BVL no 2310915).
The following endpoints and effect values have been identified as relevant for the quantitative risk assessment according to the current EFSA Guidance Document:

Concerning the acute risk to birds, it should be considered that large number of acute studies in birds without any mortality at limit doses are available. EFSA guidance document 1438/2009 indicates that "it is permissible to extrapolate an LD_{50} value in cases where there is no mortality or a single mortality at a limit dose in an acute avian toxicity study". Using the study with the bobwhite quail with a limit dose of 2000 mg/kg bw, the extrapolation factor for no mortalities at the limit dose and 20 birds per dose group (the actual number of birds tested at this limit dose exceeded 20), the acute LD_{50} to be used in a bird risk assessment according to EFSA guidance document 1438/2009 is proposed to be 2000 x 2.167 = 4334 mg/kg bw.

Concerning the effects of glyphosate on bird reproduction, studies have been conducted with bobwhite quail [redacted] 1978, BVL no 2310921 and [redacted] 1999, BVL no 2310916) and mallard duck ([redacted] 1978, BVL no 2310923; [redacted] 1999, BVL no 2310918) for the active substance glyphosate.
The study by [redacted] (1978, BVL no 2310921) is proposed for risk assessment. A significant reduction in egg weight was observed at the highest concentration tested (1000 ppm). Therefore, a NOEC of 18.1 mg a.s./kg b.w./d was determined and agreed during the EU review process. However, changes in egg weight are not considered a standard endpoint in avian reproduction studies anymore according to guideline OECD 206 and has therefore not been adressed in other studies testing higher concentrations. Other relevant endpoints determined in the study of [redacted] (1978) did not show any unacceptable differences compared to the control treatment – including no. of eggs, no. 14 d old survivals and hatchlings weight. The differences in egg weight between control and the treatment with 1000 ppm amounted to a decrease of approx. 7.5 % (10.26 g ± 0.38 g vs. 9.48 g ± 0.47 g in control and 1000 ppm treatment, respectively). Since all parameters concerning hatchling weight and survival were not affected, it can be assumed that the observed changes in egg weight are statistically significant but do not represent a population relevant adverse effect. Therefore, this endpoint will be considered as a NOAEL of 1000 ppm (equivalent to 96.3 mg a.s./kg b.w./d) and is proposed for the assessment of the chronic risk for birds exposed to glyphosate.

Table B.2.9-1: Endpoint values used for the assessment of the risk for birds arising from the exposure to glyphosate

Time scale and type of risk assessment	Test species	Proposed endpoint	Explanation/justification
Acute toxicity	Bobwhite quail *(Colinus virginianus)*	LD_{50} = 4334 mg/kg bw	A large number of acute studies in birds without any mortality at limit doses were submitted. According to EFSA guidance document 1438/2009 the LD_{50} value was extrapolated and is proposed to be LD_{50} = 4334 mg/kg bw
Reproductive toxicity (long-term)	Bobwhite quail (*Colinus virginianus*)	NOAEL = 1000 ppm corr: 96.3 mg/kg bw/d	A NOAEL of 1000 ppm, corresponding to 96.3 mg/kg/bw/d is proposed.

The risk assessment is based on the intended uses of glyphosate acid in the product MON 52276 covering several crops in pre-planting, pre-emergence, pre-harvest and row-application in orchards and vineyards. Regarding the proposed indication pre-planting (post-emergence of weeds), no bare soil scenario is considered as general scenario since the mode of action of glyphosate is via uptake by green tissues of leaves and stems of treated plants. Therefore, a leaf development is assumed in the assessment scenario.

The maximum cumulative application rate per year is set to 4.32 kg glyphosate/ha. The maximum application rate per treatment is 2.16 kg glyphosate/ha, except for spot applications in orchards and vines where the maximum application rate is 2.88 kg glyphosate/ha.

Based on the screening assessment step, the calculated TER values resulting from an exposure of birds to glyphosate reach the acceptability criteria TER ≥ 10, according to Regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2. for acute effects. The results of the assessment indicate an acceptable risk for birds due to uptake of contaminated food after use of MON 52276 in the indications according to the label.

Based on Tier 1 assessment step, the calculated TER values for the long-term risk resulting from an exposure of birds to glyphosate according to the intended use in oilseed rape (late (with seeds); rapeseed, mustard seed, linseed; BBCH 88-99) and cereals (cereals, late season) as well as in orchards (vines including citrus & and tree nuts, post emergence of weeds; 28 days interval between applications with spot treatment round of trunks or application to the intra-rows) achieve the acceptability criteria TER ≥ 5 according to Regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2. for long-term effects. The results of the assessment indicate an acceptable risk for birds due to the intended use according to the label.

For the intended use in the scenario "all crops (all seeded or transplanted crops)" with an application rate of max. 2 x 2160 g a.s./ha the decline of glyphosate residue in grass was used in the risk assessment for birds feeding on grass foliage. Based on refined assessment step, the calculated TER values for the long-term risk resulting from an exposure of birds to glyphosate achieved the acceptability criteria TER ≥ 5.

In addition to their diet, birds may also be exposed to glyphosate via drinking water. For glyphosate, the ratio of highest application rate (4320 g a.s./ha) to lowest relevant endpoint (NOAEL = 96.3 mg a.s./kg bw/d) indicated an acceptable risk from exposure to contaminated drinking water without the need for further calculations.

The metabolite AMPA has been tested in several toxicity studies which demonstrated that it is of lower toxicity than glyphosate acid (see Volume 1, chapter 2.6.8). Moreover, most of the parent glyphosate is eliminated unchanged and only a small amount (less than 1% of the applied dose) is transformed to aminomethylphosphonic acid (AMPA). Therefore it can be concluded that the risk to birds will be acceptably low and no further quantitative risk assessment is conducted. Since the log K_{ow} values of glyphosate is logP < -3.2 (pH 2-5, 20 °C), the active substance is deemed to have a negligible potential to bioaccumulate in animal tissues.

Overall, the risk to birds from the intended uses of glyphosate is considered to be acceptable.

Terrestrial vertebrates

For the first EU peer reviewed evaluation of glyphosate in 2001, a large number of toxicity studies were submitted that had been conducted with either glyphosate acid or its salts. For the current re-evaluation of glyphosate, several additional studies in rats, mice and rabbits with administration of glyphosate acid were provided (see Vol.3, B.6.2.1). For further details please refer to Volume 3, B.6.2.1.

The acute mammalian toxicity of glyphosate is low in the species tested. The oral LD_{50} was above 2000 mg/kg bw. General signs of oral intoxication were breathing difficulties, reduced

activity, ataxia, piloerection, convulsions and hunched posture. For further details please refer to Volume 3, B.6.2.1. For risk assessment an LD_{50} >2000 mg/kg bw is proposed. For further details please refer to Volume 3, B.6.2.1.

The developmental toxicity and teratogenicity of glyphosate were tested in several studies in rats and rabbits. In general rabbit proved to be more sensitive than rats. The lowest NOAEL for developmental effects was 50 mg/kg bw/day, based on post-implantation losses at 200 mg/kg bw/day in the test species rabbit. In other studies the NOEAL were consistently below 200 mg/kg bw/day due to post-implanation losses and late embryonic death (please refer to RAR, Vol 1, chapter 2.6.7.2.2.). As population relevance can not be excluded due to the clinical parameters observed, an overall NOAEL of 50 mg/kg bw/day is proposed for risk assessment.

Based on the presumptions of Tier 1, the calculated TER values for the risk resulting from an exposure of mammals to the active substance glyphosate according to the GAP of the formulation MON 52276 reach the acceptability criteria TER ≥ 10, according to Regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2. The results of the assessment indicate an acceptable risk for mammals.

For the serial application of 2 ×max. 720 g a.s./ha and once max. 2880 ga.s./ha the results of the worse case assessment indicate a low risk for small herbivorous mammals due to uptake of contaminated food.

Based on Tier 1 assessment step, the calculated TER values for the long-term risk resulting from an exposure of mammals to glyphosate do not achieve the acceptability criteria TER ≥ 5. The results of the assessment indicate an unacceptable risk for small herbivorous mammals due to the intended uses according to the label. A refined risk assessment was considered necessary.
The refinement for the long-term risk is achieved via the consideration of the glyphosate decay in plant material over time. Based on refined assessment step, the calculated TER values for the long-term risk resulting from an exposure of mammals to glyphosate achieved the acceptability criteria TER ≥ 5 for the intended uses in “all crops”, “crop maturity in cereals”, “crop maturity in oilseed rape”.
For the post emergence treatment of weeds orchard crops (vines including citrus & tree nuts, intrarow & spot treatment) the calculated TER values for the long-term risk resulting from an exposure of mammals to glyphosate do not achieve the acceptability criteria TER ≥ 5. Nevertheless, risk can be mitigated as the results of the assessment with a serial application of 3 × max.1440 g a.s./ha indicate a acceptable risk for small herbivorous mammals due to uptake of contaminated food.

In addition to their diet, mammals may also be exposed to glyphosate via drinking water. For glyphosate, the ratio of highest application rate (4320 g a.s./ha) to lowest relevant endpoint (NOAEL = 50 mg a.s./kg bw/d) indicated an acceptable risk from exposure to contaminated drinking water without the need for further calculations.

The metabolite AMPA has been tested in several toxicity studies which demonstrated that it is of lower toxicity than glyphosate acid (see Vol 1, chapter 2.6.8). Moreover, most of the parent glyphosate is eliminated unchanged and only a small amount (less than 1 % of the applied dose) is transformed to aminomethylphosphonic acid (AMPA). Therfore it can be concluded that the risk to mammals will be acceptably low and no further quantitative risk assessment is

conducted. Since the log K_{ow} values of glyphosate is logP < -3.2 (pH 2-5, 20 °C), the active substance is deemed to have a negligible potential to bioaccumulate in animal tissues.

RMS considers the provision of a scientifically sound ecological risk assessment to be coupled to the assessment of the impact of herbicide use on the diversity of terrestrial non-target species due to indirect effects via food web interference. The effects trace back to the intended effect of the herbicides – eliminating competing plants – which is virtually the same in all broad spectrum herbicides. Even though these effects are not substance-specific but rather caused by the use of herbicides per se, the RMS considers it necessary to address this issue in the context of the evaluation of the active substance glyphosate since it represents the far the most extensively used herbicides. Glyphosate can actually be regarded as the most significant herbicide with indirect effects on terrestrial vertebrates (and invertebrates) of the agricultural landscape. The scientific evidence regarding negative impacts of the use of herbicides on terrestrial vertebrates is mostly confined to the farmland birds (see DEFRA, 2005; [redacted] 2013), whereas information with respect to farmland mammals is very limited. Previous studies on potentially affected farmland bird species such as Grey Partridge demonstrate the population relevance of indirect effects of herbicides. For instance in the study of [redacted] (2001), a relationship between pesticides, food availability, breeding performance and population size has been fully demonstrated with herbicides being the main determining pesticide group. Chick survival is the key factor determining population development and Grey Partridge chicks are highly dependent on invertebrate prey abundance in arable crops, mainly cereals, where they feed on insects and other arthropods along the edges ([redacted] 1971; [redacted] 1985). The impact of the herbicide use via food chain interactions seems to be the most relevant indirect effect of PPPs although the interference with habitat quality (e. g. cover) and resulting changes in the predation risk might also play a significant role for some species or groups. For those species that depend on arable land as habitat, thus potentially affected by the PPP use due to interference with the food availability or habitat quality, the consideration of indirect effects in the risk regulation of PPPs can be regarded necessary from both the scientific as well as from the legal perspective. In previous evaluations of active substances, this kind of effect has not been assessed, and standardized assessment methods are not yet available. However, according to the new data requirements (Regulation (EU) No 283/2013) the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects via alteration of the food web, shall be considered. In the opinion of the RMS it is not a feasible and sustainable option to ignore indirect effects. Effective and reasonable risk mitigation measures are available (compensation measures, especially cropped no-spray zones, fallow land and flowering margins) but yet have to be established in the regulation of PPP. As the severity of indirect effects of herbicide use on farmland bird (and mammal) species diversity strongly depends on agricultural and landscape properties, an assessment considering all different conditions all over the EU is hardly possible. Thus, we limit to describe the high potential of glyphosate and other broad spectrum herbicides to cause indirect effects and to highlight the need for risk mitigation measures by the member states, proposing compensation measures as a suitable tool.

2.9.2 Summary of effects on aquatic organisms

The toxicity of glyphosate as the acid, the isopropylamine (IPA) salt, the potassium (K) salt and its metabolites AMPA and HMPA to aquatic organisms as well as the representative formulation in the present approval renewal of glyphosate (MON 52276) was investigated in a series of laboratory studies with representative species from different trophic levels of the

aquatic food chain (i.e. fish, aquatic invertebrates, algae and aquatic plants). A summary of the relevant acute and long-term endpoints, representing the worst case for key species are presented in the table below:

Table B.2.9-2: Endpoints for aquatic species (most sensitive species of each group) relevant for the quantitative risk assessment

Species	Substance/ Test item	Test design	Toxicity	Endpoint	Safety factor
			(mg test item/L)		
O. mykiss	Glyphosate acid	acute	38	mortality	100
L. macrochirus	Glyphosate IPA-salt	acute	>1000 741 a.s.	mortality	100
O. mykiss	Glyphosate K-salt	acute	>2573 1227 a.s	mortality	100
O. mykiss	AMPA	acute	520	mortality	100
O. mykiss	MON 52276	acute	> 989 > 306 a.s.	mortality	100
B. rerio	Glyphosate acid	long-term	1 (recalc. by RMS)	mortality	10
P. promelas	AMPA	long-term	12	hatching success, fry survival, length and weight	10
Daphnia magna	Glyphosate acid	acute	40	immobilization	100
Daphnia magna	Glyphosate IPA salt	acute	930	immobilization	100
Daphnia magna	Glyphosate K-salt	acute	592 278 a.s. (recalc. by RMS)	immobilization	100
Daphnia magna	AMPA	acute	690	immobilization	100
Daphnia magna	HMPA	acute	>100	immobilization	100
Daphnia magna	MON 52276	acute	676 209 a.s.	immobilization	100
Daphnia magna	Glyphosate acid	long-term	12.5 (recalc. by RMS)	reproduction	10
Daphnia magna	AMPA	long-term	15	reproduction	10
S. costatum	Glyphosate acid	chronic	11	biomass	10
			18	growth rate	10
A.flos-aquae	Glyphosate acid	chronic	8.5	biomass	10
			22	growth rate	10
P.subcapitata	Glyphosate IPA salt	chronic	9.25	biomass	10
			31	growth rate	10
D.subspicatus	AMPA	chronic	89.8	biomass	10
			452	growth rate	10
P.subcapitata	HMPA	chronic	> 115	biomass	10
			> 115	growth rate	10
P.subcapitata	MON 52276	chronic	178	biomass	10
			393	growth rate	10
Lemna gibba	Glyphosate acid	chronic	12	frond count	10
Lemna minor	Glyphosate-IPA salt	chronic	25.5	frond count	10
Lemna gibba	HMPA	chronic	> 123	frond count	10
Lemna gibba	MON 52276	chronic	21	frond count	10
M.aquaticum	MON 77973 Glyphosate acid	chronic	12.3	fresh weight, relative increase	10
M.aquaticum	MON 52276	chronic	4.4	fresh weight, relative increase	10
M.aquaticum	AMPA	chronic	31.1	root length, relative increase	10

The principal metabolite of glyphosate in water/sediment system is AMPA. The maximum amounts of AMPA detected were 16 % (water phase), 19 % (sediment) and up to 27 % (total system) of the total glyphosate applied. This indicates that there is a potential for exposure to

glyphosate for sediment-dwelling organisms. However, the NOEC values from the long-term *Daphnia* test with glyphosate acid are well above 0.1 mg/L, indicating low toxicity to aquatic invertebrates. According to the GD on Aquatic Ecotoxicology SANCO/3268/2001 rev.4, specific toxicity studies on sediment-dwellers should therefore not be necessary.

As worst case covering all intended uses, PEC_{SW} and PEC_{Sed} were derived for pre-emergence application of glyphosate to various field crops and for post-weed emergence use of glyphosate to the soil and trunks of pome/stone fruit trees representing the intended use in orchard crops, vines including citrus & nut trees (= perennial crops).

Table B. 2.9-3 Calculated TER values referring to FOCUS Step 1 and 2 are provided in the following table:

Scenario	**App. rate (g/ha)**	**PEC_{sw} (µg/L)**	**Fish**		**Invertebrates**		**Algae**	**Aquatic plants**
			acute	prolonged	acute	prolonged		
			O. mykiss	*B. rerio*	*D. magna*		*A. flos-aquae*	*M. aquaticum* (MON52276)
			LC_{50} (µg/L)	**NOEC (µg/L)**	**EC_{50} (µg/L)**	**NOEC (µg/L)**	**E_bC_{50} (µg/L)**	**E_bC_{50} (µg/L)**
			38000	1000	40000	12500	8500	4400
			TER					
FOCUS Step 1 Not crop specific	1×4320	104.81	363	~~53~~ 9.5	382	119	81	42
FOCUS Step 2 North EU (Oct-Feb)	2×2160	~~23.58~~ 23.38	~~1612~~ 1625	~~237~~ 42	~~1696~~ 1711	~~530~~ 535	~~360~~ 364	188
North EU (Mar - May) (Jun-Sep)	2×2160	18.49	2055	~~303~~ 54	2163	676	460	240
South EU	2×2160	~~19.30~~ 19.14	~~1969~~ 1985	~~290~~ 52	~~2073~~ 2090	~~648~~ 653	~~440~~ 444	230
TER criterion			100	10	100	10	10	10

Comparison of calculated TER values with the respective acceptability criteria clearly shows that the risk for aquatic organisms is acceptable.

2.9.3 Summary of effects on arthropods

2.9.3.1 Effect on bees

The 2001 EU glyphosate evaluation concluded that the hazard quotient values for intended uses of glyphosate are well below 50, indicating a low risk to honeybees according to the EPPO risk assessment scheme. In order to reevaluate glyphosate a total of seven laboratory toxicity studies with technical or formulated glyphosate, a tunnel test and a study about the honeybee brood development with technical glyphosate were submitted. The results from new

laboratory toxicity studies demonstrate that glyphosate, glyphosate salts and MON 52276 have very low acute contact and oral toxicity to honeybees with LD50 values around or higher than 100 µg a.s./bee. The calculated HQ values show an acceptable risk for honeybees due to the intended use of the lead formulation MON 52276 according to the label.

Additionally a bee brood study was performed following established methodology which demonstrates that glyphosate poses no chronic risk to bee brood as well at worst case field exposure levels.

2.9.3.2 Effects on other arthropod species

Several studies on the effect of glyphosate formulations on non-target arthropods were assessed during the first EU evaluation of glyphosate in 2001 and are summarised in SANCO/6511/VI/99-final. These studies were evaluated in the monograph but were not submitted with the renewal dossier and are not documented in detail in this Review Assessment Report. Additional studies with the lead formulation MON 52276 have been conducted with *Aphidius rhopalosiphi*, *Typhlodromus pyri* and *Aleochara bilineata* to meet the data requirements that are acceptable for an updated non-target arthropod risk assessment. The following endpoints and effect values have been identified as in principle relevant for the quantitative risk assessment:

Table B.2.9-4: Toxicity of MON 52276 to non-target arthropods submitted for the present application for renewal of approval for glyphosate

Species	Substance	System	Results
Aphidius rhopalosiphi	MON 52276	Extended laboratory (whole plant), 3D	LR_{50} > 16.0 L product/ha (5760 g a.s./ha)
Typhlodromus pyri	MON 52276	Extended laboratory (leaf discs), 2D	ER_{50} ≥ 12.0 L product /ha (4320 g a.s./ha)
Aleochara bilineata	MON 52276	Extended Laboratory (soil)	ER_{50} > 12.0 L product /ha (4320 g a.s./ha)

With regard to the endpoints from the extended laboratory tests regarding lethal and sublethal effects and the predicted rates of glyphosate in-field as well as off-field, all calculated HQ values remain below the acceptability criterion. The calculated HQ values show an acceptable risk for non-target arthropods due to the intended use of the lead formulation MON 52276 according to the label.
Apart from potential direct (i.e. toxicological) effects, non-target arthropods can be affected indirectly by the use of herbicides. These effects trace back to the intended effect of the herbicides – eliminating competing plants – and the interruption of the food web going along with it. Considering the strong evidence that the use of herbicides (including glyphosate) considerably contributes to the ongoing loss of biodiversity in farmland invertebrate and vertebrate non-target species additional risk mitigation measures might be regarded at member state level depending on the agricultural and landscape conditions.

2.9.4 Summary of effects on non-target soil meso- and macrofauna

Earthworms

For evaluation of acute risk of glyphosate acid on *Eisenia fetida*, we propose to use the endpoint LC_{50} > 5600 mg a.s./kg dry soil from the newly submitted test. For the evaluation of the long-term risk, a new chronic study was submitted. In this study no statistically significant effects were observed. Therefore the proposed endpoint is a NOEC = 1000 mg MON0139/kg dry soil, corresponding to 473 mg of glyphosate acid.
AMPA was classified as a major metabolite occurring in relevant amounts (1 x ≥ 10% of application rate in soil. For AMPA, in a new acute toxicity study the LC_{50} of AMPA was determined to be > 1000 mg AMPA/kg dry substrate. A new study concerning the long-term toxicity of AMPA towards earthworms was performed. For this study a re-evaluation of the deriven endpoints was performed by the RMS. A NOEC of 131.9 mg/kg was recalculated for biomass deviation and number of juveniles, due to a significant reduction in offspring number observed in treatment concentrations higher than 131.9 mg AMPA/kg dry soil. The 56-day no-observed-effect-concentration of AMPA was 131.9 mg/kg regarding earthworm reproduction (number of juvenils).

MON 52276 is the leading formulation in the Annex I re-registration dossier of glyphosate. A study with MON 52276 was conducted leading to an LC_{50}>1250 mg/kg soil dry soil (IPA-salt) corresponding to >388 mg a.s/kg dry soil.

According to the GAP, glyphosate containing plant protection products are intended to be applied at maximum application rate of 2 x 2160 g a.s./ha. The maximum application rate is supposed to be 4.32 kg/ha glyphosate in any 12 month period across use categories.
TER values were calculated for glyphosate and the metabolite AMPA for a worst-case scenario with an application rate of 4.32 kg/ha glyphosate. The results of the assessment indicate an acceptable acute and long-term risk for earthworms due to the intended use according to the label.

Soil mesofauna

New studies have been conducted exposing *Hypoaspis aculeifer* and *Folsomia candida* to glyphosate IPA salt and AMPA, respectively. The following endpoints and effect values have been identified as in principle relevant for the quantitative risk assessment:

Table B.2.9-5: Toxicity of glyphosate IPA-salt and its metabolite AMPA to soil mites and springtails

Species	Substance	Design	Endpoints (mg/kg dry soil)
Hypoaspis aculeifer	Glyphosate IPA-salt	14 d chronic	EC_{50} > 1000; > 472.8 a.e NOEC=1000; 472.8 a.e.
Folsomia candida	Glyphosate IPA-salt	28 d chronic	EC_{50} >1000; > 587 a.e. NOEC= 1000; 587 a.e.
Hypoaspis aculeifer	AMPA	14 d chronic	EC_{50} > 320 NOEC=320 mg/kg dry soil
Folsomia candida	AMPA	28 d chronic	EC_{50} >315 NOEC= 315

The TER values calculated using worst-case PEC_{SOIL} values for glyphosate acid and its metabolite AMPA - the maximum application rate is supposed to be 4.32 kg/ha glyphosate in any 12 month period across use categories - exceeded the relevant triggers, indicating that the risk to soil macro- and mesofauna acceptable.

2.9.5 Summary of effects on soil nitrogen transformation

MON 52276 is the representative formulation in the current EU review of glyphosate. MON 52276 as well as the metabolite AMPA caused no significant effects > 25 % on soil microflora respiration and soil nitrogen transformation processes. Based on laboratory testing with MON 52276, the Annex VI trigger value of > 25 % effects after 28 days was not exceeded at concentrations of 1× and 5× the maximum recommended annual use rate for 4.32 kg a.s./ha. Therefore, the use of MON 52276 according to the proposed use pattern can be considered not to result in any unacceptable adverse effects for soil micro-organisms.
During the revision of the active substance glyphosate for the renewal of Annex I inclusion (AIR2), the Rapporteur Member State (RMS) Germany requested the glyphosate task force (GTF) to submit a new N-transformation study following the OECD guideline 116, with the active substance glyphosate acid. MON 77973 (96.59% Glyphosate Acid), when applied at 6.62 and 33.1 mg a.e./kg dry soil, is not expected to cause significant long-term effects on the soil nitrogen transformation process at concentrations ≤ 33.1mg a.e./kg dry soil (≤ 23kg/ha). There was no significant difference between the treatment rates of 6.62 and 33.1 mg a.e./kg dry soil and the control treatment for nitrate production from Day 14 to 28.
~~For the active ingredient glyphosate effects on the nitrogen cycle test could not be assessed due to an invalid study according to OECD guideline 216. According to the OECD 216 guideline the test concentrations recommended are the maximum predicted environmental concentration (PEC) and five times that concentration. Sufficient data was submitted to evaluated the risk of the representative formulation MON 52276.~~

~~Nevertheless, for the evaluation of the active substance glyphosate acid, further data will have to be generated to assess the risk for non-target micro-organisms. This is necessary as the active substance glyphosate acid is considered persistent in soil (the maximum DT_{50} value of unnormalised field dissipation studies of 116.4 days exceeds the trigger value of 60 days for soil).~~

2.9.6 Summary of effects on terrestrial non-target higher plants

In the first EU peer review evaluation of glyphosate in 2001, no risk assessment for terrestrial plant was performed, but studies on seed germination and seedling emergence and one study on vegetative vigour were evaluated. New studies on seedling emergence and vegetative vigour of terrestrial non-target plants have been carried out with glyphosate acid and the lead formulation MON 52276.
The potential effects of glyphosate acid on seedling emergence could not suffuciently demonstrated as the study submitted is not considered as valid by the RMS.
So far, for risk assessment in the national and EU plant protection product authorization process, the lowest ER_{50} values in a vegetative vigour test after 21 days was observed for tomato plants and the endpoint was calculated to be 0.146 kg a.s./ha for dry weight. The new vegetative vigour study of glyphosate acid on non-target terrestrial plants did not include the most sensitive species tomato. The lowest ER_{50} values in the new study was calculated after 28 days for oilseed rape to be 0.149 kg glyphosate acid/ha and 0.150 kg glyphosate acid/ha, respectively for visual damage assessment and plant dry weight. The risk might not be reliably predicted and the assessement based on the endpoint for the active ingredient glyphosate acid can be considerd on a preliminary basis. The former EU endpoint $ER_{50,\ 21\ day}$ for tomato plants = 0.146 kg a.s./ha for dry weight is proposed for preliminary risk assessment.

During the revision of the active substance glyphosate for the renewal of Annex I inclusion (AIR2), the Rapporteur Member State (RMS) Germany requested the glyphosate task force (GTF) to submit a new vegetative vigor study following the OECD guideline 227, with the representative formulation MON52276. RMS sees uncertainties concerning the acceptability oft the study, because recommendations of the OECD guideline 227 were neglected and therefore the toxicity of the representative formulation MON52276 might be understimated due to unfauvorable environmental condidtions. These concerns could not be finalised due to a lack of further studies according to OECD227. Still the current assessment does not permit the conclusion that differently formulated plant protection products containing the active substance will not have any harmful effect on non-target plants.

Nevertheless, this study displays the only dataset provided for the representative formulation MON 52276 and therefore, includes information about the relevance of the formulants. In general toxicity studies with the commercial product are more appropriate than studies with the active ingredient only for the assessment of the effects on non-target plants.

RMS proposes that concerned Member States shall request the submission of additional studies according to OECD227 with the commmercial products particular during product authorziation, in order to evaluate specific sensitive endpoints corresponding to the product and to determine adequate bufferstrips for the protection of non–target plants in off- field areas.

In the case of the intended use on mature crops before harvest, it must be pointed out that the desiccation scenario is not considered by the current assessment based on FOCUS (2001). For the particular situation of the siccation scenario, drift rates based on FOCUS (2001) might not mirror the height of the plants and finaly might underestimate exposure. Therefore, the following uncertainties must be taken into account by MS and the possible underestimation of exposure might be considered:

The height of the sprayed crop has an influence on the drift pattern of a plant protection product to the non-target habitats (e.g. Stallinga, H., J.M.G.P. Michielsen & J.C. van de Zande, 1999. Effect van gewashoogte op de drift bij een bespuiting in een graangewas. Instituut voor Milieu- en Agritechniek, IMAG-DLO Nota P99-71, Wageningen. 1999). The spray driftability is probably increasing the higher the equipment is distant from the ground level and the higher the canopy level is. In general, this issue can be reflected by higher drift deposition values.
A study on standard spray drift deposition curves (van de Zande, J.C., D. Rautmann, H.J. Holterman & J.F.M. Huijsmans (2013): Joined spray drift curves for boom sprayers in The Netherlands and Germany. Draft Report, Plant Research International, Wageningen UR, Business Unit Agrosystems, Report 526, 72pp.) for a reference situation with a bare soil /short crop situation (crop height lower than 20 cm) and a cropped situation reports that separate spray drift curves can be described for the bare soil vs. cropped situation. The difference between the two situations vary –but can be roughly determined to be at least factor 2 higher in the cropped situation than on bare soil. In the case for crop canopy >50cm or special crop heights in vineyards, orchard and hops basic drift deposition values have been modified as well. Using the basic drift deposition values for field crops would probably underestimate risk to non target plants, when application in cereals is performed in BBCH 89 before harvest. Therefore, RMS proposed to mirror the height of the crop and the potential higher drift deposition in order to reduce risk to non-target plants in the off-crop habitat (The exposure scenario “ground crops x 2” was proposed).

However, as recommended by the PPR panel (EFSA Journal 2014;12(7):3800) the current assessment based on FOCUS (2001) is followed, because new drift curves are currently not harmonised. It is acknowledged that new drift values will be implemented in the nearest future. For sake of harmonisation, it was concluded that the present assessment should be based on the current agreed FOCUS values.

RMS highlights the importance of ecotoxicological assessment towards non-target plants for each formulation during product authorisation, not only for the single active substance.

~~For the evaluation of the representative plant protection product MON 52276, further data were generated. It can not be excluded that the formulants in the product enhance toxicity. A valid study assessing effects on non-target plants is required for the plant protection product. The test shall provide the ER_{50} values of the plant protection product to non-target plants.~~

~~It is evident from the available PPP authorization data, that the glyphosate-containing formulated products are typically about a factor of 3 more toxic than the active ingredient itself (confidential data not shown). In order to follow a precautionary principle, in a preliminary risk assessment for non-target plants, the acceptability criterion is modified as follows when assessing glyphosate-based formulated products: TER ≥ 15 instead of TER ≥5.~~

Based on the predicted rates of glyphosate in off-field areas, the TER values describing the risk for non-target plants following exposure to glyphosate indicate acceptable risks providing that the following risk mitigation measures are taken into account:

Table B.2.9-6: Proposed risk mitigation measures for the achievement of an acceptable risk for non-target plants in off-field areas

~~**Intended uses**~~	~~**Application rate (g a.s./ha)**~~	~~**Buffer strip (m) without drift reduction**~~	~~**Buffer strip (m) with x % drift reduction**~~
~~Orchard crops, vine including citrus & tree nuts*~~	~~1 x 2880~~	~~10 m~~	~~1m-90%~~
	~~1 x 2160~~	~~10 m~~	~~1m-90%~~
	~~3 x 1440~~	~~10 m~~	~~1m-90%~~
~~All crops (all seeded and transplanted crops)~~	~~2 × 2160~~	~~trigger not reached~~	~~5m-75%~~
	~~2 × 1440~~	~~trigger not reached~~	~~5m-75%~~
	~~1 × 1440~~	~~10m~~	~~1m-90%~~
	~~1 × 1080~~	~~10m~~	~~1m-90%~~
~~Cereals, Oilseeds (pre-harvest) **~~	~~1 x 2160~~	~~trigger not reached~~	~~5m-90%~~
	~~1 × 1440~~	~~trigger not reached~~	~~5m-75%~~
	~~1 × 1080~~	~~trigger not reached~~	~~5m-75%~~
	~~1x 720 g~~	~~10 m~~	~~1m-90%~~
Intended uses	**Application rate (g a.s./ha)**	**Buffer strip (m) without drift reduction**	**Buffer strip (m) with x % drift reduction**
Orchard crops, vine including citrus & tree nuts*	1 x 2880	10 m	1 m-90%
	1 x 2160	10 m	1 m-90%
	3 x 1440	10 m	1 m-90%
All crops (all seeded and transplanted crops)	2 × 2160	trigger not reached	5m-75%
	2 × 1440	trigger not reached	5m-75%
	1 × 1440	10m	1 m-90%
	1 × 1080	10m	1 m-90%
Cereals, Oilseeds (pre-harvest) ~~**~~	1 x 2160	trigger not reached	5m-75%
	1 × 1440	10m	1 m-90%
	1 × 1080	10m	1 m-90%
	1x 720 g	5m	1 m-75%

***** Because applications are made round base of trunk and to the intra-rows , (inner strips between two trees within a row), application rates per ha are expressed per 'unit of treated surface area' the actual application rate per ha orchard or vineyard will only be 50 % of the reported rate.

** In the case of the intended use on mature crops before harvest, the drift rate to be considered should mirrow the height of the plants. The exposure scenario "ground crops x 2" was chosen.

~~**Intended uses**~~	~~**Application rate (g a.s./ha)**~~	~~**Buffer strip (m) without drift reduction**~~	~~**Buffer strip (m) with 90% drift reduction**~~
~~Orchard crops, vine including citrus & tree nuts*~~	~~1 x 2880~~	~~5 m~~	~~-~~
	~~1 x 2160~~	~~5 m~~	~~-~~
	~~3 x 1440~~	~~5 m~~	~~-~~
~~All crops (all seeded and transplanted crops)~~	~~2 × 2160~~	~~10 m~~	~~-~~
	~~2 × 1440~~	~~10 m~~	~~-~~
	~~1 × 1440~~	~~5 m~~	~~-~~
	~~1 × 1080~~	~~5 m~~	~~-~~
~~Cereals, Oilseeds (pre-harvest) *~~	~~1 x 2160~~	~~trigger not reached~~	~~5m~~
	~~1 × 1440~~	~~10 m~~	~~-~~
	~~1 × 1080~~	~~10 m~~	~~-~~
	~~1x 720 g~~	~~5 m~~	~~-~~

For the intended use 'orchard crops, vine including citrus & tree nuts' the exposure towards the off-field area was reduced to 50 % of the reported rate of the actual application rate per ha, when applications are made round base of trunk and to the intra-rows or inner strips between two trees within a row. With an application rate at 1 x 2880 g a.s./ha, 1 x 2160 g a.s./ha, 3 x 1440 g a.s./ha (applic. rate /treated area), corresponding to an application rate of 50 % based on cropped area, the acceptability criteria are not met. The risk for non-target plants arising from this intended use are considered acceptable only, when including the risk mitigation measure in terms of a 10m buffer strip. If 90 % drift reduction nozzles and a buffer strip of 1 m are used, acceptable risk is achieved. Especially in these cases it appears necessary to protect off-field areas by a fixed distances to protect non target plants and biodiversity. Plant protection products must not be applied to the limit of the in-field areas in the case of strip-application but only inner strips between two trees should be treated. Only a inner strip application combined with the risk mitigation measures buffestrip and drift reducing nozzles can protect the function of the off-field area.

For the intended use 'all crops' for all seeded and transplanted crops with an application rate at 2 x 2160 g a.s./ha, 2 x 1440 g a.s./ha the acceptability criteria are not met. The risk for non-target plants arising from this intended use are considered acceptable including risk mitigation measures including drift reduction and buffer strips. If 75 % drift reduction nozzles and a buffer strip of 5 m are used, acceptable risk is achieved. For the intended uses with an application rate at 1 x 1440 g a.s./ha and 1 x 1080 g a.s./ha risk can be mitigated with 10 m buffer strips.

For the intended use 'pre-harvest' in cereals and oilseeds acceptability criteria are not met without any risk mitigation measures.
For the cereals scenario with an application rate of max. 1 x 2160 g a.s./ha a buffer of 5 m including 75% drift reduction nozzles should be applied in order to protect non target plants when using drift rates based on FOCUS (2001). For further information concerning the uncertainties and possible underestimation of risk in this particular scenario please refer to the introduction of this chapter. If 90 % drift reduction nozzles are used the application rate 1 x 1440 g a.s./ha and 1 x 1080 g a.s./ha pose an acceptable risk The risk for non-target plants arising from this intended use are considered acceptable without drift reduction only for the application of max. 1 x 720 g a.s/ha and a buffer of 5 m.

~~For the intended use 'pre-harvest' in cereals and oilseeds acceptability criteria are not met with one application rate at 1 x 2160 g a.s./ha, 1 x 1440 g a.s./ha, 1 x 1080 g a.s./ha The risk for non-target plants arising from this intended use are considered unacceptable without drift reduction. If 90 % (application rate 1 x 2160 g a.s./ha) or 75 % (application rate 1 x 1440 g a.s./ha, 1 x 1080 g a.s./ha) drift reduction nozzles and a buffer strip of 5 m are used, acceptable risk is achieved. For the cereals scenario with an application rate of max. 1 x 720 g a.s/ha we propose a buffer of 10 m.~~

A late herbicide application before harvest time poses additional ecological risks for surface water, ground water and for non-target species. We refer to Directive 2009/128/EC of the European Parliament and of the Council that establishes a framework for Community actions to achieve a more sustainable use of pesticides (Plant Protection Framework Directive). Directive 2009/128/EC commit Member States to adopt national action plans (NAP) for sustainable use of plant protection products in Article 4. The aims are to further reduce the risks and impacts to human health and the natural environment associated with the use of pesticides and to limit the application of pesticides to the necessary degree. Member States might consider the appropriateness of additional herbicide uses, especially when addressing sustainable use of plant protection products according to Directive 2009/128/EC.

The protection aims according to Regulation EC 1107/2009 – the absence of unacceptable effects on biodiverstity and the ecosystem – do not explicitly differentiate between in- and off-field habitats. Considering the flora species as an integral part of the biodiversity of non-target species and the strong evidence that the use of herbicides including glyphosate contributes to the ongoing loss of biodiversity in farmland invertebrate and vertebrate (especially farmland bird) species additional risk mitigation measures might be regarded at member state level depending on the agricultural and landscape conditions.

2.9.7 Summary of effects on biological methods for sewage treatment

Measurements of the oxygen consumption of glyphosate acid in activated sludge resulted in EC_{50}-values of > 100 mg/L. Glyphosate acid did not exhibit any significant symptoms up to the highest test concentrations. Because glyphosate acid has shown a low bactericidal activity a risk to biological sewage treatment is not expected.

2.9.8 Summary of product exposure and risk assessment

The risk assessment for birds and mammals is based on the active ingredient glyphosate acid in the product MON 52276 for the intended uses in several crops including pre-planting, pre-emergence, pre-harvest and row-application in orchards and vineyards.

The results of the assessment indicate an acceptable acute risk for birds due to uptake of contaminated food after use of MON 52276 in the indications according to the label. Based on refined assessment step for the long-term risk (decline of glyphosate on grass), the calculated TER values for the long-term risk resulting from an exposure of birds to glyphosate also achieved the acceptability criteria TER ≥ 5.

The calculated TER values for the acute risk assessment resulting from an exposure of small herbivorous mammals to glyphosate do not achieve the acceptability criteria TER ≥ 10 with the application of 2160 g a.s./ha for the grassland scenario. Risk can be mitigated with

application of max.1440 g a.s./ha indicating an acceptable risk for small herbivorous mammals.

For the serial application of 2 × max. 720 g a.s./ha and 1 × max. 2880 ga.s./ha the results indicate a low risk for small herbivorous mammals due to uptake of contaminated food. However, in orchards, as glyphosate is not applied to the grassy alleys between the tree lines (or the foliage of the trees) there might be other non contaminated vegetation for herbivores to graze. Moreover the LD_{50} > 2000 mg/kg/day represents a worse case assumption for the use in acute oral wildlife risk assessment.

The calculated TER values for the long-term risk resulting from an exposure of small herbivorous mammals to glyphosate do not achieve the acceptability criteria TER ≥ 5 with all application rates for the grassland scenario and with the maximum application rates for the post emergence treatment of weeds in orchard crops. For the use in orchard crops risk can be mitigated with a serial application of 3 × max.1440 g a.s./ha indicating an acceptable risk for small herbivorous mammals.

Comparison of calculated TER values with the respective acceptability criteria shows that the risk for aquatic organisms is acceptable.

The calculated HQ values show an acceptbale risk for non-target arthropods due to the intended use of the lead formulation MON 52276 according to the label.

During the revision of the active substance glyphosate for the renewal of Annex I inclusion (AIR2), the Rapporteur Member State (RMS) Germany requested to submit a new N-transformation study following the OECD guideline 116, with the active substance glyphosate acid. MON 77973 (96.59% Glyphosate Acid), when applied at 6.62 and 33.1 mg a.e./kg dry soil, is not expected to cause significant long-term effects on the soil nitrogen transformation process at concentrations ≤ 33.1mg a.e./kg dry soil.

During the revision of the active substance glyphosate for the renewal of Annex I inclusion (AIR2), the Rapporteur Member State (RMS) requested to submit a new vegetative vigor study following the OECD guideline 227, with the representative formulation MON 52276. Uncertainties remain concerning the acceptability oft the study. RMS can not exclude the possibility that the sensitivity of the species is underestimated, as recommendatiosn of the guidelines have not been fully considered. Nevertheless, this study displays the dataset for the representative formulation and therefore includes information about the relevance of the formulants. In general, toxicity studies with the commercial product are more appropriate than studies with the active ingredient only for the assessment of the effects on non-target plants.

In conclusion, RMS highlights the importance of toxicological assessment towards non target plants not only of the single active substance, but also of the different formulations during product authorization.

Depending on the agricultural and ecological conditions, the use of gyphosate and other broad spectrum herbicides may affect the populations of non-target terrestrial arthropod and vertebrate (especially farmland bird) species via trophic interactions. These effects trace back to the intended effect of the herbicides – eliminating competing plants – and the interruption of the food web going along with it.

2.10 Classification and labelling

2.10.1 Proposals for the classification and labelling of the active substance

The only proposed classification and labelling of the active substance glyphosate (acid) is for eye irritating properties. A respective justification is given in section 2.6.2.

According to Directives 67/548/EEC, glyphosate acid is to be labelled and classified with **Xi ('Irritant')** and **R41 ('Risk of serious damage to eyes'),** *i.e.*, the current classification should be maintained.

Corresponding to the GHS criteria, the appropriate classification and labelling **is 'Irreversible effects on the eye / serious damage to eyes (Category 1)´, H318**.

Substance	Species	Test design	Toxicity (mg /L)
Glyphosate acid	*Oncorhynchus mykiss*	acute	38.0
	Brachydanio rerio	Long-term	1
	Daphnia magna	acute	40.0
		Long-term	12.5
	Skeletonema costatum	acute	18
		Long-term	1.82
	Lemna gibba	acute	12.0
		Long-term	3.0
Biodegradability:	Classified as not readily biodegradable (see 2.8.2.3)		

Based on Commission Regulation 790/2009 (amending EC regulation 1272/2008 (CLP))

Hazard Symbol(s):	GHS09	
Classification:	Chronic 2,	
Signal word(s):	none	
Hazard statement:	H411	Toxic to aquatic life with long lasting effects
Precautionary statement:	P273, P391 P501	

2.10.2 Proposals for the classification and labelling of preparations (Annex IIIA 11.3 and 11.4)

For the preparation MON 52276 the following classification/labelling is proposed in accordance with Directives 67/548/EEC and 1999/45/EC:

None

According to the criteria given in Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008, the following classification/labelling for toxicological hazards of the preparation is proposed:

Based on Commission Regulation 790/2009 (amending EC regulation 1272/2008 (CLP))

Hazard Symbol(s):	GHS09	
Classification:	Chronic 2,	
Signal word(s):	none	
Hazard statement:	H411	Toxic to aquatic life with long lasting effects
Precautionary statement:	P273, P391 P501	

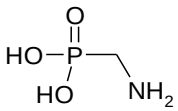
‘15.8 percent of the mixture consist of ingredients of unknown inhalation toxicity.’

2.11 Relevance of metabolites in groundwater

2.11.1 STEP 1: Exclusion of degradation products of no concern

The following metabolite was identified in soil degradation studies as potentially relevant for groundwater, due to the exceedance of the pertinent trigger values on formation in soil.

Table 2.11-1: Metabolites detected in soil degradation studies which fulfil the criteria according to SANCO/221/2000- rev.10-final (2003)

Metabolite	Stucture/ molecular formula	Molecular weight (g/mol)	maximal occurrence in soil (%) after n days (reference)
AMPA Aminomethylphosphonsäure	 CH_6NO_3P	111.04	53.8 % after 271 days (Schulz 1992, BVL no 1932133 / trial Menslage)

This chemical structure does not fulfil the criteria for being excluded as a degradation product of no concern (inorganic compound not containing a heavy metal; short aliphatic chain without alerting chemical moieties; non-toxic natural product). Hence, this metabolite has to be considered further in Step 2 of the tiered relevance assessment.

2.11.2 STEP 2: Quantification of potential groundwater contamination

Based on $FOCUS_{GW}$ calculations with the relevant input parameters, a potential to exceed groundwater concentrations of 0.1 µg/L was not identified for the metabolite AMPA, therefore it has not to be considered further in Step 3 of the tiered relevance assessment.

2.11.3 STEP 3: Hazard assessment – identification of relevant metabolites

2.11.3.1 STEP 3, Stage 1: screening for biological activity

-/-

2.11.3.2 STEP 3, Stage 2: screening for genotoxicity

-/-

2.11.3.3 STEP 3, Stage 3: screening for toxicity

-/-

2.11.4 STEP 4: Exposure assessment – threshold of concern approach

-/-

2.11.5 STEP 5: Refined risk assessment

-/-

2.11.6 Overall conclusion

In the PEC_{GW} simulation with FOCUS_PELMO 4.4.3 the metabolite AMPA does not show a potential to exceed groundwater concentrations of 0.1 µg/L. Therefore no further consideration of metabolite AMPA is needed in the relevance assessment of groundwater metabolites.

2.12 Consideration of isomeric composition in the risk assessment

2.12.1 Identity and physical chemical properties

Such consideration is not needed. Different isomers of glyphosate do not exist.

2.12.2 Methods of analysis

Such consideration is not needed. Different isomers of glyphosate and N-acetylglyphosate do not exist.

2.12.3 Mammalian toxicity

Glyphosate is not relevant concerning its isomeric composition.

2.12.4 Operator and Worker exposure

Glyphosate is not relevant concerning its isomeric composition.

2.12.5 Residues and Consumer risk assessment

Glyphosate and its metabolites are not relevant concerning their isomeric composition.

2.12.6 Environmental fate

The common name glyphosate designates an isomeric pure compound. Hence, data to assess a possible different environmental fate of stable glyphosate isomers are not required and were not submitted.

2.12.7 Ecotoxicology

The common name glyphosate designates an isomeric pure compound. Hence, data to assess a possible different environmental fate of stable glyphosate isomers are not required and were not submitted.

2.13 Residue definitions

2.13.1 Definition of residues for exposure/risk assessment

Soil
Glyphosate and its metabolite AMPA are potentially relevant compounds due to their occurrence at significant levels > 10 % in soil degradation studies.

The ecotoxicity of AMPA to soil organisms is comparable to the toxicity of the parent compound.

Surface water and sediment
Glyphosate and its metabolites AMPA and HMPA are potentially relevant compounds for further risk assessment in surface water, following the criteria from the Guidance Document on Aquatic Ecotoxicology (2002) due to their occurrence at significant levels in water/ sediment studies. The metabolite AMPA was detected in water phase and sediment phase with > 10 %, whereas the metabolite HMPA was detected only in water phase with 10 %.

Regarding surface water monitoring across Europe, glyphosate has been analyzed in almost 75000 surface water samples from about 4000 sites (from 1993-2011) and was detected in 33 % of samples, with 23 % above 0.1 µg/L. The maximum concentrations of glyphosate acid found in surface water reached from 1.3 to 370 µg/L. The highest glyphosate values in surface water were detected in Sweden (370 µg/L), Ireland (186 µg/L) and Belgium (139 µg/L). The main metabolite AMPA has been analysed in about 56700 samples from nearly 3000 sites (1997-2011) and was detected in 54 % of samples, with 46 % above 0.1 µg/L and maximum concentrations reaching from 0.22 to > 200 µg/L (Horth, 2012, BVL no 2310291).

The toxicity of AMPA and HMPA to aquatic organisms is lower than the toxicity of the parent. These metabolites are not ecotoxicologically relevant for the aquatic environment.

Groundwater
Glyphosate and its metabolite AMPA are potentially relevant compounds due to their occurrence at significant levels > 10 % in soil degradation studies. For both compounds the modelled concentration in groundwater did not exceed the trigger of 0.1 µg/L.
Regarding groundwater monitoring across Europe (Horth, 2012, BVL no 2310291), glyphosate and AMPA have been increasingly analysed and occasionally detected. Glyphosate has been analyzed in 66662 samples from about 675 sites (1993-2010) and detected in 1 % of samples, with 0.64 % above 0.1 µg/L; AMPA has been analyzed in 51652 samples from 1345 sites (1993 - 2011) and detected in 2.6 % of samples, with 0.77 % above 0.1 µg/L. The highest numbers of glyphosate detections have been reported from Denmark (4.7 µg/L) and France (24 µg/L). Findings exceeding the limit concentration 0.1 µg/l have also been measured in groundwater aquifers in Austria, Ireland, The Netherlands and the UK. Detailed groundwater monitoring studies demonstrating that glyphosate (at least partly) exceeded 0.1 µg/l are available from Italy (Calliera et al., 2011, BVL no 2310280), Germany

(Schmidt and Reichert, 2006, BVL no 2310282), The Netherlands (Franke et al., 2010, BVL no 2310284), Sweden (Carter and Pepper, 2005, BVL no 2310285), France (Anonymous, 2012, BVL no 2310289) and Spain (Sanchís et al., 2012, BVL no 2537361).

The toxicity of AMPA to aquatic organisms is lower than the toxicity of the parent. AMPA is not ecotoxicologically relevant for the groundwater compartment.

Plant and animal commodities
Sum of glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA, all expressed as glyphosate equivalents.

2.13.2 Definition of residues for monitoring

Soil
Parent glyphosate, AMPA.

Surface water and sediment
Parent glyphosate, AMPA.

AMPA is proposed as relevant residue for monitoring following precautionary principles resulting from the frequent detections in surface waters and the widespread intended uses of glyphosate in almost all crops.

Groundwater
Parent glyphosate, AMPA.

AMPA is proposed as relevant residue for monitoring following precautionary principles resulting from the frequent detections in groundwater and surface waters, the possible groundwater contamination path via bank filtration and the widespread intended uses of glyphosate in almost all crops..

Air
No criteria for definition of a relevant residue in air are available. By default, the relevant residue for the air compartment is the active substance glyphosate.

Plant and animal commodities

Residue definition for monitoring purposes in sweet corn, lentils, oilseeds rape, soya beans and maize (non-tolerant and tolerant, all modifications):
Sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate equivalents

Residue definition for monitoring purposes in other plant commodities:
Glyphosate

Residue definition for monitoring purposes in animal commodities:
Sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate

Level 3

Glyphosate

SUMMARY AND CONSIDERATION WITH RESPECT TO THE APPROVAL CRITERIA OF REGULATION (EC) No 1107/2009

IDENTIFICATION OF DATA GAPS, PROPOSED CONDITIONS, RISK MANAGEMENT MEASURES, ISSUES THAT COULD NOT BE FINALISED AND CRITICAL AREAS OF CONCERN

PROPOSED DECISION

Proposal on acceptability against the approval criteria

3 Proposed decision with respect to the application

3.1 Background to the proposed decision

3.1.1 Proposal on acceptability against the approval criteria – Article 4 and Annex II of Regulation (EC) No 1107/2009

3.1.1.1 Article 4				
		Yes	No	
i)	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.	X		See Level 2 above
3.1.1.2 Submission of further information				
		Yes	No	
i)	It is considered that a complete dossier has been submitted		X	
ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because: (a) the data requirements have been amended or refined after the submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.	X		~~The assessment of the relevance of certain impurityies in the technical material and/or specification needs to be clarified. The requested information is considered to be confirmatory in nature.~~ The submission of further information on analytical methods of residues is required in order to get a complete data base to enable an evaluation according to EU Guidance Document SANCO/825/00 rev. 8.1, 16/11/2010. The submission of further information on toxicology is required to increase confidence in the decision. The submission of further information on residues is required for formal reasons in order to use all available data for an

Proposal on acceptability against the approval criteria

				appropriate risk assessment. The submission of a study assessing the effects of the active substance towards microorganisms and a study with the representative plant protection product MON 52276 assessing the effects on non-target plants is considered to be confirmatory in nature, as required to increase confidence in the decision.
3.1.1.3 Restrictions on approval				
		Yes	No	
	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.	X		Minimum degree of purity of the active substance: ≥ 950 g/kg
3.1.1.4 Criteria for the approval of an active substance				
Dossier				
		Yes	No	
	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).	X		Sufficient data was submitted to establish the ADI and the AOEL. ~~An ARfD is not considered necessary by the RMS. If, however, EFSA or individual MS would wish to set an ARfD and provide convincing arguments that such a reference dose was in fact needed, the existing huge database will certainly allow to derive an appropriate figure. No further data would have to be generated for this purpose.~~ In the Pesticides Peer Review 125 expert meeting (February 2015) MS concluded to derive an ARfD. The data submitted was considered sufficient.
	It is considered that the dossier contains the information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on feed or	X		Sufficient data to derive residue definition in plant and animal commodities. Representative uses supported with sufficient supervised field trial data. No dietary intake concern. For desiccation of oilseeds (linseed, rapeseed, mustard seed)

Proposal on acceptability against the approval criteria

	food crops or leads indirectly to residues in food or feed). In particular it is considered that the dossier: (a) permits any residue of concern to be defined; (b) reliably predicts the residues in food and feed, including succeeding crops (c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.			supervised field trial data were not sufficient for an evaluation.
	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.	X		
Efficacy				
		Yes	No	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	X		Glyphosate is highly effective against the majority of annual and perennial mono- and dicotyledonous weeds. Herbicides containing glyphosate are used in agriculture as foliar sprays, at post-emergence of weeds in a wide range of arable crops (seed-ed and transplanted) to control a broad spectrum of weeds. The renewal submission only involves a selection of representative crop-related uses covering the majority of the volumes applied. Uses in the representative GAP include applications at pre-

Proposal on acceptability against the approval criteria

				planting, post-planting but pre-emergence of crops and post-harvesting of all crops. Pre-harvest uses in cereals, oilseeds and pulses are for desiccation and annual and perennial weed control. Other uses include annual and perennial weed control in orchard crops and vines including olives, citrus fruits and nuts and for grassland renovation. Herbicides containing glyphosate are used at different rates in the EU in agriculture, horticulture, viticulture, forestry, orchards, plantation crops, amenities, home gardening, greenhouses, on aquatic areas, on hard surface areas, on railways, along roads, and on non-cultivated areas. According to the listed uses the application rate ranged from 0.72 to 2.88 kg as/ha. The application rate varies across the EU depending on the type and time of application and also on the weed species which are present on the treated area, weed growth stages or the crops which should be managed. Because of the uptake through the leaves, the best efficacy can be achieved if the application is on well developed foliage and especially for perennial weeds, in a period with sugar translocation to roots or other underground parts. One application per growing season is normally used and the weed plants treated at the recommended rate will not start growing again. Symptoms will be seen after 10 to 14 days after application.

Proposal on acceptability against the approval criteria

Relevance of metabolites				
		Yes	No	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.	X		Refer to Volume 3, chapters B.6, B.8 and B.9.
Composition				
		Yes	No	
	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.	X		Refer to Volume 1, Level 2 and Volume 4.
	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.	X		~~Open~~
	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted			
Methods of analysis				
		Yes	No	
	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly	X		Sufficent analytical methods are available.

Proposal on acceptability against the approval criteria

	calibrated, accurate and precise.			
	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.		X	The following data gaps are identified: 1. A confirmatory method for glyphosate in animal fat and kidney/liver. 2. A confirmatory method for N-acetyl-glyphosate in dry plant materials and those with high water and high fat content. 3. A confirmatory method for N-acetyl-glyphosate in all kinds of animal matrices. 4. A confirmatory method for glyphosate and AMPA is soil
	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		

Proposal on acceptability against the approval criteria

Impact on human health				
Impact on human health - ADI, AOEL, ARfD				
		Yes	No	
	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.	X		Both the ADI and AOEL were derived from the overall NOAEL for maternal and developmental toxicity in the rabbit that was set at 50 mg/kg bw/day. The (pregnant) rabbit proved more vulnerable than other species. This NOAEL is lower than those obtained in the long-term studies in rodents or in short-term studies in rodents or dogs. When the usual assessment factor of 100 is applied, the resulting **ADI is 0.5 mg/kg bw**. For AOEL setting, the low oral absorption of approximately 20% must be taken into account for correction. Thus, a **systemic AOEL of 0.1 mg/kg bw/day** is proposed. ~~An ARfD for glyphosate is not warranted because this substance is of low acute toxicity and because there is no evidence that a single dose would be sufficient to induce toxic effects that were observed in studies with repeated administration.~~ In the Pesticides Peer Review 125 expert meeting (February 2015) the original proposal of the RMS not to establish an ARfD was not confirmed. MS experts were concerned about the maternal toxicity, including mortality, in the developmental toxicity studies in the rabbit. Post-implantation losses were observed in two of the 7 studies in rabbits. In one an increase in post-implantation losses was seen at the mid and high dose levels of 200 and 400 mg/kg bw/day with an NOAEL of 50 mg/kg bw/day. This dose had been used to establish an overall NOAEL for developmental toxicity in the rabbit and was considered the most appropriate

Proposal on acceptability against the approval criteria

				basis to derive the ARfD. Using the safety factor of 100, an **ARfD of 0.5 mg/kg bw** was established.
Impact on human health – proposed genotoxicity classification				
		Yes	No	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as mutagen category 1A or 1B.**		X	No, glyphosate is devoid of a genotoxic potential (see 2.6.4).
Impact on human health – proposed carcinogenicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogen category 1A or 1B.**		X	No, classification and labelling for carcinogenicity is not warranted. This is based on a large number of long-term studies in rats did not reveal any evidence of carcinogenicity. In the mouse, a higher incidence of malignant lymphoma was observed in one out of five carcinogencity studies at an exaggerated dose level in a strain with high background incidence of this tumour type. See section 2.6.5 for justification that classification is not needed. Epidemiological studies in the whole did not provide evidence of carcinogenicity in man.

Proposal on acceptability against the approval criteria

ii)	Linked to above classification proposal. As became evident from exposure estimations exposure of humans towards the active substance, safener or synergist in MON 52276, under realistic proposed conditions of use, is not negligible irrespective of which toxicological endpoint is concerned.			
Impact on human health – proposed reproductive toxicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 1A or 1B.**		X	In various two-generation studies in rats, there was no impact on fertility or reproductive performance (see 2.6.6). No classification and labelling for this endpoint is needed.
ii)	Linked to above classification proposal. As became evident from exposure estimations exposure of humans towards the active substance, safener or synergist in MON 52276, under realistic proposed conditions of use, is not negligible irrespective of which toxicological endpoint is concerned.			

Proposal on acceptability against the approval criteria

Impact on human health – proposed endocrine disrupting properties classification				
		Yes	No	
i)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **carcinogenic category 2 and toxic for reproduction category 2 and on that basis shall be considered to have endocrine disrupting properties**		X	See above. No classification for carcinogenicity or reproductive toxicity is proposed.
ii)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **toxic for reproduction category 2 and** in addition the RMS considers the substance **has toxic effects on the endocrine organs and on that basis shall be considered to have endocrine disrupting properties**		X	No toxic effects on hormone producing or directly horomone-dependant organs have been noted in the huge number of toxicological studies.
iii)	Linked to either i) or ii) immediately above. As became evident from exposure estimations exposure of humans towards the active substance, safener or synergist in MON 52276, under realistic proposed conditions of use, is not negligible irrespective of which toxicological endpoint is concerned.			

Proposal on acceptability against the approval criteria

Fate and behaviour in the environment				
Persistent organic pollutant (POP)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.		X	On consideration of all available data on the characteristic of glyphosate, the substance does not fulfil the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1. In particular, glyphosate fulfils the criteria for persistence[1)], but neither the criteria for for long-range transport potential[2)] nor for bioaccumulation[3)]. 1) On the basis of the maximum DT_{50} value in water/sediment studies (total system) of 301.2 days glyphosate fulfils the P criterion of POP and exceeds the trigger of both, water phase (60 days) and sediment phase (180 days). This approach represents a conservative assessment. Using the geometric mean DT_{50} value (total system) of 74.5 days (range DT_{50}: 13.8 - > 301.2 days) from five water/sediment systems the trigger value of 60 days for water would be exceeded, whereas the trigger of 180 days for sediment would not be exceeded. Because of the very rapid adsorption of glyphosate to the sediment phase the total geomean DT_{50} value of 74.5 days would be compared to the sediment trigger of 180 days. Therefore glyphosate would not fulfil the P criterion of POP on the basis of the geomean DT_{50} value. The maximum $DegT_{50}$ of un-normalised field dissipation studies of 116.4 days does not exceed the trigger value of 180 days for soil. The P-criterion is not fulfilled.

				In soil laboratory degradation studies, the maximum DT_{50} value of ~~133.8~~ 427.8 days would ~~not~~ exceed the trigger value of 180 days for soil. Therefore, glyphosate would ~~not~~ fulfil the P-criterion of POP. This approach represents a conservative assessment. Using the geometric mean DT_{50} value of ~~18.7~~ 19.7 days (range 3.6 - ~~133.8~~ 427.8 days, n = ~~17~~ 15) the trigger value of 180 days for soil would not be exceeded. The P criterion would also not be fulfilled. 2) The low vapour pressure of 1.31×10^{-5} Pa (25°C) and the calculated atmospheric half-life < 2 d indicate a low potential of glyphosate for long-range transport via air. 3) With a log $P_{o/w}$ < -3.2 glyphosate is not a lipophilic compound. No testing on bioconcentration in fish is legally required. but a bioconcentration study has been conducted with different aquatic organsism which achieved a bioconcentration factor max. 10, which is far below the Annex VI BCF trigger value of 1000. Bioaccumulation of glyphosate due to bioconcentration in fat tissues or exceedance of the BCF trigger value of 5000 are unlikely.
Persistent, bioaccumulative and toxic substance (PBT)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.		X	On consideration of all available data on the characteristic of glyphosate, the substance does not fulfil the criteria of a persistent, bioaccumulative and toxic (PBT) as laid out in Regulation 1107/2009 Annex II Section 3.7.2. In particular, glyphosate fulfils the criteria for persistence[1)], but neither the criteria for bioaccumulation[2)] nor for (eco)toxicity[3)]. 1) On the basis of the maximum DT_{50} value in water/sediment studies (total system) of 301.2 days glyphosate fulfils the P criterion of PBT and exceeds the trigger of both, water and

Proposal on acceptability against the approval criteria

				sediment phase. This approach represents a conservative assessment. Using the geometric mean DT_{50} value (total system) of 74.5 days (range DT_{50}: 13.8 - > 301.2 days) from five water/sediment systems the trigger value of 40 days for water would be exceeded and the trigger of 120 days for sediment would not be exceeded. Because of the very rapid adsorption of glyphosate to the sediment phase the total geomean DT_{50} value of 74.5 days would be compared to the sediment trigger of 120 days. Therefore, glyphosate would not fulfil the P criterion of PBT on the basis of the geomean DT_{50} value. The maximum $DegT_{50}$ of un-normalised field dissipation studies of 116.4 days does not exceed the trigger value of 120 days for soil. The P-criterion is not fulfilled in soil. In soil laboratory degradation studies the maximum DT_{50} value of ~~133.8~~ 427.8 days would exceed the trigger value of 120 days for soil. Therefore, glyphosate would fulfil the P-criterion of PBT. This approach would represent a conservative assessment. Using the geometric mean DT_{50} value of ~~18.7~~ 19.7 days (range 3.6 - ~~133.8~~ 427.8 days, n = ~~17~~ 15) the trigger value of 120 days for soil would not be exceeded. The P criterion would not be fulfilled. 2) With a log $P_{o/w}$ < -3.2 glyphosate is not a lipophilic compound. No testing on bioconcentration in fish is legally required. but a bioconcentration study has been conducted with different aquatic organsism which achieved a bioconcentration factor max. 10, which is far below the Annex VI BCF trigger value of 1000. Bioaccumulation of glyphosate due to bioconcentration in fat tissues or

Proposal on acceptability against the approval criteria

				exceedance of the BCF trigger value of 5000 are unlikely. 3) The lowest available NOEC for aquatic organisms amounts to 1.0 mg/L and is thus above the pertinent trigger value of 0.01 mg/L.
Very persistent and very bioaccumulative substance (vPvB).				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a a very persistent and very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.		X	On consideration of all available data on the characteristic of glyphosate, the substance does not fulfil the criteria of a very persistent and very bioaccumulative (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3. In particular, glyphosate fulfils the criteria for persistence[1)], but not the criteria for bioaccumulation[2)]. 1) On the basis of the maximum DT_{50} value in water/sediment studies (total system) of 301.2 days glyphosate fulfils the P criterion of vPvB and exceeds the trigger of both, water (60 days) and sediment phase (180 days). This approach represents the conservative assessment. Using the geometric mean DT_{50} value (total system) of 74.5 days (range DT_{50}: 13.8 - > 301.2 days) from five water sediment systems the trigger value of 60 days for water would be exceeded, whereas the trigger of 180 days for sediment would not be exceeded. Because of the very rapid adsorption of glyphosate to the sediment phase the total geomean DT_{50} value of 74.5 days would be compared to the sediment trigger of 180 days. Therefore glyphosate would not fulfil the P criterion of vPvB on the basis of geomean DT_{50} value. The maximum $DegT_{50}$ of un-normalised field dissipation studies of 116.4 days does not exceed the trigger value of 180 days for soil. The P-criterion is not fulfilled.

				In soil laboratory degradation studies the maximum DT_{50} value of ~~133.8~~ 427.8 days would ~~not~~ exceed the trigger value of 180 days for soil. Therefore, glyphosate would ~~not~~ fulfil the P-criterion of vPvB. This approach represents the conservative assessment. Using the geometric mean DT_{50} value of ~~18.7~~ 19.7 days (range 3.6 - ~~133.8~~ 427.8 days, n = = ~~17~~ 15) the trigger value of 180 days for soil would not be exceeded. The P criterion would also not be fulfilled. 2) With a log $P_{o/w}$ < -3.2 glyphosate is not a lipophilic compound. No testing on bioconcentration in fish is legally required. but a bioconcentration study has been conducted with different aquatic organsism which achieved a bioconcentration factor max. 10, which is far below the Annex VI BCF trigger value of 1000. Bioaccumulation of glyphosate due to bioconcentration in fat tissues or exceedance of the BCF trigger value of 5000 are unlikely.
Ecotoxicology				
		Yes	No	
	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.	X		*Birds* The calculated TER values for the acute and long-term risk resulting from an exposure of birds to glyphosate reach the acceptability criteria TER ≥ 10 and TER ≥ 5 for acute and long-term effects, repectively. The results of the assessment indicate an acceptable risk for birds due to uptake of contaminated food in the representative uses/use scenarios. *Mammals* The calculated TER values for the acute and long-term risk resulting from an exposure of mammals to reach the accep-

				tability criteria TER ≥ 10 and TER ≥5 for acute and long-term effects, repectively for the following representative uses/use scenarios: - " all crops", - "crop maturity in cereals" - "crop maturity in oilseed rape". For use in orchard crops (vines including citrus & tree nuts, intrarow & spot treatment) the calculated TER values for the long-term risk resulting from an exposure of mammals to glyphosate do not achieve the acceptability criteria TER ≥ 5 with the maximum application rates. Nevertheless, risk can be mitigated with a serial application of 3 times 1440 g a.s./ha. For use in grassland the calculated TER values for the long-term risk resulting from an exposure of mammals to glyphosate do not achieve the acceptability criteria TER ≥ 5 with all application rates. *Aquatic organisms* The risk for aquatic organisms is acceptable in the representative uses/use scenarios. *Arthropods* With regard to the endpoints from the extended laboratory tests regarding lethal and sublethal effects and the predicted rates of glyphosate in-field as well as off-field, all calculated HQ values remain below the acceptability criterion. The calculated HQ values show an acceptable risk for non-target arthropods due to the intended use of the lead formulation MON 52276 in the representative uses/use scenarios.

Proposal on acceptability against the approval criteria

				Soil macro and mesofauna TER values were calculated for glyphosate and the metabolite AMPA for a worst-case scenario with a max.annual application rate of 4.32 kg/ha glyphosate. The results of the assessment indicate an acceptable acute and long-term risk for earthworms and soil macro-organisms other than earthworms due to the representative uses/use scenarios. *Soil microbes* MON 77973 (96.59% Glyphosate Acid), when applied at 6.62 and 33.1 mg a.e./kg dry soil, is not expected to cause significant long-term effects on the soil nitrogen transformation process at concentrations ≤ 33.1mg a.e./kg dry soil.Based on laboratory testing with MON 52276, the Annex VI trigger value of > 25% effects after 28 days was not exceeded at concentrations of 1× and 5× the max. recommended annual use rate for 4.32 kg a.s./ha. Therefore, the use of MON 52276 according to the proposed use pattern can be considered not to result in any unacceptable adverse effects for soil micro-organisms. *Non target plants* Based on the predicted rates of glyphosate in off-field areas, the TER values describing the risk for non-target plants following exposure to glyphosate indicate acceptable risks providing that the following risk mitigation measures are proposed:

Proposal on acceptability against the approval criteria

Intended uses	Application rate (g a.s./ha)	Buffer strip (m) without drift reduction	Buffer strip (m) with x % drift reduction
Orchard crops, vine including citrus and tree nuts	1 x 2880	10 m	1m-90%
	1 x 2160	10 m	1m-90%
	3 x 1440	10 m	1m-90%
All crops (all seeded and transplanted crops	2 × 2160	trigger not reached	5m-75%
	2 × 1440	trigger not reached	5m-75%
	1 × 1440	10m	1m-90%
	1 × 1080	10m	1m-90%
Cereals, Oilseeds (pre-harvest)	1 x 2160	trigger not reached	5m-~~90~~ 75%
	1 × 1440	~~trigger not reached~~ 10 m	1m-90% ~~5m-75%~~
	1 × 1080	~~trigger not reached~~ 10 m	1m-90% ~~5m-75%~~
	1 x 720	~~10~~ 5 m	1m-~~90~~ 75%

For several intended uses the acceptability criteria is not met. The risk for non-target plants arising from these intended uses are considered unacceptable without drift reduction.

Sewage treatment

Measurements of the oxygen consumption of glyphosate acid in activated sludge resulted in EC_{50}-values of > 100 mg/L. Glyphosate acid did not exhibit any significant symptoms up to the highest test concentrations. Because glyphosate acid has shown a low bactericidal activity a risk to biological sewage treatment is not expected.

Proposal on acceptability against the approval criteria

	It is considered that, on the basis of the assessment of Community or internationally agreed test guidelines, the substance **HAS** endocrine disrupting properties that may cause adverse effects on non-target organisms.		X	
	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.			N/A
	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or synergist: — will result in a negligible exposure of honeybees, or — has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.	X		Risk assessment for other pollinators than bees (e.g. bumblee bees or solitary bees) will be performed as soon as the agreed guidance for the assessment is available on EU level.
Residue definition				
		Yes	No	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.	X		Definition of the residue for plant commodities Residue definition for enforcement purposes in sweet corn, lentils, oilseeds rape, soya beans and maize (non-tolerant and tolerant, all modifications): sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate

Proposal on acceptability against the approval criteria

				Residue definition for enforcement purposes in other plant commodities: glyphosate Residue definition for dietary intake purposes in plant commodities: sum of glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA, all expressed as glyphosate equivalents (For the future generation of residue data N-acetly-glyphosate and N-acetyl-AMPA are only mandatory analytes in GAT-modified crops.) Definition of the residue for animal commodities Residue definition for enforcement purposes in animal commodities: sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate Residue definition for dietary intake purposes in animal commodities: sum of glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA, all expressed as glyphosate equivalents (For the calculation of the maximum dietary burden for the purpose of MRL setting, only glyphosate and N-acetyl-glyphosate need to considered, since the reformation of both analytes from AMPA or N-acetyl-AMPA is unlikely).

Proposal on acceptability against the approval criteria

				Definition of residues for monitoring *Soil* Glyphosate, AMPA *Surface water and sediment* Glyphosate, AMPA, HMPA *Groundwater* Glyphosate, AMPA *Air* Not defined.
Fate and behaviour concerning groundwater				
		Yes	No	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		*Glyphosate* PEC_{GW} < 0.1 µg/L in all nine FOCUS scenarios - The risk for groundwater is acceptable with no limitations for all intended uses. *AMPA* PEC_{GW} < 0.1 µg/L in in all nine FOCUS scenarios - The risk for groundwater is acceptable with no limitations for all intended uses. *Overall conclusion* The risk for groundwater from the application of glyphosate is acceptable with no limitations for all intended uses.

Proposal - candidate for substitution

3.1.2 Proposal - Candidate for substitution

Candidate for substitution				
		Yes	No	
	It is considered that the active substance shall be approved as a candidate for substitution		X	On consideration of all available data on the characteristic of glyphosate, the active substance does not fulfil two of the three criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2. In particular, glyphosate does fulfil the criteria for persistence, but neither for bioaccumulation nor for (eco)toxicity.

3.1.3 Proposal – Low risk active substance

Low-risk active substances				
		Yes	No	
	It is considered that the active substance **shall be considered of low risk.** In particular it is considered that the substance **should NOT be classified or proposed for classification** in accordance with Regulation (EC) No 1272/2008 as at least one of the following: — carcinogenic, — mutagenic, — toxic to reproduction, — sensitising chemicals, — very toxic or toxic, — explosive, — corrosive. In addition it is considered that **the substance is NOT**: — persistent (half-life in soil more than 60 days), — has a bioconcentration factor higher than 100, — is deemed to be an endocrine disrupter, or — has neurotoxic or immunotoxic effects.		X	According to CLP, corrosive effects and severe eye damage are no longer distinguished. Glyphosate has to be classified and labelled for severe eye irritation/damage (Cat. 1, H318). Therefore, it cannot be considered to be of low risk. Glyphosate is considered persistent in soil. The maximum DT_{50} value of unnormalised field dissipation studies of 116.4 days exceeds the trigger value of 60 days for soil.

3.1.4 List of studies to be generated, still ongoing or available but not evaluated

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
3.1.4.1 Identity of the active substance or formulation				
3.1.4.2 Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				
~~Spectra of relevant impurities~~	~~Not relevant~~			
3.1.4.3 Data on uses and efficacy				
3.1.4.4 Data on handling, storage, transport, packaging and labelling				
3.1.4.5 Methods of analysis				
A confirmatory method for glyphosate in animal fat and kidney/liver.	Relevant, basic data set for all active substances!			

Level 3 – List of studies to be generated, still ongoing or available but not evaluated

A confirmatory method for *N*-acetyl-glyphosate ~~in all kind of plant materials~~ in dry plant materials and those with high water and high fat content and in all kinds of animal matrices.	Relevant, basic data set for all active substances!			
A confirmatory method for glyphosate and AMPA in soil.	Relevant, basic data set for all active substances!			
3.1.4.6 Toxicology and metabolism				
~~KIIA, 5.5:~~	~~In the carcinogenicity studies in mice by [redacted] (1997, ASB2012-11493, notifier: Arysta) and by [redacted] (2009, ASB2012-11492, notifier: Nufarm), a certain increase in the incidence of malignant lymphoma in high dose males (6/50 as compared to 2/50 in the control in the first and 5/51 vs. 0/51 in the second study) was noted. The differences did not gain statistical significance. However, for more reliable assessment, historical control data from the performing laboratories should be provided.~~			

Level 3 – List of studies to be generated, still ongoing or available but not evaluated

3.1.4.7 Residue data				
Metabolism in GAT-modified crops, metabolism in livestock animals with N-acetyl-glyphosate, hydrolysis stability of N-acetly-glyphosate and N-acetyl-AMPA	The introduction of GAT-modified crops influences the residue definition in plant and animal commodities.	The relevant studies were submitted to the EU for an import tolerance and evaluated by EFSA in 2009 (RMS DE). However, the applicant is no member of the current task force, thus these studies are no part of the dossier submitted. Since the GAT-modification provides crucial information for a harmonised definition of the residue, the data have to be taken into account in the RAR. It needs to be clarified, if reference to the 2009 EU-evaluation by EFSA is sufficient in this case or if accessability of the studies by the Task Force is required.		
3.1.4.8 Environmental fate and behaviour				
3.1.4.9 Ecotoxicology				
~~Submission of supplemental studies on acute and chronic toxicity of glyphosate to aquatic organisms cited by FAO specifications and evaluations (2000/2001) for glyphosate.~~ ~~Rationale: For aquatic toxicity additional end points are cited, which were not submitted with the supplementary dossier.~~	~~Relevant, basic data set for risk assessment. Studies might provide supportive evidence and might replace relevant end points for risk calculation.~~			

Level 3 – List of studies to be generated, still ongoing or available but not evaluated

~~Submission of a study according to OECD 216 with maximum predicted environmental concentration (PEC) and five times that concentration.~~	~~Relevant, basic data set for risk assessment. For the evaluation of the active substance glyphosate acid further data would have to be generated for the purpose of risk assessment. The submitted nitrogen cycle test is considered not valid according to OECD guideline 216.~~	~~A study assessing effects of the active substance towards microorganisms is required.~~		
~~Submission of a study with the representative plant protection product MON 5227 6 according to OECD 227 including *Lycopersicum esculentum*. The test shall provide the ER50 values of the plant protection product to non-target plants.~~	~~Relevant, basic data set for risk assessment. For the evaluation of the representative plant protection product MON 52276, the study submitted is not considered to be valid and acceptable.~~	~~A study assessing effects on non-target plants is required for the plant protection product, as the risk cannot be reliably predicted on the basis of the active substance data.~~		

3.1.5 Issues that could not be finalised

An issue is listed as an issue that could not be finalised where there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) No 546/2011, and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

Area of the risk assessment that could not be finalised on the basis of the available data	**Relevance in relation to representative use(s)**
~~Assessment of the relevance of impurities in technical material and/or specification (besides impurities 2, 11, 20, 21)~~	~~*All uses*~~
Assessment of the toxicological equivalence of the tested materials with the specification for certain sources.	*All uses*
~~Safe maximum levels for relevant impurities cannot be derived based on the currently available data.~~	~~*All uses*~~
~~Effects of the active substance towards microorganisms.~~	~~*All uses*~~
~~Effects of the plant protection product on non-target plants.~~	~~*All uses*~~
Biodiversity: Regarding effects on biodiversity including indirect effects via trophic interaction, no quantitative assessment methods are established. However, risks for non-target organisms, in particular farmland bird species, were identified based on the evaluation of existing field studies.	*All uses*

3.1.6 Critical areas of concern

An issue is listed as a critical area of concern:
(a) where the substance does not satisfy the criteria set out in points 3.6.3, 3.6.4, 3.6.5 or 3.8.2 of Annex II of Regulation (EC) No 1107/2009 and the applicant has not provided detailed evidence that the active substance is necessary to control a serious danger to plant health which cannot be contained by other available means including non-chemical methods, taking into account risk mitigation measures to ensure that exposure of humans and the environment is minimised, or
(b) where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) 546/2011, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

Critical area of concern identified	Relevance in relation to representative use(s)
Biodiversity: The use of gyphosate and other broadspectrum herbicides may affect populations of non-target terrestrial arthropod and vertebrate (especially farmland bird) species via trophic interactions. Member states should pay attention to such potential indirect effects. Depending on the agricultural and ecological conditions, Member States may consider adequate risk mitigation measures.	*All uses*
Non target plants: RMS highlights the importance of toxicity studies with the commercial product for ecotoxicological assessment towards non target plants. During product authorization, Member States should additionally consider the use of a dataset for the respective formulation in order to include toxicity information of co-formulants.	

3.1.7 Overview table of the concerns identified for each representative use considered

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in 3.3.1, has been evaluated as being effective, then 'risk identified' is not indicated in this table.)

All columns are grey as the material tested in the toxicological studies has not been demonstrated to be representative of the technical specification.

Representative use		All crops (Pre planting) All seeded or transplanted crops	All crops (Post planting/ pre emergence) All seeded or transplanted crops	Cereals (Pre-harvest) Wheat, rye, triticale	Cereals (Pre-harvest) Barley and oats	Oilseeds (Pre-harvest) Rapeseed, mustard seed, linseed	Orchard crops, vines, including citrus & tree nuts (Post-emergence of weeds)	Orchard crops, vines, including citrus & tree nuts (Post-emergence of weeds) ULV
Operator risk	Risk identified							
	Assessment not finalised							
Worker risk	Risk identified							
	Assessment not finalised							
Bystander risk	Risk identified							
	Assessment not finalised							
Consumer risk	Risk identified							
	Assessment not finalised							

Overview table of the concerns identified

Representative use		All crops (Pre planting) All seeded or transplanted crops	All crops (Post planting/ pre emergence) All seeded or transplanted crops	Cereals (Pre-harvest) Wheat, rye, triticale	Cereals (Pre-harvest) Barley and oats	Oilseeds (Pre-harvest) Rapeseed, mustard seed, linseed	Orchard crops, vines, including citrus & tree nuts (Post-emergence of weeds)	Orchard crops, vines, including citrus & tree nuts (Post-emergence of weeds) ULV
Risk to wild non target terrestrial vertebrates	Risk identified	X*	X*					
	Assessment not finalised							
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified							
	Assessment not finalised							
Risk to aquatic organ-isms	Risk identified							
	Assessment not finalised							
Groundwater exposure active substance	Legal para-metric value breached							
	Assessment not finalised							
Groundwater exposure metabolites	Legal para-metric value breached							
	Parametric value of 10µg/L[(a)] breached							

Overview table of the concerns identified

Representative use		All crops (Pre planting) All seeded or transplanted crops	All crops (Post planting/ pre emergence) All seeded or transplanted crops	Cereals (Pre-harvest) Wheat, rye, triticale	Cereals (Pre-harvest) Barley and oats	Oilseeds (Pre-harvest) Rapeseed, mustard seed, linseed	Orchard crops, vines, including citrus & tree nuts (Post-emergence of weeds)	Orchard crops, vines, including citrus & tree nuts (Post-emergence of weeds) ULV
	Assessment not finalised							
Comments/Remarks								

The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.
(a): Value for non relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003
* For use in grassland the calculated TER values for the long-term risk resulting from an exposure of mammals to glyphosate do not achieve the acceptability criteria TER ≥ 5 with all application rates.

3.1.8 Area(s) where expert consultation is considered necessary

It is recommended to organise a consultation of experts on the following parts of the assessment report:

Area(s) where expert consultation is considered necessary	Justification

3.1.9 Critical issues on which the Co-RMS did not agree with the assessment by the RMS

Points on which the co-rapporteur Member State did not agree with the assessment by the rapporteur member state. Only the points relevant for the decision making process should be listed.

Issue on which Co-RMS disagrees with RMS	Opinion of Co-RMS	Opinion of RMS
None		

3.2 Proposed decision

It is proposed that:

[redacted]

[redacted]

[redacted]

[redacted]

- [redacted]
- [redacted]
- [redacted]

[redacted]

[redacted]
[redacted]
[redacted]
[redacted].

[redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

3.3 Rational for the conditions and restrictions to be associated with any approval or authorisation(s), as appropriate

3.3.1 Particular conditions proposed to be taken into account to manage the risks identified

Proposed condition/risk mitigation measure	Relevance in relation to representative use(s)
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

APPENDICES

Appendix 1 - Guidance documents used in this assessment

[List of Guidance documents used in the conduct of the evaluation and risk assessment.]

Appendix 2 - Reference list

List [in the conventional format] any references specifically cited in Volume 1 (i.e references to underpinning documents such as PPR-Panel Opinions, EFSA conclusions, national documents etc.).

Renewal Assessment Report

18 December 2013

Revised
29 January 2015

Glyphosate

Volume 3

Annex B.2

Physical and chemical properties

RMS: Germany **Co-RMS: Slovakia**

Version history (Volume 3, chapter B.2)

Date	**Reason for revision**
19 December 2013	Initial Renewal Assessment Report
29 January 2015	Updated following RAR peer review, taking into account the reporting table, the evaluation table and additional data

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B.2 Physical and chemical properties

B.2.1 Physical and chemical properties of the active substance (Annex IIA 2)

In Table B.2.1-1 the physical and chemical properties of the active substance glyphosate (parent) and the variants isopropyamine salt (IPA), ammonium salt and potassium salt are given. Information with respect to GLP is given in the chapter "References relied on".

The variant glyphosate dimethylamine salt (DMA) is formed during the formulation process and therefore the physical and chemical data are not required. However, attempts to isolate the DMA salt showed that a hard glass is formed when the solvent is removed. The isolation of pure glyphosate DMA salt was not possible since the product was unsuitable to purification by recrystallisation. Nevertheless, several studies were performed with a solution of 62.1 % glyphosate DMA salt (GF-1667). These studies have been not evaluated and are listed in Table B.2.1-1 only for the sake of completeness.

Table B.2.1-1: Summary of the physical and chemical properties of the active substance glyphosate

Section (Annex point)	Study	Method	Test material/ Purity	Results	Conclusion/ Comment	Reference
B.2.1.1.1 (IIA 2.1.1)	Melting point, freezing point or solidification point	OECD 102 (melt microscope)	parent 99.9 %	189.5 °C ± 0.5 °C	acceptable (DAR)	Dommröse (1989) CHE9600605
		OECD 102 (capillary method)	IPA 98.0 %	110 – 113 °C	acceptable (DAR)	Schneider (1993) CHE9600659
		EEC A.1 (DSC method)	98.1 %	143 – 164 °C		Krips (1995) CHE9600606
		OECD 102 (capillary method)	NH_4 salt 97.9 %	Decomposition at temperatures > 190 °C without melting.	acceptable (DAR)	Cowlyn (1993) CHE9600608

Section (Annex point)	Study	Method	Test material/ Purity	Results	Conclusion/ Comment	Reference
		EEC A.1 (capillary method)	K salt 98.4 %	219.8 °C	acceptable	Midgley (2007) BVL no 2308949
B.2.1.1.2 (IIA 2.1.2)	Boiling point			Not applicable because glyphosate decomposes during melting.	acceptable (DAR)	
B.2.1.1.3 (IIA 2.1.3)	Temperature of decomposition or sublimation	OECD 102	parent 99.9 %	199 °C	acceptable (DAR)	Dommröse (1989) CHE9600605
		OECD 102, EEC A.1	99.6 %	Pure glyphosate decomposes at about 200 °C.	acceptable	Wollerton, Husband (1997) CHE2001-61
		OECD 102, EEC A.1	96.9 %	Glyphosate technical material decomposes at about 200 °C.	additional information as technical as was used.	Wollerton, Husband (1997) CHE2001-63
		EEC A.1 (DSC method)	IPA 98.1 %	> 282 °C	acceptable (DAR)	Krips (1995) CHE9600606
		OECD 102 (capillary method)	NH_4 salt 97.9 %	> 190 °C	acceptable (DAR)	Cowlyn (1993) CHE9600608
		EEC A.1	K salt 98.4%	223.9 °C	acceptable	Midgley (2007) BVL no 2308951
		OPPTS 830.6316	DMA salt 62.1 %	Exothermic decomposition begins at approximately 280 °C.	additional information	Alvis (2005) BVL no 2308953

Section (Annex point)	Study	Method	Test material/ Purity	Results	Conclusion/ Comment	Reference
B.2.1.2 (IIA 2.2)	Relative density	OECD 109 EEC A.3 (pyknometer)	parent 99.5 %	D^{20}_{4} = 1.704	acceptable (DAR)	Verbist (1998) CHE9600609
		OECD 109 EEC A.3 (pyknometer)	99.6 %	D^{20}_{4} = 1.70	acceptable	Wollerton, Husband (1997) CHE2001-61
		OECD 109 EEC A.3 (pyknometer)	96.9 %	D^{20}_{4} = 1.69	not acceptable (technical as)	Wollerton, Husband (1997) CHE2001-63
		OECD 109 (pyknometer)	IPA 98.3 %	D^{20}_{4} = 1.482	acceptable (DAR)	Bates (1992) CHE9600611
		OECD 109 (pyknometer)	NH_4 salt 97.9 %	D^{22}_{4} = 1.433	acceptable (DAR)	Cowlyn (1993) CHE9600608
		OECD 109	K salt 98.4 %	D^{20}_{4} = 1.845	acceptable	Midgley (2007) BVL no 2308955
		OECD 109	DMA salt 62.1 %	D^{22}_{4} = 1.2597	additional information	Alvis (2005) BVL no 2308957

Section (Annex point)	Study	Method	Test material/ Purity	Results	Conclusion/ Comment	Reference
B.2.1.3.1 (IIA 2.3.1)	Vapour pressure	OECD 104 (vapour pressure balance method)	parent 98.6 %	1.31 x 10^{-5} Pa at 25 °C	acceptable (DAR)	Robson (1991) LUF9500151
		Herz-Knudsen effusion method	IPA technical grade	2.1 x 10^{-6} Pa at 25 °C	acceptable (DAR) The result was corrected. In the DAR the unit should read hPa.	Tria (1990) LUF9500152
		OECD 104 (gas saturation method)	98.0 %	1.3 x 10^{-6} Pa at 25 °C 0.7 x 10^{-6} Pa at 20 °C	acceptable (DAR)	Schneider (1993) LUF950097
		OECD 104 (vapour pressure balance method)	NH_4 salt 97.9 %	9 x 10^{-6} Pa at 25 °C	acceptable (DAR) The purity in the DAR was related to glyphosate.	Cowlyn (1993) LUF9500154
		OECD 104, EEC A4 effusion method	K salt 91.8 %	< 5.8 x 10^{-3} Pa at 25 °C < 1.5 x 10^{-3} Pa at 20 °C	acceptable	Oudhoff (2012) BVL no 2308959

Section (Annex point)	Study	Method	Test material/ Purity	Results	Conclusion/ Comment	Reference
B.2.1.3.2 (IIA 2.3.2)	Volatility, Henry's law constant	Calculation	parent	2.1 x 10^{-7} Pa m^3 mol^{-1} (25 °C)	acceptable (DAR)	See methods in B.2.1.3.1 and B.2.1.6
			IPA	4.6 x 10^{-10} Pa m^3 mol^{-1} (25 °C)	acceptable (DAR)	
			NH_4 salt	1.2 x 10^{-8} Pa m^3 mol^{-1} (25 °C)	acceptable (DAR)	
			K salt	< 1.31 x 10^{-6} Pa m^3 mol^{-1} (25 °C) (calculated from vapour pressure < 5.8 x 10^{-3} Pa and water solubility 918.7 g/L) < 3.38 x 10^{-7} Pa m^3 mol^{-1} (20 °C) (calculated from vapour pressure < 1.5 x 10^{-3} Pa and water solubility 918.7 g/L)	acceptable	Annex II, Document M
B.2.1.4.1 (IIA 2.4.1)	Appearance: physical state	Visual assessment	parent 99.6 %	solid	acceptable	Wollerton, Husband (1997) CHE2001-61
			96.9 %	solid	additional information as technical as was used.	Wollerton, Husband (1997) CHE2001-63
			IPA 96.7 %	powder	acceptable (DAR)	Krips (1995) CHE9600613
			NH_4 salt 97.9 %	crystalline powder	acceptable (DAR)	Cowlyn (1993) CHE9600608
			K salt 98.4 %	crystalline solid	acceptable	Midgley (2007) BVL no 2308961

Section (Annex point)	Study	Method	Test material/ Purity	Results	Conclusion/ Comment	Reference
			DMA salt 62.1 %	liquid	additional information	Alvis (2005) BVL no 2308963
B.2.1.4.2 (IIA 2.4.1)	Appearance: colour	Visual assessment	parent 99.6 %	white	acceptable	Wollerton, Husband (1997) CHE2001-61
			96.9 %	white	additional information as technical as was used.	Wollerton, Husband (1997) CHE2001-63
			IPA 98.1 %	white	acceptable (DAR)	Krips (1995) CHE9600613
			NH_4 salt 97.9 %	white	acceptable (DAR)	Cowlyn (1993) CHE9600608
			K salt 98.4 %	white	acceptable	Midgley (2007) BVL no 2308961
			DMA salt 62.1 %	yellow	additional information	Alvis (2005) BVL no 2308963
B.2.1.4.2 (IIA 2.4.2)	Appearance: odour	Olfactory assessment	parent 99.6 %	no characteristic odour	acceptable	Wollerton, Husband (1997) CHE2001-61
			96.9 %	no characteristic odour	not acceptable (technical as)	Wollerton, Husband (1997) CHE2001-63

Section (Annex point)	Study	Method	Test material/ Purity	Results	Conclusion/ Comment	Reference
			IPA 96.7 %	no characteristic odour	acceptable (DAR)	Krips (1995) CHE9600613
			NH_4 salt	–	not submitted (as in the DAR)	–
			K salt 98.4 %	No specific odour was reported during the evaluation of appearance.	acceptable	Midgley (2007) BVL no 2308965
			DMA salt 62.1 %	waxy odour	additional information	Alvis (2005) BVL no 2308967
B.2.5.1.5 (IIA 2.5.1)	Spectra of the active substance	UV/VIS OECD 101	parent 99.6 %	$\varepsilon < 10$ L mol^{-1} cm^{-1} ($\lambda > 290$ nm)	acceptable	Wollerton, Husband (1997) CHE2001-61
			99.5 %	No maximum in the range 200-340 nm	acceptable (DAR)	Sorensen, Bjorholm (1992) CHE9600614
			–	No maximum in the range 200-340 nm	acceptable (DAR)	Frings (1990) CHE9600674
			IPA 98.1 %	No maximum in the range 220-550 nm	acceptable (DAR)	Verhoeven (1995) CHE9600615
			98 %	No maximum in the range 220-550 nm	acceptable (DAR)	Schneider (1993) CHE9600681
			NH_4 salt 97.9 %	No maximum in the range 220-800 nm.	acceptable (DAR)	Cowlyn (1993) CHE9600608

Section (Annex point)	Study	Method	Test material/ Purity	Results	Conclusion/ Comment	Reference
			K salt 91.8 %	No maximum in the range 200-900 nm at pH 1, pH 5 and pH 13.	acceptable	Oudhoff (2012) BVL no 2308969
		UV/VIS, IR, ^{1}H-NMR, MS	parent	Spectra are consistent with the assigned structure of glyphosate.	acceptable	Wollerton, Husband (1997) CHE2001-61
			IPA 98.1 %	Spectra are consistent with the assigned structure of the material.	acceptable (DAR)	Verhoeven (1995) CHE9600615 CHE9600616
			98.1 %	^{1}H-NMR spectrum is consistent with the assigned structure of the material.	acceptable (DAR)	Krips (1995) CHE9600617
			98.1 %	Mass spectra are consistent with the assigned structure of the material.	acceptable	Van Schöll (1995) BVL no 2308975
			NH_4 salt 97.9 %	Spectra are consistent with the assigned structure of the material.	acceptable (DAR)	Cowlyn (1993) CHE9600608
			97.5 %	Mass spectra are consistent with the assigned structure of the material.	acceptable	Oudhoff (2012) BVL no 2308977
			K salt 91.82 %	UV/VIS, IR and mass spectra are consistent with the assigned structure of the material.	acceptable	Oudhoff (2012) BVL no 2308969
			91.82 %	^{1}H-NMR spectrum is consistent with the assigned structure of the material.	acceptable	Janssen (2012) BVL no 2308973

Section (Annex point)	Study	Method	Test material/ Purity	Results	Conclusion/ Comment	Reference
B.2.1.5.2 (IIA 2.5.2)	Spectra of relevant impurities	UV/VIS, IR, NMR, MS	100 %	UV/VIS and IR spectra are consistent with given structure of *N*-nitroso-glyphosate.	acceptable ~~The purity was confirmed by the applicant in the reporting table.~~	Triska (2011) BVL no 2308985 Horton (2013) BVL no 2716044
				Spectra are consistent with given structure of formaldehyde.	acceptable (DAR)	Sorensen, Bjorholm (1992) CHE9600621
				The IR spectrum is consistent with given structure of formaldehyde.	acceptable	Triska (2011) BVL no 2308987
				Spectra are consistent with given structure of *N*-nitroso-glyphosate.	acceptable (DAR)	Snoddy (1995) CHE9600620
B.2.1.6 (IIA 2.6)	Solubility in water	OECD 105 EEC A 6 (flask method)	parent 99.5 %	10.5 g/L at 20 °C (pH 1.90 – 1.98)	acceptable (DAR)	Ochsenbein (1990) CHE9600622
		OECD 105 (flask method)	IPA 98.1 %	1050 g/L at 20 °C and pH 4.3 (pure water) 627 g/L at pH 3.9 (in acidic medium) 990 g/L at pH 6.2 (in alkaline medium) When the pH was raised to 6.5 by addition of sodium hydroxide a precipitate was formed. Thus, no determination at pH-values greater than 6.5 is possible.	acceptable (DAR)	Vogels (1995) CHE9600608

Section (Annex point)	Study	Method	Test material/ Purity	Results	Conclusion/ Comment	Reference
		OECD 105 EEC A 6 (flask method)	NH_4 salt 97.9 %	144 g/L at 20 °C and pH 3.2	acceptable (DAR)	Cowlyn (1993) CHE9600608
		OECD 105 EEC A 6 (flask method)	K salt 98.4 %	918 g/L (distilled water, 20 °C) 923 g/L (pH 4, 20 °C) 919 g/L (pH 7, 20 °C) 902 g/L (pH 9, 20 °C)	acceptable	Midgley (2007) BVL no 2308989
B.2.1.7 (IIA 2.7)	Solubility in organic solvents	Shake flask method	parent 98.6 %	acetone 0.078 g/L dichloromethane 0.233 g/L ethyl acetate 0.012 g/L hexane 0.026 g/L methanol 0.231 g/L propan-2-ol 0.020 g/L toluene 0.036 g/L all values at 20 °C	acceptable (DAR)	Robson (1991) CHE9600625
		OECD 105 (flask method)	96.9 %	acetone < 0.6 mg/L 1,2-dichloroethane < 0.6 mg/L ethyl acetate < 0.6 mg/L heptane < 0.6 mg/L methanol 10 mg/L octan-1-ol < 0.6 mg/L xylenes < 0.6 mg/L acetonitrile 0.8 mg/L all values at 20 °C	acceptable	Wollerton, Husband (1997) CHE2001-63

Section (Annex point)	Study	Method	Test material/ Purity	Results	Conclusion/ Comment	Reference
		EEC A.6	IPA 98.0 %	acetone < 0.05 g/L ethyl acetate < 0.05 g/L hexane < 0.05 g/L methanol 19.86 g/L dichloromethane < 0.05 g/L toluene < 0.05 g/L all values at 23 °C	acceptable (DAR)	Schneider (1993) CHE9600686
		Shake flask method	98.0 %	dichloromethane 0.184 g/L methanol 15.88 g/L all values at 20 °C	acceptable	Bates (1992) CHE9300107
		OECD 105 EEC A.6	NH_4 salt > 98 %	acetone 2.3 mg/L ethyl acetate < 1.3 mg/L heptane < 1.3 mg/L methanol 159 mg/L 1,2-dichloroethane < 1.3 mg/L *p*-xylene < 1.3 mg/L all values at 20 °C	acceptable	Bates (1999) BVL no 2308991
		EEC A.6	K salt 98.2 %	acetone < 10.2 mg/L dichloromethane < 10.2 mg/L ethyl acetate < 10.2 mg/L heptane < 10.2 mg/L methanol 217 mg/L toluene < 10.2 mg/L all values at 20 °C	acceptable	Midgley (2007) BVL no 2308993

Section (Annex point)	Study	Method	Test material/ Purity	Results	Conclusion/ Comment	Reference
B.2.1.8 (IIA 2.8)	Partition coefficient (*n*-octanol/water)	OECD 107 (scintillation counter)	parent 97.4 %	log $K_{o/w}$ < -3.4 at 20 °C	acceptable (DAR)	Burgener (1990) CHE9600626
		US EPA Guide-line CG-1400	99.9 %	log $K_{o/w}$ < -3.2 at 25 °C (at pH buffers from 5 to 9)		Leiber (1987) CHE9600627
		EEC A.8	IPA 98.1 %	log $K_{o/w}$ < -5.4 at 20 °C (at pH buffers 4.3 and 6.2)	acceptable (DAR)	De Vries (1995) CHE9600628
		OECD 107, EEC A.8	NH_4 salt 97.9 %	log $K_{o/w}$ < -3.7 at 20 °C (at pH 3.16)	acceptable (DAR)	Cowlyn (1993) CHE9600608
		EEC A.8 (shake flask method)	K salt 91.8 %	log $K_{o/w}$ < -0.7 at 20 °C (at pH 3.16)	acceptable	Oudhoff (2012) BVL no 2308995

Section (Annex point)	Study	Method	Test material/ Purity	Results	Conclusion/ Comment	Reference
B.2.1.9.1 (IIA 2.9.1)	Hydrolysis rate	US EPA 161-1	parent 96.6 [^{14}C-1-methane label]	After an incubation time of 30 days at 25 °C, no hydrolysis products were detected in the test solutions and no significant amount of volatile products were observed in the absorption traps (< 0.1 %), In the pH range 5 to 9 tested glyphosate is stable towards hydrolysis.	acceptable (DAR)	Burgener (1990) WAS9500229
		BBA-Guideline: "Prüfung des Verhaltens von PSM im Wasser" (Merkblatt 55)	99.0 %	For none of the three pH values (5, 7 and 9) there was hydrolysis to be seen within time of investigation.	acceptable (DAR)	Schneider (1991) WAS9500136
		EEC A 7	IPA 99.0 %	At pH 4, 7 and 9 a decrease in concentration less than 10 % was observed after 5 days. Glyphosate is hydrolytically stable. The test was performed at 50 °C.	acceptable (DAR)	Leeijen (1995) WAS9500283
B.2.1.9.2 (IIA 2.9.2)	Direct photo-transformation				not required, molar absorption is below 10 L mol^{-1} cm^{-1} at λ > 290 nm	

Section (Annex point)	Study	Method	Test material/ Purity	Results	Conclusion/ Comment	Reference
B.2.1.9.3 (IIA 2.9.3)	Quantum yield of direct photo-degradation				not required, molar absorption is below 10 L mol–1 cm–1 at λ > 290 nm	
B.2.1.9.4 (IIA 2.9.5)	Dissociation constant (pK_a)	OECD 112 (potentiometric titration)	parent 97.5 %	pK_{a1} = 2.72 (25 °C) pK_{a2} = 5.63 pK_{a3} = 10.2	acceptable (DAR)	Wells (1995) WAS9500224
		OECD 112 (titration)	99.0 %	pK_{a1} = 2.34 (20 °C) pK_{a2} = 5.73	acceptable (DAR)	Leeijen (1995) WAS9500284
		OECD 112 (titration)	IPA 98.1 %	pK_{a1} = 5.77 (20 °C) pK_{a2} = 2.18	not required (DAR)	Vogels (1995) WAS9500223
		OECD 112 (titration)	NH_4 salt 97.51 %	pK_a = 5.5 (20 °C)	not required	Oudhoff (2012) BVL no 2309003
B.2.1.10 (IIA 2.10)	Estimated photochemical oxidative degradation	Atkinson calculation AOPWIN (v1.65)	parent	DT_{50} = 1.6 h k = 79.0 $\cdot 10^{-12}$ cm s^{-1} (OH-radical conc.: 1.5 $\cdot 10^{6}$ cm^{-3}) Since no olefinic and acetylanic moieties are present in glyphosate reactions with ozone are not to be expected and no ozone reaction estimation is possible.	acceptable (DAR)	De Vries (1995) LUF9500083

Section (Annex point)	Study	Method	Test material/ Purity	Results	Conclusion/ Comment	Reference
		Atkinson calculation AOPWIN (v1.91)	IPA	DT_{50} = 1.38 h k = 93.0 $\cdot 10^{-12}$ cm s^{-1} (OH-radical conc.: 1.5 $\cdot 10^{6}$ cm^{-3})	acceptable	Mehrsheikh (2012) BVL no 2309005
		Atkinson calculation AOPWIN (v1.91)	NH_4 salt	DT_{50} = 1.72 h k = 74.7 $\cdot 10^{-12}$ cm s^{-1} (OH-radical conc.: 1.5 $\cdot 10^{6}$ cm^{-3})	acceptable	Mehrsheikh (2012) BVL no 2309005
		Atkinson calculation AOPWIN (v1.91)	K salt	DT_{50} = 1.72 h k = 74.7 $\cdot 10^{-12}$ cm s^{-1} (OH-radical conc.: 1.5 $\cdot 10^{6}$ cm^{-3})	acceptable	Mehrsheikh (2012) BVL no 2309005
		Atkinson calculation AOPWIN (v1.91)	DMA salt	DT_{50} = 1.66 h k = 77.2 $\cdot 10^{-12}$ cm s^{-1} (OH-radical conc.: 1.5 $\cdot 10^{6}$ cm^{-3})	additional information	Mehrsheikh (2012) BVL no 2309005
B.2.1.11.1 (IIA 2.11.1)	Flammability	EEC A.10	parent 98.7 %	Glyphosate is not highly flammable under the conditions of this test.	acceptable (DAR)	Van Helvoirt (1989) CHE9600629
		EEC A.10	IPA 96.7 %	Glyphosate IPA salt is not highly flammable under the conditions of this test.	acceptable (DAR)	Krips (1995) CHE9600630
		EEC A.10	NH_4 salt 97.9 %	Glyphosate NH_4 salt is not highly flammable under the conditions of this test.	acceptable	Cowlyn (1993) CHE9600608

Section (Annex point)	Study	Method	Test material/ Purity	Results	Conclusion/ Comment	Reference
		EEC A.10	K salt 98.4 %	Glyphosate K salt is not highly flammable under the conditions of this test.	acceptable	Oudhoff (2012) BVL no 2309007
		OPPTS 830.6315	DMA salt 62.1 %	Glyphosate DMA salt is not highly flammable (flash point > 100 °C).	additional information	Alvis (2005) BVL no 2309009
B.2.1.11.2 (IIA 2.11.2)	Auto-flammability	EEC A.16	parent 96.9 %	Glyphosate technical material does not self-ignite below the upper limit of the test (400 °C). The relative self-ignition temperature could not be determined.	acceptable	Wollerton, Husband (1997) CHE2001-63
		EEC A.16	IPA 96.7 %	Glyphosate IPA salt is not auto-flammable.	acceptable (DAR)	Krips (1995) CHE9600631
		EEC A.16	NH_4 salt 97.9 %	Glyphosate NH_4 salt is not auto-flammable.	acceptable (DAR)	Cowlyn (1993) CHE9600608
		EEC A.16	K salt 88.3 %	Glyphosate K salt has a self-ignition temperature of 348 °C.	acceptable ~~Study missing~~ ~~It was confirmed by the applicant in the reporting table that a study will be available by the end of 2013.~~	Brekelmans (2013) BVL no 2716050
		EEC A.15	DMA salt 62.1 %	Glyphosate DMA salt is not auto-flammable.	additional information	Turner (2012) BVL no 2309011

Section (Annex point)	Study	Method	Test material/ Purity	Results	Conclusion/ Comment	Reference
B.2.1.12 (IIA 2.12)	Flash point			Not applicable – material does not have a melting point below 40 °C.		
		OPTS 830.6315	DMA salt 62.1 %	Flash point was determined to be > 100 °C.	additional information	Alvis (2005) BVL no 2309013
B.2.1.13 (IIA 2.13)	Explosive properties	Statement	parent	From the structural formula of glyphosate technical it can be concluded that the substance is not explosive. The substance does not contain any chemically instable or highly energetic groups that might lead to an explosion.	acceptable	Van Helvoirt (1994) CHE9600798
		OECD 113	purity not stated	No endothermic or exothermic decomposition is observed in nitrogen or air below 150 °C.	additional information, not in compliance with EEC A.14	Jackson (2002) BVL no 2309015
		EEC A.14	IPA 96.7 %	Non-explosive in all trials for shock, friction and thermal sensitivity.	acceptable (DAR) decomposition of the test substance by friction	Krips (1995) CHE9600633
		EEC A.14	NH_4 salt 97.9 %	Non-explosive in all trials for shock, friction and thermal sensitivity.	acceptable (DAR) decomposition of the test substance by friction	Cowlyn (1993) CHE9600608

Section (Annex point)	Study	Method	Test material/ Purity	Results	Conclusion/ Comment	Reference
		UN Recommen-dations on the Transport of Dangerous Goods (DSC)	K salt 91.8 %	The test substance has no explosive properties.	acceptable	Oudhoff (2012) BVL no 2309017
		EEC A.14 OPTS 830.6315	DMA salt 60.8 % 62.1 %	Not explosive The formulation was not impact sensitive. The thermal explodability results showed exothermic decomposition beginning at approximately 280 °C.	additional information additional information	Turner (2012) BVL no 2309021 Alvis (2005) BVL no 2309019
B.2.1.14 (IIA 2.14)	Surface tension	OECD 115, EEC A 5 OECD 115, EEC A.5	parent 98.6 % 96.9 %	73.0 mN/m (9.45 g/L H_2O solution,20 °C) 72.2 mN/m (1 g/L H_2O solution, 20 °C)	acceptable (DAR) acceptable	Robson (1991) CHE9600634 Wollerton, Husband (1997) CHE2001-63
		OECD 115, EEC A.5	IPA 96.7 %	72.8 mN/m (1 g/L H_2O solution, 20 °C)	acceptable (DAR)	Krips (1995) CHE9600637
		OECD 115, EEC A.5	NH_4 salt 97.9 %	71.2 mN/m (1 g/kg H_2O solution, 22 °C) 71.2 mN/m (0.502 g/kg H_2O solution, 22 °C)	acceptable (DAR)	Cowlyn (1993) CHE9600608
		OECD 115, EEC A.5	K salt 91.8 %	72.7 mN/m (1 g/L H_2O solution, 20 °C)	acceptable	Oudhoff (2012) BVL no 2309023

Section (Annex point)	Study	Method	Test material/ Purity	Results	Conclusion/ Comment	Reference
		OECD 115, EEC A.5	DMA salt 60.8 %	74.5 mN/m in the neat formulation (25 °C). 73.0 mN/m in the neat formulation (40 °C).	additional information	Turner (2012) BVL no 2309025
B.2.1.15 (IIA 2.15)	Oxidising properties	EEC A.17	parent 96.9 %	Glyphosate technical material is not classified as an oxidising substance.	acceptable	Wollerton, Husband (1997) CHE2001-63
		EEC A.17	IPA 96.7 %	Glyphosate IPA salt is not an oxidising substance.	acceptable (DAR)	Krips (1995) CHE9600635
		EEC A.17	NH_4 salt 97.9 %	Glyphosate NH_4 salt is not an oxidising substance.	acceptable	Cowlyn (1993) CHE9600608
		Statement	K salt	Glyphosate potassium salt has no oxidising properties.	acceptable	Oudhoff (2012) BVL no 2309027
		EEC A.21	DMA salt 60.8 %	Glyphosate DMA salt is not an oxidising substance.	additional information	Comb (2012) BVL no 2309029

B.2.1.16 Summary of data presented under points B.2.1.1 to B.2.1.15

Glyphosate and its salts (IPA, K, NH_4) are water soluble solids at room temperature without any explosive or oxidising properties.

B.2.2 Physical, chemical and technical properties of the plant protection products (Annex IIIA 2)

MON52276 (containing 360 g/L glyphosate as 486 g/L isopropylamine salt of glyphosate, SL)

Information with respect to GLP are given in the chapter "References relied on".

Table B.2.2-1: Summary of the physical, chemical and technical properties of the plant protection product

Section (Annex point)	Study	Method	Results	Comment/ Conclusion	Reference
B.2.2.1.1 (IIIA 2.1)	Appearance: colour	EPA Guideline 63.2 Visual assessment	light yellow (Munsell 5Y 8.5/10) clear yellow	acceptable Additional data submitted to confirm the data from the first EU-evaluation	Alexander et al (1992) CHE9600653 Bates (2001) BVL no 2315951
B.2.2.1.2 (IIIA 2.1)	Appearance: odour	EPA Guideline 63.4 Olfactory assessment	earthy odour of amine	acceptable Additional data submitted to confirm the data from the first EU-evaluation	Alexander et al (1992) CHE9600653 Bates (2001) BVL no 2315951

Section (Annex point)	Study	Method	Results	Comment/ Conclusion	Reference
B.2.2.1.3 (IIIA 2.1)	Appearance: physical state	EPA Guideline 63.3 Visual assessment	oily liquid homogeneous liquid free from visible suspended matter and sediment	acceptable Additional data submitted to confirm the data from the first EU-evaluation	Alexander et al (1992) CHE9600653 Bates (2001) BVL no 2315951
B.2.2.2.1 (IIIA 2.2.1)	Explosive properties	EEC A 14 (Koenen tube)	The test substance is not explosive.	acceptable (The DAR contains a case based on thermo-dynamic data)	Alexander et al (1992) CHE9600653

Section (Annex point)	Study	Method	Results	Comment/ Conclusion	Reference
B.2.2.2.2 (IIIA 2.2.2)	Oxidising properties	Statement	MON 52276 has no oxidizing properties.	Acceptable	Brekelmans (2012) BVL no 2716873
		~~EEC A 17 (Glyphosate IPA salt, 96.7 %)~~	~~The technical active ingredient is not oxidizing. Since it is the largest formulation component and since the formulation is water based MON 52276 is considered to have no oxidising properties.~~	~~Although, no different conclusion is accepted, a case or the respective testing should be submitted for the formulation. The current case is based on a study for the a.s., only.~~ ~~It was confirmed by the applicant in the reporting that a study (expert judgement) would be available.~~	~~See B.2.1.15, Krips~~

Section (Annex point)	Study	Method	Results	Comment/ Conclusion	Reference
B.2.2.3.1 (IIIA 2.3.1)	Flash point		The requirement is not applicable as the formulation does not contain any flammable solvents.	acceptable	
B.2.2.3.2 (IIIA 2.3.2)	Flammability		Not required in the case of liquid preparations.		
B.2.2.3.3 (IIIA 2.3.2)	Auto-flammability	EEC A 15	440 ± 5 °C	acceptable	Alexander et al (1992) CHE9600653
B.2.2.4.1 (IIIA 2.4.1)	Acidity/alkalinity	CIPAC MT 75.1	pH of the test item (undiluted) = 4.90 Therefore, the determination of the acidity is not triggered.	acceptable	Bates (2001) BVL no 2315953
B.2.2.4.2 (IIIA 2.4.2)	pH	US EPA Guideline 63.12, ASTM Method E 70-90 CIPAC MT 75.2	1 % solution: 4.67 at 20 °C 2 % solution: 4.67 at 20 °C 5 % solution: 4.67 at 20 °C 4.83 (1 % in double distilled water at room temperature)	acceptable Additional data submitted to confirm the data from the first EU-evaluation	Alexander et al (1992) CHE9600653 Bates (2001) BVL no 2315955
B.2.2.5.1 (IIIA 2.5.1)	Kinematic viscosity	OECD 114 (capillary viscometer)	63.77 mm^2/s at 20 °C 25.28 mm^2/s at 40 °C	acceptable	Van Hoeylandt, Roels (2011) BVL no 2315957

Section (Annex point)	Study	Method	Results	Comment/ Conclusion	Reference
B.2.2.5.2 (IIIA 2.5.2)	Dynamic viscosity	OECD 114 (rotation viscometer)	65 mPa s at 21 °C	acceptable	Alexander et al (1992) CHE9600653
B.2.2.5.3 (IIIA 2.5.3)	Surface tension	EEC A 5 (ring method)	40.8 mN/m (2 % w/v) 39.8 mN/m (5 % w/v)	acceptable According to the criteria mentioned in EEC A5, the preparation should be regarded as surface active.	Alexander et al (1992) CHE9600653
B.2.2.6.1 (IIIA 2.6.1)	Relative density	EEC A 3 (pycnometer method) OECD 109	1.1694 g/cm^3 at 20 °C 1.166 g/cm^3 at 20 °C	acceptable Additional data submitted to confirm the data from the first EU-evaluation	Alexander et al (1992) CHE9600653 Bates (2001) BVL no 2315959
B.2.2.6.2 (IIIA 2.6.2)	Bulk (tap) density		Not required in the case of liquid preparations.		

Section (Annex point)	Study	Method	Results		Comment/ Conclusion	Reference
B.2.2.7.1 (IIIA 2.7.1)	Stability after storage for 14 days at 54 °C	CIPAC MT 46	Stable, shelf life expected to exceed 2 years on ambient temperature testing.		acceptable	Alexander et al (1992) CHE9600653
			Before storage:	After storage:		
		Colour	Munsell 5 Y 8.5/10	Munsell 5 Y 8/12		
		Physical state	Oily liquid	Oily liquid		
		Odour	Earthy	Earthy		
		Active substance content (% recovery of glyphosate acid)	97.5	96.8		
		Dissolution	25 s (2 % at 21 °C) 38 s (5 % at 21 °C)	33 s (2 % at 21 °C) 24 s (5 % at 21 °C)		
		pH	4.67 (1 % at 20 °C) 4.64 (2 % at 20 °C) 4.55 (5 % at 20 °C)	4.70 (1 % at 21 °C) 4.60 (2 % at 21 °C) 4.50 (5 % at 21 °C)		
		Relative density	1.1694 at 20 °C	1.1697 at 20 °C		

Section (Annex point)	Study	Method	Results		Comment/ Conclusion	Reference
		Persistent foam	2 % at 21 °C: after 10 sec 69 mL after 1 min 63 mL after 3 min 58 mL after 12 min 19 mL 5 % at 21 °C: after 10 sec 79 mL after 1 min 73 mL after 3 min 55 mL after 12 min 21 mL	2 % at 21 °C: after 10 sec 81 mL after 1 min 75 mL after 3 min 68 mL after 12 min 61 mL 5 % at 21 °C: after 10 sec 84 mL after 1 min 78 mL after 3 min 57 mL after 12 min 32 mL		
		Viscosity	65 ± 2 mPa s at 21 °C	65 ± 2 mPa s at 21 °C		
		Dilution storage stability (% recovery of glyphosate acid)	98.0	113.3		
		CIPAC MT 47.2	Before storage for 14 days at 54 °C:	After storage for 14 days at 54 °C:	acceptable	Van Hoeylandt, Roels (2013) BVL no 2741216
			8 % in CIPAC water D: after 10 sec 24 mL after 1 min 0 mL after 3 min 0 mL after 12 min 0 mL	8 % in CIPAC water D: after 10 sec 52 mL after 1 min 4 mL after 3 min ring after 12 min 0 mL		

Section (Annex point)	Study	Method	Results		Comment/ Conclusion	Reference
B.2.2.7.2 (IIIA 2.7.2)	Stability after storage for other periods and/or temperatures		Not relevant as the formulation is stable at 54 °C.			
B.2.2.7.3 (IIIA 2.7.3)	Minimum content after heat stability testing		Not relevant as the formulation is stable at 54 °C.			
B.2.2.7.4 (IIIA 2.7.4)	Effect of low temperature on stability	CIPAC MT 39.2	No separation after storage at 0 °C for 48 h, or after attaining room temperature.		Additional information as the current FAO specification requires 7 d at 0 °C	Alexander et al (1992) CHE9600653
		CIPAC MT 39.2	Stable for 7 days at 0 °C.		acceptable	Dieudonné (2000) BVL no 2315961
			Before storage:	After storage:		
		Visual assessment	free-flowing, homogeneous clear yellow liquid	free-flowing, homogeneous clear yellow liquid		
		CIPAC MT 75.2 (pH)	4.83 (1 % in double distilled water)	4.85 (1 % in double distilled water)		
		CIPAC MT 75 (pH)	4.9 (undiluted)	4.9 (undiluted)		

Section (Annex point)	Study	Method	Results		Comment/ Conclusion	Reference
		CIPAC MT 41	Homogeneous liquid, no separated material (5 % v/v at 20 °C).	Homogeneous liquid, no separated material (5 % v/v at 20 °C).		
B.2.2.7.5 (IIIA 2.7.5)	Shelf life following storage at ambient temperature	GIFAP Monograph 17	Stable for two years at 20 °C in the original packaging (HDPE)		acceptable	Dinwoodie (1994) CHE9600654
			Before storage:	After storage:		
		Colour	Munsell 5 Y 8.5/10	Munsell 7.5 Y 8.5/10		
		Physical state	Oily liquid	Oily liquid		
		Odour	Earthy	Earthy		
		Active substance content (% recovery of glyphosate acid)	97.5	99.4		
		Dissolution	25 s (2 % at 21 °C) 38 s (5 % at 21 °C)	11.4 s (2 % at 23 °C) 11.8 s (5 % at 23 °C)		
		pH	4.67 (1 % at 20 °C) 4.64 (2 % at 20 °C) 4.55 (5 % at 20 °C)	4.59 (1 % at 21 °C) 4.53 (2 % at 21 °C) 4.45 (5 % at 21 °C)		
		Relative density	1.1694 at 20 °C	1.1712 at 20 °C		

Section (Annex point)	Study	Method	Results		Comment/ Conclusion	Reference
		Persistent foam	2 % at 21 °C: after 10 sec 69 mL after 1 min 63 mL after 3 min 58 mL after 12 min 19 mL 5 % at 21 °C: after 10 sec 79 mL after 1 min 73 mL after 3 min 55 mL after 12 min 21 mL	2 % at 20 °C: after 10 sec 69 mL after 1 min 67 mL after 3 min 63 mL after 12 min 48 mL 5 % at 20 °C: after 10 sec 70 mL after 1 min 68 mL after 3 min 58 mL after 12 min 47 mL		
		Viscosity	65 ± 2 mPa s at 21 °C	63 ± 3 mPa s at 22 °C		
		GIFAP Monograph 17	Stable for two years at 20 °C in the original packaging (HDPE)		Additional data submitted to confirm the data from the first EU-evaluation	Bates (2001) BVL no 2315963
			Before storage:	After storage:		
		CIPAC 284/SL/(M)/3 (HPLC)	30.4 % w/w	30.1 % w/w		
		Visual assessment	clear yellow homogeneous liquid free from visible suspended matter and sediment	yellow homogeneous liquid free from visible suspended matter and sediment		

Section (Annex point)	Study	Method	Results		Comment/ Conclusion	Reference
		Olfactory assessment	odour of amine	odour of amine		
		CIPAC MT 75.2 (pH)	4.83 (1 % in double distilled water)	4.91 (1 % in double distilled water)		
		CIPAC MT 75.1 (pH)	4.90 (undiluted)	4.76 (undiluted)		
		CIPAC MT 41	Homogeneous liquid, no separated material (5 % v/v at 20 °C).	Homogeneous liquid, no separated material (5 % v/v at 20 °C).		
B.2.2.8.1 (IIIA 2.8.1)	Wettability		Not applicable			

Section (Annex point)	Study	Method	Results	Comment/ Conclusion	Reference
B.2.2.8.2 (IIIA 2.8.2)	Persistent foaming	CIPAC MT 47	5 % w/v: after 10 sec 79 mL after 1 min 73 mL after 3 min 55 mL after 12 min 21 mL 2 % w/v: after 10 sec 69 mL after 1 min 63 mL after 3 min 58 mL after 12 min 19 mL	acceptable	Alexander et al (1992) CHE9600653

Section (Annex point)	Study	Method	Results	Comment/ Conclusion	Reference
		CIPAC MT 47.2	5 % v/v in CIPAC water D: after 10 sec 35 mL after 1 min 0 mL after 3 min 0 mL after 12 min 0 mL 2 % v/v in CIPAC water D: after 10 sec 46 mL after 1 min 0 mL after 3 min 0 mL after 12 min 0 mL	acceptable It should be noted that according to the FAO Manual the test concentration should correspond to the highest rate of use. According to the list of representative uses the highest rate is 8 %. ~~It was confirmed by the applicant in the reporting that a study will be conducted if requested.~~	Van Hoeylandt, Roels (2011) BVL no 2315965

Section (Annex point)	Study	Method	Results	Comment/ Conclusion	Reference
		CIPAC MT 47.2	8 % v/v in CIPAC water D: after 10 sec 24 mL after 1 min 0 mL after 3 min 0 mL after 12 min 0 mL	acceptable	Van Hoeylandt, Roels (2013) BVL no 2716875
B.2.2.8.3.1 (IIIA 2.8.3.1)	Suspensibility		Not applicable		
B.2.2.8.3.2 (IIIA 2.8.3.2)	Spontaneity of dispersion		Not applicable		
B.2.2.8.4 (IIIA 2.8.4)	Dilution stability	CIPAC MT 41	Homogeneous liquid, no separated material (5 % v/v at 20 °C).	acceptable	Bates (2001) BVL no 2315967
B.2.2.8.5.1 (IIIA 2.8.5.1)	Dry sieve test		Not applicable		
B.2.2.8.5.2 (IIIA 2.8.5.2)	Wet sieve test		Not applicable		
B.2.2.8.6.1 (IIIA 2.8.6.1)	Size distribution of particles		Not applicable		
B.2.2.8.6.2 (IIIA 2.8.6.2)	Nominal size range of granules		Not applicable		
B.2.2.8.6.3 (IIIA 2.8.6.3)	Dust content		Not applicable		

Section (Annex point)	Study	Method	Results	Comment/ Conclusion	Reference
B.2.2.8.6.4 (IIIA 2.8.6.4)	Particle size of dust		Not applicable		
B.2.2.8.6.5 (IIIA 2.8.6.5)	Friability and attrition characteristics of granules		Not applicable		
B.2.2.8.7.1 (IIIA 2.8.7.1)	Emulsifiability		Not applicable		
B.2.2.8.7.2 (IIIA 2.8.7.2)	Emulsion stability		Not applicable		
B.2.2.8.7.3 (IIIA 2.8.7.3)	Re-emulsifiability		Not applicable		
B.2.2.8.7.4 (IIIA 2.8.7.4)	Stability of dilute emulsions		Not applicable		
B.2.2.8.7.5 (IIIA 2.8.7.5)	Stability of emulsions		Not applicable		
B.2.2.8.8.1 (IIIA 2.8.8.1)	Flowability		Not applicable		
B.2.2.8.8.2 (IIIA 2.8.8.2)	Pourability (including rinsed residue)		Not applicable		

Section (Annex point)	Study	Method	Results	Comment/ Conclusion	Reference
B.2.2.8.8.3 (IIIA 2.8.8.3)	Dustability following accelerated storage		Not applicable		
B.2.2.9.1 (IIIA 2.9.1)	Physical compatibility of tank mixes		According to the current Doc M not recommended on product label.		
B.2.2.9.2 (IIIA 2.9.2)	Chemical compatibility of tank mixes		According to the current Doc M not recommended on product label.		
B.2.2.10.1 (IIIA 2.10.1)	Distribution (seed treatment)		Not relevant.		
B.2.2.10.2 (IIIA 2.10.2)	Adherence (seed treatment)		Not relevant.		

B.2.2.16 Summary and evaluation of data presented under points B.2.2.1 to B.2.2.15 (IIIA 2.16)

MON52276 is a SL formulation without any explosive or oxidising properties that is stable for two years under the conditions applied (original container, at 20 °C).

B.2.3 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIA 2.1.1 KIIA 2.1.3 KIIA 2.2 KIIA 2.4.1 KIIA 2.4.2 KIIA 2.6 KIIA 2.7 (OECD)	Midgley, B., de Ryckel, B.	2007	Physico-chemical properties of glyphosate potassium salt MSL0021012 GLP: Y, published: N 2308949 / 2308951 / 2308955 / 2308961 / 2308965 / 2308989 / 2308993	Y	EGT
KIIA 2.1.3 KIIA 2.2 KIIA 2.4.1 KIIA 2.4.2 KIIA 2.5.1 (OECD)	Wollerton, C.; Husband, R.	1997	Glyphosate acid: Physical and Chemical Properties of Pure Material RJ2400B GLP: Y, published: N CHE2001-61	Y	SYD
KIIA 2.1.3 KIIA 2.2 KIIA 2.4.1 KIIA 2.4.2 KIIA 2.7 KIIA 2.11.2 KIIA 2.14 KIIA 2.15 (OECD)	Wollerton, C.; Husband, R.	1997	Glyphosate acid: Physical and Chemical Properties of Technical Material RJ2401B GLP: Y, published: N CHE2001-63	Y	SYD
KIIA 2.3.1 KIIA 2.5.1 KIIA 2.8.1 KIIA 2.11.1 KIIA 2.13 KIIA 2.14 KIIA 2.15 (OECD)	Oudhoff, K.A.	2012	Determination of physico-chemical properties of glyphosate potassium salt 497741 GLP: Y, published: N 2308959 / 2308969 / 2308995 / 2309007 / 2309017 / 2309023 / 2309027	Y	EGT
KIIA 2.5.1 (OECD)	Janssen, H.M.	2012	Determination of 1H NMR spectrum 497740 GLP: N, published: N 2308973	Y	EGT

[1] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIA 2.5.1 (OECD)	Van Schöll, I.	1995	Determination of the mass spectrum of MON 77209 pure V 95.110 GLP: N, published: N 2308975	N	EGT
KIIA 2.5.1 KIIA 2.9.5 (OECD)	Oudhoff, K.A.	2012	Determination of Physico-chemical properties of glyphosate ammonium salt; MS spectrum and dissociation constant MSL0023949 GLP: Y, published: N 2308977 / 2309003	Y	EGT
KIIA 2.5.2 (OECD)	Triska, J.J.	2011	UV/VIS spectral analysis and IR (infrared) spectral analysis of N-nitrosoglyphosate (NNG) PCH-2011-0666 GLP: Y, published: N 2308985	Y	EGT
KIIA 2.5.2 (OECD)	Triska, J.J.	2011	IR (infrared) spectral analysis of Formaldehyde PCH-2011-0667 GLP: Y, published: N 2308987	Y	EGT
KIIA 2.5.2 (OECD)	Horton, S.	2013	Analytical reference standard, Certificate of analysis , Expiration extension - NNG 3034 GLP: N, published: N 2716044	Y	EGT
KIIA 2.5.2 (OECD)	Bjorholm, T.; Sorensen, E.V.	1992	Characterisation of formaldehyde (CAS No. 50-00-0) ca. 20% in water and 10% methanol, Merck 4025, batch No. 847225 REF 062-01 GLP: Y, published: N 2716046 / CHE9600621	N	EGT
KIIA 2.5.2 (OECD)	Snoddy, C.B.	1995	Spectral information for N-nitrosoglyphosate, Lot No. NPD-9205-4204-A AA016556 GLP: N, published: N 2717476 / CHE9600620	N	EGT
KIIA 2.7 (OECD)	Bates, C.	1999	The solubility of sodium and ammonium glyphosate salts in organic solvents MLL31266 GLP: Y, published: N 2308991 /	N	EGT
KIIA 2.10 (OECD)	Mehrsheikh, A.	2012	Atmospheric Oxidation of Glyphosate Salts - Atkinson Calculation Excel MSL0024050 GLP: N, published: N 2309005	Y	EGT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIA 2.11.2 (OECD)	Brekelmans, M.J.C.	2013	Determination of the relative self-ignition temperature of glyphosate potassium salt MSL0025288 GLP: N, published: N 2716050	Y	EGT
KIIA 2.13 (OECD)	Jackson, W.A.	2002	Thermal stability/ Stability in Air - 759acid HT02/075 GLP: Y, published: N 2309015	Y	EGT
KIIIA1 2.1 KIIIA1 2.4.1 KIIIA1 2.4.2 KIIIA1 2.6.1 KIIIA1 2.7.5 KIIIA1 2.8.4 (OECD)	Bates, C.	2001	Long term storage stability at ambient temperature of MON 52276 (glyphosate SL): analysis after 2 years storage at room temperature MSL-17439 GLP: Y, published: N 2315951 / 2315953 / 2315955 / 2315959 / 2315963 / 2315967	N	EGT
KIIIA1 2.2.2 (OECD)	Midgley, B.; Brekelmans, M.J.C.	2012	Statement on the oxidizing properties of MON 52276 MSL0024643 GLP: N, published: N 2716873	Y	EGT
KIIIA1 2.5.1 KIIIA1 2.8.2 (OECD)	Van Hoeylandt, M., Roels, M.	2011	Kinematic viscosity and persistent foaming properties of MON 52276 MSL 0023798 GLP: N, published: N 2315957 / 2315965	Y	EGT
KIIIA1 2.7.1 KIIIA1 2.8.2 (OECD)	Van Hoeylandt, M.; Roels, M.	2013	Persistent foaming properties of MON 52276 MSL0025280 GLP: N, published: N 2741216 / 2716875	Y	EGT
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Codes of owner

EGT European Glyphosate Task Force AIR 2
SYD Syngenta Agro GmbH

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Volume 3

Annex B.3

Data on application
and further information

RMS: Germany **Co-RMS: Slovakia**

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B.3 Data on application and further information

B.3.1 Data on application relevant to the active substance (Annex IIA 3.1 to 3.6)

B.3.1.1 Function

Herbicide

B.3.1.2 Effects on harmful organisms

Glyphosate is a non-selective herbicidal active substance, belonging to the chemical class of glycines, with no or only low soil residual activity. Glyphosate is taken up by the leaves and other green parts of the plant and is translocated systemically (apoplastic and symplastic) in the whole plant, also in underground parts like roots, rhizomes or stolons. Glyphosate is adsorbed immediately by soil particles and rapidly inactivated and degraded by soil microorganisms. Glyphosate kills the plant by blocking the shikimic acid pathway which leads to a deficient protein biosynthesis. Glyphosate binds to and blocks the activity of its target enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), an enzyme of the aromatic amino acid biosynthetic pathway. The inhibition of the enzyme prevents the plant from synthesising the essential aromatic amino acids needed for protein biosynthesis. EPSPS is present in all plants, bacteria and fungi, but not animals. Action at the shikimic acid pathway is unique to glyphosate and the absence of this pathway in animals is an important factor of its low animal toxicity.

B.3.1.3 Field of use

Herbicides containing glyphosate are commonly used for the control of annual and perennial mono- and dicotyledonous weeds and woody plants in agriculture, horticulture, viticulture, forestry, orchards, plantation crops, amenities, home gardening and greenhouses. Furthermore it is used for weed control on aquatic areas, on hard surfaces, on railways, along roads and on non-cultivated areas. Besides weed control, herbicides containing glyphosate are also used for the control of suckers, for the destruction of grassland before renewal and for harvest management (desiccation) in various crops. Over the last decades an increasing use of glyphosate was observed. An increase in glyphosate use is expected over the next few years. Glyphosate applications should be avoided wherever possible and appropriate. However, it is important that label recommendations are followed and rates are not reduced below recommendations.

Glyphosate is believed to be the world`s most used herbicidal active substance with over 600 thousand tonnes used annually. Since 1996, the already high levels of glyphosate usage have led to a strong increase with the adoption of genetically modified glyphosate-resistant crops.

Glyphosate inhibits 5-*enol*-pyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme in the aromatic amino acid biosynthetic pathway. Because of its broad-spectrum and potent weed control and favorable environmental characteristics, attempts to engineer glyphosate resistance have been intensive in the past few decades.
Some crops have been genetically engineered to be resistant to glyphosate. Such crops allow farmers to use glyphosate as a post-emergence herbicide against both broadleaf and cereal

weeds, but the development of similar resistance in some weed species is emerging as a costly problem. Genetically modified crops have become the norm in the United States. For example, in 2010, 70 % of all the maize that was planted was herbicide-resistant; 78 % of cotton, and 93 % of all soybeans. In 1996, genetically modified soybeans were made commercially available and introduced in the United States. Current glyphosate-resistant crops include soybean, maize, sorghum, canola, alfalfa, and cotton, with wheat still under development. Herbicide-tolerant crops were grown mainly in USA, Canada, Argentina, Brazil, South Africa, Australia and Paraguay.
The use of at least three different mechanisms has conferred glyphosate resistance in normally sensitive crop species. Early work focused on progressive adaptation of cultured plant cells to stepwise increases in glyphosate concentrations. The resulting cells were resistant to glyphosate because of EPSPS overexpression, EPSPS gene amplification, or increased enzyme stability. Further work aimed to achieve resistance by transforming plants with glyphosate metabolism genes. An enzyme from a soil microorganism, glyphosate oxidoreductase (*GOX*), cleaves the nitrogen– carbon bond in glyphosate yielding aminomethylphosphonic acid. This is actually not of importance. Another metabolism gene, glyphosate *N*-acetyl transferase (*gat*), acetylates and deactivates glyphosate. A third mechanism, and the one found in all currently commercial glyphosate-resistant crops, is the insertion of a glyphosate-resistant form of the EPSPS enzyme. Several researchers have used site-directed mutagenesis or amino acid substitutions of EPSPS. However, the most glyphosate-resistant EPSPS enzyme to date has been isolated from *Agrobacterium* spp. strain CP4 and gives high levels of resistance in plants.

The approval for growing genetically modified crops will lead to an increase in glyphosate use in the EU. Taking into consideration past experience (e.g. in the US) with the use of glyphosate in resistant crops, recent developments are especially important for a sustainable use of glyphosate.

B.3.1.4 Harmful organisms

Glyphosate controls the most important annual dicotyledonous species, for example *Chenopodium album*, biennial species such as *Cirsium* spp. and perennial dicotyledonous weeds such as *Rubus* spp. In addition glyphosate controls annual monocotyledonous species such as *Alopecurus myosuroides* and perennial monocotyledonous weeds including *Sorghum halepense* and *Elytrigia repens* in stubble, cereals, peas, beans, oilseed rape, flax, mustard, orchards, pastures, forestry and industrial weed control.
For some species efficacy is not sufficient.

B.3.1.5 Mode of action

Glyphosate is an organic phosphorus compound, belonging to the chemical class of glycines, with no or low soil residual activity. Herbicides containing glyphosate differ in the salt formulation. Glyphosate may be present as glyphosate-ammonium-salt, as glyphosate-isopropylamine-salt or as glyphosate-potassium-salt. Glyphosate is a non-selective herbicidal active substance. Glyphosate is taken up by the leaves and other green parts of the plant and is translocated systemically (apoplastic and symplastic) in the whole plant, also in underground parts like roots, rhizomes or stolons. Glyphosate uptake through the roots is negligible because the active substance is strongly adsorbed in the soil. The extensive adsorption of

glyphosate together with a rapid degradation in soil are the principal deactivation and dissipation mechanisms in the soil environment.

In plants, glyphosate inhibits the shikimic acid pathway. Glyphosate binds to and blocks the activity of its target enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), an enzyme of the aromatic amino acid biosynthetic pathway. The inhibition of the enzyme prevents the plant from synthesising the essential aromatic amino acids (e.g. phenylalanine, tyrosine, tryptophane) needed for protein biosynthesis. This reduces the production of protein in the plant, and inhibits plant growth. EPSPS is present in all plants. It leads to an accumulation of the amino acids glutamine, glutamic acid, shikimic acid and ammonia. As a consequence of missing aromatic amino acids the formation of phenolic compounds is inhibited (e.g. lignin, flavanoids).

First signs of wilting occur in annual weeds 4 days and in perennial weeds 7 to 10 days after herbicide application. Leaf symptoms are usually detected 7 to 14 days after application, while a complete death of the plant takes up to 30 days. As light affects the metabolism via photosynthesis, a higher activity in plants means a better distribution of glyphosate and thus greater herbicidal effect. Increasing temperatures result in increased biochemical activity, and thus in an increased rate of efficacy. Optimum temperatures are 10 to 20 °C. High humidity affects the quality of the leaf surface and thus promotes the uptake of the herbicide.

Plant metabolism studies have been conducted on numerous crops. The only significant metabolite in plants was aminomethylphosphonic acid (AMPA). AMPA is not biologically active within soils.
Reference to B.3.2.2 and B.3.2.8.

B.3.1.6 Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies

The resistance risk assessment submitted by the glyphosate task force in prinicipal follows the EPPO Standard 1/213.

Mechanism of resistance
To date there are different confirmed mechanisms of resistance including target-site resistance and metabolic resistance.
Across all herbicide groups, target-site mutations and metabolism are the most common resistance mechanisms. Target-site mutations are alterations in the amino acid sequence of a targeted protein such that the function of the protein in not altered, but the ability of the herbicide to bind to the protein is affected, thus limiting the capacity of the herbicide to kill the plant. Target-site mutations generally result in ‘high levels’ of resistance (i.e. 1000X) and are the common resistance mechanism for the ALS inhibitor and ACCase inhibitor groups. (Note: 1000 X means the biotype is not affected by a herbicide rate that is 1000 times greater than the rate that would normally kill a biotype). In contrast, glyphosate-resistant weed species with target-site mutations demonstrate relatively ‘weak’ resistance (i.e. 2-3X). Target-site mutation has been found in *Lolium* species and in *Eleusine indica*. In most plants with target-site resistance, a change of amino acid 106 from proline to serine, alanin or threonine results in a slightly resistant target enzyme (EPSPS) that allows plants to survive commercial rates of glyphosate.
A rare second target-site mechanism is gene amplification. In a resistant individual with amplified EPSPS gene copies present on multiple chromosomes, there are high levels of

EPSPS expression and the normal glyphosate rate cannot inhibit all of the available EPSPS. This mechanism has been found in *Amaranthus palmeri* from Georgia and imparts high-level glyphosate resistance.
Another possible resistance mechanism is overexpression of EPSPS. This resistance type is based on a two to threefold elevated EPSPS expression and enzyme activity and was found in glyphosate-resistant *L. rigidum*. In the investigated biotypes, EPSPS from glyphosate-resistant and –susceptible plants were equally sensitive to glyphosate. The elevated expression was not due to gene amplification, because the EPSPS gene copy number in *L. rigidum* was examined using DNA blot hybridisations, and glyphosate-resistant lines did not have an increased EPSPS gene copy number in comparison with glyphosate-susceptible lines.
Several types of non-target site mechanisms have been defined for glyphosate of which most are based on reduced translocation of the active ingredient in resistant plants. Reduced translocation has been found in *Lolium* spp., *Conyza* spp. and *Sorghum halapense*. Possible mechanisms of reduced translocation include; (1) reduced movement of glyphosate as a result of a hypersensitive effect (rapid tissue necrosis) of leaf tissue treated with glyphosate (*Ambrosia* sp.), (2) reduced translocation to rhizome/root tissue (*Sorghum* sp.), and (3) accumulation/sequestration of glyphosate in the vacuole preventing lethal concentrations getting into plastids, the site of the shikimic acid pathway (*Lolium* sp). In most cases, a reduced translocation was inherited as a single, nuclear gene trait.

The mechanism of action of a gene providing tolerance to glyphosate in glyphosate-tolerant crops is different to the genes identified as conferring target-site resistance. For example, the commercial glyphosate-tolerant gene from Monsanto, obtained from a bacterial strain of Agrobacterium sp., produces an EPSPS enzyme (CP4 EPSPS) that has less binding affinity to glyphosate than plant EPSPS. This fact combined with the high production of CP4 EPSPS in the crop accounts for the high level of tolerance found in crops containing the gene.

Evidence of resistance and cross-resistance

Whereas glyphosate was first introduced as a commercial herbicide in 1974 under the trade name of Roundup, the first documented case of glyphosate resistance was reported 22 years later in the case of a *Lolium* species from an Australian orchard in 1996, based on an intensive selection pressure over 15 years with two or three applications per year. Since 1996, several additional resistant weed populations have been identified, encompassing cases from all over the world, including Europe. Currently (Nov 2014) the online database of Ian Heap (www.weedscience.org) lists 31 plant species for which resistance has been documented. In the following table, the documented cases of weed resistance to glyphosate are summarised (Table B.3.1-1). Biotypes indicated with a '*' show multiple resistance mechanisms.

Table B.3.1-1: Glycine-resistant weeds by species and country (source: www.weedscience.org)

Weed species	**Country**	**Years with documented cases till Sept 2012**
Amaranthus palmeri	USA	2005, 2006, 2007, 2008*, 2009*, 2010, 2011, 2012*, 2013*, 2014*
Amaranthus quitensis	Argentina	2013
Amaranthus spinosus	USA	2012
Amaranthus tuberculatus (Syn. rudis)	USA	2005*, 2006*, 2007, 2009, 2010, 2011*, 2012
Ambrosia artemisiifolia	USA	2004, 2006*, 2007, 2008, 2010*, 2013, 2014
	Canada	2012*
Ambrosia trifida	USA	2004, 2005, 2006*, 2007, 2008*, 2009, 2010, 2011*
	Canada	2008, 2011*
Bidens pilosa	Mexico	2014
Brachiaria eruciformis	Australia	2014
Bromus diandrus	Australia	2011
Chloris elata	Brazil	2014
Chloris truncata	Australia	2010
Conyza bonariensis	South Africa	2003
	Spain	2004
	Brazil	2005
	Israel	2005
	Colombia	2006
	USA	2007, 2009*
	Greece	2010
	Portugal	2010
	Australia	2010, 2011
Conyza canadensis	USA	2000, 2001, 2002, 2003*, 2005, 2006, 2007*, 2009, 2011, 2013
	Brazil	2005
	China	2006
	Spain	2006
	Czech Republic	2007
	Canada	2010, 2011*

Weed species	**Country**	**Years with documented cases till Sept 2012**
	Italy	2011
	Portugal	2011
	Greece	2012
Conyza sumatrensis	Spain	2009
	Brazil	2010, 2011*
	Greece	2012
Cynodon hirsutus	Argentina	2008
Digitaria insularis	Brazil	2008, 2010
	Paraguay	2005, 2006, 2008
Echinochloa colona	Australia	2007, 2009, 2010
	Argentina	2008
	USA	2008
Eleusine indica	Malaysia	1997*
	Colombia	2006
	China	2010
	USA	2010, 2011
	Argentina	2012
	Malaysia	2014*
Hedyotis verticillata	Malaysia	2005
Kochia scoparia	USA	2007, 2009, 2011, 2012, 2013*
	Canada	2012*
Leptochloa virgata	Mexico	2010
Lolium perenne ssp. multiflorum	USA	2004, 2005, 2008, 2010*, 2012, 2014
	Chile	2001, 2002*, 2006*, 2007*
	Brazil	2003, 2006, 2010*
	Spain	2006
	Argentina	2007, 2010*
	Japan	2011
	Italy	2012*
	New Zealand	2012
Lolium perenne	Argentina	2008
	New Zealand	2012
	Portugal	2013
Lolium rigidum	Australia	1996, 1997, 1999*, 2003, 2008*, 2010*, 2013*

Weed species	**Country**	**Years with documented cases till Sept 2012**
	USA	1998
	South Africa	2001, 2003*
	France	2005
	Israel	2007*
	Italy	2007
	Spain	2006
Parthenium hysterophorus	Colombia	2004
Plantago lanceolata	South Africa	2003
Poa annua	USA	2010, 2011, 2013
Sonchus oleraceus	Australia	2014
Sorghum halepense	Argentina	2005, 2006
	USA	2007, 2008, 2010
Urochloa panicoides	Australia	2008

Looking at the situation in Europe in more detail, currently 16 cases of resistance are known, encompassing six different weed species, the dicotyledonous *Conyza bonariensis*, *C. canadensis* and *C. sumatrensis*, as well as the monocotyledonous *Lolium perenne*, *Lolium perenne spp. multiflorum* and *L. rigidum*. Table B.3.1-2 gives an overview of the reported cases of glyphosate resistance in Europe.

Table B.3.1-2: Glyphosate-resistant biotypes in Europe and cropping situations in which resistance has been observed (source: www.weedscience.org)

Genus and Species	**Reported**	**Country**	**Situation**
Conyza bonariensis	2004	Spain	Orchards
	2010	Greece	Orchards
	2010	Portugal	Orchards
Conyza canadensis	2006	Spain	Orchards
	2007	Czech Republic	Railways
	2011	Italy	Orchards
	2011	Portugal	Olive
	2012	Greece	Grapes/Orchards
Conyza sumatrensis	2009	Spain	Orchards
	2012	Greece	Grapes/Orchards
Lolium perenne ssp. multiflorum	2006	Spain	Orchards
	2012	Italy	Wheat
Lolium perenne	2013	Portugal	Grapes

Genus and Species	Reported	Country	Situation
Lolium rigidum	2005	France	Asparagus, orchards and vineyards
	2006	Spain	Orchards
	2007	Italy	Orchards and vineyards

With one exception (*Lolium perenne ssp. Multiflorum* in wheat in Italy), resistant, resistant biotypes have been found exclusively in orchards, vineyards or olive groves (Italy, Spain and France) or on railways (Czech Republic). All cases have been reported from the last ten years, indicating a growing impact of glyphosate resistance. The most serious problem seems to be *Lolium* species with reported cases from four different countries including a high number of affected sites, although the surface area affected remains small at < 1 %. Up to now no cases of resistant weeds in annual crops were found in the EU. Apart from one case (*C. canadensis)* in the Czech Republic, Northern and Central Europe are not affected by glyphosate resistance up to now.
For all glyphosate-resistant weed species from Europe, research has shown that these particular biotypes may also be cross-resistant to other HRAC Group G herbicides. Multiple resistance seems to be a serious issue in the case of some glyphosate-resistant weeds outside Europe where resistance to up to four different herbicide groups (different modes of action) has been detected.

Analysis of the inherent risk
Also, the above listed cases of glyphosate resistance show clearly that a high resistance risk for glyphosate has to be considered, although the impact in Central Europe is still comparatively low, at least in annual cropping systems. However, the increasing numbers of resistant populations worldwide including populations with multiple resistance to other MoA indicate a considerable and increasing resistance risk for glyphosate. In addition, the number of resistance cases in Europe has rapidly increased over the last few years.
A high risk situation is evident for some of the weed species controlled by glyphosate such as *Lolium* spp. and *Conyza* spp. which have already evolved resistance in Europe. These target species can be considered as being high risk species as they have evolved resistance to many different MoA. *Lolium* spp. and *Conyza* spp. are commonly found in perennial cropping systems and in non-cropped situations including railways.
In consideration of the increasing number of resistance cases worldwide and also in Europe and according to increasing glyphosate use, the inherent risk has to be assessed as being medium.

Analysis of the agronomic risk
According to the EPPO Standard 1/213(2), the analysis of the inherent risk of the active substance is followed by an assessment of the agronomic risk. However, the agronomic risk is predominantly influenced by the agricultural practices and the cropping systems the formulated herbicide product is going to be applied in and will therefore vary greatly between different Member States. An assessment of the agronomic risk therefore has to be conducted at national level with regard to the respective application types and frequency.

However, one can differentiate between the following agronomic situations under which glyphosate is applied:

- Annual cropping systems
- Perennial cropping systems (orchards, olive groves, vineyards)

- Non-crop situations (amenities, railways)

Most cases of glyphosate resistance have been recorded in the USA with considerable numbers also in South America and Australia where glyphosate is frequently applied throughout the year in both perennial (orchards, vineyards) and annual cropping systems (Roundup Ready crop varieties). The fact that all resistant biotypes in Europe have been found in perennial systems indicates that a high risk applies especially for situations in which glyphosate is applied several times a year e.g. on railways or in non-crop situations. However, the frequency of glyphosate application in annual cropping systems has also increased in many European Member States due to the adaption of reduced tillage systems and the increase in weed biotypes resistant to other MoA such as ACCase and ALS inhibitors. In many cropping situations with major resistance problems, glyphosate constitutes an essential part of the annual weed control and is therefore frequently applied in inter-crop situations.

However, the design of the respective crop rotations and the associated frequency of glyphosate application may differ in the various Member States: a national assessment of the agronomic risk is therefore recommended. Compared to arable crops, there therefore seems to be a higher risk for resistance development in perennial systems, under non-crop situations or on railways, as glyphosate may be used as the only herbicide. The implementation of Good Agricultural Practises reduces the agronomic risk.

B.3.2 Data on application relevant to the plant protection product (Annex IIIA 3)

B.3.2.1 Field of use envisaged

Herbicides containing glyphosate are used in agriculture, horticulture, viticulture, forestry, orchards, plantation crops, amenities, home gardening, greenhouses, on aquatic areas, on hard surfaces, on railways, along roads, and on non-cultivated areas. The representative uses in the Annex I renewal include the control of annual, biennial and perennial mono- and dicotyledonous weeds in all seeded or transplanted crops (pre-planting; post-planting pre-emergence of crop), cereals and oilseeds (pre-harvest desiccation), orchards, vines, citrus fruits and nuts. Representative countries (UK, Sweden, France) of the three regulatory zones (central, north, south) are mentioned for representative uses. In all other countries of the EU, glyphosate is authorised for a large number of uses. The main drivers for global glyphosate use in recent years have been the absence of till farming, and outside the EU the development and cultivation of crops genetically modified to be tolerant of glyphosate.

B.3.2.2 Effects on harmful organisms

Glyphosate is a non-selective herbicidal active substance, belonging to the chemical class of glycines, with no soil residual activity. Glyphosate is taken up by the leaves and other green parts of the plant and is translocated systemically (apoplastic and symplastic) in the whole plant, also in underground parts like roots, rhizomes or stolons. Glyphosate is adsorbed immediately by soil particles and rapidly inactivated and degraded by soil micro-organisms. Glyphosate kills the plant by blocking the shikimic acid pathway which leads to a deficient protein biosynthesis. Glyphosate binds to and blocks the activity of its target enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), an enzyme of the aromatic amino acid bio-

synthetic pathway. The inhibition of the enzyme prevents the plant from synthesizing the essential aromatic amino acids needed for protein biosynthesis. EPSPS is present in all plants, bacteria, and fungi, but not animals. Action at the shikimic acid pathway is unique to glyphosate and the absence of this pathway in animals is an important factor of its low animal toxicity.
Reference to B.3.1.5 and B.3.2.8.

B.3.2.3 Details of intended use

Glyphosate is highly effective against the majority of annual and perennial mono- and dicotyledonous weeds. Herbicides containing glyphosate are used in agriculture as foliar sprays, at post-emergence of weeds in a wide range of arable crops (seeded and transplanted) to control a broad spectrum of weeds. The renewal submission only involves a selection of representative crop-related uses covering the majority of the volumes applied. Uses in the representative GAP include applications at pre-planting, post-planting but pre-emergence of crops and post-harvesting of all crops. Pre-harvest uses in cereals, oilseeds and pulses are for desiccation and annual and perennial weed control. Other uses include annual and perennial weed control in orchard crops and vines including olives, citrus fruits and nuts and for grassland renovation.

B.3.2.4 Application rate

Herbicides containing glyphosate are used at different rates in the EU in agriculture, horticulture, viticulture, forestry, orchards, plantation crops, amenities, home gardening, greenhouses, on aquatic areas, on hard surface areas, on railways, along roads, and on non-cultivated areas. According to the listed uses the application rate ranged from 0.72 to 2.88 kg as/ha. The application rate varies across the EU depending on the type and time of application and also on the weed species which are present on the treated area, weed growth stages or the crops which should be managed. Because of the uptake through the leaves, the best efficacy can be achieved if the application is on well developed foliage and especially for perennial weeds, in a period with sugar translocation to roots or other underground parts. One application per growing season is normally used and the weed plants treated at the recommended rate will not start growing again. Symptoms will be seen after 10 to 14 days after application.

B.3.2.5 The maximum recommended field rate of the herbicide and the corresponding rate of the active substance per hectare concentration of active substance in material used

According to the listed uses the application rate ranged from 0.72 to 2.88 kg as/ha. The number of treatments ranged from 1 to 3 per year. The maximum application rate over a 12-month period is stated at 4.32 kg as/ha.

B.3.2.6 Method of application

There are various methods of application of glyphosate including tractor-mounted hydraulic sprayers, handheld sprayers including rotary atomisers and knapsack sprayers, wiper applications, injection systems, droplet applicators and cut-stump treatments for trees. Spray volumes

range between 100 and 400 L/ha for most uses. This is in line with the GAP supported for the renewal of glyphosate.

B.3.2.7 Number and timing of applications and duration of protection

The number of treatments ranged from 1 to 3 per year. The maximum application rate over a 12-month period should not exceed 4.32 kg as/ha. In agriculture and horticulture glyphosate is mainly used at pre-emergence or pre- and post-harvest. In other uses glyphosate can be used during the whole vegetation period.

B.3.2.8 Necessary waiting periods or other precautions to avoid phytotoxic effects on succeeding crops

In the normal crop rotation no effects are expected on any succeeding crops. Glyphosate is an organic phosphorus compound with no soil residual activity. In soils, glyphosate will be adsorbed quickly onto soil particles and inactivated. This was once thought to mean that glyphosate is not biologically active within soils. But it can become unbound again in small amounts. What impact this has on plants and soil microorganisms has not been completely clarified. In soils (field), DT_{50} varies from 3 to 174 days depending on edaphic and climatic conditions. The major metabolite in soil is aminomethylphosphonic acid (AMPA).

Findings have shown that glyphosate can be transferred from the roots of target plants to the rhizosphere and non-target plants can also be influenced (e.g. reduced absorption of micronutrients – Mn and Fe deficiency). Glyphosate is a strong chelator to various divalent cations such as Ca, Fe, Cu and Mn. The available scientific data suggest that the strong affinity of glyphosate and its metabolite AMPA to most soils prevents the uptake of these compounds by root systems of non-target plants.
Glyphosate binds micronutrients in the soil and can cause micronutrient deficiencies in plants that increase their susceptibility to disease, especially on soils with pure nutrient content. It can reduce the plant`s production of lignin and phenolic compounds, which are also important for disease resistance.
Glyphosate can reduce some beneficial organisms such as saprophytic fungi that decompose dead plant material and are important for soil fertility. Studies have shown that glyphosate stimulates the growth of a number of fungal pathogens that cause diseases in crops.

There is a risk of crop damage using glyphosate in direct drilling systems. To avoid phytotoxic effects on crops in pre-emergence uses seeds must be totally covered with soil. If the herbicide comes into contact with seed during application, germination and plant growth are influenced negatively. Considering label recommendations, a glyphosate application is possible up to several days after sowing.
Reference to B.3.1.5 and B.3.2.2.

B.3.2.9 Proposed instructions for use

Glyphosate is a non-selective herbicidal active substance, belonging to the chemical class of glycines. It is active against a range of annual and perennial mono- and dicotyledonous weeds. Glyphosate is sprayed 1 to 3 times during the growing season. The maximum total rate per 12-month period is stated at 4.32 kg as/ha. In most cases, products are applied using trac-

tor-mounted or knapsack, ground-directed sprayers. Typical spray volumes are between 200 to 400 L/ha.

B.3.3 Summary of data on application

B.3.3.1 List of uses evaluated for Annex-I-inclusion

List of representative uses evaluated (Glyphosate)

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	L/ha product* min max	water L/ha min max	g as/ha min max		
All crops** (all seeded or trans-planted crops)	EU		F	Emerged annual, peren-nial and bien-nial weeds	SL	360g/L	Spray	Pre planting of crop	1-2	21d (see remark)	1-6	100-400	0.36-2.16		Spring & autumn after harvest (incl. stubble and/or seedbed prep.) For all crops: Maximum applica-tion rate 4.32 kg glyphosate/ha in any 12 month period across use catego-ries, equivalent to the sum of pre-plant, pre-harvest and post harvest stubble applications The interval between applications is dependent on new weed emergence after the first treat-ment, relative to the time of planting the crop.
All crops** (all seeded crops)	EU		F	Emerged annual, peren-nial and bien-nial weeds	SL	360g/L	Spray	Post planting/ pre emergence of crop	1		1-3	100-400	0.36-1.08		

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	L/ha product* min max	water L/ha min max	g as/ha min max		
Cereals (pre-harvest) wheat, rye, triticale,	EU		F	Emerged annual, perennial and biennial weeds	SL	360g/L	Spray	Crop maturity < 30 % grain moisture	1		2-6	100-400	0.72-2.16	7	Maximum application rate 4.32 kg glyphosate/ha in any 12 month period across use categories, equivalent to the sum of pre-plant, pre-harvest and post harvest stubble applications. Pre-harvest uses in all crops include uses for weed control (higher doses) and harvest aid, sometimes referred to as desiccation (lower doses). The critical GAP is the high dose recommended used for weed control.
Cereals (pre-harvest) barley and oats	EU		F	Emerged annual, perennial and biennial weeds	SL	360g/L	Spray	Crop maturity < 30 % grain moisture	1		2-6	100-400	0.72-2.16	7	
Oilseeds (pre-harvest) rapeseed, mustard seed, linseed	EU		F	Emerged annual, perennial and biennial weeds	SL	360g/L	Spray	Crop maturity < 30 % grain moisture	1		2-6	100-400	0.72-2.16	14	
Orchard crops, vines, including citrus & tree nuts	EU		F	Emerged annual, perennial and biennial weeds	SL	360g/L	Spray	Post emergence of weeds	1-3	28d	2-8	100-400	0.72-2.88	N/A	Stone & pome fruit, olives Applications to avoid contact with tree branches. Maximum cumulative application rate 4.32 kg glyphosate/ha in any 12 month period Note: Because applications are

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	L/ha product* min max	water L/ha min max	g as/ha min max		
															made to the intra-rows (inner strips between the trees within a row), application rates per ha are expressed per 'unit of treated surface area' the actual application rate per ha orchard or vineyard will roughly only be 33%
Orchard crops, vines, including citrus & tree nuts	EU		F	Emerged annual, perennial and biennial weeds	SL	360g/L	(ULV) Sprayer or Knapsack use (spot treatment)	Post emergence of weeds	1-3	28d	2-8	0-400	0.72-2.88		Stone & pome fruit, olives Applications made round base of trunk [0.0 L/ha water addresses ULV application of the undiluted product] Maximum cumulative application rate 4.32 kg glyphosate/ha in any 12 month period Note: Because applications are made round base of trunk and to the intra-rows , (inner strips between two trees within a row),

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	L/ha product* min max	water L/ha min max	g as/ha min max		
															application rates per ha are expressed per 'unit of treated surface area' the actual application rate per ha orchard or vineyard will roughly only be 33 %-50 %

Remarks:
(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*eg.* fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) eg. biting and suckling insects, soil born insects, foliar fungi, weeds
(d) eg. wettable powder (WP), watersoluble granule (WG)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, eg. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, eg. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(i) g/kg or g/L
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) The minimum and maximum number of application possible under practical conditions of use must be provided
* former information on kg as/hL replaced by RMS
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions
** Crops including but not restricted to: root & tuber vegetables, bulb vegetables, stem vegetables, field vegetables (fruiting vegetables, brassica vegetables, leaf vegetables and fresh herbs, legume vegetables), pulses, oil seeds, potatoes, cereals, and sugar- & fodder beet; before planting fruit crops, ornamentals, trees, nursery plants etc.

B.3.3.2 Recommended methods and precautions concerning handling, storage, transport or fire (Annex IIA 3.7)

Reference:
Anonymous (2011), Safety data sheet – Glyphosate Acid Wetcake,
Part of Anonymous (2011), Doc MIIA Section 1, Point 3, EGT (BVL no 2312084)

B.3.3.2.1 Handling

Avoid contact with skin and eyes.
Wear suitable protective clothing, gloves and eye/face protection.
When using do not eat, drink or smoke.
Wash hands immediately after handling.
Wash contaminated clothing before re-use.
Do not mix, store or apply this product in galvanised steel or unlined steel containers (except stainless steel) as the material will react with such containers to produce hydrogen, an explosive gas. Should be mixed, stored and applied in stainless steel, aluminium, fibreglass, plastic or plastic lined steel containers.
Do not re-use supply container.
Thoroughly clean equipment with water after use.
Do not contaminate drains, sewers and water ways when disposing of equipment rinse water.
Emptied packages retain product residue and dust.

B.3.3.2.2 Storage

Maximum storage temperature: 50 °C
Compatible materials for storage: stainless steel, fibreglass, plastic, glass lining
Incompatible materials for storage: galvanised steel, unlined mild steel
Keep out of reach of children.
Keep away from food, drink and animal feed.
Keep container tightly closed and in a well ventilated place.
Keep only in the original container.

B.3.3.2.3 Transport

The data provided in this section is for information only. Please apply the appropriate regulations to properly classify your shipment for transportation.

Land transport ADR/RID:	Environmentally hazardous substance, solid, N.O.S. UN No.: UN3077; Class: 9; Kemler: 90; Packing Group: III
Maritime transport IMDG:	Environmentally hazardous substance, solid, N.O.S. UN No.: UN3077; Class: 9; Packing Group: III MARINE POLLUTANT
Air transport IATA:	Environmentally hazardous substance, solid, N.O.S. UN No.: UN3077; Class: 9; Packing Group: III MARINE POLLUTANT

B.3.3.2.4 Fire fighting measures

Extinguishing media:	Recommended: water, foam, dry chemical, carbon dioxide (CO2)
Unusual fire and explosion hazards:	Minimise use of water to prevent environmental contamination.
Hazardous products of combustion:	Carbon monoxide (CO), phosphorus oxides (P_xO_y), nitrogen oxides (NO_x)
Fire fighting equipment:	Self-contained breathing apparatus. Equipment should be thoroughly decontaminated after use.

B.3.3.3 Procedures for destruction or decontamination (Annex IIA 3.8)

Spillages are contained by adsorbing into earth or sand. Sweep up into container (do not store in galvanised steel or unlined steel containers) for disposal. Collect waste onto earth. Shovel up into covered containers for disposal. Burn in licensed incinerator. Do not emty into drains. Do not contaminate waterways. Wear suitable clothing when handling active substance.

B.3.3.4 Controlled incineration

Complete pyrolysis of glyphosate occurs after 15 minutes at 800 °C. The major product is carbon dioxide, other volatiles included acetonitrile, alkylpyrazines and alkylpyridines.

B.3.3.5 Others

See B.3.3.3 above

B.3.3.6 Emergency measures in the case of an accident (Annex IIA 3.9)

Reference:
Anonymous (2011), Safety data sheet – Glyphosate Acid Wetcake,
Part of Anonymous (2011), Doc MIIA Section 1, Point 3, EGT (BVL no 2312084)

<u>First-aid measures:</u>

General information:	This product is not an inhibitor of cholinesterase. Treatment with atropine and oximes is not indicated.
After eye contact:	If in eyes, hold eye open and rinse slowly and gently for 15 – 20 minutes. Remove contact lenses, if present, after first 5 minutes, then continue rinsing. Call a poison control center or doctor for treatment advice.
After skin contact:	Take off contaminated clothing. Rinse skin immediately with plenty of water for 15 – 20 minutes. Call a poison control center or doctor for treatment advice.

After inhalation:	If inhaled, move person to fresh air. If person is not breathing, call emergency number or ambulance, then give artificial respiration, preferably mouth-to-mouth, if possible. Call a poison control center or doctor for treatment advice.
After ingestion:	Call a poison control center or doctor immediately for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to do so by the poison center or doctor. Do not give anything by mouth to an unconscious person.

Accidental release measure:

Personal precautions:	Keep upwind of spillage.
Environmental precautions:	Small quantities: Low environmental hazard. Large quantities: Minimise spread. Notify authorities.
Methods for cleaning up:	Small quantities: Flush spill area with water. Large quantities: Dig up heavily contaminated soil. Collect in containers for disposal. Flush residues with small quantities of water. Minimise use of water to prevent environmental contamination.

Glyphosate disperses quickly and partitions into the sediment phase where it is biodegraded. In addition glyphosate is removed from water by most routine/conventional water treatment steps. The presence of glyphosate in surface water sources for the production of drinking water has been observed and research has been done to evaluate its removal in treatment processes commonly used for drinking water production from surface water. The performance of commonly used water treatment has been reviewed by Hall and Camm, 2007 (see doc K, Annex IIIA, 9.6.4).

B.3.4 Further information on the plant protection product (Annex IIIA 4)

B.3.4.1 Packaging and compatibility with the preparation (OECD IIIA 4.1)

B.3.4.1.1 Description and specification of the packaging and materials used in packaging, size, capacity, size of openings, types of closure and seals (OECD IIIA 4.1.1)

The following containers are available for professional use:

1 L bottle:	Material:	HDPE
	Shape/size:	Round and rectangular
	Opening:	63 mm
	Closure:	HDPE screw cape top with induction seal
5 L, 10 L container:	Material:	HDPE
	Shape/size:	Rectangular
	Opening:	63 mm
	Closure:	HDPE screw cape top with induction seal
20 L container:	Material:	HDPE
	Shape/size:	Rectangular
	Opening:	61 mm
	Closure:	HDPE screw cape top with foam seal
60 L, 120 L, 200 L drum:	Material:	HDPE
	Shape/size:	Drum
	Opening:	50.8 mm (2 inches)
	Closure:	Screw in plugs
640 L, 1000 L IBC:	Material:	HDPE
	Shape/size:	Rigid IBC

B.3.4.1.2 Suitability of packaging and closures: strength; leakproofness; resistance to normal transport and handling (OECD IIIA 4.1.2)

Reference:
Midgley (2012), Packaging suitability testing of MON 52276 with 1-litre high density polyethylene bottles, MPW 1536, EGT (BVL no 2315969)

A 1.0 litre HDPE commercial pack was tested in the physical and chemical properties and storage stability tests (reference section AIII 2.1 & 2.2; Bates, 2001 & Alexander, 1992). At the start of the test and after 14 days accelerated storage and after 2 year storage the packaging material showed no deterioration, had not been altered by the test item, and there was no sign of contamination on the outer surface or of leakage during shaking or turning. This small pack is of the same material as the larger packs and is considered representative of the complete range.
The data from Alexander, 1992 and Bates, 2001 relevant to the suitability and resistance of the packaging was summarised in Midgley, 2012 (BVL no 2315969, see reference above).
All the containers listed in 4.1.1 are UN approved for substances which are non-classified. MON 52276 is not classified for transport following ADR regulations.
In addition the packaging of MON 52276 has been tested to meet transport requirements. The suitability of the packaging, including closures, in terms of strength, leakage and resistance to normal transport and handling has been determined according to ADR Methods 3552, 3553, 3554, 3555, 3556 and 3558 as part of the normal manufacturing procedures.
Reference:
Verbist (1993), Packaging MON 52276, MOD (CHE9700144)

Packaging has to be found to be suitable using appropriate methods.

B.3.4.1.3 Resistance of the packaging material to its contents (OECD IIIA 4.1.3)

Dokument M:
Shelf life testing has been conducted under accelerated ageing conditions and up to 2 years at 20 °C, using representative standard HDPE packaging. The results showed no concerns (see B.3.5.1.2).

DAR:
Shelf life testing according to GIFAP Technical Monograph has been carried out at Inveresk Research International using HDPE packaging. No adverse reaction was reported by the laboratory and the product reviewed stable for two years of storage.

B.3.4.2 Procedures for cleaning application equipment (OECD IIIA 4.2)

It is essential to thoroughly clean out the entire sprayer system, using a detergent cleaner. As glyphosate is a water soluble compound normal detergent washing of clothing should remove any contamination.

B.3.4.2.1 Procedures for cleaning application equipment and protective clothing (OECD IIIA 4.2.1)

It is essential to thoroughly clean out the entire equipment and protective clothing: sprayer system, using a detergent cleaner. Equipment and clothing cleaning should take place in designated areas or in the field avoiding surface water contamination.

B.3.4.2.2 Effectiveness of the cleaning procedures (OECD IIIA 4.2.2)

Reference:
Van Den Heuvel (2001), Pressure rinsing of the 20L MONAGL container according to the CEN directive EN 12761-3:2001, MPW 1066, EGT (BVL no 2315971)

The product is soluble in water. It can be removed from surfaces with water.

The effectiveness of the cleaning of containers was demonstrated by rinsing of a 20L MONAGL container (2 bar during 15 s, rotating tank cleaning nozzle). An efficiency of >99.99 % was observed.

B.3.4.3 Re-entry periods, necessary waiting periods or other precautions to protect man, livestock and the environment (OECD IIIA 4.3)

The following safety intervals as defined in OECD IIIA point 4.3 are adequately covered by information described in chapters mentioned below.
- pre-harvest interval for each relevant crop
  see chapters B.7.4 and B.7.10
- re-entry period for livestock to areas to be grazed
  see chapters B.7.4 and B.7.10
- re-entry period for man to crops, building or spaces treated
  see chapter B.6.14
- withholding period from animal feeding stuffs
  see chapters B.7.4 and B.7.10
- waiting period between application and handling to treated products
  see chapters B.7.4 and B.7.10
- waiting period between last application and sowing or planting succeeding crops
  see chapter B.3.2.8.

B.3.4.4 Statement of the risks arising from the recommended methods, precautions and handling procedures to minimise those risks, relating to (OECD IIIA 4.4)

Reference:
Anonymous (2012), Safety data sheet – MON 52276, MOT (BVL no 2315945)
Part of Anonymous (2011), Doc MIIA Section 1, Point 3, EGT (BVL no 2312084)

B.3.4.4.1 Warehouse storage (OECD IIIA 4.4.1)

Good industrial practice in housekeeping and personal hygiene should be followed.

Safe handling:	When using do not eat, drink or smoke. Wash hands thoroughly after handling or contact. Wash contaminated clothing before re-use. Thoroughly clean equipment after use. Do not contaminate drains, sewers and water ways when disposing of equipment rinse water. Emptied containers retain vapour and product residue. Follow labelled warnings even after container is emptied.
Storage:	Minimum storage temperature: -15 °C Maximum storage temperature: 50 °C Compatible materials for storage: stainless steel, fibreglass, plastic, glass lining Incompatible materials for storage: galvanised steel, unlined mild steel Keep out of reach of children. Keep away from food, drink and animal feed. Keep only in the original container. Partial crystallisation may occur on prolonged storage below the minimum storage temperature. If frozen, place in warm room and shake frequently to put back into solution. This formulation can be stored for 2 weeks to 3 weeks at temperatures colder than -20 °C without impact. If the temperature remains below -20 °C for longer the water phase of the formulation may freeze. Should this occur allow the product to warm and it will return to its original homogeneous state. We recommend that customers follow the typical use instructions which state that the container should be agitated (shaken) prior to pouring.

B.3.4.4.2 User level storage (OECD IIIA 4.4.2)

See recommendation described under B.3.4.4.1.

B.3.4.4.3 Transport (OECD IIIA 4.4.3)

Land transport ADR/RID:	Not regulated
Maritime transport IMDG:	Not regulated
Air transport IATA:	Not regulated

The data provided in this section is for information only. Please apply the appropriate regulations to properly classify your shipment for transportation.

B.3.4.4.4 Fire (OECD IIIA 4.4.4)

Extinguishing media:	Recommended: water, foam, dry chemical, carbon dioxide (CO2)
Unusual fire and explosion hazards:	Minimise use of water to prevent environmental contamination.
Hazardous products of combustion:	Carbon monoxide (CO), phosphorus oxides (P_xO_y), nitrogen oxides (NO_x)
Fire fighting equipment:	Self-contained breathing apparatus. Equipment should be thoroughly decontaminated after use.

B.3.4.4.5 Protective clothing and equipment proposed for use in storage, transport or in the event of fire – nature (OECD IIIA 4.4.5)

Engineering measures:	No special requirement when used as recommended.
Respiratory protection:	No special requirement when used as recommended.
Hand protection:	If repeated or prolonged contact: Wear chemical resistant gloves. Chemical resistant gloves include those made of waterproof materials such as nitrile, butyl, neoprene, polyvinyl chloride (PVC), natural rubber and/or barrier laminate.
Eye protection:	No special requirement when used as recommended. Wear chemical resistant googles
Skin and body protection:	No special requirement when used as recommended. Wear professional clothing (cotton coverall), water- or chemical resistant clothing and sturdy footwear.

B.3.4.4.6 Protective clothing and equipment proposed for use in storage, transport or in the event of fire – characteristics (OECD IIIA 4.4.6)

Please refer to B.3.4.4.5.

B.3.4.4.7 Sufficient data to evaluate suitability and effectiveness of the protective clothing and equipment under realistic conditions of use (OECD IIIA 4.4.7)

Please refer to B.3.4.4.5.

B.3.4.4.8 Procedure to minimise the generation of waste (OECD IIIA 4.4.8)

Only purchase and store quantities of product required for the short term in order to minimise the generation of waste. Do not open larger containers than is necessary for immediate requirements. Do not mix a volume of spray solution greater than is required for immediate use.

B.3.4.4.9 Information on combustion products likely to be generated in the event of fire (OECD IIIA 4.4.9)

Materials to avoid/ Reactivity:	Reacts with galvanised steel or unlined mild steel to produce hydrogen, a highly flammable gas that could explode.
Chemical stability:	Stable under normal conditions of handling and storage.
Oxidizing properties:	None
Hazardous products of combustion:	Carbon monoxide (CO), phosphorus oxides (P_xO_y), nitrogen oxides (NO_x)

B.3.4.5 Detailed procedures for the use in the event of an accident during transport, storage or use (OECD IIIA 4.5)

Reference:
Anonymous (2012), Safety data sheet – MON 52276, MOT (BVL no 2433956)

B.3.4.5.1 Containment of spillages (OECD IIIA 4.5.1)

Person-related precaution measures:	Use personal protection.
Environment precautions:	Small quantities: Low environmental hazard. Large quantities: Minimise spread. Keep out of drains, sewers, ditches and water ways. Notify authorities.
Methods for cleaning up/taking up:	Place leaking containers in oversize leakproof drums for transport. Small quantities: Flush spill area with water. Large quantities: Absorb in earth, sand or absorbent material. Dig up heavily contaminated soil. Collect in containers for disposal. Flush residues with small quantities of water. Minimise use of water to prevent environmental contamination.

B.3.4.5.2 Decontamination of areas, vehicles and buildings (OECD IIIA 4.5.2)

Please refer to B.3.4.5.1

B.3.4.5.3 Disposal of damaged packaging, adsorbents and other materials (OECD IIIA 4.5.3)

Product:	Recycle if appropriate facilities/equipment available. Burn in special, controlled high temperature incinerator. Dispose of as hazardous industrial waste. Keep out of drains, sewers, ditches and water ways. Follow all local/regional/national/international regulations.
Package:	See the individual container label for disposal information. Empty packaging completely. Triple or pressure rinse empty containers. Pour rinse water into spray tank. Ensure packaging cannot be reused. Do not re-use containers. Store for collection by approved waste disposal service. Emptied containers product residue. Observe all labelled safeguards until container is cleaned, re-conditioned or destroyed. Follow all local/regional/national/international regulations.

B.3.4.5.4 Protection of emergency workers and bystanders (OECD IIIA 4.5.4)

Use the recommended personal protective equipment.

B.3.4.5.5 First aid measures (OECD IIIA 4.5.5)

After inhalation:	Remove to fresh air and seek medical attention immediately.
After eye contact:	Flush immediately with plenty of water for at least 15 minutes. If easy to do, remove contact lenses. Seek medical attention if irritation persists.
After skin contact:	Take off contaminated clothing, wristwatch, jewellery. Wash affected skin with plenty of water. Wash clothes and clean shoes before re-use.
After ingestion:	Immediately offer water to drink. Never give anything by mouth to an unconscious person. Do not induce vomiting unless directed by medical personnel. If symptoms occur, get medical attention. Seek medical attention immediately and show label, container or this datasheet.
Over exposure:	No symptoms diagnostic of systemic poisoning are apparent with this product.
Guide to doctor:	No specific antidote known. Treat symptomatically. The product does not inhibit cholinesterase and treatment with atropine or oximes is not indicated.

B.3.4.6 Neutralisation procedures (e.g. reaction with alkali to form less toxic compounds) for use in the event of accidental spillages (OECD IIIA 4.6)

B.3.4.6.1 Details of proposed procedures for small quantities (OECD IIIA 4.6.1)

The product have a neutral pH. Therefore no special measures for neutralisation are required.

Spillages are contained by adsorbing into earth or sand. Sweep up into container (do not store in galvanised steel or unlined steel containers) for disposal. Collect waste onto earth. Shovel up into covered containers for disposal. Burn in licensed incinerator. Do not emty into drains. Do not contaminate waterways. Wear suitable clothing when handling active substance.

B.3.4.6.2 Evaluation of products of neutralisation (small quantities) (OECD IIIA 4.6.2)

See B.3.4.6.1 above

B.3.4.6.3 Procedures for disposal of neutralised waste (small quantities) (OECD IIIA 4.6.3)

See B.3.4.6.1 above

B.3.4.6.4 Details of proposed procedures for large quantities (OECD IIIA 4.6.4)

See B.3.4.6.1 above

B.3.4.6.5 Evaluation of products of neutralisation (large quantities) (OECD IIIA 4.6.5)

See B.3.4.6.1 above

B.3.4.6.6 Procedures for disposal of neutralised waste (large quantities) (OECD IIIA 4.6.6)

See B.3.4.6.1 above

B.3.4.7 Pyrolytic behaviour of the active substance under controlled conditions at 800 °C and the content of polyhalogenated dibenzo-p-dioxins in the products of pyrolysis (OECD IIIA 4.7)

Complete pyrolysis of glyphosate occurs after 15 minutes at 800 °C. The major product is carbon dioxide, other volatiles included acetonitrile, alkylpyrazines and alkylpyridines.

B.3.4.8 Disposal procedures for the plant protection product (OECD IIIA 4.8)

See B.3.4.5.3 above

B.3.4.8.1 Detailed instructions for safe disposal of the plant protection product and its packaging (OECD IIIA 4.8.1)

Collect in dry containers and close securely. Dig up heavily contaminated soil and place in drums. Treat as chemical waste. Flush spill area with water.

The product can be disposed safely by burning in a high temperature incinerator equipped with efficient gas scrubbing. Incineration products are likely to be carbon dioxide, Phosphates, nitrates and water. Please compy with the national or regional authorities. Consideration of content of halogens is not relevant given the molecular structure of glyphosate. Unused undiluted product and contaminated un-rinsed packaging should be treated as hazardous waste, and should be disposed of by controlled incineration or according to local regulations. Where large quantities of unused product are concerned, consult the supplier.

B.3.4.8.2 Methods other than controlled incineration for disposal: detailed description of such methods; data to establish their effectiveness and safety (OECD IIIA 4.8.2)

Not relevant.

No other methods are proposed to dispose of the formulation. Consult the supplier where greater quantities have to be disposed of.

B.3.4.9 Other/special studies (OECD IIIA 4.9)

No other/special studies.

B.3.5 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIIA1 4.1	Midgley, B.	2012	Packaging suitability testing of MON 52276 with 1-litre high density polyethylene bottles MPW 1536 GLP: N, published: N 2315969	Y	EGT
KIIIA1 4.2.2	Van Den Heuvel, F.	2001	Pressure rinsing of the 20L MONAGL container according to the CEN directive EN 12761-3:2001 MPW 1066 GLP: N, published: N 2315971	Y	EGT

[1] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIIA1 4.4 KIIIA1 4.5	Anonymous	2012	Safety data sheet - MON 52276 GLP: N, published: N 2315945 / 2433956	Y	MOT

Codes of owner

EGT European Glyphosate Task Force AIR 2
MOT Monsanto Agrar

Renewal Assessment Report

18 December 2013

NEW
29 January 2015

Glyphosate

Volume 3

Annex B.3 (Appendix)

Evaluation of open literature regarding efficacy

RMS: Germany **Co-RMS: Slovakia**

Version history (Volume 3, chapter B.3)

Date	**Reason for revision**
19 December 2013	Initial Renewal Assessment Report
29 January 2015	New Appendix created following RAR peer review, taking into account the reporting table, the evaluation table and additional data

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B Rapporteur Member State summary, evaluation and assement of the data and information

B.3 Data on application and further information

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The following studies have not been submitted specifically for the efficacy section of the RAR but were reviewed in order to check for their relevance regarding the specific annex points.

1. Succeeding crops

The following studies focus on the effect of glyphosate on succeeding crops. The submitted documents provide no new scientific evidence. A change or amendment in the assessmnent is not necessary.

Jaskulski & Jaskulska, 2011

Author(s)	**Year**	**Study title**
Jaskulski, D., Jaskulska, I.	2011	Effect of pre-harvest glyphosate application on grain germination and emergence of winter wheat self-sown plants Progress in Plant Protection/Postepy w Ochronie Roslin 51(2) Language: polish
Study type		Research article
Guidelines		Not stated
Guideline deviations		Not applicable
GLP		Not stated

Abstract
The aim of performed research was to determine the effect of pre-harvest application of glyphosate on the germination and emergence of winter wheat grain stored for two years, considering limiting the risk of the occurrence of self-sown plants. It was found that glyphosate had an unfavourable effect on the germination energy of grain and growth of radicles, and the effect persisted during the period of grain storage. After 24 months also its germinability decreased. Winter wheat emergence and seedling growth were limited by the plantation desiccation using 2 kg of glyphosate per ha.

KLIMISCH EVALUATION

1. Relevance of study:	**Not be in rating, paper in polish language**
2. Reliability of study:	**Not be in rating, paper in polish language**
3. Klimisch code:	**Not be in rating, paper in polish language**

Author(s)	**Year**	**Study title**
Tesfamariam, T., Bott, S., Römheld, V., Neumann, G.	2009	Fate of glyphosate stored in weed residues and the potential of phytotoxicity for following crops The Proceedings of the International Plant Nutrition Colloquium XVI, Department of Plant Sciences, UC Davis, UC Davis http://escholarship.org/uc/item/6b02p0xt

Study type		Research article
Guidelines		Not stated
Guideline deviations		Not applicable
GLP		Not stated

Tesfamariam et al, 2009

Abstract
Glyphosate, a broad spectrum, non-selective herbicide, is the world´s most important and widely used herbicide. The globally increasing adoption of no-till or reduced tillage systems is becoming a driving force for enhanced glyphosate use. In such systems, glyphosate is applied pre-sowing for weed control and glyphosate may remain in root and shoot residues. To evaluate potential risks associated with glyphosate residues, a pot experiment was conducted under controlled greenhouse conditions with two contrasting soils: weakly buffered acidic Arenosol and highly buffered Luvisol. Glyphosate was supplied as glyphosate enriched rye grass straw (1.2 g DM kg-1 soil) prior to sowing sunflower as a non-target plant. Several physiological parameters such as intracellular shikimate accumulation as a metabolic indicator for glyphosate toxicity, biomass production and micronutrient status were analyzed. Detrimental effects on sunflower plants linked to glyphosate toxicity were observed only in the Arenosol but not in the Luvisol. This is most probably related to the difference in soil properties. The detoxification capacity of the finetextured Luvisol, with a high clay content, was high enough for an adequate immobilization and inactivation of glyphosate. On the sandy Arenosol, the level of glyphosate supply exceeded the detoxification capacity. In addition to the difference in detoxification capacity, differences in nutrient bio-availability might also have aggravated the observed inhibition of nutrient acquisition. Thus, the findings suggest the importance of weed residues in transferring glyphosate from target to nontarget plants, particularly in no-till or reduced tillage systems, with the consequence of detrimental effects on following crop plants.

MATERIALS AND METHODS

1. Test material:

Test item:	Glyphosate
Active substance(s):	Glyphosate
Surfactant:	Not stated
Description:	Not stated
Source of test substance:	Not stated
Lot/Batch #:	Not stated
Purity:	Not stated

2. Vehicle and/or positive control: None

3. Test organism:

Species:	Rye grass (Lolium perenne L.) as target plant; Sunflower (Helianthus annuus L.) as non target plant

4. Test system:

Study type:	Research article; greenhouse study
Guideline:	Not stated
GLP:	Not stated
Guideline deviations:	-
Duration of study:	27 days from glyphosate treatment on rye

	grass to final analysis.
Test conditions:	Target plant rye grass in nutrient solution was sprayed with 15.6 mL of 28.4 mM glyphosate solution, 12 h later harvested and mixed soil for sunflower seeds. 7 seeds were directly sown in pots of 500 g soil (with or without rye grass). 10 days after sowing, 5 seedling were taken for shikimate analysis and 26 days after application, 2 plants were taken for mineral analysis.
Replicates per concentration:	4
Organisms per replicate:	7 sunflower seeds/pot
Parameters measured:	shikimate analysis 10 days after application, mineral analysis 26 days after application.
Test concentrations:	Rye grass leaf surface area was calculated to be 7802 cm2 per pot and therefore each pot was treated with 15.6 mL of a 28.4 mM solution of glyphosate corresponding to recommended application rate of 200 L of 28.4 mM glyphosate solution per ha.
Application:	not stated
Application devices:	not stated
Application verification:	not stated
Analytical determination of test concentrations:	not stated

5. Environmental conditions:

Temperature:	Not stated
Photoperiod:	Not stated
Light intensity	Not stated
Soil texture, organic matter, pH:	Not stated

6. Observations/analyses:

Measurements:	
Statistics:	The results shown in the text, tables and figures are expressed as the mean ± standard error (SEM). No further information Differences among treatment groups were tested by one way analysis of variance (ANOVA). The statistical analysis was carried out using the software program BioEstat 5.0 and GraphPad Prism 5.

KLIMISCH EVALUATION

1. Relevance of study: **Relevant with restrictions**

	Comment:	This publication is a proceedings paper, summarizing a research presented at the conference. Therefore, it does not sufficiently describe the experimental details and can be taken into account for conclusion on environmental risks with restrictions. The aspect of glyphosate stored in weed residues and the potential of phytotoxicity for following crops was already assessed in the original RAR.
2. Reliability of study:		**Not Reliable**
	Comment:	Documentation of material and methods is poor and kept to a minimum. It remains unknown what glyphosate formulation was used. Formulation is of unknown content of active substance, adjuvants or surfactants. No validation of concentrations of glyphosate in the soil/rhizosphere.
3. Klimisch code:		**Klimisch rating of 3**

Truta et al., 2010

Author(s)	**Year**	**Study title**
Truta, E., Vochita, G., Rosu, C.M., Zamfirache, Olteanu, Z.	2010	Evaluation of roundup-induced toxicity on genetic material and on length growth of barley seedlings Acta Biologica Hungaria 62(3), 290-301. DOI: 10.1556/ABiol.62.2011.3.8
Study type		Research article
Guidelines		Not stated
Guideline deviations		Not stated
GLP		Not stated

Abstract
The study was performed in order to evaluate Roundup-induced genotoxic effects in *Hordeum vulgare* L. cv *Madalin* root meristems and to analyze herbicide impact on length growth of barley seedlings. Caryopses were treated for 3 hours and 6 hours with 0.1%, 0.5%, 1.0% and 2.0% Roundup solutions (v/v), containing 0.36 mg ml–1, 1.8 mg ml–1, 3.6 mg ml–1 and 7.2 mg ml–1 glyphosate active ingredient. Mitotic index decreased in both exposure times with concentration increase. In 3-h treatment, its average values decreased from 4.73±0.31% to 1.51±0.43%, whereas in 6-h treatment this parameter declined from 3.86±0.92% to 0.62±0.15%. The highest ana-telophase aberration rates were noted in 3-h treatments (8.91%, 9.19%, 9.47%, 11.25%, comparatively to control – 5.99%). Roundup enhanced the number of metaphase disturbances proving its noxious effect on normal functioning of mitotic spindle. Seedling growth was negatively influenced at all tested concentrations in both exposure times.

The length decreased as concentration increased, so that the average length is 7.5– 9 times smaller than in control at the maximum concentration, in both exposures.

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup
Active substance(s):	Glyphosate
Surfactant:	Not stated
Description:	Not stated
Source of test substance:	Not stated
Lot/Batch #:	Not stated
Purity:	Not stated

2. Vehicle and/or positive control: none

3. Test organism:

Species:	Hordeum vulgare L., cultivar Madalin
Age of test organisms at study initiation:	Age of the study organisms is not mentioned.

4. Test system:

Study type:	Research article presents effects of glyphosate on length growth of barley seedlings.
Guideline:	Not stated
GLP:	Not stated
Guideline deviations:	-
Duration of study:	7 days.
Test conditions:	Petri dishes were covered and maintained at 20 °C. Filter paper was daily moistened with distilled water.
Replicates per concentration:	No information
Organisms per replicate:	-
Parameters measured:	<u>Cytogenetic analysis</u> Root tips were squashed in a drop of 45% acid acetic, and then analyzed at Nikon Eclipse 600 microscope and photographed at 100× objective, with Nikon Cool Pix 950 digital camera. Five slides (*n* = 5) in each variant and 10 fields for each preparation were microscopically analyzed, to estimate the mitotic index and chromosome aberrations. <u>Length growth</u> After 7-d growth, shoot length of barley seedlings was measured from culms base to the tip of the longest leaf. As for MI, inhibitory rate (%) was calculated
Test concentrations:	Commercial formulation of Roundup is a liquid water-soluble organophosphorus herbicide, containing glyphosate [N

	(phosphonomethyl)glycine], C3H8NO5P – as active ingredient. In field, Roundup is applied at concentrations ranging from 0.26 to 1.152% a.i.. In the experiment, from commercial formulation containing 360 g/L glyphosate, water solutions of Roundup were prepared (0.1%, 0.5%, 1.0% and 2.0%, v/v), to the following concentrations of active ingredient: 0.36, 1.80, 3.60, respectively, 7.20 mg ml^{-1}.
Application:	not stated
Application devices:	not stated
Application verification:	not stated
Analytical determination of test concentrations:	not stated

5. Environmental conditions:

Temperature:	Not stated
Photoperiod:	Not stated
Light intensity	Not stated
pH:	Not stated

6. Observations/analyses:

Measurements:	Cytogenetic analysis, length growth
Statistics:	Data were expressed as mean ± standard error of the means (±SE) for all groups of investigated parameters. Statistical analysis also consisted in calculation of Pearson's correlation coefficient (*r*) and of regression equation in the case of two studied variables: shoot length – MI; MI – herbicide concentration; shoot length – herbicide concentration. The *r* coefficient is a measure of the strength of linear dependence between two variables, while the regression equation mainly evidences the relations of cause-effect type existing between the two variables. Formula of this equation is $(y) = a + bx$, where *x* and *y* are the variables, *b* is the slope of the regression line, and a represents the intercept point of the regression line and the *y* axis. To calculate and to graphically represent the statistical parameters, the Microsoft Office Excel 2003 software of Windows XP operating system was used.

KLIMISCH EVALUATION

1. Relevance of study: **Not relevant**

	Comment:	Evaluation of roundup-induced toxicity on genetic material and on length growth of barley seedlings are not in the focus of the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant for the assessment of glyphosate efficacy.
2. Reliability of study:		**Not Reliable**
	Comment:	Not all relevant details on the investigation are reported in the present publication. Studies were not carried out according to OECD guideline and under GLP conditions.
3. Klimisch code:		**Klimisch rating of 3**

2. Weed recognition

The following studies focus on weed recognition before and after herbicide application. Image analysis and sensor based weed recognition is not in the focus of the efficacy evaluation for glyphosate.

Ali et al., 2013

Author(s)	**Year**	**Study title**
Ali, A., Streibig, J.C., Duus, J., Andreasen, C.	2013	Use of Image Analysis to Assess Color Response on Plants Caused by Herbicide Application Weed Technology 2013 27:604–611
Study type		Research article
Guidelines		Not stated
Guideline deviations		Not stated
GLP		Not stated

Abstract
In herbicide-selectivity experiments, response can be measured by visual inspection, stand counts, plant mortality, and biomass. Some response types are relative to nontreated control. We developed a nondestructive method by analyzing digital color images to quantify color changes in leaves caused by herbicides. The range of color components of green and nongreen parts of the plants and soil in Hue, Saturation, and Brightness (HSB) color space were used for segmentation. The canopy color changes of barley, winter wheat, red fescue, and brome fescue caused by doses of a glyphosate and diflufenican mixture, cycloxydim, diquat dibromide, and fluazifop-p-butyl were described with a log-logistic dose response model, and the relationship between visual inspection and image analysis was calculated at the effective doses that cause 50% and 90% response (ED50 and ED90, respectively). The ranges of HSB components for the green and nongreen parts of the plants and soil were different. The relative potencies were not significantly different from one, indicating that visual and image analysis estimations were about the same. The comparison results suggest that image analysis

can be used to assess color changes of plants in response to some herbicides and may have the potential to provide an objective measurement of symptoms.

MATERIALS AND METHODS

1. Test material:

Test item:	Glyphosate, others
Active substance(s):	Glyphosate, others
Surfactant:	Not stated
Description:	Not stated
Source of test substance:	Not stated
Lot/Batch #:	Not stated
Purity:	Not stated

2. Vehicle and/or positive control: none

3. Test organism:

Species:	Weeds
Age of test organisms at study initiation:	Age of the study organisms is not mentioned.

4. Test system:

Study type:	Research article presents the Use of Image Analysis to assess color response on plants caused by herbicide application
Guideline:	Not stated
GLP:	Not stated
Guideline deviations:	-
Duration of study:	Images were taken 14 d after application (DAA) of herbicides
Test conditions:	Greenhouse experiments The temperature was kept at 15/13 C day/night temperatures. The pots were placed on tables with automatic watering, supplying nutrients in the form of liquid fertilizer (N–P–K: 5-1-4 plus Mg and S). Three weeks after germination, the pots were sprayed with diquat dibromide, when the plants were at 15 scale (BBCH) stage of development . The herbicide was sprayed (47, 94, 188, 375, 751, 1,502, 3,004 g ai ha_1) with a pot sprayer. Field experiments Spring barley and winter wheat were grown in strips that were 30 m by 1.5 m and were separated by controlled strips that were 1.5 m wide, with two replicates for each herbicide. The selected strips were sprayed at the 15 BBCH stage of development with various herbicides.
Replicates per concentration:	3
Organisms per replicate:	-
Parameters measured:	Non destructive method by analyzing digital

	color images to quantify color changes in leaves caused by herbicides length growth.
Test concentrations:	The herbicide was sprayed (47, 94, 188, 375, 751, 1,502, 3,004 g ai ha_1) with a pot sprayer.
Application:	not stated
Application devices:	pot sprayer
Application verification:	not stated
Analytical determination of test concentrations:	not stated

5. Environmental conditions:

Temperature:	15/13 C day/night in the greenhouse experiment.
Photoperiod:	Not stated
Light intensity	Not stated
pH:	Not stated

6. Observations/analyses:

Measurements:	digital color images
Statistics:	Dose–response curves were fitted for each herbicide and species. A nonlinear, log-logistic model was used to describe the response. The percentage of change in the green color of the crops y, estimated by visual ratings or image analysis, was regressed on the doses. The analyses were done in an open-source statistical software R, version 2.10.1 (R Development Core Team), and the drc add-on package (www.r-project. org).

KLIMISCH EVALUATION

1. Relevance of study:	**Not relevant**
Comment:	Use of Image Analysis to Assess Color Response on Plants Caused by Herbicide Application is not in the focus of the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant for the assessment of glyphosate efficacy.
2. Reliability of study:	**Not reliable**

Comment: Not all relevant details on the investigation are reported in the present publication.

Studies were not carried out according to OECD guideline and under GLP conditions.

3. Klimisch code: **Klimisch rating of 3**

Streibig et al., 2014

Author(s)	**Year**	**Study title**
Streibig, J.C., et al.	2014	Sensor-based assessment of herbicide effects. Weed Research 54, 223-233. DOI: 10.1111/wre.12079
Study type		Research article
Guidelines		Not stated
Guideline deviations		Not stated
GLP		Not stated

Abstract
Non-destructive assessment of herbicide effects may be able to support integrated weed management. To test whether effects of herbicides on canopy variables could be detected by sensors, two crops were used as models and treated with herbicides at BBCH 20 using a logarithmic sprayer. Twelve days after spraying at BBCH 25 and 42 days after sowing, nine sensor systems scanned a spring barley and an oilseed rape field experiment sown at different densities and sprayed with increasing field rates of glyphosate and tribenuron-methyl. The objective was to compare ED50s for crops and weeds derived by the different sensors in relation to crop density and herbicides. Although sensors were not directly developed to detect herbicide symptoms, they all detected changes in canopy colours or height and crop density. Generally ED50s showed the same pattern in response to crop density within herbicide, but there were marked differences between barley and oilseed rape. We suggest that the results of comparing the various sensor outputs could become a stepping stone to future standardisation for the benefit of the research and development of sensors that will detect herbicide effect on crops and weeds, particularly at the most vulnerable stages of development of the canopy.

MATERIALS AND METHODS

1. Test material:

Test item: Glyphosate, others
Active substance(s): Glyphosate, others
Surfactant: Not stated
Description: Not stated
Source of test substance: Not stated
Lot/Batch #: Not stated
Purity: Not stated

2. Vehicle and/or positive control: none

3. Test organism:

Species:	Spring barley, oilseed rape
Age of test organisms at study initiation:	Age of the study organisms is not mentioned.

4. Test system:

Study type:	Research article presents sensor-based assessment of herbicide effects
Guideline:	Not stated
GLP:	Not stated
Guideline deviations:	-
Duration of study:	
Test conditions:	Two field experiments, one with spring barley (Hordeum vulgare L.) and another one with oilseed rape (Brassica napus ssp. oleifera (DC.) Metgz), were carried out. The experiments were a split plot with species as main plot and seed densities (barley: 0, 75, 150, 300 and 450 seeds m^2; oilseed rape: 0, 20, 40, 80 and 120 seeds m^2) as subplots of 3 m by 35 m and sprayed at BBCH 20. The spatial distribution of the different crop densities (subplots) was randomly distributed within their main plots, and the natural weed infestation was included in the canopy; plots with no crops only had a weed canopy.
Replicates per concentration:	-
Organisms per replicate:	-
Parameters measured:	Nondestructive method by analyzing digital color images to quantify color changes in leaves caused by herbicides<u>Length growth.</u>
Test concentrations:	Glyphosate (Glyphosate 360; 360 g a.i. L^{-1}) and tribenuron-methyl (express ST; 500 g a.i. kg^{-1}) wereapplied with a special tractor-mounted logarithmic sprayer to the subplots with a 6 m half-life decrease of the maximumdose of 4.3 kg glyphosate ha^{-1} and 30 g tribenuron-methyl ha^{-1}. The spray volume was 150 L ha^{-1}.
Application:	not stated
Application devices:	logarithmic sprayer
Application verification:	not stated
Analytical determination of test concentrations:	not stated

5. Environmental conditions:

Temperature:	15/13 C day/night in the greenhouse experiment.
Photoperiod:	Not stated

Light intensity	Not stated
pH:	Not stated

6. Observations/analyses:

Measurements:	Sensor-based assessment of herbicide effects with different sensors.
Statistics:	Sensor output was analysed within crop, density and herbicide. All data were normalised between 1 and 0 in the response and dose, respectively, to easily assess the regression quality among sensors, particularly the upper limit D and the lower limit C. The dose–response curves were in most instances described by a log-logistic model.

KLIMISCH EVALUATION

1. Relevance of study:		**Not relevant**
	Comment:	Use of Image Analysis to Assess Color Response on Plants Caused by Herbicide Application is not in the focus of the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant for the assessment of glyphosate efficacy.
2. Reliability of study:		**Not reliable**
	Comment:	Not all relevant details on the investigation are reported in the present publication. Studies were not carried out according to OECD guideline and under GLP conditions.
3. Klimisch code:		**Klimisch rating of 3**

3. Non-target plants

The following studies focus on the influence of glyphosate application on non-target plants. The documents are checked for their relevance for the issue of adjacent crops although this is not an annex point that has to be addressed in the renewal assessmernt of glyphosate. As for any herbicide there is a potential impact on adjacent crops by spray drift. The documents submitted on the topic of adjacent crops provide no new scientific evidence. A change or amendment in the assessmnent is not necessary.

Saunders et al., 2013

Author(s)	**Year**	**Study title**
Saunders, L.E., Koontz, M.B., Pezeshki, R.	2013	Root-zone glyphosate exposure adversely affects two ditch species. Biology 2, 1488-1496 DOI: 10.3390/biology2041488
Study type		Research article
Guidelines		Not stated
Guideline deviations		Not stated
GLP		Not stated

Abstract
Glyphosate, one of the most applied herbicides globally, has been extensively studied for its effects on non-target organisms. In the field, following precipitation, glyphosate runs off into agricultural ditches where it infiltrates into the soil and thus may encounter the roots of vegetation. These edge-of-field ditches share many characteristics with wetlands, including the ability to reduce loads of anthropogenic chemicals through uptake, transformation, and retention. Different species within the ditches may have a differential sensitivity to exposure of the root zone to glyphosate, contributing to patterns of abundance of ruderal species. The present laboratory experiment investigated whether two species commonly found in agricultural ditches in southcentral United States were affected by root zone glyphosate in a dose-dependent manner, with the objective of identifying a sublethal concentration threshold. The root zone of individuals of *Polygonum hydropiperoides* and *Panicum hemitomon* were exposed to four concentrations of glyphosate. Leaf chlorophyll content was measured, and the ratio of aboveground biomass to belowground biomass and survival were quantified. The findings from this study showed that root zone glyphosate exposure negatively affected both species including dose-dependent reductions in chlorophyll content. *P. hydropiperdoides* showed the greatest negative response, with decreased belowground biomass allocation and total mortality at the highest concentrations tested.

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup ProDry
Active substance(s):	Glyphosate
Surfactant:	Not stated
Description:	Not stated
Source of test substance:	Not stated
Lot/Batch #:	Not stated
Purity:	Not stated

2. Vehicle and/or positive control: none

3. Test organism:

Species: *Polygonum hydropiperoides* and *Panicum hemitomon*

Age of test organisms at study initiation: Not stated

4. Test system:

Study type: Research article presents results on glyphosate effects after run off on two species commonly found in agricultural ditches.

Guideline: Not stated

GLP: Not stated

Guideline deviations: -

Duration of study: 21 days after glyphosate exposure

Test conditions: Laboratory experiments
Following collection, plants were standardized by cutting individuals to 15 cm stem and 10 cm root and then were potted in PVC pots (60 cm h × 5 cm d) containing washed commercial play sand, limiting the adsorption of glyphosate onto organic matter. Plants were maintained for 4 weeks in a climate-controlled greenhouse (20/31 °C) without supplemental lighting. Plants were watered daily with tap water and received weekly fertilizer applications at a rate of 1.25 g/L 20-20- (Scotts MiracleGrow Company, Marysville, OH, USA).
Following the maintenance period, individuals were transferred to a laboratory equipped with supplemental light on a 16-h photoperiod, illuminated by four 400 W high pressure sodium and four 400 W metal halide lamps in water-cooled ballasts, providing approximately 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density at the leaf canopy level.

Replicates per concentration: *Polygonum hemitomon* plants (N = 24) and 10 *Panicum hydropiperoides* plants (N = 40)

Organisms per replicate: 6 *Polygonum hemitomon* plants and 10 *Panicum hydropiperoides*

Parameters measured: Leaf chlorophyll content index (CCI) was recorded prior to treatment initiation and daily thereafter for the study duration using a chlorophyll content meter (CCM-200, Opti Sciences, Tyngsboro, MA, USA).

Test concentrations: four glyphosate concentrations (0, 10, 1,000, 10,000 mg/L glyphosate)

Application:	During exposure, 100 mL glyphosate solution of the appropriate concentration was introduced to the top of the substrate and allowed to infiltrate for two hours, after which the substrate was rinsed with 500 mL deionized water.
Application devices:	not stated
Application verification:	not stated
Analytical determination of test concentrations:	not stated

5. Environmental conditions:

Temperature:	Plants were maintained for 4 weeks in a climate-controlled greenhouse (20-31 °C)
Photoperiod:	Not stated
Light intensity	1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density
pH:	Not stated

6. Observations/analyses:

Measurements:	Leaf chlorophyll content index (CCI) was recorded prior to treatment initiation and daily thereafter for the study duration using a chlorophyll content meter (CCM-200, Opti Sciences, Tyngsboro, MA, USA). Following the study termination on day 21, plants were divided into above- and below-ground tissue and dried in an oven at 70 °C until a constant weight was reached, then the dry weights were recorded. These dry weights were used to calculate the ratio of aboveground biomass to belowground biomass.
Statistics:	Differences in means for pre-exposure and post-exposure CCI were analyzed using a repeated measures analysis of variance (ANOVA) with two sampling dates and four levels of glyphosate treatment as independent factors. Differences in means for root:shoot ratios and for differences among treatment groups for survival for each species were analyzed for each species using a one-way ANOVA with four levels of glyphosate exposure as the independent factor. Significant differences were followed by Tukey`s post-hoc comparison. Differences were considered significant at $\alpha<0.05$.

KLIMISCH EVALUATION

1. Relevance of study:		**No rating**
	Comment:	Glyphosate effects after run off on species commonly found in agricultural ditches is not in the focus of the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant for the assessment of glyphosate efficacy. Using good agricultural practice, there is no expected impact on non target plants.
2. Reliability of study:		**Reliable with restrictions**
	Comment:	Studies were not carried out according to OECD guideline and under GLP conditions.
3. Klimisch code:		**Klimisch rating of 2**

Allison et al., 2013

Author(s)	**Year**	**Study title**
Allison, J.E., Boutin, C., Carpenter, D.	2013	Influence of soil organic matter on the sensitivity of selected wild and crop species to common herbicides. Ecotoxicology 22, 1289-1302 DOI: 10.1007/s10646-013-1116-3
Study type		Research article
Guidelines		Not stated
Guideline deviations		Not stated
GLP		Not stated

Abstract
Current regulatory protocols for assessing herbicide effects on plants rely heavily on the use of crops grown under controlled greenhouse conditions to indicate risks to wild vegetation. Guideline test protocols call for low levels of soil nutrients, approximately 3% organic matter (OM), to test the worst-case scenario for non-target species growing in poor soils. However, species sensitivity to herbicides may be affected by growing conditions, especially soil nutrient levels. The purpose of this study was to assess the impact of high and low soil OM content on the sensitivity of plants to several common agricultural herbicides (glyphosate, chlorimuron ethyl and dicamba). Ten plant species (wild and crop) with high (nitrophilous) or low (non-nitrophilous) affinity for nitrogen were grown under greenhouse conditions in soil with two levels of OM (3 and 9 %) and were exposed to seven doses of the three herbicides in concurrent experiments. Results showed that most species were more sensitive to glyphosate under high OM conditions while chlorimuron ethyl and dicamba results were mixed with respect to OM levels. Overall, 15 species-herbicide combinations were more sensitive in high OM soil, while 11 were more sensitive in low OM soil. No clear pattern in sensitivity

emerged among nitrophilous and non nitrophilous species. Several species showed a difference in response at herbicide levels expected to reach non target habitats adjacent to crop fields through drift (5 % commonly and 25 % occasionally). In terms of regulatory testing, guidelines may need alteration to allow testing with nutrient levels that more accurately reflect natural environments.

MATERIALS AND METHODS

1. Test material:

Test item:	Vision (Monsanto Canada Inc., Mississauga, ON, Canada), containing 356 g acid equivalent (a.e.) L^{-1} glyphosate and two other herbicides
Active substance(s):	Glyphosate
Surfactant:	Not stated
Description:	Not stated
Source of test substance:	Not stated
Lot/Batch #:	Not stated
Purity:	Not stated

2. Vehicle and/or positive control: none

3. Test organism:

Species:	*Lactuca sativa var., Raphanus sativus var., Solanum lycopersicum, Lolium perenne, Clinopodium vulgare, Potentilla recta, Koeleria macrantha, Chenopodium album, Urtica dioica, Poa palustris*

4. Test system:

Study type:	Research article presents results on the influence of soil organic matter on the sensitivity of selected wild and crop species to common herbicides.
Guideline:	Guidelines established by the Organization for Economic Cooperation and Development OECD (2006) and by the United States Environmental Protection Agency USEPA (2012) call for soils containing no more than 3 % OM (approximately 1.5 % OC) for chemical testing involving terrestrial plants.
GLP:	Not stated
Guideline deviations:	-
Duration of study:	At 28 days, all aboveground plant material was harvested
Test conditions:	Experiments were conducted in a greenhouse module between January and June 2012. Average daily temperatures ranged between 16.4 ± 2.5 and 39.6 ± 6.2 °C and photosynthetic active radiation measured between 285 (cloudy day) to 1951 lmol photons m^{-2} s^{-1} (sunny day) during a 16:8 h day: night lightcycle.

Replicates per concentration:	Doses, including controls, consisted of five replicates for a total of 35 plants for each of the low and high OM soil treatments.
Organisms per replicate:	-
Parameters measured:	At 28 days, all aboveground plant material was harvested. Necrotic tissue resulting from herbicide exposure was removed and the remaining tissue was bagged and dried for a minimum of 3 days at approximately 70 °C before weighing.
Test concentrations:	Doses selected for application were based on label recommendations for ground equipment. Glyphosate label recommendations are 3–6 L (1,068–2,136 g a.e. ha^{-1}) per hectare to control herbaceous species
Application:	Seedlings were sprayed at the 2- to 4-true leaf stage, between 14 and 32 days after transplant. Species were sprayed over an 11 week period while attempting to mix nitrophilous, non nitrophilous and crop species in the greenhouse. Low and high OM soil treatments were sprayed on the same day wherever possible, with the same herbicide preparation.
Application devices:	Herbicides were applied using a track-spray booth (de Vries Manufacturing, Hollandale, MN, USA) outfitted with a TeeJet 8002E flat-fan spray nozzle.
Application verification:	not stated

5. Environmental conditions:

Temperature:	Average daily temperatures ranged between 16.4 ± 2.5 °C and 39.6 ± 6.2 °C and photosynthetic active radiation measured between 285 (cloudy day) to 1951 lmol photons m^{-2} s^{-1} (sunny day) during a 16:8 h day: night lightcycle.
Photoperiod:	Not stated
Light intensity	285 (cloudy day) to 1951 lmol photons m^{-2} s^{-1} (sunny day)
Organic matter (OM):	a low OM soil containing 3:3.6 potting soil:silica sand (approximately 3 % OM) and a high OM soil containing 3:1 potting soil:silica sand (approximately 9 % OM) both by volume

6. Observations/analyses:

Measurements:	Plants were grouped by size and distributed

across doses to prevent size bias. Initial height/diameter measurements taken the day of spray indicated starting size uniformity across doses for each soil treatment (ANOVA or Kruskal–Wallis; p[0.05 for all herbicide-species-soil treatment combinations). At 28 days, all aboveground plant material was harvested. Necrotic tissue resulting from herbicide
exposure was removed and the remaining tissue was bagged and dried for a minimum of 3 days at approximately 70 °C before weighing. Control plants grown in low OM soil (n = 15, all herbicide controls combined) and in high OM soil (n = 15) were used to verify or establish nitrogen affinity of the different plant species. Soil samples were harvested from every species at every stage of the experimental process: transplant, spray and harvest. At transplant, a small scoop of soil was bagged after every 20 pots were filled to obtain a cross section of the soil mix. At spray, soil was collected from each of ten of the extra pots from the low and ten from the high OM treatments.
At harvest, soil samples were taken from all control pots and all pots in the highest dose (72.4 %) for each herbicide and soil treatment. Samples from each species were combined and OM content determined through LOI.

Statistics: Data were analysed using Systat 13 Version 13.00.05 (Systat Software Inc., Chicago, IL, USA) or R version 2.13.0 (The R Foundation Statistical Computing, Vienna, Austria). Student t tests were used to verify significant differences among soil characteristics. Categorization of species as non-nitrophilous and nitrophilous was verified by Mann–Whitney comparison of unsprayed control plant biomass between low and high OM treatments at the end of the experiment.

KLIMISCH EVALUATION

1. Relevance of study: **Not relevant**

Comment: Influence of soil organic matter on the sensitivity of selected wild and crop species to common herbicides (e.g. glyphosate) is not in the focus of the efficacy evaluation for glyphosate. The study

can therefore not be classified as relevant for the assessment of glyphosate efficacy.

Using good agricultural practice, there is no expected impact on non target plants.

2. Reliability of study: **Reliable with restrictions**

Comment: Not all relevant details on the investigation are reported in the present publication.

Studies were not carried out according to OECD guideline and under GLP conditions.

3. Klimisch code: **Klimisch rating of 2**

Boutin et al., 2014

Author(s)	**Year**	**Study title**
Boutin, C., Strandberg, B., Carpenter, D., Mathiassen, S.K., Thomas, P.J.	2014	Herbicide impact on non-target plant reproduction: What are the toxicological and ecological implications? Environmental Pollution 185, 295-306
Study type		Research/Review article
Guidelines		Not stated
Guideline deviations		Not stated
GLP		Not stated

Abstract
Declining plant diversity and abundance have been widely reported in agro-ecosystems of North America and Europe. Intensive use of herbicides within cropfields and the associated drift in adjacent habitats are partly responsible for this change. The objectives of this work were to quantify the phenological stages of non-target plants in in-situ field situations during herbicide spray and to compare plant susceptibility at different phenological stages. Results demonstrated that a large number of non-target plants had reached reproductive stages during herbicide spray events in woodlots and hedgerows, both in Canada and Denmark where vegetation varies considerably. In addition, delays in flowering and reduced seed production occurred widely on plants sprayed at the seedling stage or at later reproductive periods, with plants sprayed at reproductive stages often exhibiting more sensitivity than those sprayed as seedlings. Ecological risk assessments need to include reproductive endpoints.

MATERIALS AND METHODS

1. Test material:

Test item: Different herbicides
Active substance(s): Glyphosate and others

Surfactant:	Not stated
Description:	Not stated
Source of test substance:	Not stated
Lot/Batch #:	Not stated
Purity:	Not stated

2. Vehicle and/or positive control: none

3. Test organism:

Species:	104 species were identified across the three woodlots during the three years of the study.

4. Test system:

Study type:	Research article presents results on to quantify the phenological stages of non-target plants in in-situ field situations during herbicide spray and to compare plant susceptibility at different phenological stages.
Guideline:	Not stated
GLP:	Not stated
Guideline deviations:	-
Duration of study:	-
Test conditions:	Vegetation/phenology surveys Greenhouse experiment
Replicates per concentration:	6 in greenhouse experiment
Organisms per replicate:	-
Parameters measured:	Number and proportion of species at flowering stages during spray events. Differences in the number of species affected by herbicide spray. EC50 (effective concentration resulting in a 50% decrease as compared to the controls) was used to examine herbicide effects. Dry vegetative biomass and reproductive parameters (seed production or measurable equivalent). Dose response curves were estimated for each plant species.
Test concentrations:	Not stated for glyphosate
Application:	Not stated for vegetation/phenology surveys and greenhouse experiments
Application devices:	Not stated
Application verification:	Not stated

5. Environmental conditions:

Temperature:	Not stated
Photoperiod:	Not stated
Light intensity	Not stated

6. Observations/analyses:

Measurements:	Number and proportion of species at flowering stages during spray events. Differences in the number of species affected by herbicide spray. EC50 (effective concentration resulting in a 50% decrease as compared to the controls) was used to examine herbicide effects. Dry vegetative biomass and reproductive parameters (seed production or measurable equivalent). Dose response curves were estimated for each plant species.
Statistics:	In EXP1 and EXP2, two-way ANOVAs were used to assess the number and proportion of species at flowering stages during spray events. In EXP3, EXP4 and EXP5, KruskaleWallis and Conovan-Inman post-hoc tests were used for significance between doses. Chi-square tests, Student t-tests and ManneWhitney U tests were conducted to assess differences in the number of species affected by herbicide spray (EXP1) and in flowering between organic and conventional systems (EXP2). In all cases, the assumptions of normality and homogeneity of variance were tested. The EC50 (effective concentration resulting in a 50% decrease as compared to the controls) was used to examine herbicide effects. In EXP3 and EXP4, EC50s were calculated using non-linear regressions when the data met the assumptions of normality and homogeneity of variance, else the nonparametric ICPIN method was used. Dry vegetative biomass and reproductive parameters (seed production or measurable equivalent) were used separately in each calculation. Similarly in EXP6 and EXP7, the EC50 using fresh weight and seed production (number of seeds) were analysed with non-linear regressions using log-logistic dose response models. For each herbicide, dose response curves were estimated for each plant species and growth stage using two endpoints (biomass and seed production). Fitness of model was verified using an F-test for lack of fit, comparing the residual sum of squares.

KLIMISCH EVALUATION

1. Relevance of study:		**Not relevant**
	Comment:	Herbicide impact on non-target plant is not in the focus of the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant for the assessment of glyphosate efficacy. Using good agricultural practice, there is no expected impact on non target plants.
2. Reliability of study:		**Not reliable**
	Comment:	Not all relevant details on investigation are reported in the present publication. This concerns the following aspects (e.g.): Some data on test conditions are missing non-GLP, non-guideline study that meets scientific principles.
3. Klimisch code:		**Klimisch rating of 3**

Costa et al., 2012

Author(s)	**Year**	**Study title**
Costa, A.C.P.R. da, Costa, N.V. da, Rocha Pereira, M.R., Martins, D..	2012	Simulated drift effect of glyphosate in different parts of Eucalyptus grandis plants Ciencias Agrarias Londrina 33, 5, 1663-1672 DOI: 10.5433/1679-0359.2012v33n5p1663
Study type		Research
Language		Portuguese
Guidelines		Not stated
Guideline deviations		Not stated
GLP		Not stated

Abstract
The present work aimed to evaluate the effects of simulated drift of glyphoste on *Eucalyptus grandis*, through the application of low doses in different parts of the plant. The experimental design was a randomized block design with five replications. The treatments were glyphosate application at 0; 30; 60; 90 e 120 g a.e. ha-1 of the commercial formulation Scout®. Three forms of application were used: applying on leaf, on stem, and on the entire plant (leaf + stem). For leaf application, stems were covered with plastic ribbons to protect them from the solution; the same was made with plants that were sprayed on stems, covering leaf with plastic bag. The application was carried out in an armed stationary spray tips XR 11002 VS, with 183 KPa pressure in volume of 200 L ha-1. The eucalyptus plants receiving applications in leaves and whole plant (leaves + stem) showing effects of intoxication are more intense

about the plants that received the stem applications only. However, there may be increases in height growth and total dry mass of eucalyptus plants in applications of 30 g a.e. ha^{-1} glyphosate.

KLIMISCH EVALUATION

1. Relevance of study: **Not be in rating, paper in Portuguese language**

2. Reliability of study: **Not be in rating, paper in Portuguese language**

3. Klimisch code: **Not be in rating, paper in Portuguese language**

Hatterman-Valenti, 2014

Author(s)	**Year**	**Study title**
Hatterman-Valenti, H.M.	2014	Simulated glyphosate drift to potato mother plants and effect on daughter tubers used for seed production. Weed Technology 28, 253-258
Study type		Research
Guidelines		Not stated
Guideline deviations		Not stated
GLP		Not stated

Abstract
Field trials were conducted to evaluate the effect of simulated glyphosate drift rates applied to two non-irrigated potato cultivars in the late tuber bulking stage grown for potato seed production. Mother plants receiving a sublethal glyphosate application had similar yields regardless of the year or cultivar. 'Red LaSoda' seed pieces visually evaluated the following spring had more sprout inhibition from glyphosate-treated plots. With 'Russet Burbank', only seed pieces from mother plants that received more than 18 g ha^{-1} glyphosate showed sprout inhibition. Total yield reductions the year following glyphosate treatments were attributed to fewer and smaller tubers from plants that did not emerge or were delayed in emergence. Red LaSoda seed pieces from mother plants receiving $\geq$71 g ha^{-1} glyphosate yielded less than the nontreated. In 2005, Russet Burbank seed pieces from mother plants receiving $\geq$35 g ha^{-1} glyphosate had reduced yields, whereas in 2006, all glyphosate treatments reduced total yield compared with the nontreated.

MATERIALS AND METHODS

1. Test material:

Test item: Roundup Weather Max, Monsanto Company

Active substance(s):	Glyphosate
Surfactant:	Not stated
Description:	Not stated
Source of test substance:	Not stated
Lot/Batch #:	Not stated
Purity:	Not stated

2. Vehicle and/or positive control: none

3. Test organism:

Species:	potato cultivar, 'Russet Burbank' and 'Red LaSoda'.

4. Test system:

Study type:	Research article presents results on glyphosate drift to potato mother plants and effect on daughter tubers used for seed production
Guideline:	Not stated
GLP:	Not stated
Guideline deviations:	-
Duration of study:	Planted May 20 and May 24 Harvest October
Test conditions:	Field trials
Replicates per concentration:	-
Organisms per replicate:	-
Parameters measured:	Plant Injury, tuber yield, Sprout inhibition
Test concentrations:	rate at 0, 18, 35, 71, 141, and 282 g ae ha^{-1}; or 0.021, 0.042, 0.083, 0.167, and 0.33 the recommended rate of 846 g ha^{-1} for a preharvest application in small grains.
Application:	use of a backpack compressed-CO2 sprayer equipped with 8002 XR flat-fan nozzles (Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60188) calibrated to deliver 180 L ha_1 at a pressure of 276 kPa.
Application devices:	backpack compressed-CO2 sprayer
Application verification:	Not stated

5. Environmental conditions:

Temperature:	Not stated
Photoperiod:	Not stated
Light intensity	Not stated

6. Observations/analyses:

Measurements:	Plant Injury, tuber yield, Sprout inhibition
Statistics:	All data were subjected to ANOVA with the use of PROC GLM procedure in SAS 9.1 (SAS

Circle, P.O. Box 8000, Cary, NC 25712). Percent inhibition data were subjected to a square-root transformation prior to analysis. Because transformed data did not change the ANOVA results, the nontransformed data were used. Data were pooled across years where appropriate. Mean comparisons were performed where appropriate with the use of Fisher's protected LSD test at P ≤ 0.05.

KLIMISCH EVALUATION

1. Relevance of study:		**Not relevant**
	Comment:	Herbicide impact on non-target plant is not in the focus of the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant for the assessment of glyphosate efficacy. Using good agricultural practice, there is no expected impact on non target plants.
2. Reliability of study:		**Reliable with restrictions**
	Comment:	Studies were not carried out according to OECD guideline and under GLP conditions.
3. Klimisch code:		**Klimisch rating of 2**

Hensley et al., 2013

Author(s)	**Year**	**Study title**
Hensley, J.B., Webster, E.P., Blouin, D.C., Harrel, D.L., Bond, J.A.	2013	Response of rice to drift rates of glyphosate applied at low carrier volumes Weed Technology 27, 257-262
Study type		Research
Guidelines		Not stated
Guideline deviations		Not stated
GLP		Not stated

Abstract
Field studies were conducted near Crowley, LA in 2005 through 2007 to evaluate the effects of simulated herbicide drift on 'Cocodrie' rice. Each application was made with the spray volume varying proportionally to herbicide dosage based on a constant spray volume of 234 L ha^{-1} and a glyphosate rate of 863 g ae ha^{-1}. The 6.3%, 54 g ha^{-1}, herbicide rate was applied at a spray volume of 15 L ha^{-1}, and the 12.5%, 108 g ha^{-1}, herbicide rate was applied at a spray volume of 29 L ha^{-1}. Compared with the nontreated, glyphosate applied at one tiller, panicle

differentiation (PD), and boot resulted in increased crop injury. The greatest injury was observed on rice treated at the one-tiller timing. Applications of glyphosate at one tiller, PD, and boot reduced plant height at harvest and primary and total crop yield. Rice treated at primary crop maturity was not affected by glyphosate applications.

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup Weather Max, Monsanto Company
Active substance(s):	Glyphosate
Surfactant:	Not stated
Description:	Not stated
Source of test substance:	Not stated
Lot/Batch #:	Not stated
Purity:	Not stated

2. Vehicle and/or positive control: none

3. Test organism:

Species:	rice cultivar 'Cocodrie'.

4. Test system:

Study type:	Research article presents results on glyphosate drift to rice plants
Guideline:	Not stated
GLP:	Not stated
Guideline deviations:	-
Duration of study:	rice was drill seeded March 28 to April 17 in 2005 through 2007, rating date on primary crop rice injury 7, 14, 21, and 28 d after treatment
Test conditions:	Field trials, rice cultivar 'Cocodrie' was drill seeded March 28 to April 17 in 2005 through 2007. Plots consisted of 12 to 18–cm spaced rows 6 m long.
Replicates per concentration:	two-factor factorial arrangement of treatments in a randomized complete block with a nontreated added for comparison and four replications
Organisms per replicate:	Not stated
Parameters measured:	Plant Injury, plant height, yield, stem and panicle counts
Test concentrations:	simulated drift rates of 6.3 and 12.5% of the labeled usage rate of 863 g ae ha^{-1}, or 54 and 108 g ha^{-1}, respectively.
Application:	Tractormounted CO2-pressurized sprayer calibrated to deliver a constant carrier volume with speed adjusted to vary application rate and equipped with Teejet TX-2 Conejet 800033 nozzles (Teejet Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60187).

Application devices:	Tractormounted CO2-pressurized sprayer
Application verification:	Not stated

5. Environmental conditions:

Temperature:	Not stated
Photoperiod:	Not stated
Light intensity	Not stated

6. Observations/analyses:

Measurements:	Plant Injury, plant height, yield, stem and panicle counts
Statistics:	All data were subjected to the Mixed Procedure of SAS. Year and replications (nested within year) were considered random effects. Application timing, rate, and evaluation date were considered fixed effects. Considering year as a random effect permits inferences about treatments over a range of environments. Type III statistics were used to test all possible effects of fixed factors and leastsquare means were used for mean separation at the 5% probability level ($P \leq 0.05$).

KLIMISCH EVALUATION

1. Relevance of study: **Not relevant**

Comment: Herbicide impact on non-target plant is not in the focus of the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant for the assessment of glyphosate efficacy.

Using good agricultural practice, there is no expected impact on non target plants.

2. Reliability of study: **Reliable with restrictions**

Comment: Studies were not carried out according to OECD guideline and under GLP conditions.

3. Klimisch code: **Klimisch rating of 2**

Mateos-Naranjo & Perez-Martin, 2013

Author(s)	Year	Study title
Mateos-Naranjo, E., Perez-Martin, A.	2013	Effects of sub-lethal glyphosate concentrations on growth and photosynthetic performance of non-target species *Bolboschoenus maritimus* Chemosphere 93, 2631-2638
Study type		Research
Guidelines		Not stated
Guideline deviations		Not stated
GLP		Not stated

Abstract
Glyphosate use has increased over the last decades for the control of invasive plant species in wetland ecosystems. Although glyphosate has been considered 'environmentally' safe, its repeated use could increase the toxicological risk derived from diffuse pollution of surface and groundwater on non-target vegetation. A glasshouse study was designed to determine the effect produced by the addition of different sub-lethal doses of glyphosate herbicides (5–30 mg L^{-1}) to the nutrient solution on the growth and photosynthetic apparatus of *Bolboschoenus maritimus*. Although *B. maritimus* plants were able to grow and survive after 20 d of exposure to glyphosate, the presence of this herbicide affected their growth, through a direct interaction with the root system. Particularly, at 30 mg L^{-1} glyphosate, *B. maritimus* showed ca. 30% of biomass decrease. The reduction in *B. maritimus* growth was due to a decrease in net photosynthetic rate (A), which ranged between values ca. 11.5 and 5.5 lmol m^{-2} s^{-1} CO_2 for the control and the highest glyphosate treatment, respectively. The response of A to glyphosate could be largely accounted for by non-stomatal limitations, since stomatal conductance was similar in all glyphosate treatments. Thus, A decrease was prompted by the negative impact of herbicide on photochemical (PSII) apparatus, the reduction in the absorption of essential nutrients, the reduction of photosynthetic pigments and possibly the reduction in Rubisco carboxilation capacity. Moreover, glyphosate excess caused photoinhibitory damage. In conclusion, in this study we have shown that herbicide water pollution could be a source of indirect phytotoxicity for *B. maritimus*.

MATERIALS AND METHODS

1. Test material:

Test item:	Glimur LS
Active substance(s):	Glyphosate
Surfactant:	Not stated
Description:	Not stated
Source of test substance:	Not stated
Lot/Batch #:	Not stated
Purity:	Not stated

2. Vehicle and/or positive control: none

3. Test organism:

Species: *Bolboschoenus maritimus*

4. Test system:

Study type: Research article presents results of sub-lethal glyphosate concentrations on growth and photosynthetic performance of non-target

	species
Guideline:	Not stated
GLP:	Not stated
Guideline deviations:	-
Duration of study:	All the measurements were made on day 20 after glyphosate application
Test conditions:	glasshouse study Seeds were subjected to an alternating diurnal regime of 16 h of light (photon flux rate, 400–700 nm, 35 µmol m^{-2} s^{-1}) at 25 °C and 8 h of darkness at 12 °C, for a month in a germinator (ASL Aparatos Científicos M-92004, Madrid, Spain). Seedlings were planted in individual plastic pots (9 cm high x 11 cm diameter) filled with vermiculite and placed inside a glasshouse with minimum–maximum temperatures of 21–25 _C, 40–60% relative humidity and natural daylight (minimum and maximum light flux: 200 and 1000 lmol m^{-2} s^{-1}, respectively).
Replicates per concentration:	eight
Organisms per replicate:	4-6 live tillers
Parameters measured:	Growth analysis, relative growth rate, Gas exchange measurements, Leaf water content, Photosynthetic pigments, Measurement of chlorophyll fluorescence, Chemical analysis of plant samples
Test concentrations:	0, 5, 10, 20 and 30 mg L^{-1} Plants were subjected to glyphosate for 20 d.
Application:	addition of different sub-lethal doses of glyphosate herbicides (5–30 mg L^{-1}) to the nutrient solution
Application devices:	unspecified
Application verification:	Not stated

5. Environmental conditions:

Temperature:	16 h of light at 25 °C and 8 h of darkness at 12 °C
Photoperiod:	16 h of light, 8 h of darkness
Light intensity	photon flux rate, 400–700 nm, 35 µmol m^{-2} s^{-1}

6. Observations/analyses:

Measurements:	Growth analysis, relative growth rate, gas exchange measurements, leaf water content, photosynthetic pigments,chlorophyll fluorescence, chemical analysis of plant samples
Statistics:	Statistical analysis was carried out using Statistica v. 6.0 (Statsoft Inc.). Pearson coefficients (r) were calculated to assess

correlation between different variables. Data were analysed by means of a one-way analysis of variance (F-test). Data were first tested for normality with the Kolmogorov Smirnov test and for homogeneity of variance with the Brown–Forsythe test. Significant test results were followed by Tukey tests for identification of important contrasts. Differences between measurements of fluorescence at dawn and midday were compared by the Student test (t- test).

KLIMISCH EVALUATION

1. Relevance of study: **Not relevant**

Comment: Herbicide impact on non-target plant is not in the focus of the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant for the assessment of glyphosate efficacy.

Using good agricultural practice, there is no expected impact on non target plants.

2. Reliability of study: **Reliable with restrictions**

Comment: Studies were not carried out according to OECD guideline and under GLP conditions.

3. Klimisch code: **Klimisch rating of 2**

Author(s)	**Year**	**Study title**
Rebessi, A.C., Medina, A.F., Pereira, B., Franc, D., Santos, D.F., Mendes, F., Rodrigues, G., Nascimento e Silva, J.H., Greve, L.F., Ciribello, L.F., Marchini, L., Felix, M., Navarro, M., Chinelatto, M.R., Ovidio, T.T., de Brito, N.N.	2011	Study of chronic toxicity of the glyphosate herbicide in seeds of corn, okra and arugula. Engenharia Ambiental. Espirito Santo do Pinhal 8, 2, 17-27.
Study type		Research
Language		Portuguese
Guidelines		Not stated
Guideline deviations		Not stated

GLP		Not stated

Rebessi et al., 2011

Abstract
Glyphosate is an agrochemical from the group of phosphonates, which is widely used as a herbicide. Although it kills almost any plant, it can be used to eliminate weeds during the growth of crops that are resistant to glyphosate. This research aimed to evaluate the toxicological effects of the herbicide in a study using as test organism seeds of maize (*Zea mays*), okra (*Abelmoschus esculentus* (L.) Moench) and arugula (*Eruca sativa*). The toxicological testing was to use a paper towel dampened with a sample of glyphosate and twenty seeds evenly distributed in containers with lids, left in a dry and airy place, and watered once a day for 5 days. It was possible to check the toxic effect of glyphosate for corn seeds at concentrations above 90 mg.L^{-1}, for okra seeds at concentrations above 75 mg L^{-1} and arugula seeds in concentrations above 2 mg L^{-1}. Thus it was concluded that the seeds studied showed a high sensitivity with the possibility to be applied in toxicity testing, representing a simple and low cost.

KLIMISCH EVALUATION

1. Relevance of study:	**Not be in rating, paper in Portuguese language**
2. Reliability of study:	**Not be in rating, paper in Portuguese language**
3. Klimisch code:	**Not be in rating, paper in Portuguese language**

Bergfield, 2013

Author(s)	**Year**	**Study title**
Bergfield, A.	2013	MON 52276: Effects on the vegetative vigor of non-target terrestrial plants (Tier II) Project identification: ABC Study No. 80477 Performing laboratory: ABC Laboratories, Inc., Columbia, Missouri 65202 Not public, the document is for use only by the regulatory authority to which it has been submitted by Monsanto Company
Study type		Research
Guidelines		OECD 227
Guideline deviations		Not stated
GLP		Yes

Abstract
A Tier II vegetative vigor non-target study was conducted with MON 52276 having an analyzed purity of 30.45 wt% glyphosate acid. The test protocol was based on the OECD Guideline for the Testing of Chemicals, Guideline 227, Terrestrial Plant Test: Vegetative Vigour Test. Four species of monocotyledonous plants and six species of dicotyledonous were selected either from the listing in OECD 227 or were requested by the Study Sponsor. The monocotyledons tested were corn – *Zea mays*, oat – *Avena sativa*, onion – *Allium cepa*, and wheat – *Triticum aestivum*. The dicotyledons tested were cucumber – *Cucumis sativus*, oilseed rape – *Brassica napus*, radish – *Raphanus sativus*, soybean – *Glycine max*, sunflower – *Helianthus annus*, and tomato – *Lycopersicon esculentum*. Testing was conducted at seven rates of up to 1,280 g a.e./hectare. The test substance was suspended in deionized water and diluted with deionized water to produce application solutions of the appropriate concentrations to achieve the target application rates. Serial dilutions from the primary application solution were prepared in deionized water in a geometric series separated by a factor of 2.0 to achieve the application spray solutions. The spray solutions were applied to the test system using an overhead track sprayer. The apparatus was calibrated prior to use to provide the proper spray volume and to evenly cover the surface of the test system. The target application volume was 100 liters of application solution per hectare. A negative control (deionized water) was tested for each test species. Data collected included percent survival, visual injury (i.e., phytotoxicity), shoot length, and replicate shoot fresh weight at termination. The data were analyzed using analysis of variance to compare treatments to the control to determine the no effect concentration (NOEC). Regression analysis was used to estimate the effective rate to elicit a 25 and 50% detrimental effect (ER25, and ER50) for length and weight data. The NOEC, ER25, and ER50 rates (g a.e./hectare) for the effects of MON 52276 on plant survival, shoot height, and shoot weight from the vegetative vigor test are summarized in the following table.

MATERIALS AND METHODS

1. Test material:

Test item:	MON 52276
Active substance(s):	Glyphosate
Surfactant:	Not stated
Description:	Not stated
Source of test substance:	Not stated
Lot/Batch #:	Not stated
Purity:	Not stated

2. Vehicle and/or positive control: none

3. Test organism:

Species:	*Zea mays, Avena sativa, Allium cepa, Triticum aestivum, Cucumis sativus, Brassica napus, Raphanus sativus, Helianthus annus, Lycopersicon esculentum*

4. Test system:

Study type:	Research article presents effects of glyphosate on non target plant.
Guideline:	OECD Guideline for the Testing of Chemicals, Guideline 227, Terrestrial Plant Test: Vegetative Vigour Test

GLP:	yes
Guideline deviations:	-
Duration of study:	The plants were grown for approximately 2-5 weeks
Test conditions:	greenhouse study
Replicates per concentration:	4
Organisms per replicate:	5
Parameters measured:	numbers of live plants present and cumulative mortality; phytotoxicity ratings (i.e., visual injury assessments); fresh weight; dose response (ER25 and ER50)
Test concentrations:	The applications produced target application rates of 0 (control), 20, 40, 80, 160, 320, 640, and 1,280 g a.e./ha.
Application:	The test substance treatments were dosed by spraying the control or appropriate test substance spray solution onto the foliage of the seedlings and surface of the soil within the test pots.
Application devices:	Overhead track sprayer (De Vries Manufacturing). The single nozzle sprayer was equipped with a TeeJet 4001 E nozzle and operated at 40 psi.
Application verification:	Not stated

5. Environmental conditions:

Temperature:	Average temperature and relative humidity was 24.7°C and 52%, respectively.
Photoperiod:	16-hr light to 8-hr dark
Light intensity	Natural sunlight supplemented with 600 watt high pressure sodium grow lights. During the test period, the average daily photosynthetically active radiation (PAR) was 170 µEs-1 m^{-2} (daily accumulated PAR was 10 E/m2) with an average 16 hours light.

6. Observations/analyses:

Measurements:	numbers of live plants present and cumulative mortality; phytotoxicity ratings (i.e., visual injury assessments); fresh weight; dose response (ER25 and ER50)
Statistics:	Mean percent survival, mean visual rating, mean shoot height, and total shoot fresh weight and their standard deviations were determined for each replicate, test substance rate, and plant species. All Statistical computations were performed using SAS Version 9.3. Continuous data (length, weight) was analyzed using analysis of variance

(ANOVA). If monotonicity was found, the NOEC was determined using Jonckheere-Terpstra. The NOEC for quantal data (survival) for species less than 100% was determined by Cochran-Armitage. If monotonicity was not determined then pair-wise testing was performed using Dunnett or Dunn for continuous data, after Shapiro-Wilk and Levene testing for normality and homogeneity of variance respectively, and Fisher's Exact test for quantal data.

KLIMISCH EVALUATION

1. Relevance of study:		**Not relevant**
	Comment:	Herbicide impact on non-target terrestrial plants is not in the focus of the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant for the assessment of glyphosate efficacy. Using good agricultural practice, there is no expected impact on non target plants.
2. Reliability of study:		**Reliable without restrictions**
	Comment:	Studies were carried out according to OECD guideline and under GLP conditions.
3. Klimisch code:		**Klimisch rating of 1**

Literature reviews

Gomes et al., 2014

Author(s)	Year	Study title
Gomes, M.P., Smeldbol, E., Chalifour, A., Henault-Ethier, L., Labrecque, M., Lepage, L., Lucotte, M., Juneau, P.	2014	Alteration of plant physiology by glyphosate and its by-product aminomethylphosphonic acid: an review Journal of Experimental Botany 65, 17, 4691-4703. DOI: 10.1093/jxb/eru269

Abstract
It is generally claimed that glyphosate kills undesired plants by affecting the 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS) enzyme, disturbing the shikimate pathway. However, the mechanisms leading to plant death may also be related to secondary or indirect effects of glyphosate on plant physiology. Moreover, some plants can metabolize glyphosate to aminomethylphosphonic acid (AMPA) or be exposed to AMPA from different environmental matrices. AMPA is a recognized phytotoxin, and its co-occurrence with glyphosate could modify the effects of glyphosate on plant physiology. The present review

provides an overall picture of alterations of plant physiology caused by environmental exposure to glyphosate and its metabolite AMPA, and summarizes their effects on several physiological processes. It particularly focuses on photosynthesis, from photochemical events to C assimilation and translocation, as well as oxidative stress. The effects of glyphosate and AMPA on several plant physiological processes have been linked, with the aim of better understanding their phytotoxicity and glyphosate herbicidal effects.

In the review by Gomes et al. (2014), most of the cited literature (108 references) was addressed to the effects of glyphosate on plant physiology. The influence of glyphosate and its metabolite aminomethylphosphonic acid (AMPA) on physiological processes is described. References from the years 1963 to 2013 are being shown. Most references (85) are from the period 2000 to 2013 (63 ≥2005).

1. Relevance of study:		**Not relevant**
	Comment:	Effects of glyphosate on plant physiology is not in the focus of the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant for the assessment of glyphosate efficacy.

4. Glyphosate-resistant crops

The following studies focus on the effect of glyphosate application on different plant processes in glyphosate-resistant soybeans. Transgenic-crops such as glyphosate-resistant soybeans are not in the focus of the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant for the assessment of glyphosate efficacy.

Zobiole et al., 2012

Author(s)	Year	Study title
Zobiole, L.H.S. Silvério de Oliveira Junior, R. Constantin, J. Kremer, R.J. Biffe, D.F.	2012	Amino acid application can be an alternative to prevent glyphosate injury in glyphosate-resistant soybeans Journal of Plant Nutrition Volume: 35 Pages: 268-287 DOI: 10.1080/01904167.2012.636130

Abstract
Glyphosate-resistant (GR) soybeans have continuously increased; however, this expansion significantly increased the use of glyphosate and therefore, in some cases, has resulted in injury symptoms observed in GR soybean, known as “yellow flashing”. Previous reports of interference of glyphosate with nutrient availability and utilization by GR soybean may be linked to this injury symptom. Also, because glyphosate interferes with amino acid synthesis, supplementation with exogenous aminoacids may help GR soybean recover from adverse effects of glyphosate. Therefore, an experiment was designed to evaluate different amino acid concentrations. Near-isogenic and GR soybean varietieswere grown in the greenhouse in two soils with and without glyphosate at different rates and amino acids were foliarly applied with and without glyphosate. In general, the photosynthetic variables, nutrient contents, and shoot

and root dry biomass parameters were affected by glyphosate, however, use of amino acid formulations suppressed harmful effects of glyphosate on these parameters.

MATERIALS AND METHODS

1. Test material:

Test item:	Not specified: commercial formulation
Active substance(s):	Glyphosate
Surfactant/Adjuvant:	Not stated
Description:	Not stated
Source of test substance:	Not stated
Lot/Batch #:	Not stated
Purity:	480 g a.s./L
Test item:	Commercial formulation of amino acid
Active substance(s):	Alanine: 0.889%, Arginine: 0.145%, Aspartic Acid: 1.484%, Glutamic Acid: 2.533%, Glycine: 0.155%, Isoleucine: 0.131%, Leucine: 0.205%, Lysine: 0.184%, Phenylalanine: 0.109%, Serine: 0.137%, Threonine: 0.144%, Tryptophan: 0.175%, Tyrosine: 0.086%, Valine: 0.220%; N 2%, P_2O_5 17%
Surfactant:	Not stated
Description:	Not stated
Source of test substance:	Ajipower, Ajinomoto, Sao Paulo, Brazil
Lot/Batch #:	Not stated
Purity:	Not stated
Test item:	Non-commercial formulation of amino acid
Active substance(s):	Alanine: 1.101%, arginine: 0.179%, aspartic acid: 1.838%, glutamic acid: 3.136%, glycine: 0.191%, isoleucine: 0.162%, leucine: 0.253%, lysine: 0.227%, phenylalanine: 0.136%, serine: 0.170%, threonine: 0.178%, tryptophan: 0.175%, tyrosine: 0,106%, valine: 0.272%; N 4%, P_2O_5 19%, Mn 5%, Zn 2%, B 1%
Surfactant:	Not stated
Description:	Not stated
Source of test substance:	Ajinomoto, Sao Paulo, Brazil
Lot/Batch #:	Not stated
Purity:	Not stated
2. Vehicle and/or positive control:	Control plants and non-transgenic GR parental line soybean did not receive glyphosate

3. Test organism:

Species:	Soybean *Glycine max* L.
Cultivar:	BRS 133 (normal) and BRS-245 GR (glyphosate resistant) cultivars
Source:	Not stated
Crop growth stage at treatment:	25 DAS (days after sowing) = V4 growth stage (four-leaf stage)
Holding conditions prior to test:	Seeds were inoculated with a culture of *Bradyrhizobium elkanii*, strains SEMIA 587 and SEMIA 5019 (5 x 109 bacteria per gram) at 300 mL/100 kg seeds.

4. Test system:

Study type:	Greenhouse under controlled conditions
Guideline:	None
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	58 days from sowing to final measurement at R1 growth stage.
Treatments:	Totally 10 treatments combined in a factorial scheme (3 x 3) +1: one factor are glyphosate test concentrations and second factor is aminon acid (AA) treatment with commercial formulation Ajipower, non-commercial formulation and without AA treatment; and 1 non-GR/non-applied treatment.
Test conditions:	2 Experiments plants were sprayed outside the greenhouse at 26°C, 85% relative humidity and wind speed between 5 and 8 km/h under open sky without cloudiness. After application, plants were returned to greenhouse, irrigated to keep the soil moist, and hand weeded for weed control. Measurements were conducted immediately before and after treatment and at R1 growth stage.
Replicates:	Four replicates in two different soil types
Individuals per replicate:	1 plant per experimental unit/pot
Test concentrations:	0, 1200 and 2400 g glyphosate ha^{-1} 0,5 L commercial formulation of amino acid ha^{-1} 0,5 L non-commercial formulation ha^{-1}
Application:	Plants were sprayed outside the greenhouse
Application devices:	Backpack sprayer with SF110.02 nozzles, under 2 kg cm^{-2} of carbon dioxide (CO2) at 190 L ha^{-1}
Application verification:	not stated

5. Environmental conditions:

Test medium / Soil at study site	Horizon A of Typic Hapludox soil (exp. 1) and Rhodic Ferralsol soil (exp. 2)
Weather conditions / Temperature / relative humidity:	25–35:20–22◦C day/night
Photoperiod:	Natural daylight conditions (November 2008 – March 2009)
Light intensity	Natural daylight conditions
Study site:	23°25'S, 51°57'W – State University of Maringá
pH:	5.70 (exp. 1) and 5.30 (exp. 2)
Organic matter (Corg):	25.82 g/dm^3 (exp. 1) and 7.82 g/dm^3 (exp. 2)
Soil textural fractions / extractable micronutrient concentrations:	Experiment 1: 76% clay, 15% sand Al: 0.0, Ca: 8.71, Mg: 3.22, K: 1.13 cmolc/ dm3 P: 18.00, S: 9.61, Fe: 64.43, Zn: 13.87, Cu: 25.61, Mn: 156.40 mg/dm^3. Experiment 2: 20% clay, 72% sand Al: 0.0, Ca: 1.85, Mg: 1.24, K: 0.46 cmolc/ dm3 P: 18.00, S: 9.61, Fe: 64.43, Zn: 13.87, Cu: 25.61, Mn:

156.40 mg/dm^3.

6. Observations/analyses:

Measurements: All parameters were evaluated at the last fully expanded trifolium immediately before and after treatment applications (25 DAS; 40 DAS, respectively) and at R1 growth stage (58 DAS) the measures were taken at
the diagnostic leaf

Parameters measured: Net photosynthesis, soil-plant analysis development (SPAD), nutrient contents in diagnostic leaves, shoot and root dry biomass

Statistics: Analyses of the effects of glyphosate and amino acids amendments were determined by Scott Knott groupment test to 5% probability by SISVAR variance analysis software (Ferreira 1999). All treatments were compared with non-GR parental line by Dunnet test to 5% probability by PROC GLM (SAS Institute, Cary, NC, USA).

KLIMISCH EVALUATION

1. Relevance of study: **Not relevant**

Comment: Transgenic-crops such as glyphosate-resistant soybeans are not in the focus of the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant for the assessment of glyphosate efficacy.
It is not clear if the glyphosate formulation tested includes co-formulants and if application rates are relevant for uses in the EU.

2. Reliability of study: **Not reliable**

Comment: No information is given about the used formulation of glyphosate neither the sources.
Detailed information about source of soybean seeds not given.
Application rate may vary greatly due to manual use of a backpack sprayer and wind conditions during application outside.

3. Klimisch code: **Klimisch rating of 3**

Author(s)	**Year**	**Study title**
Zobiole, L.H.S. Kremer, R.J. Silvério de Oliveira Junior, R. Constantin, J.	2012	Glyphosate effects on photosynthesis, nutrient accumulation, and nodulation in glyphosate-resistant soybean Journal of Plant Nutrition and Soil Science

		Volume: 175 Pages: 319-330 DOI: 10.1002/jpln.201000434

Zobiole et al., 2012

Abstract
Previous greenhouse studies have demonstrated that photosynthesis in some cultivars of first- (GR1) and second-generation (GR2) glyphosate-resistant soybean was reduced by glyphosate. The reduction in photosynthesis that resulted from glyphosate might affect nutrient uptake and lead to lower plant biomass production and ultimately reduced grain yield. Therefore, a field study was conducted to determine if glyphosate-induced damage to soybean (Glycine max L. Merr. cv. Asgrow AG3539) plants observed under controlled greenhouse conditions might occur in the field environment. The present study evaluated photosynthetic rate, nutrient accumulation, nodulation, and biomass production of GR2 soybean receiving different rates of glyphosate (0, 800, 1200, 2400 g a.e. ha^{-1}) applied at V2, V4, and V6 growth stages. In general, plant damage observed in the field study was similar to that in previous greenhouse studies. Increasing glyphosate rates and applications at later growth stages decreased nutrient accumulation, nodulation, leaf area, and shoot biomass production. Thus, to reduce potential undesirable effects of glyphosate on plant growth, application of the lowest glyphosate rate for weed-control efficacy at early growth stages (V2 to V4) is suggested as an advantageous practice within current weed control in GR soybean for optimal crop productivity.

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup Weather Max®
Active substance(s):	Glyphosate (as potassium salt)
Surfactant:	Not stated
Description:	Not stated
Source of test substance:	Monsanto Company
Lot/Batch #:	Not stated
Purity:	540 g a.e. L^{-1}

2. Vehicle and/or positive control: no-glyphosate-application (control) treatment

3. Test organism:

Species:	Glyphosate-resistant soybean (*Glycine max* L. Merr.)
Cultivar:	Asgrow AG3539
Source:	Not stated
Crop growth stage at treatment:	Applications at different growth stages: V2 (10 DAS – days after sowing), V4 (20 DAS) and V6 (34 DAS).
Holding conditions prior to test:	Seeds were inoculated with a culture of *Bradyrhizobium japonicum* (SEMIA 5079) and *B. elkanii* SEMIA 587 (> 109 cells per gram) at 500 g/20 kg seeds.

4. Test system:

Study type:	Field experiment
Guideline:	Non
GLP:	No

Guideline deviations:	Not applicable
Duration of study:	48 days after sowing to final measurement at R1 growth stage.
Treatments:	Totally 10 treatments combined in a factorial scheme (3 x 3) +1: one factor are 3 glyphosate test concentrations and second factor was the application timing at 3 different growth stages; and 1 no-glyphosate application control treatment.
Replicates:	Two sites with four replications
Test concentrations:	0, 800 (i.e. label rate for region of study site), 1200 (maximum labelled rate for other regions) and 2400 (virtually "worst-case scenario" to promote injuries) g a.s./ha
Application:	Plants at different growth stages identified during the season, including V2 (10 DAS - days after sowing), V4 (20 DAS), and V6 (34 DAS), were sprayed with glyphosate.
Application devices:	Backpack sprayer with SF110.02 nozzles, under 2 kg cm^{-2} of carbon dioxide (CO2) at 190 L ha^{-1}
Application verification:	not stated

5. Environmental conditions:

Study site:	Sanborn Field (38°56″N, 92°28′W) at the University of Missouri, Columbia, MO
Weather conditions / Temperature / relative humidity:	25-27°C, 80-89% relative humidity at applications, 24-32°C, 78-82% relative humidity at assessments.
Test medium / Soil at study site	Mexico silt loam (fine, smectitic, mesic Aeric Vertic Epiaqualfs)
Photoperiod:	natural daylight conditions (August 2009 – September 2009)
Light intensity	natural daylight conditions
pH:	5.7
Organic matter (Corg):	31 g/kg
Fertilizer:	22 kg P/ha and 42 kg K/ha

6. Observations/analyses:

Measurements:	At R1 growth stage (48 DAS), the last fully expanded trifoliate (diagnostic leaf) was randomly selected from four plants in the middle of the two center rows, which represented 38, 28, and 12 d after glyphosate application at V2, V4, and V6 growth stages, respectively. The **photosynthetic rate** was recorded using an infrared gas analyzer (IRGA; Li-Cor, LI 6400XT, Lincoln, NE, USA) and calculated using the equations of von Caemmerer and Farquhar (1981). Two evaluations were carried out between 1100h and 1200h under different weather conditions. The first assessment was on a cloudy day, with the air temperature between 24°C and 26°C, relative humidity between 86% and 88%, and photosynthetic

photon-flux density (PPFD) between 100 and 140 lmol m^{-2} s^{-1} at the top of the leaf canopy. In contrast, the second evaluation was on a sunny day, no clouds, with the air temperature ranging between 29°C and 32°C, relative humidity between 78% and 82%, and PPFD between 1700 and 1900 lmol m^{-2} s^{-1} at the top of the leaf canopy.

The **SPAD** (Minolta SPAD-502 meter) evaluations were taken on these same diagnostic leaves, whereby the meter was placed randomly on leaf mesophyll tissue avoiding the veins. Three SPAD readings were taken per leaflet of the terminal leaflet of the diagnostic leaf and averaged to provide a single SPAD unit.

Nutrient accumulation

Immediately after collecting the SPAD and photosynthetic rate measurements at R1 growth stage, the upper three leaves consisting of the diagnostic trifoliate and the leaves from the nodes above and below the diagnostic leaf (including petioles) were collected from each of the four sampled plants.

Leaves were washed in deionized water, packed in paper bags, and dried in an oven at 60°C–70°C and weighed after 48 h when a constant dry weight was achieved. The mineral composition (P, K, Ca, Mg, S, Zn, Mn, Fe, Cu) of the leaves was determined by complete perchloric nitric digestion (6 : 1), and B concentration was obtained after dry digestion. All elements, except N, were measured using an AES Perkin Elmer ICP (inductively coupled plasma) spectrophotometer. Nitrogen was determined using the Kjeldahl method, after complete sulfuric acid digestion. The data of nutrient accumulation per plant were calculated based on total shoot (leaves, stems, and petioles) dry weight and nutrient concentrations in the leaves of the upper plant parts. Leaves and petioles comprise approximately 90% of the dry biomass of soybean through the V6 growth stage, and because leaves are the dominant nutrient sinks, nutrient contents were expressed on an aboveground plant basis.

Leaf area, nodulation, and biomass

The leaf area was measured using a leaf-area meter (Delta T. Devices) per entire four plants per plot and then averaged to obtain a total area (cm2 $plant^{-1}$). After these assessments, shoots were clipped at the soil surface and roots were carefully removed from soil, washed under running water and nodules were removed and counted. Immediately after nodule counting, the roots, shoots, and nodules were then

	placed in separate paper bags and transferred to an oven at 60°C–70°C for 48 h after which dry weights were determined.
Parameters measured:	Photosynthetic rate, soil-plant analysis development (SPAD), nutrient accumulation, nodulation, leaf area, shoot and root biomass production
Statistics:	The data errors passed the normality test of Shapiro and Wilk (1965), and because there was homogeneity of error variances, the data for the two repeated experiments were combined. No transformations were necessary. Data were subjected to ANOVA by SAS statistical program (SAS, 2006), and when F values were significant ($p < 1\%$), regression analyses were conducted and equations were adjusted using the polynomial model $y = a + bx + cx^{05}$ by SigmaPlot 10.0 statistical package (SPSS, 2000).

KLIMISCH EVALUATION

1. Relevance of study:		**Not relevant**
	Comment:	Transgenic-crops such as glyphosate-resistant soybeans are not in the focus of the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant. It is not clear if application rates are relevant for uses in the EU. Since only the formulation and not glyphosate alone was tested, study is only relevant for the Roundup formulation.
2. Reliability of study:		**Not reliable**
	Comment:	Detailed information about source of soybean seeds not given. Application rate may vary greatly due to manual use of a backpack sprayer and wind conditions during application outside. Only the lowest test concentration of 800 g a.s./ha comply with labelled single application rate for crops in the region of study sites. The two higher rates do not represent a realistic exposure scenario even not a worst-case.
3. Klimisch code:		**Klimisch rating of 3**

Author(s)	**Year**	**Study title**
Zobiole, L.H.S.	2012	Nutrient Accumulation in Conventional and

Oliveira Junior, R.S. Constantin, J. Oliveira Junior, A. Castro, C. Oliveira, F.A. Kremer, R.J. Moreira, A. e Romagnoli, L.M.		Glyphosate-Resistant Soybean under Different Types of Weed Control (English title) Planta Daninha Volume: 30 No: 1 Pages: 75-85 DOI: 10.1590/S0100-83582012000100009

Zobiole et al., 2012

Abstract
The cultivation of soybean-Glycine max (Roundup Ready® – RR) has increased and little has been reported on the influence of glyphosate on the nutritional status of the plants. The aim of this work was to compare nutrient accumulation at different phenological stages between the cultivars BRS 184 (conventional) and BRS 243 RR (transgenic), with the same crop cycle, under different weed management systems (hand weed and herbicide). Nutrient accumulation and dry matter in conventional soybean was superior to that in the glyphosate-treated RR soybean, indicating that a higher level of nutrients might be required for the RR cultivars to achieve physiological efficiency and a new fertilizer recommendation for RR crops may be considered, due to the reduced nutritional efficiency imposed by glyphosate.

KLIMISCH EVALUATION

1. Relevance of study:		**Not assignable**
2. Reliability of study:	Comment:	**Not assignable** Paper is published in Spanish and the English abstract does not contain sufficient information to assess the relevance and reliability of the study
3. Klimisch code:		**Klimisch rating of 4**

5. Plant communities

The following studies focus on the effect of glyphosate on plant community composition and dynamics. Both are not annex points that have to be addressed in the renewal assessment of active substances. As for any herbicide there is a potential impact on plant communities and their population dynamics. The documents submitted on this topic provide no new scientific evidence. A change or amendment in the assessmnent is not necessary.

Ahrens & Auer, 2012

Author(s)	Year	Study title
Ahrens, C.W. Auer, C.A.	2012	Annual Glyphosate Treatments Alter Growth of Unaffected Bentgrass (*Agrostis*) Weeds and Plant Community Composition. PLoS ONE

		Volume: 7 Issue: 12 DOI: 10.1371/journal.pone.0050643

Abstract
Herbicide resistance is becoming more common in weed ecotypes and crop species including turfgrasses, but current gaps in knowledge limit predictive ecological risk assessments and risk management plans. This project examined the effect of annual glyphosate applications on the vegetative growth and reproductive potential of two weedy bentgrasses, creeping bentgrass (CB) and redtop (RT), where the glyphosate resistance (GR) trait was mimicked by covering the bentgrass plants during glyphosate application. Five field plots were studied in habitats commonly inhabited by weedy bentgrasses including an agricultural hayfield, natural meadow, and wasteland. Results showed that annual glyphosate treatment improved bentgrass survivorship, vegetative growth, and reproductive potential compared with bentgrass in unsprayed subplots. In the second year of growth, RT plants had an 86-fold increase in flower number in glyphosate-treated subplots versus controls, while CB plants had a 20-fold increase. At the end of the three year study, plant community composition had changed in glyphosate-treated subplots in hayfield and meadow plots compared to controls. Soils in subplots receiving glyphosate had higher nitrate concentrations than controls. This is the first study to mimic the GR trait in bentgrass plants with the goal of quantifying bentgrass response to glyphosate selection pressure and understanding the impacts on surrounding plant communities.

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup Pro
Active substance(s):	Glyphosate
Surfactant:	Not stated
Description:	Not stated
Source of test substance:	Monsanto Company, St. Louis, MO
Lot/Batch #:	Not stated
Purity:	Not stated
2. Vehicle and/or positive control:	Control subplots received no weed management during the experiment.
3. Test organism:	
Species:	Creeping bentgrass (*Agrostis stolonifera*) and redtop (*Agrostis gigantea* Roth.)
Cultivar:	Conventional (non-genetically engineered) *Agrostis stolonifera* ‘Penn A-4’ and *Agrostis gigantea* ‘unspecified’
Source:	*Agrostis gigantea*: Des Moines Forage and Turf Seed Corporation, Ankeny, IA
Crop growth stage at treatment:	Not stated
Holding conditions prior to test:	*Agrostis* plants were grown at the university research farm with biweekly mowing (1.27 cm height) and two fertilization treatments at a rate of 0.45 kg per 0.0093 ha for N, P and K (19-19-19, Lesco fertilizer, Troy, MI). In May, 2008, small plugs (3 cm x 3 cm) of acclimated *Agrostis* plants were transplanted into

	the center of 100 subplots (20 subplots x 5 plots).

4. Test system:

Study type:	Field experiment
Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	Three years
Treatments:	A 262 factorial experiment was set up in each plot using a randomized complete block design with two management regimes (annual glyphosate spray or non-sprayed control) and two weedy, non-native bentgrass species (*Agrostis stolonifera* and *Agrostis gigantea*).
Replicates:	Five
Test concentrations:	1.69 kg Roundup Pro® per hectare
Application:	Plots sprayed once per year on June 24–26th. *Agrostis* plants within the sprayed plots were temporarily covered with white plastic bags (0.5 mm thickness) for 10–15 minutes to mimic the herbicide resistance trait. Control subplots received no weed management during the experiment.
Application devices:	Hand-held wand with a single boom Teejet XR8004VS nozzle (Wheaton, IL) and a backpack sprayer (SP system backpack, Santa Monica, CA).
Application verification:	not stated

5. Environmental conditions:

Study site:	Mansfield, Connecticut in the Lower New England Ecoregion, subecoregion Southern New England Coastal Hills and Plains. The plot names and locations were: Meadow 1, 41 499 30.3099 N, 72 149 12.9199 W; Meadow 2, 41 499 30.7199 N, 72 149 18.4399 W; Hayfield 1, 41 469 58.3099 N, 72 139 07.5999 W; Hayfield 2, 41 469 55.8099 N, 72 139 10.5699 W; and Wasteland, 41 499 01.2599 N, 72 139 10.5699 W. Plots were not fertilized, irrigated or mowed during the 3 years of the study, and there was no known history of herbicide use at the study sites.
Weather conditions / Temperature / relative humidity:	Yearly rainfall totals from November 1 to October 31 were: 2008, 375 cm; 2009, 310 cm; 2010, 304 cm. Mid-summer rainfall was: June, 2008, 10.8 cm; July, 2008, 11.5 cm; June, 2009, 14.5 cm; July, 2009, 19.5 cm; June, 2010, 11.1 cm; July, 2010, 8.1 cm. Mid-summer mean maximum temperatures (C*u*) were: June, 2008, 24.2; July, 2008, 26.4; June, 2009, 21.1; July, 2009, 24.1; June, 2010, 24.8; July, 2010, 27.8.
Test medium / Soil at study site	sandy loam or loamy sand
Photoperiod:	natural daylight conditions

Light intensity	natural daylight conditions
pH:	5.64-6.07
Organic matter (Corg):	Not stated
Fertilizer:	No fertilizer applied

6. Observations/analyses:

Measurements:	Measurements of bentgrass growth were taken monthly (May-October) for three years including: surface area covered, maximum leaf height, number of tillers (stolons), number of panicles, and number of flowers. All vascular plant species in each subplot were identified and monitored during the three year study. At the end of the third year (November, 2010), bentgrass survival and above-ground plant biomass were recorded. Subplots with *Agrostis* plants were randomly assigned to two treatment groups: annual glyphosate treatment or control (no herbicide).
Parameters measured:	Surface area covered, maximum leaf height, number of tillers (stolons), number of panicles, number of flowers, bentgrass survival and above-ground plant biomass.
Statistics:	At the end of the experiment, dependent variables were examined using analyses of variance (SAS ver. 9). Soil nutrient levels were analyzed as possible covariates. Data from all subplots were combined to analyze treatment, year (treated as a random variable) and bentgrass species interaction effects. Survivorship was analyzed using a probit model to determine the significance of glyphosate application. Probit analysis was chosen because this type of regression analysis was developed for biological systems involving quantal responses (e.g. death) to differing dosages of a toxin or a stress (e.g. glyphosate application). To assess changes in plant community composition, subplots were graphed on a two-axis graph using non-multidimensional scaling. The Bray-Curtis Dissimilarity between each subplot was calculated and the layout was based on the lowest amount of stress between actual dissimilarity and graphed dissimilarity. Confidence intervals (99%) were calculated and drawn for both control and sprayed subplot groups within each plot. If the confidence intervals did not overlap, then the communities in the subplot treatments (control vs. sprayed) were considered to be different

KLIMISCH EVALUATION

1. Relevance of study:		**Relevant with restrictions**
	Comment:	The issue of glyphosate-resistant weed species and the agronomic consequences were already assessed in the original RAR.
2. Reliability of study:		**Reliable with restrictions**
	Comment:	No information given on the amount of active ingredient (glyphosate) in Roundup Pro. Application rate may vary greatly due to manual use of a backpack sprayer and wind conditions during application outside.
3. Klimisch code:		**Klimisch rating of 2**

Damgaard et al., 2013

Author(s)	Year	Study title
Damgaard, C. Strandberg, B. Mathiassen, S.K. Kudsk, P.	2013	The Effect of Nitrogen and Glyphosate on Survival and Colonisation of Perennial Grass Species in an Agro-Ecosystem: Does the Relative Importance of Survival Decrease with Competitive Ability? PLoS ONE Volume: 8 Issue: 4 DOI: 10.1371/journal.pone.0060992

Abstract
The ecological success of a plant species is typically described by the observed change in plant abundance or cover, but in order to more fully understand the fundamental plant ecological processes, it is necessary to inspect the underlying processes of survival and colonization and how they are affected by environmental conditions. A general ecological hypothesis on the effect of environmental gradients on demographic parameters is proposed and tested. The hypothesis is that decreasing fitness or competitive ability along an environmental gradient is associated with an increasing importance of survival for regulating the abundance of the species. The tested hypothesis is related to both the stress gradient hypothesis and whether the importance of competition increases along productivity gradients. The combined effect of nitrogen and glyphosate on the survival and colonization probability of two perennial grass species, *Festuca ovina* and *Agrostis capillaris*, which are known to differ in their responses to both glyphosate and nitrogen treatments, is calculated using pin-point cover data in permanent frames. We found that the relative importance of survival increased with the level of glyphosate for the glyphosate sensitive *A. capillaris* and decreased for the glyphosate tolerant *F. ovina*. Likewise, increasing levels of nitrogen increased the importance of survival for the relative nitrophobic *F. ovina*. Consequently, the proposed hypothesis was corroborated in this specific study. The proposed method will enable predictions of the effects of agricultural practices on community dynamics in a relatively simple setup eliminating the need to quantify all the interaction among the species in the plant community. The method will be immediately useful for the regulation of noncultivated buffer

strips between agricultural fields and semi-natural and natural biotopes such as hedgerows and waterways.

MATERIALS AND METHODS

1. Test material:

Test item:	RoundupBio®
Active substance(s):	Glyphosate
Surfactant:	Not stated
Description:	Not stated
Source of test substance:	Monsanto Crop Science, Denmark
Lot/Batch #:	Not stated
Purity:	360 g/L glyphosate as a isopropylamine salt
2. Vehicle and/or positive control:	Not stated
3. Test organism:	
Species:	*F. ovina* and *A. capillaris* are perennial grasses. They both have a caespitose growth form, but the turfs formed by *F. ovina* generally are much denser than those formed by *A. capillaris*. In contrast to *A. capillaris* which is deciduous, *F. ovina* is winter green and productive through the winter as long as temperatures are above zero. Furthermore, *F. ovina* has narrow, curled and waxy leaves, whereas *A. capillaris* has broader, flat leaves without any wax cover. *F. ovina* has previously been shown to be more tolerant to glyphosate than *A. capillaris*, whereas *A. capillaris* is a more nitrophilous species than *F. ovina* (the Ellenberg N values of the two species are 4 and 1, respectively).
Cultivar:	Not stated
Source:	Not stated
Crop growth stage at treatment:	Not stated
Holding conditions prior to test:	Thirty-one selected grassland plant species covering different life form strategies (CRS strategies) were sown in spring 2001.

4. Test system:

Study type:	Field experiment
Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	Three years
Treatments:	Thirty-one selected grassland plant species covering different life form strategies (CRS strategies) were sown in spring 2001. The experimental manipulations were set up as a completely randomized block design with 10 replicates of twelve treatments. Each replicate plot was 7 m x 7 m with a buffer zone of 1.5 m surrounding the plot. A buffer zone of 10 m separated the experiment from the surrounding

	vegetation. The buffer zones were also sown with the seed mixture. The treatments included 4 glyphosate treatments and 3 nitrogen treatments (0, 25 and 100 kg N/ha) applied in a full factorial design.
Replicates:	10
Test concentrations:	0; 14.4; 72 and 360 g glyphosate/ha. The applied glyphosate doses were equal to 0, 1, 5 and 25%, respectively, of the dose recommended for pre- and post-harvest treatment against perennial weeds, which is the most common use of glyphosate in Denmark.
Application:	The plots were treated by glyphosate for the first time on 24 August 2001. Since then, glyphosate and fertilizer treatments were carried out once every year in mid-May.The wind speed on the days selected for spraying was very low (0–2 m/s).
Application devices:	Experimental spraying equipment was used. The boom was fitted with Lurmark Low-drift LD 015 Green nozzles operated at a pressure of 2.0 bars delivering a spray volume of 300 L/ha.
Application verification:	not stated

5. Environmental conditions:

Study site:	Djursland, Denmark
Weather conditions / Temperature / relative humidity:	not stated
Test medium / Soil at study site	Former agricultural field on dry, nutrient poor sandy soil. The field laid fallow for a couple of years prior to the start of the experiment in 2001.
Photoperiod:	natural daylight conditions
Light intensity	natural daylight conditions
pH:	not stated
Organic matter (Corg):	Not stated
Fertilizer:	All 120 plots received phosphorus (53 kg/ha), potassium (141 kg/ha), sulphur (50 kg/ha) and copper (0.7 kg/ha) every year.

6. Observations/analyses:

Measurements:	In order to study the effect of nitrogen and glyphosate on *F. ovina* and *A. capillaris*, one permanent 0.5 m x 0.5 m quadrate was placed within each of the 120 plots in 2007. The quadrate was not placed randomly, but in such a way that both *F. ovina* and *A. capillaris* were noticeably abundant in the quadrate. Local presence-absence data of the two species were determined by the pin-point method using a pin-point frame with the same dimension as the quadrate. The frame had 25 pin-positions regularly placed at a distance of 10 cm. At each position, a sharply pointed pin with a diameter of 0.5 mm was passed vertically through the vegetation and

	presence-absence data of the two species were recorded for each pin. The sampling was performed three times a year for three years in the period 2007–2009, but in this analysis, the sampling that was made in the spring approximately two weeks after the herbicide application was used.
Parameters measured:	Presence-absence of *F. ovina* and *A. capillaris*
Statistics:	The quantitative effect of survival and colonization processes on population growth of individuals was summarized using population models and the elasticity (the relative contribution of different demographic parameters on population growth rates) was calculated.

KLIMISCH EVALUATION

1. Relevance of study:		**Not relevant**
	Comment:	Plant population dynamics are not in the focus of the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant for the assessment of glyphosate efficacy.
2. Reliability of study:		**Reliable without restrictions**
	Comment:	Well documented non-GLP, non-guideline study that meets scientific principles.
3. Klimisch code:		**Klimisch rating of 1**

Marshall et al., 2013

Author(s)	**Year**	**Study title**
Marshall, J. Brown, V.K. Boatman, N.D. Lutman, P.J.W. Squire, G.R. Ward, L.K.	2003	The role of weeds in supporting biological diversity within crop fields Weed Research 2003 43, 77–89 Volume: 43 Pages: 77-89 DOI: 10.1046/j.1365-3180.2003.00326.x

Abstract
Weeds are major constraints on crop production, yet as part of the primary producers within farming systems, they may be important components of the agroecosystem.

Using published literature, the role of weeds in arable systems for other above-ground trophic levels are examined. In the UK, there is evidence that weed flora have changed over the past century, with some species declining in abundance, whereas others have increased. There is also some evidence for a decline in the size of arable weed seedbanks. Some of these changes reflect improved agricultural efficiency, changes to more winter-sown crops in arable rotations and the use of more broad-spectrum herbicide combinations. Interrogation of a database of records of phytophagous insects associated with plant species in the UK reveals that many arable weed species support a high diversity of insect species. Reductions in abundances of host plants may affect associated insects and other taxa. A number of insect groups and farmland birds have shown marked population declines over the past 30 years. Correlational studies indicate that many of these declines are associated with changes in agricultural practices. Certainly reductions in food availability in winter and for nestling birds in spring are implicated in the declines of several bird species, notably the grey partridge, Perdix perdix. Thus weeds have a role within agroecosystems in supporting biodiversity more generally. An understanding of weed competitivity and the importance of weeds for insects and birds may allow the identification of the most important weed species. This may form the first step in balancing the needs for weed control with the requirements for biodiversity and more sustainable production methods.

In the review by Marshall et al. (2003), most of the cited literature (60 references) was addressed tothe role of weds in agro-ecosystems. Altough the effect of herbicide application on weed species diversity is addressed, the specific influence of glyphosate in specific is not covered. Most references (52) are from the period 1990 to 2001.

1. Relevance of study:		**Not relevant**
	Comment:	The influence of glyphosate in specific is not covered in the review. The study can therefore not be classified as relevant for the assessment of glyphosate efficacy.

Pfleeger et al., 2014

Author(s)	**Year**	**Study title**
Pfleeger,T. Blakeley-Smith, M. Lee, E.H. King, G. Plocher, M. Olszyk, D.	2014	Effects of single and multiple applications of glyphosate or aminopyralid on simple constructed plant communities. Environmental Toxicology and Chemistry Volume: 33 Issue: 10 DOI: 10.1002/etc.2686

Abstract
To determine effects of multiple applications of herbicides on small constructed plant communities, *Prunella vulgaris* L.var. *lanceolata* Fern, *Festuca roemeri* (Pavlick) Alexeev, *Clarkia amoena* (Lehm.) Nels., and *Cynosurus echinatus* L. were grown together in small field plots. Plants were treated with glyphosate at target concentrations of 0x, 0.01x, 0.1x, and 0.2x a field application rate (FAR) of 1122 g ha^{-1} active ingredient (a.i.) for 3 yr in 1 location, and for 2 yr in a second location. Plants also were treated with aminopyralid at 0x, 0.037x, 0.136x, and 0.5x FAR of 123 g ha^{-1} a.i. for 2 yr in 2 locations. Plants received 1, 2, or 3

applications of each herbicide each year. Species and community responses depended on herbicide concentration and number of applications. With glyphosate, plant volume (modified formula for a cone) tended to decrease for all species (especially *C. echinatus*), and the decreases generally became larger with more applications. Plant communities exposed to the 2 greatest concentrations initially differed from controls but then appeared to recover. With aminopyralid, *C. amoena* was essentially eliminated from the communities, especially at the 2 greatest FARs, whereas the other 3 species tended to have significant increases in volume, especially at the 2 smallest FARs. With aminopyralid, increasing numbers of applications produced variable results, and the plant community volume never tended to recover.

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup Original
Active substance(s):	Glyphosate
Surfactant:	All herbicide treatments except for the "0" no spray control (no application) treatment, included Preference (nonionic surfactant blend; WinField Solutions) at 0.5% v/v.
Source of test substance:	Monsanto Company, St. Louis, MO USA
Lot/Batch #:	Not stated
Purity:	41% active ingredient
Test item:	Milestone
Active substance(s):	Aminopyralid
Surfactant:	All herbicide treatments except for the "0" no spray control (no application) treatment, included Preference (nonionic surfactant blend; WinField Solutions) at 0.5% v/v.
Source of test substance:	Dow AgroSciences
Lot/Batch #:	Not stated
Purity:	40.6% active ingredient
2. Vehicle and/or positive control:	Not stated
3. Test organism:	
Species:	New populations of seedlings (ca. 500 individuals in 2007 and 2008 and 150 in 2006) of *Prunella vulgaris* L.var. *lanceolata* Fern. (self-heal), *Festuca roemeri* (Pavlick) Alexeev (Roemer's fescue), *Cynosurus echinatus* L. (bristly dogstail grass), and *Clarkia amoena* (Lehm.) Nels. (farewell to spring) were started each year in a greenhouse and then transplanted to the field. Three of the species are native to Oregon (*C. amoena*, *P. vulgaris*, and *F. roemeri*), whereas *C. echinatus* was introduced to the United States. Two species are forbs (C. amoena and *P. vulgaris)*, and 2 (*C. echinatus* and *F. roemeri*) are grasses. Two species are annuals (*C. amoena* and *C. echinatus*), and 2 (*P. vulgaris* and *F. roemeri*) are perennials. Based on greenhouse studies, *P. vulgaris* was the most sensitive of the 4 species to glyphosate,

	showing the greatest reduction in height. *Clarkia amoena* was the only 1 of the 4 species to show a height reduction with aminopyralid, whereas *C. echinatus* showed a slight increase in height.
Cultivar:	Not stated
Source:	Not stated
Crop growth stage at treatment:	Not stated
Holding conditions prior to test:	After approximately 21 d of growth in the greenhouse, plants were moved to a lath house for several more days to harden off prior to planting at the farms. Transplanting took place in mid-April.

4. Test system:

Study type:	Field experiment
Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	Three years
Treatments:	There were 154 plots at the Botany Farm in 2006 and 200 plots at each farm in 2007 and 2008. Two herbicides, glyphosate and aminopyralid were applied 1, 2, or 3 times per growing season. A restricted randomization design that balanced initial plant size across treatments was used to allocate 1/3 of the herbicide-treated plots to a single application, 1/3 of the herbicide-treated plots to 2 applications, and 1/3 of the herbicide-treated plots to 3 applications. These 3 spray events occurred each year and were 2 wk apart. In 2006, only glyphosate was used and only at the Botany Farm. The first herbicide application was in early May each year.
Replicates:	There were 14 replicate plots per treatment in 2006 and 10 replicate plots for each treatment and farm in 2007 and 2008.
Test concentrations:	Glyphosate was applied at 0x, 0.01x (11.2 g ha^{-1}), 0.1x (112.2 g ha^{-1}), and 0.2x (224.4 g ha^{-1}) of the field application rate of 1122 g ha^{-1} a.i. (832 g ha^{-1} acid phosphate). Aminopyralid was applied at target concentrations of 0x, 0.037 x (4.6 g ha^{-1}), 0.136 x (16.7 g ha^{-1}), and 0.5 x (61.5 g ha^{-1}) of the field application rate, with a field application rate of 123 g ha^{-1} a.i.
Application:	Concentrations of 0.01x to 0.1x field application rate would represent levels at or just outside the field edge, whereas vhigher concentrations were used for comparison purposes to represent concentrations likely to affect plants.
Application devices:	Herbicides were applied using a unicycle plot sprayer (Mater Engineering) equipped with a single spray nozzle (TeeJet TP80015; Spraying Systems) on a hand-held wand.

Application verification:	not stated

5. Environmental conditions:

Study site:	The experiments were conducted over several years in fields at 2 Oregon State University experimental farms (Botany and Hyslop) in the Willamette Valley (OR, USA). Each year, a new field was chosen adjacent to the previous year's fields, and new experimental plots were created.
Weather conditions / Temperature / relative humidity:	not stated
Test medium / Soil at study site	not stated
Photoperiod:	natural daylight conditions
Light intensity	natural daylight conditions
pH:	Not stated
Organic matter (Corg):	Not stated
Fertilizer:	Each plot was fertilized with 10 g of Osmocote 14-14-14 slow release fertilizer

6. Observations/analyses:

Measurements:	Each plant's height and width were measured the week before the first herbicide treatment (first sampling period) and every 2 wk thereafter during the growing season (second through sixth sampling periods in 2006, and through the seventh sampling period in 2007 and 2008). The end of the growing season was determined as the time when plant growth ceased and plants began to dry up and senesce. For *C. echinatus* at both sites in 2007, no measurements were made at the seventh sampling period because all plants died. Height was measured from the ground to the tallest point of living tissue. Plant width was recorded at the broadest diameter of the plant (w1), and a second width was taken perpendicular to the first (w2). Plant volume was determined by using a modification of the formula of a cone.
Parameters measured:	Plant height and width, plant volume
Statistics:	For each herbicide, species, and sampling period, the weekly plant measurements of plant volume at the 2 farms and replicate experiments across years were first combined and analyzed with univariate analysis of covariance (ANCOVA) using SAS PROC GLM. The ANCOVA included terms for the main effects of year, farm, target application rate, and number of applications and their interactions. The log of initial plant volume, taken at the first sampling period prior to the first application of an herbicide treatment, was used as a covariate. Assuming that treatment effects and standard deviations were proportional to plant size, a log transformation was applied to the plant

volume data to test for treatment differences and to stabilize the variance. To be able to analyze for dead plants with 0 volume, a small number (0.001) was added to all volumes prior to log transformation. Because treatment variances were 0 or near 0 for treated plants displaying high mortality rates, a weighted ANCOVA with weights proportional to the inverse of the treatment variance was required to normalize the data to unit variance. A weight of 10 000 was used when the treatment variance was 0 (i.e., all treated plants died). Control plots were not sprayed with either herbicide or surfactants. To obtain a balanced ANCOVA, the control plots for each farm were randomly assigned to the 3 application periods with 5 plots, 5 plots, and 4 plots for the first, second, and third applications, respectively, in 2006 and 3 plots, 3 plots, and 4 plots for the 3 application periods, respectively, in 2007 and 2008.

Because of many significant interactions involving year and farm (data not shown), the ANCOVA was also run separately for each herbicide, species, year, farm, and sampling period to test for the simple effects of an herbicide treatment. Statistical analyses were performed using the GLMprocedure of the SAS/STAT software, Ver 9.2 of the SAS System (SAS Institute). Ordinations were performed on total plant volume data for the Botany Farm in 2007 by herbicide and herbicide concentration separately in PC Ord, Ver 6.0. Prior to importing the data into PC Ord, the means from the 10 replicates were taken for each treatment within an herbicide but with replicates omitted where data were missing. The data were then square root transformed. After importation into PC Ord, data were relativized (p¼1) by rows to reduce nonconstant variances.

Nonmetric multidimensional scaling (NMS) was used to visualize how the communities changed over time following the experimental treatments. Each point on the ordinations represented plant community composition for each treatment group per sample period. The ordinations included successional vectors that connected each treatment group at each sampling period. Points that were close to one another were more similar in species composition based on volume than points that were further apart. A 2-dimensional solution was selected using Sorensen distance, a random starting configuration, and 250 runs with real data, as suggested by McCune and Grace. Twodimensional outcomes were selected for a more informative graphics display. A multiresponse

permutation procedure (MRPP) was used following the NMS. This was a nonparametric test of the hypothesis of no difference between 2 or more a priori groups. A priori groups were defined by the number of herbicide applications: no herbicide (control), 1 time, 2 times, or
3 times. The data were partitioned and analyzed separately by herbicide, field application rate, and sampling period. The MRPP tested the hypothesis of no significant difference in plant communities with the different numbers of applications and herbicide concentrations at the first (prior to treatments), fourth (after all treatments), and sixth (last sampling with all 4 species) sampling periods, with A (chance corrected within-group agreement) and p (likelihood that an observed difference is because of chance) values given for differences.

KLIMISCH EVALUATION

1. Relevance of study:		**Not relevant**
	Comment:	Plant population dynamics are not in the focus of the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant for the assessment of glyphosate efficacy.
2. Reliability of study:		**Reliable without restrictions**
	Comment:	Well documented non-GLP, non-guideline study that meets scientific principles.
3. Klimisch code:		**Klimisch rating of 1**

Akamatsu et al., 2014

Author(s)	Year	Study title
Akamatsu , F. Makishima, M. Taya, Y. Nakanishi , S. Miwa, J.	2014	Evaluation of glyphosate application in regulating the reproduction of riparian black locust (*Robinia pseudoacacia* L.) after clear-cutting, and the possibility of leaching into soil Landscape and Ecological Engineering Volume: 10 Pages: 47-54 DOI: 10.1007/s11355-013-0215-x

Abstract
Black locust - an invasive alien species in riparian forests - is becoming more prevalent in many rivers of eastern Japan. Riparian black locust forests are typically cut down to maintain

river-flow capacity. However, such forests often reproduce rapidly by stump sprouting and root suckering and regenerate by germination. Thus, more effective riparian forest management approaches are required. To regulate the reproduction of black locust forests after clear-cutting, we examined the regrowth-inhibiting effects of glyphosate herbicide application to stumps, in accordance with current river management protocol (i.e., winter logging operation). Further, we investigated the concentrations of glyphosate leaching into the soil at a depth of 30 cm in a riparian area of the Tenryu River. Our results showed that glyphosate application to stumps completely inhibited stump sprouting but not root suckering or seedling germination. The glyphosate concentration leaching into the soil reached a maximum (2.6 ± 0.7 mg kg-1, mean ± standard error) on day 1 after the application, and subsequently declined to below the detection limit on day 2. Thus, the rapid degradation of glyphosate was confirmed, despite the fact that the herbicide leached into the soil after application to the stumps. The glyphosate application has limited effectiveness against root suckering and germination of riparian black locust forests after clear-cutting in winter, in accordance with the current river management protocol.

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup Maxload
Active substance(s):	Glyphosate
Surfactant:	Not stated
Source of test substance:	Nissan Chemical Industries Ltd., Tokyo, Japan
Lot/Batch #:	Not stated
Purity:	Not stated

2. Vehicle and/or positive control: Not stated

3. Test organism:

Species:	*Robinia pseudoacacia* L. (black locust)
Cultivar:	Not stated
Source:	Not stated
Crop growth stage at treatment:	Trees were cut down before application.
Holding conditions prior to test:	Trees were cut down before application.

4. Test system:

Study type:	Field experiment
Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	One year
Treatments:	To examine the regrowth-inhibiting effects of glyphosate application to black locust stumps and the concentrations of glyphosate leaching into soil, a glyphosate treatment plot (50 x 50 m) and a control plot (20 x 20 m) were established approximately 100 m apart. The plots were subdivided into 100 and 16 quadrats, respectively, each measuring 5 x 5 m.
Replicates:	None
Test concentrations:	Not stated.
Application:	Using a paintbrush, adequate amounts of undiluted solution of a herbicide, the active ingredient of which is the potassium salt of glyphosate were applied to

	stumps of trees in the treatment quadrats. The paintbrush method was used rather than aerial application in an attempt to minimize the environmental impact.
Application devices:	Paintbrush
Application verification:	not stated

5. Environmental conditions:

Study site:	Riverbank located in Komagane City, Nagano Prefecture, 180 km from the mouth of the Tenryu River 35°43'44''N, 137°58'06''E; 560 m above sea level (a.s.l.); riverbed slope, 1/226.
Weather conditions / Temperature / relative humidity:	During the past decade, the mean annual precipitation and mean annual temperature recorded at Ina weather station (located 10 km from the study site) were 1551 mm and 11.6 °C, respectively.
Test medium / Soil at study site	not stated
Photoperiod:	natural daylight conditions
Light intensity	natural daylight conditions
pH:	Not stated
Organic matter (Corg):	Not stated
Fertilizer:	Not stated

6. Observations/analyses:

Measurements:	**Plant measurements** In February 2012, the diameter of all black locust trees within the treatment and control quadrats at breast height over the bark (DBH at a height of 1.3 m) were measured and the trees cut down. At the end of June 2012, the numbers of stump sprouts, root suckers, and seedlings in the treatment and control quadrats were counted. As a preliminary investigation, 100 shoot samples were dug up to distinguish root suckers and seedlings. **Analysis of glyphosate in soil** To investigate time-dependent changes in concentrations of glyphosate leaching into soil after glyphosate application treatment, four stumps from each treatment and control quadrat, i.e., eight stumps were randomly selected. In addition, soil samples of approximately 500 g at a depth of 30 cm and a distance of 50 cm from each stump on five occasions were collected: before glyphosate application and on days 1, 2, 5, and 7 after treatment to yield 40 total samples. At this depth, it was anticipated that glyphosate would leach out of the roots of black locust stumps to which the compound had been applied with a paintbrush. The collected soil samples were passed through a sieve to obtain particles with a diameter of <2 mm for glyphosate analysis. Glyphosate in the sieved samples was extracted and

	derivatized according to a method described by the Ministry of Health, Labour and Welfar. Subsequent quantitative analysis of the samples was conducted according to the following method outlined by the Ministry of the Environment: To an extraction vessel, 4 g (wet weight) of sample and 10 ml of 1 % aqueous ammonia were added and shaken for 30 min. After centrifugation, the supernatant was transferred to another container. To the residual mixture, 10 ml of 1 % aqueous ammonia was added, shaken for 30 min, and centrifuged; the supernatant was then added to the container. To the extract in the container, 1 % aqueous ammonia was added to give a total volume of 40 ml. After filtration of the extract (40 ml) with a filter u0.45 lm), 5 ml of the extract was concentrated on a rotary evaporator to remove aqueous ammonia. The residual liquid was transferred to a stoppered test tube, and ultrapure water was added to the tube to give a total volume of 5 ml. Next, 0.25 ml of 0.1 mol l^{-1} sodium tetraborate solution, 2.6 ml of 0.1 % 9-fluorenylmethyl chloroformate (FMOC) in acetone, and 0.25 g of sodium chloride (NaCl) were added to the tube. The tube was shaken for 10 min and left at room temperature for 30 min for derivatization. After the addition of 4 ml of ethyl acetate to the obtained derivatized solution, the solution was shaken for extraction, and the aqueous layer was separated and used for quantitative analysis. The glyphosate concentrations in samples were determined using high-performance liquid chromatography (HPLC) (LC-20AD, Shimadzu Corporation, Kyoto, Japan) equipped with a fluorometric detector (RF-10AXL, Shimadzu). Separation was performed using an octyl-bonded silica column (150 9 4.6 mm internal diameter, particle size 5 lm; Inertsil C8-3, GL-Sciences Inc., Tokyo, Japan). The mobile phase was acetonitrile/water (20/80, v/v) containing 0.1 M monopotassium phosphate (KH2PO4) (pH 2.5) as a pH regulator. The mobile-phase flow rate was 1.0 ml min^{-1}, and the detector wavelength was 315 nm. Under these conditions, the retention time for glyphosate was 4.4 min. The glyphosate concentration in each sample was expressed on a soil dry weight basis.
Parameters measured:	Stump sprouts, root suckers, and seedlings

Statistics: Differences between treatment and control quadrats were independently analyzed by t test to compare DBH of black locust and densities of stump sprouts, root suckers, and seedlings. The relationship between DBH and the number of stump sprouts and between densities of stumps and root suckers or seedlings were assessed using Spearman's rankcorrelation coefficient. The significance level of all statistical tests was assessed at a = 0.05. We performed all statistical analyses using R ver. 2. 15.00 software (R Development Core Team 2012).

KLIMISCH EVALUATION

1. Relevance of study: **Not relevant**

Comment: The use of glyphosate to manage riparian forests and the leaching of glyphosate in soil are not addressed in the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant for the assessment of glyphosate efficacy.

2. Reliability of study: **Not reliable**

Comment: No information given on the amount of active ingredient (glyphosate) in the used formulation. The application method of glyphosate (application via a paintbrush) is not reliable as no information on the amount of herbicide applied can be obtained.

3. Klimisch code: **Klimisch rating of 3**

Chauhan & Abugho, 2012

Author(s)	Year	Study title
Chauhan, B.S. Abugho, S. B.	2012	Threelobe Morningglory (*Ipomoea triloba*) Germination and Response to Herbicides Weed Science Volume: 60 Pages: 199-204 DOI: 10.1614/WS-D-11-00137.1

Abstract
Experiments were conducted in the laboratory and screenhouse to determine the effects of scarification; alternating day/ night temperatures; light, salt, and water stress; seed burial

depth; and rice residue on seed germination and seedling emergence of threelobe morningglory, and to evaluate the response of this weed to commonly available POST herbicides in the Philippines. Germination was stimulated by seed scarification, suggesting that inhibition of germination in this species is mainly due to the hard seed coat. Germination of the scarified seeds was not influenced by the tested temperatures (alternating day/night temperatures of 25/15, 30/20, and 35/25 °C) and light. The concentrations of sodium chloride, ranging from 0 to 250 mM, did not influence germination of the scarified seeds of threelobe morningglory. The osmotic potential required for 50% inhibition of maximum germination was 20.35 MPa, although some seeds germinated at 20.6 MPa. Seedling emergence was greatest for the seeds placed on the soil surface (96%), and emergence declined with increased burial depth in soil. The burial depth required for 50% inhibition of maximum emergence was 2.8 cm. No seedlings emerged from a burial depth of 6 cm or greater. Residues of up to 6 Mg ha21 on the soil surface did not influence seedling emergence of threelobe morningglory. The herbicide 2,4-D at 400 g ai ha21 provided excellent control of threelobe morningglory when applied at the four-leaf (100%) and six-leaf (97%) stages. However, at the eight-leaf stage, percent control was reduced to 67% and herbicide rate had to be increased twofold to achieve 95% control. The information gained from this study could contribute to developing components of integrated weed management strategies for threelobe morningglory. Soil inversion by tillage to bury weed seeds below their maximum depth of emergence and early application of an effective POST herbicide could serve as important tools for managing threelobe morningglory.

MATERIALS AND METHODS

1. Test material:

Test item:	Not stated
Active substance(s):	Glyphosate and other active ingredients tested
Surfactant:	Not stated
Source of test substance:	Monsanto Phis. Inc., Muntinlupa City, Philippines
Lot/Batch #:	Not stated
Purity:	Not stated

2. Vehicle and/or positive control: Not stated

3. Test organism:

Species:	*Ipomoea triloba* (threelobe morningglory)
Source:	Seeds of threelobe morningglory were collected in March 2011 from rice fields at the International Rice Research Institute (IRRI) farm, Philippines. Seeds collected from at least 150 plants were bulked and cleaned.
Crop growth stage at treatment:	Four-, six-, and eight-leaf stage.
Holding conditions prior to test:	Twenty scarified seeds were planted on the soil surface in plastic trays (8 by 8 by 5 cm) and covered with a thin layer of soil.

4. Test system:

Study type:	Laboratory and screenhouse
Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	Not stated
Treatments:	The POST herbicide treatments included (among

	others) glyphosate.
Replicates:	All experiments were conducted two times using a randomized complete block design with four replicates.
Test concentrations:	250 and 500 g glyphosate/ha
Application:	Threelobe morningglory seedlings were sprayed at the four-, six-, and eight-leaf stages, and there were control treatments for each leaf stage in which herbicides were not sprayed.
Application devices:	Herbicides were applied using a research track sprayer (DeVries Manufacturing, Hollandale, MN) that delivered 210 L ha21 spray solution at a spray pressure of 140 kPa. Flat nozzles (Teejet 80015) were used in the sprayer.
Application verification:	Not stated

5. Environmental conditions:

Study site:	Experiments were conducted at the laboratory and screenhouse facilities of the IRRI, Los Banos, Philippines.
Weather conditions / Temperature / relative humidity:	Not stated
Test medium / Soil at study site	31 : 37 : 32% sand : silt : clay
Photoperiod:	Not stated
Light intensity	Not stated
pH:	Not stated
Organic matter (Corg):	Not stated
Fertilizer:	Not stated

6. Observations/analyses:

Measurements:	Seedling survival was determined 14 d after herbicide application with the criterion of at least one green leaf on the plant. The shoot (leaf and stem) of the plants were dried in an oven at 70 C for 72 h to measure dry biomass and expressed as percent control.
Parameters measured:	Seedling survival, shoot dry biomass
Statistics:	The data from the two ‘‘experiments/ runs’’ were combined for analysis because there were no interaction effects of treatment and experiment. Data variance was visually inspected by plotting residuals to confirm homogeneity of variance before statistical analysis. Herbicide data were subjected to an ANOVA and means were separated using LSD at P 5 0.05 (GenStat 13th Edition, VSN International Ltd., U.K.).

KLIMISCH EVALUATION

1. Relevance of study: **Not relevant**

Comment: The weed species *Ipomoea triloba* does not occur in Europe and is therefore not a target species of glyphosate in Europe. The study can therefore not be classified as relevant for the European assessment of glyphosate efficacy.

2. Reliability of study: **Not reliable**

Comment: It remains unknown what glyphosate formulation was used. Formulation is of unknown content of active substance, adjuvants or surfactants.
The information on light and temperature regime for the herbicide experiments is not sufficient.

3. Klimisch code: **Klimisch rating of 3**

Pfleeger et al., 2012

Author(s)	Year	Study title
Pfleeger,T. Blakeley-Smith, M. King, G. Lee, E.H. Plocher, M. Olszyk, D.	2012	The effects of glyphosate and aminopyralid on a multi-species plant field trial Ecotoxicology Volume: 21 Pages: 1771-1787 DOI: 10.1007/s10646-012-0912-5

Abstract
In the United States, the US EPA has the responsibility for the registration of pesticides. For the protection of nontarget terrestrial plants this requires two simple greenhouse tests (seedling emergence and vegetative vigor), each done with ten species grown individually. Indications of unacceptable effects levels equivalent to environmental exposure can lead to field testing which is not well-defined. Our objective was to develop a regional field test that is simple, economical, geographically flexible and with endpoints of ecological significance and compare the results with the standard greenhouse tests. Three native Oregon plant species were grown together with an introduced species. The experiment was replicated at two locations and repeated for 3 years with glyphosate applied at 0, 0.01 (8.3 g/ha), 0.1 (83.2 g/ha), and 0.2 (166.4 g/ha) 9 FAR (Field Application Rate of 832 gm/ha acid equivalent) and 2 years with aminopyralid applied at 0, 0.037 (4.6 g/ha), 0.136 (16.7 g/ha), and 0.5 (61.5 g/ha) 9 FAR (123 g/ha acid equivalent). With glyphosate, plant height and volume decreased with increasing herbicide concentration for all species, and for nearly all farm x year combinations. With aminopyralid, one species died at nearly all concentrations, sites and years, while the effects on the other three species were less pronounced and variable. The relative rank in glyphosate sensitivity among species in the field studies differed from the ranking from greenhouse studies, with *Cynososurs echinatus* the most sensitive in the field but *Prunella vulgaris* the most sensitive in the greenhouse. With aminopyralid, sensitivity generally was similar for all species in the greenhouse as in the field. The results suggest that a simple field test can be successfully designed to investigate the ecological effects of herbicides on plant communities and supplement information gained from greenhouse tests performed in controlled environments.

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup Original
Active substance(s):	Glyphosate
Surfactant:	All herbicide treatments except for the "0" no spray control (no application) treatment, included Preference (nonionic surfactant blend; WinField Solutions) at 0.5% v/v.
Source of test substance:	Monsanto Company, St. Louis, MO USA
Lot/Batch #:	Not stated
Purity:	41% active ingredient
Test item:	Milestone
Active substance(s):	Aminopyralid
Surfactant:	All herbicide treatments except for the "0" no spray control (no application) treatment, included Preference (nonionic surfactant blend; WinField Solutions) at 0.5% v/v.
Source of test substance:	Dow AgroSciences, LLC, Indianapolis, USA
Lot/Batch #:	Not stated
Purity:	40.6% active ingredient
2. Vehicle and/or positive control:	Not stated
3. Test organism:	
Species:	*Prunella vulgaris* L.var. *lanceolata* Fern.(Self-heal) *Festuca roemeri* (Pavlick) Alexeev. (Roemer's fescue) *Cynosurus echinatus* L. (Bristly dogstail grass) *Clarkia amoena* (Lehm.) Nels. (Farewell to spring)
Cultivar:	Not stated
Source:	Not stated
Crop growth stage at treatment:	**Greenhouse experiment** Plants were treated with herbicides when they were small and at an early vegetative growth stage, approximately 24–40 days after planting, depending on growth rate of the species. Plants were small, at 25–36 mm high for the two forbs, *C. amoena* and *P. vulgaris* and 103–196 mm high for the two grasses *C. echinatus* and *F. roemerii*.
Holding conditions prior to test:	**Field experiment** *Prunella vulgaris* L.var. *lanceolata* Fern.(Self-heal) and *Festuca roemeri* (Pavlick) Alexeev. (Roemer's fescue) seeds were thinly covered and then stratified between 2 and 4 weeks at 5 °C on pre-moisten native mineral soil from the Willamette Valley in Conetainers. They were then allowed to germinate on a mist bench prior to being transferred to greenhouse benches. Seeds of *Cynosurus echinatus* L. (Bristly dogstail grass) and *Clarkia amoena* (Lehm.) Nels. (Farewell to spring) were planted similarly except omitting the stratification step. After about 21 days of the plants

growing in the greenhouse, they were moved to a lath house for several more days prior to planting at the farms. Transplanting took place in mid April. Field plots included one plant of each of the four species, with plants 10 cm apart.

Greenhouse experiment

Plants were grown in 10 cm diameter x 9.8 cm high pots, with a volume of approximately 350 ml.

4. Test system:

Study type:	Field experiment and greenhouse
Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	Three years
Treatments:	**Field experiment** Two herbicides, glyphosate and aminopyralid were applied one per growing season in May. **Greenhouse experiment** Two herbicides, glyphosate and aminopyralid were applied at different concentrations.
Replicates:	**Field experiment** There were 14 replicate plots per treatment in 2006 and 10 replicate plots for each treatment and farm in 2007 and 2008. **Greenhouse experiment** There were two replicate experiments (i.e., groups) with each herbicide rate, and six single plant (pot) replicates per treatment per experiment.
Test concentrations:	**Field experiment** Glyphosate was applied at 0, 0.01x (8.3 g ha^{-1}), 0.1 (83.2 g ha^{-1}), and 0.2x (166.4 g ha^{-1}) of the field application rate of 832 g ha^{-1} a.i. **Greenhouse experiment** Concentrations of glyphosate were 0, 0.001 (0.8 g/ha), 0.002 (1.7 g/ha), 0.01 (8.3 g/ha), 0.1 (83.2 g/ha) and 1.0 (832 g/ha) 9 FAR of 832 g/ha a.e. (w/v).
Application devices:	**Field experiment** Herbicides were applied using a unicycle plot sprayer (Mater Engineering) equipped with a single spray nozzle (TeeJet TP80015; Spraying Systems) on a hand-held wand. **Greenhouse experiment** Herbicides were applied using a spray chamber (Model RC-5000-100EP Overhead Trolley Sprayer for Reproducible Agrochemical Research, Mandel Scientific Company, Ltd, Guelph, ON, Canada) which has a laminar flow hood design in which the platform holding the plants can be adjusted to vary the distance between the nozzle (‘‘Even flat fan’’ 8002E) and the plant material. The nozzle (‘‘Even

	flat fan'' TeeJet TP8002E-VS, Spraying Systems, Wheaton, IL USA) was 43-48 cm above the plant canopy. Herbicide solutions were delivered at 233 l ha^{-1}. The same herbicide formulations were used as in the field.
Application verification:	not stated
5. Environmental conditions:	
Study site:	**Field experiment** The field experiment was conducted at two farms within 15 km of each other in the Willamette Valley River flood plain within Oregon, US. One was located at the Oregon State University, Botany and Plant Pathology Farm (Botany farm) and the other at the OSU Hyslop Crop Science Field Research Laboratory (Hyslop farm).
Weather conditions / Temperature / relative humidity:	**Field experiment** Weather data (mean monthly maximum and mean monthly minimum temperatures, total precipitation) were collected at each farm. At the Botany farm, air temperatures ranged from 8.6 to 24.3 °C and precipitation ranged from 61.5 to 86.4 mm from April to August during 2006, 2007 and 2008. While at the Hyslop farm, air temperatures ranges from 7.8 to 23.0 °C and precipitation ranged from 68.6 to 88.4 mm from April to August during 2007 and 2008. **Greenhouse experiment** Average daily environmental conditions during growth of the plants for glyphosate experiments were: photosynthetically active radiation, 188 lmol m^{-2} s^{-1}, humidity 70 %, air temperature 22.3 °C, CO_2 414 ppm.
Test medium / Soil at study site	**Field experiment** The soils on the farms were slightly different but both were well drained. The soil at the Botany farm had a higher percent sand and lower percent silt than at the Hyslop farm. The soil at the Botany farm had much less P and more K than at the Hyslop farm. **Greenhouse experiment** An artificial potting mix (OBC #3, containing mulch, peat moss, pumice, vermiculite, perlite and wetting agent) was used.
Light intensity	**Field experiment** Natural daylight conditions **Greenhouse experiment** The glyphosate experiment was conducted in late summer without supplemental artificial lights and under whitewashed glass to reduce the heat load on the greenhouse. Both of these conditions resulted in a lower light intensity.
pH:	**Field experiment**

	Botany farm: 5.4-5.9 Hyslop farm: 5.4
Organic matter (Corg):	Not stated
Fertilizer:	**Field experiment** Each plot was fertilized with 10 g of Osmocote 14-14-14 slow release fertilizer. **Greenhouse experiment** Controlled release fertilizer (Osmocote 14 x 14 x 14) provided at a rate of *4.1 g (2.5 ml by volume) per pot.

6. Observations/analyses:

Measurements:	**Field experiment** Each plant's height and width were measured every 2 wk during the growing season. Height was measured from the ground to the tallest point of living tissue. The plant was measured as it was; foliage was not straightened or lifted. Plant width was recorded at the broadest diameter of the plant (w1), and a second width was taken perpendicular to the first (w2). Plant volume was determined by using a modification of the formula of a cone. **Greenhouse experiment** The general conditions described in the EPA vegetative vigor test to assess effects to non-target plants were used. Plant height was measured the day before herbicide treatment and 13 days after treatment (except for 14 days for the first group of *P. vulgaris* plants).
Parameters measured:	Plant height and width, plant volume
Statistics:	**Field experiment** For each herbicide, the weekly plant measurements of vegetative growth at the two sites and replicate experiments across years were combined and analyzed with multivariate analysis of covariance (MANOCOVA) using SAS PROC GLM. The between-subject factors were year and site, and the within-subject factors were species and sampling date. Each analysis was done separately by pesticide initially, and by pesticide, year, species, and/or location depending upon whether there were significant interactions. Measured data were log transformed to meet the assumptions of normal distribution and constant variance required for MANOCOVA. Dunnett's multiple range test was used to determine which chemical treatments caused effects that were significantly different than those of the controls (alpha = 0.05). Controls were plots that were not sprayed with either herbicide or adjuvants. Statistical analyses were performed using SAS/STAT_ software, Version 9.2 of the SAS

System (SAS Institute Inc., Cary, NC).

Greenhouse experiment

For the greenhouse study, a restricted randomization design was used to assign the control and the aminopyralid herbicide at five rates (0, 0.001, 0.002, 0.01, and 0.1 9 FAR) and glyphosate at six rates (0, 0.001, 0.002, 0.01, 0.1, and 1 9 FAR) to the experimental units. All analyses except *C. amoena* for glyphosate used untransformed pretreatment and post treatment height measurements. In the case of *C. amoena* and glyphosate
a base 10 logarithm was used to transform pretreatment and post treatment measurements prior to analysis. Where there were occasional dead plants and no height value, the preheight value was used for the statistical analysis.

Data were analyzed using ANOVA with group, herbicide rate, and group x herbicide rate as factors, and plant height before treatment as a covariate using SAS PROC GLM in SAS/STAT_ software Version 9.2 of the SAS System (SAS Institute Inc., Cary, NC). To determine differences from the control plants, one tailed tests were performed using Dunnett's test at P<0.05 when no covariate was included and Bonferroni's test at p<0.05 when a covariate was included. For *C. echinatus* treated with aminopyralid, Bonferroni's test was used when an increase in height
(hormesis) was the alternative hypotheses of treatment< control response.

KLIMISCH EVALUATION

1. Relevance of study:		**Not relevant**
	Comment:	Plant population dynamics are not in the focus of the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant for the assessment of glyphosate efficacy. Using good agricultural practice, there is no expected impact on non target plants.
2. Reliability of study:		**Reliable without restrictions**
	Comment:	Well documented non-GLP, non-guideline study that meets scientific principles.
3. Klimisch code:		**Klimisch rating of 1**

Egan et al., 2014

Author(s)	Year	Study title
Egan, J. F. Graham, I. M. Mortensen, D. A	2014	A comparison of the herbicide tolerances of rare and common plants in an agricultural landscape Environmental Toxicology and Chemistry Volume: 33 Issue: 3 Pages: 696–702 DOI: 10.1002/etc.2491

Abstract
Declining plant biodiversity in agroecosystems has often been attributed to escalating use of chemical herbicides, but other changes in farming systems, including the clearing of seminatural habitat fragments, confound the influence of herbicides. The present study introduces a new approach to evaluate the impacts of herbicide pollution on plant communities at landscape or regional scales. If herbicides are in fact a key factor shaping agricultural plant diversity, one would expect to see the signal of past herbicide impacts in the current plant community composition of an intensively farmed region, with common, successful species more tolerant to widely used herbicides than rare or declining species. Data from an extensive field survey of plant diversity in Lancaster County, Pennsylvania, USA, were compared with herbicide bioassay experiments in a greenhouse to test the hypothesis that common species possess higher herbicide tolerances than rare species. Five congeneric pairs of rare and common species were treated with 3 commonly used herbicide modes of action in bioassay experiments, and few significant differences were found in the tolerances of rare species relative to common species. These preliminary results suggest that other factors beyond herbicide exposure may be more important in shaping the distribution and abundance of plant species diversity across an agricultural landscape.

MATERIALS AND METHODS

1. Test material:

Test item: Roundup Powermax
Active substance(s): Glyphosate and other active ingredients tested
Surfactant: Not stated
Source of test substance: Monsanto
Lot/Batch #: Not stated
Purity: 540 g a.e. L^{-1}

2. Vehicle and/or positive control: Not stated

3. Test organism:

Species: *Asclepias syriaca*
Asclepias tuberosa
Bidens frondosa
Bidens cernua
Elymus riparius
Elymus hytrix
Polygonum convolvulus
Polygonum lapathifolia
Vebena urticifolia

	Vebena hastata
Source:	Not stated
Crop growth stage at treatment:	At the 4– to 6–true leaf growth stage (4–8 wk after transplanting, depending on the genus).

4. Test system:

Study type:	Greenhouse experiment
Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	Not stated
Treatments:	Each plant was exposed to 1 of 3 herbicides: atrazine, dicamba, or glyphosate. Each congeneric contrast (one rare and one common species) was arranged as a randomized complete block experiment, with 2 species, 3 herbicides, 5 doses of each herbicide, and 4 blocks.
Replicates:	Four
Test concentrations:	The 5 doses were designed to represent the label field application rate for corn or soybean weed control (1x), nontarget exposure from a serious misapplication event (0.1x), exposure from particle drift (0.01x), and exposure from vapor drift or surface runoff (0.001x). The field application rate of glyphosate is 844 g a.e. ha^{-1}
Application devices:	Herbicide treatments were applied using a bench-top track sprayer with an 8002E nozzle calibrated to apply herbicide solution at a rate of 120 L ha^{-1} at 180 kPa.
Application verification:	Not stated

5. Environmental conditions:

Study site:	Research greenhouses of The Pennsylvania State University (University Park, PA, USA)
Weather conditions / Temperature / relative humidity:	Ambient greenhouse conditions
Test medium / Soil at study site	Commercial potting soil mix (Sunshine Mix #5)
Photoperiod:	Not stated
Light intensity	Not stated
pH:	Not stated
Organic matter (Corg):	Not stated
Fertilizer:	Soil supplemented with 4.0 g of Osmocote 19N-6P-12K fertilizer (Scotts Miracle-Gro).

6. Observations/analyses:

Measurements:	28 days after treatment, plants were clipped at soil level, oven-dried, and weighed for aboveground biomass. At 28 d after treatment in 2011, many *Verbena* spp. plants had reached peak flowering, and so we also measured inflorescence length (average of 5 inflorescences) as a measure of reproductive

	performance. For *Elymus* spp. in 2011, we also counted the number of clonal shoots emerging from rhizomes as a measure of vegetative propagation.
Parameters measured:	Aboveground biomass, inflorescence length, number of clonal shoots
Statistics:	For each measure of plant performance, log–logistic dose-response curves for each herbicide and genus combination were fitted using the package drc in the statistical program R. The 3-parameter version of the log–logistic function was used, which assumes a lower asymptote of 0. The significance of the log–logistic model was tested using F tests. In several cases in which a hormesis effect seemed plausible, Cedergreen hormesis models were fitted and compared with the 3-parameter log– logistic model using Aikiake's Information Criterion. After fitting the models, the effective dose causing on average a 5%, 25%, or 50% reduction in performance (ED_5, ED_{25}, and ED_{50} values, respectively) was calculated for each herbicide and genus combination. The delta interval feature provided by the drc package was used to calculate 95% confidence intervals for these estimates. For a given herbicide and genus combination, if these 95% confidence intervals did not overlap, this difference was interpreted as evidence that the species with a smaller effective dose value was less tolerant to the herbicide active ingredient.

KLIMISCH EVALUATION

1. Relevance of study:		**Not relevant**
	Comment:	Plant population dynamics are not in the focus of the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant for the assessment of glyphosate efficacy. Using good agricultural practice, there is no expected impact on non target plants.
2. Reliability of study:		**Not reliable**
	Comment:	Well documented non-GLP, non-guideline study that meets scientific principles.
3. Klimisch code:		**Klimisch rating of 3**

Author(s)	Year	Study title

Olszyk, D. Blakeley-Smith, M. Pfleeger,T. Lee, E.H. Plocher, M.	2013	Effects of low levels of herbicides on prairiespecies of the Willamette Valley, Oregon Environmental Toxicology and Chemistry Volume: 32 No: 11 Pages: 2542–2551 DOI: 10.1002/etc.2331

Olszyk et al., 2013

Abstract

The relative sensitivity of 17 noncrop plant species from Oregon's Willamette Valley was determined in response to glyphosate, tribenuron methyl (tribenuron), and fluazifop-p-butyl (fluazifop) herbicides. For glyphosate, *Elymus trachycaulus*, *Festuca arundinacea*, *Madia elegans*, *Potentilla gracilis*, and *Ranunculus occidentalis* were the most sensitive species, based on a concentration calculated to reduce shoot dry weight by 25% (IC25 values) of 0.02 to 0.04 x a field application rate of 1112 g active ingredient (a.i.) per hectare. *Clarkia amoena* and *Lupinus albicaulis* were the most tolerant to glyphosate, with IC25 values near the field application rate. *Clarkia amoena*, *Prunella vulgaris*, and *R. occidentalis* were the most sensitive to tribenuron, with IC25 values of 0.001 to 0.004 x a field application rate of 8.7 g a.i. ha^{-1} for shoot dry weight. Five grass species were tolerant to tribenuron with no significant IC25 values. For fluazifop, 2 native grasses, *E. trachycaulus* and *Danthonia californica*, were the most sensitive species, with IC25 values of 0.007 and 0.010 a field application rate of 210 g a.i. ha^{-1}, respectively, for shoot dry weight, while a native grass, *Festuca roemeri*, and nearly all forbs showed little or no response. These results also indicated that the 3 introduced species used in the present study may be controlled with 1 of the tested herbicides: glyphosate (*F. arundinacea*), tribenuron *(Leucanthemum vulgare*), and fluazifop (*Cynosurus echinatus*).

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup Original and two other herbicides
Active substance(s):	Glyphosate as isopropylamine salt
Surfactant:	Not stated
Source of test substance:	Monsanto
Lot/Batch #:	Not stated
Purity:	Not stated

2. Vehicle and/or positive control: Not stated

3. Test organism:

Species:
Bromus carinatus
Clarkia amoena
Collinsia grandiflora
Cynosurus echinatus
Danthonia californicac
Elymus trachycaulus
Eriophyllum lanatum
Festuca arundinacea
Festuca roemeri
Gilia capitata
Leucanthemum vulgare
Lupinus albicaulis
Madia elegans

	Potentilla gracilis *Prunella vulgaris* *Ranunculus occidentalis* *Sanguisorba occidentalis*
Cultivar:	Not stated
Source:	Not stated. Seeds purchased.
Crop growth stage at treatment:	
Holding conditions prior to test:	Initially, specific conditions were determined to induce germination for each species [19]. One species, *Lupinus albicaulis*, required a scarification process to imbibe water. Scarification was achieved by rubbing seeds for 15 s with a piece of sandpaper against a hard surface prior to cold stratification. Species which required cold stratification were started in 50-cell plastic germination trays (53 cm long x 27 cm wide tray, with each cell 5 cm x 5 cm x 6 cm high) containing OBC#1 potting mix (OBC Northwest) without fertilizer. The OBC#1 mix contains peat moss, fine perlite, washed sand, and wetting agent. Flats were put in a dark 8 °C cooler for stratification. At 5 d to 7 d after seedling emergence, individual seedlings were transplanted into plastic pots, which were 10 cm diameter x 9.8 cm high, filled with a volume of 350mL of soil. The pots were put on a regular greenhouse bench. Seeds which did not require stratification were planted directly into the pots and put on a mist bench until seedling emergence. *Danthonia californica* was planted in natural sandy loam soil because it exhibited injury symptoms from an unknown cause when grown in the OBC#3 mix. After pots were transferred to regular greenhouse benches, watering was from the bottom if pots were in saucers or large trays. When overhead watering was required (as when tops of pots had dried out), it was with a wand moved in a circular motion around the edge of each pot, being careful not to get water or soil on the leaves.

4. Test system:

Study type:	Field experiment and greenhouse
Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	Not stated
Treatments:	A regression-based experimental design was used to test for the effects of a herbicide at simulated drift levels separately for each species and each herbicide. For all herbicides there was a “0 herbicide” control treatment and a progression of herbicide levels (0.001, 0.002, 0.01, and 0.1x the field application

	rate). In addition, all glyphosate experiments had a 1.0x the field application rate treatment for all species except *Elymus trachycaulus*.
Replicates:	The herbicide response experiments for each species and herbicide were replicated in time. Each species was grown in 2 groups for each herbicide, except for tribenuron where there were 4 groups with *D. californica* and 4 groups with *L. vulgare* and for fluazifop where there were 3 groups for *P. vulgaris*. Where feasible, the experiments were replicated close together in time to reduce the effect of changes in environmental growing conditions. However, for some species and herbicides, replicate groups were weeks to months apart from each other, resulting in larger differences in environmental conditions between groups.
Test concentrations:	Glyphosate was applied based on a field application rate of 1112 g active ingredient (a.i.) ha^{-1} (835 g ha^{-1} acid glyphosate), which is the recommended label rate for high-volume broadcast ground applications to annual and perennial weed species. Test concentration were 0, 0.001, 0.002, 0.01, 0.1 and 1.0 x the field application rate
Application devices:	Herbicide solutions were applied to plants in a Research Instrument Company spray chamber (Model RC-5000-100EP Overhead Trolley Sprayer for Reproducible Agrochemical Research; Mandel Scientific). The sprayer was equipped with an even flat fan spray nozzle (TeeJet TP8002E-VS; Spraying Systems).
Application verification:	Not stated

5. Environmental conditions:

Study site:	Not stated
Weather conditions / Temperature / relative humidity:	Experimental conditions in the greenhouse were based on those recommended for the Vegetative Vigor Test. Air temperature was setat 25 °C during the day and 20 °C at night (16:8 h day/night cycle), with supplemental high-pressure sodium lighting during the day to increase light intensity during the winter months. Air temperature and CO_2 were similar across all experiments, with daily average, maximum, and minimum temperatures of 22 °C, 26 °C, and 18 °C, respectively, and daily average CO_2 of approximately 430 ppm. Because this was a greenhouse study with plants grown at different times of the year, there was considerable variation in other environmental conditions. During the winter months (most of tribenuron and fluazifop experiments) average light intensity was high at approximately 340 $mmolm^{-2}$ s^{-1} and relative humidity

	was low at approximately 45%. In contrast, during the summer months (rest of experiments) average light intensity was approximately 175mmolm^{-2} s^{-1} and relative humidity was approximately 70%.
Test medium / Soil at study site	For nearly all species, an artificial potting mix (OBC#3, containing mulch, peat moss, pumice, vermiculite, perlite, and wetting agent) was used.
Light intensity	See above
pH:	Not stated
Organic matter (Corg):	Not stated
Fertilizer:	Fertilizer (Osmocote®, 14-14-14) was provided at a rate of approximately 4.1 g (2.5mL by volume) per pot.

6. Observations/analyses:

Measurements:	Height was evaluated for each plant prior to herbicide treatment and just before harvest (14 d or 15 d between measurements). Harvest was 14 d after treatment to allow adequate time for development of shoot growth inhibition and leaf injury. The plant response endpoints considered were shoot dry weight, the most direct indicator of absolute plant growth, and height relative growth rate, indicating growth for individual plants considering the size at the time of treatment. The height relative growth rate was calculated as [(final height – original height)/original height]/days between height measurements. Shoot dry weights were determined following a final harvest. Shoots were cut off at the soil line, placed in paper bags, and then dried for 3 d at 60 °C prior to weighing on a digital balance.
Parameters measured:	Plant height, shoot dry weight, height relative growth rate
Statistics:	Analysis of variance (ANOVA) was performed for each species and herbicide to test for herbicide and replicate group effects and their interaction. Herbicide levels in the ANOVA and regression analysis were expressed as the percentage of the field application rate based on the calculated concentrations. To consider variation in size before treatment in the analysis for shoot dry weight, the height before treatment (preheight) was used as a covariate both alone and as a covariate by group interaction in an analysis of covariance (ANCOVA). Where heterogeneity of variance was severe for shoot dry-weight data (such as for *P. vulgaris*), a natural log transformation was used to stabilize variances prior to ANOVA or ANCOVA. Negative values for height relative growth rate because of a shrinkage of the plants or values for dead plants were replaced by

0 prior to
statistical analysis, and a weighted ANOVA on height relative growth rate was performed to account for heterogeneity of treatment variances. Based on the ANOVA and ANCOVA, an F-test was used to detect effects on shoot dry weight and height relative growth rate at a $p<0.05$ level of significance. Furthermore, a significant treatment by group interaction which had F values <10% of those for the treatment and/or group main effects were not considered to be important because an interaction was more likely with larger main effects. As a visual check, the interaction was ignored when the F value for interaction was low relative to that for the main effects and the herbicide response relationship was similar and equal in sign for each group. For R. occidentalis treated with glyphosate, height relative growth rate data from the no-spray plants were used as the control plants in the statistical analysis instead of the carriercontrol
0-herbicide plants because the carrier control plants' growth was reduced by an unknown nonherbicide factor relative to the other treatments. The lowest concentration below that which produced a statistically significant effect (no-observed-effect concentration [NOEC]) was calculated at the 5% level of significance for a reduction in a response in treated versus control plants using Dunnett's 1-tailed test, if no covariate was used with the analysis. For shoot dry weight, if the covariate was significant, Bonferroni's test was used to determine the NOEC. If there was a significant herbicide effect but not a significant herbicide treatment by group interaction, the NOEC value was determined across groups of plants for a species. If there was a significant rate effect and a significant rate by group interaction, a separate NOEC value was determined for each group. Post-ANOVA nonlinear regression analyses were used to calculate the inhibitory concentration of a herbicide associated with a 25% reduction in plant response (IC25, calculated as effective concentration based on Bruce and Versteeg and further redefined as IC based on USEPA). The regression analysis used the calculated concentrations of 0, 0.001, 0.002, 0.01, 0.1, and 1.0 (if present) x the field application rate for the herbicide concentrations. In the present study, point and interval estimates of the IC25 associated with a 25% reduction in shoot dry weight or height relative growth rate were calculated using the maximum likelihood approach. The 3-parameter Weibull model was used and reparameterized so that

1 of its parameters was the IC25. If there was no significant concentration by group interaction, the data for the 2 replicate groups were combined to fit a Weibull model with a common IC25 and shape parameter and either a common or separate y intercept for each group depending on whether the group effect was significant. When there was a significant concentration by group interaction, separate models were fit to data for each group. When the concentration and group effects were highly significant and the concentration by group interaction was barely significant, the interaction effect was ignored and a common 4-parameter Weibull model with separate y intercepts for each group was fit to the combined data. The nonlinear regression considered covariates for shoot dry weight if significant and weighing factors for height relative growth rate if required. Variance around IC25 values was expressed as likelihoodconfidence intervals. The PROC GLM ANOVA and PROC NLIN nonlinear regression procedures in SAS/STAT softwarewere used for these analyses (Ver 9.2 of SAS).

KLIMISCH EVALUATION

1. Relevance of study:		**Not relevant**
	Comment:	Plant population dynamics are not in the focus of the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant for the assessment of glyphosate efficacy. Using good agricultural practice, there is no expected impact on non target plants.
2. Reliability of study:		**Reliable with restrictions**
	Comment:	Glyphosate formulation is of unknown content of active substance, adjuvants or surfactants. Studies were not carried out according to OECD guideline and under GLP conditions.
3. Klimisch code:		**Klimisch rating of 2**

6. Pathogens & Beneficial Microorganism

The following studies focus on the effect of glyphosate on crop pathogens and microorganisms associated with crop species especially mycorrhiza species. All are not annex points that have to be addressed in the renewal assessment of active substances. As for several herbicides there are potential effects on on crop pathogens and beneficial microorganisms. A change or amendment in the assessmnent is not necessary.

Giovannetti et al., 2006

Author(s)	Year	Study title
Giovannetti, M. Turrini, A. Strani, P. Sbrana,C. Avio, L. Pietrangeli, B.	2006	Mycorrhizal fungi in ecological studies: Soil impact of fungicides, insecticides and herbicides Prevention Today Volume: 2 No: 1-2 Pages: 47-62 DOI: Not stated

Abstract
Biological soil monitoring involves the assessment of soil quality by monitoring living organisms in their natural environment or by toxicity laboratory tests. Soil biomonitoring allows the assessment of the biological effect linked to the bioavailable fraction of polluting substances and, as such, it plays a major role in defining quality criteria for the bioremediation of contaminated sites (Ministerial Decree 471/99) or, more generally, in the assessment of the quality of agricultural and natural soils. Due to their key role in preserving soil fertility, arbuscular mycorrhizal fungi can be considered as the main non-target microorganisms to be monitored in environmental impact assessments of pesticides used in agriculture. Experimentation was chiefly aimed at validating a model system that provides for the use of the arbuscular mycorrhizal fungus *Glomus mosseae* as a biological indicator of chemical substances applied to the soil, and consequently, of the toxicological risk associated with the man-made pollution of soil ecosystems.
The experimental tests demonstrated that spore germination and/or mycelial growth of *G. mosseae* are adversely affected by most of the substances tested and, in some cases, at much lower concentrations than those indicated for use (hormesis). The results of the research suggest that *G. mosseae* can be a valuable indicator both for assessing the environmental impact of pesticides and other pollutants and for providing useful indications for the development of new active principles with a low environmental impact.

MATERIALS AND METHODS

1. Test material:

Test item:	Risolutiv
Active substance(s):	Glyphosate
Surfactant:	Not stated
Source of test substance:	Not stated
Lot/Batch #:	Not stated
Purity:	41% active ingredient

2. Vehicle and/or positive control: Not stated

3. Test organism:

Species:	*Glomus mosseae* (IMA1)
Cultivar:	Not stated
Source:	Preserved by the Department of Crop Plant Biology at the University of Pisa
Crop growth stage at treatment:	Not stated
Holding conditions prior to test:	"Pot culture" soil (pot cultivation of the mycorrhizal fungus with a host plant) was suspended in the water, decanted and filtered through a series of sieves at least 5 times (mesh: 100 to 500 microns). Sporocarps to be used for plate assays were then isolated from the residual material obtained from the sieves.

4. Test system:

Study type:	Laboratory experiment
Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	Not stated
Treatments:	In order to analyse the impact of pesticides on (non-target) beneficial symbiotic fungi, an experimental model was devised, based on the "sandwich system" used to study the first stages of the life cycle of AM fungi. The sporocarps isolated from the soil were collected and placed on membranes of cellulose esters (Millipores). A membrane was laid on each membrane containing 14 sporocarps; the sandwiches thus obtained were placed in Petri dishes containing sterile quartz, and incubated in the dark at 25°C, in the presence of the chemical substance to be tested.
Replicates:	Not stated
Test concentrations:	73, 36, 18, 9 and 5 mg glyphosate/l
Application devices:	Not stated
Application verification:	Not stated

5. Environmental conditions:

Study site:	Not stated
Weather conditions / Temperature / relative humidity:	Incubation in the dark at 25°C
Test medium / Soil at study site	Cellulose esters (Millipores)
Light intensity	Dark
pH:	Not stated
Organic matter (Corg):	Not stated
Fertilizer:	Not stated

6. Observations/analyses:

Measurements:	After 10 days, the membranes were removed from the quartz, opened and checked for spore germination and the growth of the mycelium using Trypan blue staining (0.05 % in lactic acid).
Parameters measured:	Spore germination, growth of the mycelium

Statistics: Not stated

KLIMISCH EVALUATION

1. Relevance of study:		**Relevant with restrictions**
	Comment:	The influence of glyphosate on soil microogranismen has already been addressed in the current RAR as part of the efficacy evaluation for glyphosate.
2. Reliability of study:		**Not reliable**
	Comment:	Not all relevant details on the investigation are reported in the present publication. This concerns the following aspects: - No information on the preparation of the test solutions of the test item is given. - Data on test conditions and application are incomplete or completely missing. - No information on analytic measurement is given. - It is not clear if the glyphosate formulation tested incl. co-formulants and application rates are relevant for uses in the EU. - The term hormesis is not used in the right context in the abstract. -
3. Klimisch code:		**Klimisch rating of 3**

Sheng et al., 2012

Author(s)	Year	Study title
Sheng, M. Hamel, C. Fernandez, M.R	2012	Cropping practices modulate the impact of glyphosate on arbuscular mycorrhizal fungi and rhizosphere bacteria in agroecosystems of the semiarid prairie Canadian Journal of Microbiology Volume: 58 Pages: 990-1001 DOI: 10.1139/W2012-080

Abstract
A growing body of evidence obtained from studies performed under controlled conditions suggests that glyphosate use can modify microbial community assemblages. However, few studies have examined the influence of glyphosate in agroecosystems. We examined 4 wheat-based production systems typical of the Canadian prairie over 2 years to answer the following question: Does preseeding of glyphosate impact soil rhizosphere microorganisms? If so, do cropping practices influence this impact? Glyphosate caused a shift in the species dominating

the arbuscular mycorrhizal fungal community in the rhizosphere, possibly through the modification of host plant physiology. Glyphosate stimulated rhizobacterial growth while having no influence on saprotrophic fungi, suggesting a greater abundance of glyphosate-tolerant 5-enolpyruvylshikimate-3- phosphate synthase (EPSPS) in bacteria than in fungi. Glyphosate stimulated rhizosphere bacteria in pea but not in urea-fertilized durum wheat, which is consistent with inhibition of EPSPS tolerance to residual glyphosate through high ammonium levels. Mitigation of the effects of glyphosate on rhizosphere bacteria through tillage suggests a reduction in residual glyphosate activity through increased adsorption to soil binding sites upon soil mixing. The influence of glyphosate on Gram-negative bacteria was mitigated under drought conditions in 2007. Our experiment suggests that interactions between soil fertility, tillage, and cropping practices shape the influence of glyphosate use on rhizosphere microorganisms.

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup Weathermax
Active substance(s):	Glyphosate
Surfactant:	Not stated
Source of test substance:	Not stated
Lot/Batch #:	Not stated
Purity:	Not stated

2. Vehicle and/or positive control: Not stated

3. Test organism:

Species:	Soil rhizosphere microorganisms
Cultivar:	Not stated
Source:	Not stated
Crop growth stage at treatment:	3- to 4-leaf stage of wheat
Holding conditions prior to test:	Not stated

4. Test system:

Study type:	Field experiment
Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	This rotation experiment was initiated in 2006, and the data were collected in 2007 and 2008.
Treatments:	The 8 treatments of the experiment were factorial combinations of (1) 2 glyphosate levels: with and without; (2) 2 cropping systems levels: a rotation system consisting of growing durum wheat (*Triticum turgidum* var. *durum* Desf.) on pea (*Pisum sativum* L.) stubbles in 2007 and pea on durum wheat stubbles in 2008, and a continuous durum system, consisting of continuously growing durum wheat on durum wheat stubbles; and (3) 2 tillage levels, conservation tillage and no tillage. Cropping systems were randomized into main plots, and the combinations of glyphosate and tillage treatments were randomized into subplots. Glyphosate (450 g active ingredient ha^{-1}) formulated

	as was sprayed only in designated plots at the 3- to 4-leaf stage of wheat. A mixture of 495 g glufosinate ammonium ha^{-1} formulated as Liberty, 23 g MCPB (4-(4-chloro-2-methylphenoxy) butanoic acid) ha^{-1}, and 1.58 g MCPA (2-methyl-4- chlorophenoxyacetic acid) ha^{-1} formulated as Select (Dow AgroSciences), and the surfactant Amigo (Loveland Industries, Greeley, Colorado, USA) (0.5%, v/v) was also sprayed in all plots at that time to leave no standing vegetation. In 2006, plots assigned to the rotation treatments were seeded with pea. They were then seeded with durum wheat in 2007 and with pea in 2008. The pea cultivar used was Eclipse, which was seeded at a rate of 213 kg ha^{-1}; the durum wheat cultivar Strongfield was seeded at a rate of 101 kg ha^{-1}. The crops were seeded on 13 June 2007 and on 4 June 2008.
Replicates:	All experimental treatments were replicated in 4 blocks for a total of 32 plots, with individual plots measuring 20 m long and 10 m wide.
Test concentrations:	450 g active ingredient ha^{-1}
Application devices:	Not stated
Application verification:	Not stated

5. Environmental conditions:

Study site:	The study used a subset of 32 plots from a 48-plot field experiment located at the South Experimental Farm of the Semiarid Prairie Agricultural Research Centre in Swift Current, Saskatchewan, Canada (latitude: 50°17'N; longitude: 107°41'W; elevation: 825 m above sea level).
Weather conditions / Temperature / relative humidity:	The climate at the study site is semiarid. The monthly mean temperature and total precipitation during the 2 years of the study were monitored by a weather station located at the experimental farm.
Test medium / Soil at study site	The soil at the site was a very gently sloping (1%) Brown Chernozem of the Wood Mountain soil association with a clay loam texture and a columnar prismatic to solod profile. Wood Mountain soils developed from modified glacial till deposits including Tertiary sands, silts, clays, shales, sandstones, quartzite, and chert pebbles. In early summer 2007 the experimental soil contained on average (in mg kg^{-1}): 3.4 calcium chloride extractible SO_4-S; 2.09 Cu, 37.4 Fe, 31.5 Mn, 1.0 Zn, and 0.56 Mo extractible with DTPA–TEA (diethylene triamine pentaacetic acid – triethanol amine); 2301 Ca, 653 Mg, and 25.4 Na extractible with ammonium acetate; and 537 sodium bicarbonate extractible K. Prior to

	establishment of the experiment in the spring of 2006, the top 0–15 cm soil layer (in spring) contained (per kg of soil) 2.40 mg NO_3-N and 9.88 mg PO_4-P (bicarbonate extraction).
Light intensity	Natural light conditions.
pH:	Not stated
Organic matter (Corg):	Not stated
Fertilizer:	Seeds were placed 7.6 cm deep with a Flexicoil air drill delivering, in 2007, 30.2 kg ammonium phosphate (11–51–0) ha^{-1} in the pea and durum wheat seed furrows and 103.0 kg urea (46–0–0) ha^{-1} in a band 5 cm lateral to and 5 cm beneath the seed furrows only for the durum wheat crops. In 2008, 43.7 kg ammonium phosphate (11–51–0) ha^{-1} was applied in the pea and durum wheat seed furrows and 98.6 kg urea (46– 0–0) ha^{-1} was applied in a band to durum wheat crops.

6. Observations/analyses:

Measurements:	**Soil and root sampling** On 14 August 2007 and 18 July 2008 (dates corresponding to the milk stage of wheat development), rhizosphere soil samples were collected from the root surface according to the method of Lupwayi et al. (2004). In each plot, the roots of 10 plants were harvested and transported to the laboratory, where they were shaken to detach loose soil and carefully brushed to collect rhizosphere soil adhering to roots. The rhizosphere soil was homogenized by sieving through a 2 mm mesh prior to the collection of samples for analysis. Root samples of pea and wheat were collected in 2008. In each plot, roots were collected from 20 plants selected at random, washed gently with tap water, and dried with paper towels. The roots were stored in a cold room at 4 °C and the soil samples in a freezer at –18 °C until processing. **AM fungal colonization rate** Root samples were cleared in a boiling 10% KOH solution, stained with ink and vinegar, and examined under a dissecting microscope for determination of the percentage of AM root colonization using the gridline intersect method (Giovannetti and Mosse 1980). **DNA extraction, nested PCR, and DGGE analysis** DNA was extracted using an UltraClean Soil DNA Isolation kit (Mo Bio Laboratories Inc., Carlsbad, California, USA) with a final elution volume of 50 µL. Preliminary tests dictated the use of a 3-step PCR amplification procedure for optimal detection of AM fungi at the study site. AM fungi are scarce in

cultivated Brown Chernozems compared with less arid prairie soils, and in the investigated Brown soil, AM fungi were difficult to detect in the dry year of 2007, i.e., the first year of the study. The soil extracts were diluted 20–50 times (to approximately 10 ng μL^{-1}) and subjected to the first-round PCR using the primers GeoA2 and Geo11 to amplify an approximately 1.8 kb fragment of the 18S rRNA gene (Schwarzott and Schüßler 2001). PCR was performed in a 20 µL volume containing 2.0 µL of template DNA, 0.5 µL each of GeoA2 and Geo11 (16 pmol μL^{-1}), and 17 µL of Platinum PCR SuperMix (Life Technologies Inc., Burlington, Ontario) containing 22 U mL^{-1} complexed recombinant Taq DNA polymerase with the Platinum Taq antibody, 22 mmol L^{-1} Tris– HCl (pH 8.4), 55 mmol L^{-1} KCl, 1.65 mmol L^{-1} MgCl2, 220 µmol·L^{-1} each dNTP (deoxynucleoside triphosphate), and stabilizers. Amplification was carried out in a Veriti 96- well Fast Thermal cycler (Applied Biosystems) using the following conditions: 94 °C for 2 min; 35 cycles at 94 °C for 30 s, 59 °C for 60 s, and 72 °C for 2.0 min; and 72 °C for 10 min. The first-round PCR products were analyzed via agarose gel electrophoresis (1.0% (m/v) agarose; 100 V, 60 min) with SYBR Safe staining. The first-round PCR products showing a visible band were diluted 1:100 and used as a template in the second-round PCR assay. The second-round PCR was performed in 30 µL volume containing 2 µL of template DNA, 0.5 µL of each of AM1 and NS31-GC (at 16 pmol·µL–1), and 27 µL of Platinum PCR SuperMix (Invitrogen), as described above. AM1 (Helgason et al. 1998) and NS31-GC, which corresponds to NS31 described by Simon et al. (1992) plus a 50 GC clamp sequence described by Kowalchuk et al. (1997), produced an approximately 550 bp fragment. The thermocycling program used was as follows: 94 °C for 2 min; 35 cycles at 94 °C for 30 s, 67 °C for 60 s, and 72 °C for 60 s; and 72 °C for 10 min. The second-round PCR products were analyzed as described for the first-round PCR products.
Amplification products from the second-round PCR were
diluted 1:100 and used as the template in the third-round
PCR using the NS31-GC and the Glo1 (Cornejo et al. 2004) primers with the same reaction mixture as employed in the second-round PCR, with the exception of the primers used. The thermocycling program used was as follows: 94 °C for 2 min; 35

cycles at 94 °C for 45 s, 50 °C for 45 s, and 72 °C for 60 s; and 72 °C for 10 min. The third-round PCR products were analyzed as described for the first-round PCR products. A 20 µL aliquot of the third-round PCR products was used for DGGE analysis. The gels were cast with a Model 475 gradient former with the following characteristics: 6% (m/v) polyacrylamide (38.93:1.07 acrylamide – bis-acrylamide), 1 mm thickness, 16 cm × 16 cm area. A linear gradient of
30%–45% denaturant was used, where 100% denaturant
acrylamide is defined as containing 7 mol·L^{-1} urea and 40% formamide. Before polymerization, a 7 mL top gel with no denaturants was added to obtain well-polymerized gel slots. The PCR products were separated using DGGE with a DCode Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, California, USA) at a constant temperature of 60 °C in 1× Tris – acetic acid – EDTA buffer (TAE). Electrophoresis was performed for 10 min at 75 V, after which the voltage was lowered to 60 V for an additional 14 h. The gels were stained in 1× TAE containing 6 µL of YBR Safe stain per 30 mL of TAE and visualized using UV illumination. Gel images were digitally captured by AlphaDigiDoc RT. DGGE patterns were analyzed with BioNumerics software version 5.1 (Dong and Reddy 2010). The richness of AM fungi was determined from the number of bands belonging to AM fungi in a given rhizosphere soil sample. Recovery of DNA from DGGE gel. All of the detected DGGE bands were excised from the acrylamide gel for DNA isolation and transferred to 2 mL polypropylene tubes with approximately 20 µL ddH2O. The acrylamide gel fragments were broken up with a 1 mL micropipette tip, soaked at room temperature for 5 min, mixed, and centrifuged at 7500g for 5 min to pellet the acrylamide. A
volume of 2 µL of supernatant was used for a further PCR
amplification using the same primers and cycling conditions as employed in the third round of PCR, and the products were again separated through DGGE. PCR products showing single bands following the second DGGE run were purified for sequence analysis using the QIAquick Gel Extraction kit (Qiagen, Hilden, Germany) with a final elution volume of 30 µL. Sequencing using the primer NS31 was carried out at Plant Biotechnology Institute of the National Research Council of Canada in

	Saskatoon. Comparisons of the similarity of the partial 18S rDNA sequences were performed using the National Centre for Biotechnology Information online standard BLAST (Basic Local Alignment Search Tool) program (http://www.ncbi.nlm.nih.gov/). **Phospholipid fatty acid analysis** The methods used for PLFA analysis were as described in Nayyar et al. (2008). An evaluation of the active microbial community can be derived from PLFA patterns. Because specific groups of microorganisms contain different fatty acid profiles, it is possible to distinguish among them and evaluate microbial diversity. The specific PLFAs listed in Table 1 was used as markers for 3 microbial groups: Gram-negative bacteria, Gram-positive bacteria, and fungi. Values for Grampositive and values for Gram-negative PLFA markers were summed to yield, respectively, total Gram-negative and total Gram-positive PLFA, which were used as indicators of the biomass of bacteria in these groups. PLFA 16:1u5 is abundant in AM fungi but is also present in bacteria. The abundance of 16:1u5 in neutral lipid fatty acid was low relative to its abundance in PLFA (average neutral lipid fatty acid to PLFA ratio was 0.163), indicating that PLFA 16:1u5 was largely bacterial in our system, which precluded the use of this marker as indicator of AM fungal biomass (Frostegård et al. 2011). PLFA 18:2 is known as a good marker for soil fungi, although it is also found in eukaryotes, because fungi are more abundant than eukaryotes (Frostegård et al. 2011). The marker is particularly reliable in this study, since collecting soil samples with a brush prevented any fortuitous inclusion of visible contaminants.
Parameters measured:	Spore germination, growth of the mycelium
Statistics:	Analysis of variance was performed using the MIXED procedure with repeated measures in years to test the effects of glyphosate, tillage, cropping systems, and years as well as their interactions on the PLFA markers indicating bacterial and fungal biomasses using SAS (SAS Institute Inc. 2001). A log10 transformation was applied to the PLFA data to meet the requirements of the test for normality of residue distribution and homogeneity of variance. Treatment effects on AM fungal colonization rates, AM fungal richness, and AM fungal general diversity

were similarly tested, but only in 2008, as the low abundance of AM fungi in 2007 negatively impacted the distribution of residues. Treatment means were compared using least significant difference at the 5% level of significance. The PLFA markers that could not be success- fully transformed were not subjected to analysis of variance, but all PLFA indicators were included in a principal component analysis (PCA) (Legendre and Legendre 1998) using the library vegan (Oksanen et al. 2011) in R (R Development Core Team 2011). The dataset was standardized prior to PCA to give equal weight to the different PLFA markers. A biplot was constructed with dimensions 1 and 2 of the PCA
conducted on the data from 2008, which was the year most conducive to expressing treatment effects, to show the relationships between PLFA markers and treatments.
Multivariate analysis of variance in redundancy analysis
(Borcard et al. 2011) was conducted to analyze the effects of glyphosate, tillage, and their interaction on the community of AM fungi under continuous durum wheat cultivation and the durum–pea rotation in 2007 and 2008 using the library vegan (Oksanen et al. 2011) in R (R Development Core Team 2011). Redundancy analysis was also used to test the relationship between the AM fungi distribution and the rhizosphere soil microbial community structure, also using the library vegan in R. The Hellinger transformation was applied to the AM fungi community data prior to analysis, as recommended for presence–absence data. The difference in the AM fungal community structure in glyphosate-treated and nontreated plots was also tested using the multiresponse permutation procedure (McCune and Grace 2002) after averaging the AM fungal sequence data over cropping systems, tillage systems, and years. The AM fungal sequences detected in field plot rhizosphere soil were subjected to phylogenetic distance analysis conducted with MEGA version 4.0.2 according to their sequence similarity to reference DNA sequences selected in GenBank. A Leucostoma persoonii sequence was used as an out group to root the tree.

KLIMISCH EVALUATION

1. Relevance of study:		**Relevant with restrictions**
	Comment:	The influence of glyphosate on soil microogranismen has already been addressed in the current RAR as part of the efficacy evaluation for glyphosate.
2. Reliability of study:		**Not reliable**
	Comment:	Not all relevant details on the investigation are reported in the present publication. This concerns the following aspects: - No information on the preparation of the test solutions of the test item is given. - Data on application device, water volume etc.are completely missing. - It is not clear if the glyphosate formulation tested incl. co-formulants and application rates are relevant for uses in the EU. -
3. Klimisch code:		**Klimisch rating of 3**

Duke et al., 2012

Author(s)	Year	Study title
Duke, S.O Lydon,L. Koskinen, W.C. Moorman, T.B. Chaney, R.L. Hammerschmidt, R.	2012	Glyphosate Effects on Plant Mineral Nutrition, Crop Rhizosphere Microbiota, and Plant Disease in Glyphosate-Resistant Crops Journal of Agricultural and. Food Chemistry Volume: 60 Pages: 10375−10397 DOI: 10.1021/jf302436u

Abstract
Claims have been made recently that glyphosate-resistant (GR) crops sometimes have mineral deficiencies and increased plant disease. This review evaluates the literature that is germane to these claims. Our conclusions are: (1) although there is conflicting literature on the effects of glyphosate on mineral nutrition on GR crops, most of the literature indicates that mineral nutrition in GR crops is not affected by either the GR trait or by application of glyphosate; (2) most of the available data support the view that neither the GR transgenes nor glyphosate use in GR crops increases crop disease; and (3) yield data on GR crops do not support the hypotheses that there are substantive mineral nutrition or disease problems that are specific to GR crops.

In the review by Duke et al. (2012), most of the cited literature (232 references) was addressed to the effect of glyphosate on mineral nutrition, crop rhizosphere microbiota, and plant disease in glyphosate-resistant crops. Most references are from the period 2001 to 2012.

1. Relevance of study:		**Not relevant**
	Comment:	Transgenic-crops and associated effect of glyphosate application are not in the focus of the efficacy evaluation for glyphosate. The study can therefore not be classified as relevant for the

assessment of glyphosate efficacy.

Duke et al., 2012

Duke, S.O Lydon, L. Koskinen, W.C. Moorman, T.B. Chaney, R.L. Hammerschmidt, R.	2011	Distribution and Prevalence of Fusarium Crown Rot and Common Root Rot Pathogens of Wheat in Montana Plant Diseases Volume: 95 Pages: 1099-1108 DOI: 10.1094/PDIS-11-10-0795

Abstract
Distribution of Fusarium crown rot (FCR) and common root rot (CRR) pathogens associated with wheat (*Triticum aestivum*) in 91 fields in Montana were determined during the 2008 and 2009 crop seasons using real-time quantitative polymerase chain reaction (qPCR) and conventional isolation methods. Correlations ($P < 0.001$) were found between detection methods for both diseases. FCR was detected in 57% of the fields and CRR was detected in 93% of the fields surveyed. Percent incidence based on isolation from individual tillers was *Bipolaris sorokiniana* (15%), *F. culmorum* (13%), and *F. pseudograminearum* (8%). FCR populations were highly variable across the regions and were not detected in any fields from the Gb5 soil types of Judith Basin and Fergus counties. The spatial distributions of FCR and CRR were affected by elevation, soil type, and temperature. High FCR populations were associated with spring wheat crops rather than winter wheat based on qPCR ($P < 0.001$). FCR and CRR could produce yield losses in a range of 3 to 35%. This study is the first time that qPCR was used to survey these two pathogen groups, and the merits and weakness of qPCR relative to traditional isolation methods are discussed.

1. Test material:

Test item: Glyphosate not used as a test item
Active substance(s): Not stated
Surfactant: Not stated
Source of test substance: Not stated
Lot/Batch #: Not stated
Purity: Not stated

2. Vehicle and/or positive control: Not stated

3. Test organism:

Species: Fusarium crown rot and common root rot
Cultivar: Not stated
Source: Natural field infestation
Crop growth stage at treatment: No glyphosate treatment applied
Holding conditions prior to test: Not stated

4. Test system:

Study type: Field experiment
Guideline: Non
GLP: No
Guideline deviations: Not applicable
Duration of study: Sampled collected 2008 and 2009
Treatments: No treatments applied
Replicates: For each county, between two and nine fields were

	sampled each year. Sampled fields were randomly chosen with the help of the county extension agents of the respective counties.
Test concentrations:	No treatments applied
Application devices:	No treatments applied
Application verification:	No treatments applied

5. Environmental conditions:

Study site:	A survey was conducted involving 40 and 51 commercial wheat fields, respectively, from 10 counties throughout the northcentral and central regions of Montana. The counties sampled were Blaine, Choteau, Fergus, Glacier, Hill, Judith Basin, Liberty, Phillips, Teton, and Toole. Fields selected were over 25 ha and were separated by a minimum of 2 km.
Weather conditions / Temperature / relative humidity:	The climate at the study site is semiarid. The monthly mean temperature and total precipitation during the 2 years of the study were monitored by a weather station located at the experimental farm.
Test medium / Soil at study site	Not stated
Light intensity	Not stated
pH:	Not stated
Organic matter (Corg):	Not stated
Fertilizer:	Not stated

6. Observations/analyses:

Measurements:	**Counties surveyed and sampled** For each field, plant samples were collected within 14 days of harvest (either before or after). Samples were collected during 11 to 16 August 2008 and 10 to 25 August 2009. For each field, samples were collected at 20 sites along a 600-m diagonal transect, with each site being approximately 30 m apart. Transect direction was variable and always started at least 30 m from the margin of the field, and was representative. At each sampling site, three randomly selected tillers were collected for a total of 60 tillers per field. The selected tillers were taken to the laboratory, where they were cleaned, dried, and stored at 4°C until they could be processed using qPCR or media-based isolation. Coordinated with the field sampling, growers were surveyed about their cropping systems. Survey questions detailed wheat type (spring versus winter), cropping intensity, crop rotation, glyphosate usage, fungicide seed treatments, and nitrogen fertilizer applications. This information was used to categorize the crops and fields according to crop production factors and to determine association of these factors with pathogen populations as measured by qPCR quantification of

targeted DNA sequences.

Quantification of crown and root rot infection on field samples

For DNA extractions and qPCR quantification, 3-mm stem sections were cut from the first internode of 30 randomly selected tillers per field sample. Subsequent handling, processing, and DNA extraction of the stem sections followed previously established protocols. Real-time quantification of pathogen populations was conducted using probes dual-labeled with the 6 carboxyfluorescein fluorescent reporter dye and the 6-carboxyltetramethylrhodamine fluorescence quencher. The probe and primers used for quantification of *Fusarium* populations were previously described by Strausbaugh et al. and modified by Hogg et al.. This assay was specific for the three main species in the FCR complex: *F. culmorum*, *F. pseudograminearum*, and *F. graminearum*. The probe and primers used for *B. sorokiniana* were designed using the partial DNA sequence of the glyceraldeyde-3-phosphate dehydrogenase- like (*gpd*) gene from *B. sorokiniana* isolate ND93-1 (Gen-Bank accession EF513209.1). The probe sequence was 5′-TCCATGGAGCGAGACTGGGCGC-3′, which is located between the 377 and 398 bp of the *gpd* sequence. The forward and reverse primer sequences were 5′-GAAGGACCCCGCCAACA-3′ and 5′-CCGCTACACTCGACGACGTAGT-3′, respectively. All amplifications were carried out in a Rotor-Gene Q (Qiagen Inc., Valencia, CA) using a two-step protocol with the following thermocycling parameters: 2 min at 55°C, 10 min at 94°C, and 40 cycles of 15 s at 94°C and 60 s at 60°C. qPCR reactions (25 µl) consisted of 12.5 µl of Universal TaqMan Master Mix (PE Applied Biosystems, Foster City, CA), 2.5 µl of 2 µM TaqMan probe, 2.25 µl of a 1:1 mixture of 20 µM forward and reverse primer solutions (Integrated DNA Technologies, Inc., Coralville, IA), 2.75 µl of molecular-grade water, and 5 µl of DNA sample. Each real-time run included five
10-fold-dilution standards, ranging from 660 copies to 6.6 million copies. Standard curves produced by plotting the log of the DNA standards concentrations versus the cycle threshold (Ct) values showed strong relationships (*R* values always exceeded 0.99). Both the DNA standards and no-template control samples were run in triplicate for each real-time run. DNA field samples were run three times and the average of

the three runs was considered the level of crown and root rot infection present in the field. To avoid artifacts from primer dimmers, a cut-off value of 36 cycles was established for Ct, the cycle number at which the fluorescence generated within the reaction crossed the threshold for being significantly different from the baseline or background signal. For *Fusarium* spp., the DNA standard used for absolute quantification during qPCR was a cloned fragment of the *tri5* gene from *F. culmorum*, isolate 2223 (29). For *B. sorokiniana*, a 507-bp fragment of the *gpd* gene from isolate 2243 (collected in Chester, MT) was cloned using Invitrogen's (Carlsbad, CA) TOPO TA cloning kit as per the manufacturer's instructions. A *gpd* gene fragment was amplified using forward primer 5′-TAAAGCTGACCCTGT GTCTCAGCA-3′ and reverse primer 5′ AGAGAACCTCAATGTCGGGCTTGT-3′. Colony selections were confirmed by PCR amplification. Because the *B. sorokiniana* assay was new, its specificity and sensitivity was tested across the following plant pathogens: *F. culmorum*, *F. pseudograminearum*, *F.graminearum*, *F. sambucinum* Fuckel (teleomorph *G. pulicaris*), *F. avenaceum*, *F. acuminatum* Ellis & Everh. (teleomorph *G. acuminate* Wollenw.), *F. solani* (Mart.) Sacc., *F. oxysporum* Schltdl., *F. verticillioides* (Sacc.) Nirenberg (syn. *F. moniliforme* J. Sheld.), *F. equiseti*, *F. poae* (Peck) Wollenw., *F. sporotrichioides* Sherb. (syns. *F. tricinctum*dand *F. sporotrichiella* var. *sporotrichioides* (Sherb.) Bilaĭ), *Verticillium dahliae* Kleb., *Thielaviopsis basicola* (Berk. & Broome) Ferraris (syn. *Chalara elegans* Nag Raj & W.B. Kendr.), *Cephalosporium gramineum* Y. Nisik. & Ikata (syn. *Hymenula cerealis* Ellis & Everh.), *Gaeumannomyces graminis* var. *tritici* J. Walker, *Pythium ultimum* Trow, *Phialophora* spp., *Sclerotinia sclerotiorum* (Lib.) de Bary, *Aphanomyces cochlioides* Drechsler, *Penicillium claviforme* Bainier, *Oculimacula yallundae* (Wallwork & Spoonee) Crous & W. Gams (syn. *Tapesia yallundae*), *Rhizoctonia solani* Kühn, and *B. maydis* (Y. Nisik. & C. Miyake) Shoemaker (teleomorph = *Cochliobolus heterostrophus* (Drechsler) Drechsler; syn. *Helminthosporium maydis* Y. Nisik. & C. Miyake). For each species, 10 ng of DNA was tested via qPCR to check for nonspecific reactions and experiments were repeated once in their entirety. The only fungi amplified by

this assay were *B. maydis* and *B. sorokiniana*. Both species had similar amplification curves. To monitor inhibition in the qPCR reactions, all undiluted DNA samples were spiked with 335 pg of plasmid containing the β-*actin* gene (*mjba-1*) from *Meloidogyne javanica* (40). No inhibition was observed in any of the samples based on qPCR results.

Isolation and identification of pathogens

To determine the root and crown rot pathogens involved in the infection of the stems and to corroborate qPCR results, additional 3-mm segments were excised from the first internodes of the 30 wheat tillers used for qPCR quantification. These new segments were used to assess presence of *Fusarium* spp., *B. sorokiniana*, and other pathogen species in these tissues through culturing on a general and selective media. One-half of the segments were plated onto 50% potato dextrose agar (PDA) amended with antibiotics (100 µg of streptomycin sulfate, 100 µg of tetracycline sulfate, and 50 µg of neomycin sulfate). The other half of the stem segments were plated onto a media selective for *Bipolaris* spp., because the slow growing *B. sorokiniana* was easily overgrown by *Fusarium* mycelia on PDA.

Prior to plating, stem segments were disinfected with 0.54% sodium hypochlorite solution for 30 s, rinsed with sterile distilled water, and dried on a sterile paper towel. Five disinfected segments were placed on each media plate. Plates were incubated at 23°C and monitored daily for fungal growth. Resulting fungal colonies were hyphal tipped and placed onto PDA to develop monoxenic cultures. All resulting isolates were identified to genus using morphologic and culture characteristics. All *Fusarium* cultures were transferred to carnation leaf amended (CLA) media for identification to species using traditional species identification characteristics.

Isolates of *B. sorokiniana* and other fungi were identified under a microscope according the characteristics of their conidia and mycelia. The number and identity of fungi were recorded for each sample. These data were used to correlate relationships between isolation frequencies and the population sizes as determined by the qPCR assays. In consideration that *F. culmorum*, *F. pseudograminearum*, and *F. graminearum* are all detected by the qPCR *Fusarium* assay, these pathogens were considered as

members of the FCR complex when used to compare isolation and qPCR assay results.

Relationship between pathogen populations and grain yield

To study the relationships between disease pathogen populations and grain yield, four selected fields in 2008 and five selected fields in 2009 were intensively sampled. For 2008, intensive sampling was done in two dryland winter wheat fields and one spring wheat field in Choteau County and one irrigated spring wheat field in Phillips County. In 2009, intensive sampling occurred on two dryland winter wheat fields, one each in Phillip and Chouteau counties; two dryland spring wheat fields, one each in Glacier and Blaine counties; and a repeated sampling of the irrigated spring wheat field from Phillips County that had been sampled in 2008. Intensive sampling involved collecting 20 crown and grain samples from 20 arbitrarily selected sites along a 600-m transect within each field. For each sampling site, heads were collected from 1 m^2 to determine yield and 60 tillers collected from the corresponding area being analyzed for FCR and CRR pathogen populations by the qPCR method described above. For this article, pathogen populations are equated to disease infestation. The grain was harvested from collected heads using a Vogel thresher (Bill's Welding, Pullman, WA), cleaned, and weighed.

Environmental and spatial characterization of field infection

All fields were geo-referenced through Google Earth software (http://www.google.com/earth/index.html). In cases where no precise global positioning system (GPS) location for the field could be determined, coordinates were matched to the nearby geographical place names recognizable in the spatial database. In addition to locations, elevation for each GPS field position was registered. For each survey location, data for average growing degree-days between May and August, average annual temperatures and precipitation, growth period temperature and precipitation (April to August), general soil types, and soil water-holding capacity were extracted with ArcViewGIS (version 9.0; ESRI, Redland, CA) from the geographical information system (GIS) database MAP *Atlas* 6.0 (11). All environmental data were analyzed on either an individual pathogen species basis or as a group of species according to their environmental association using a Spearman's coefficient of rank correlation.

Parameters measured:	Crown and root rot infection on field samples, relationship between pathogen populations and grain yield, environmental and spatial characterization of field infection
Statistics:	DNA copy numbers obtained through qPCR for each disease of interest were log (x + 1) transformed to reduce variance instability within the data sets. In addition, zero values were trimmed from data sets when comparisons between results of qPCR and isolation methods were made using correlation coefficients and linear regressions. Across years, Levene's test of homogeneity of variance found variances homogenous and, therefore, the data from both years were combined. Pearson's and Spearman's correlation coefficients and linear regressions were performed to examine various relationships between disease and pathogen populations as determined by qPCR or conventional methods and other measured parameters using the PROC COR and PROC REG procedures of SAS software (version 9.2; SAS Institute, Inc., Cary, NC). A standard $\chi 2$ test was used to compare frequencies of infested fields by county obtained through isolation methods and qPCR assay for each disease. Multiregression analyses were conducted to assess additive effects or interaction between populations of FCR pathogen species and *B. sorokiniana* as quantified by qPCR and grain yield for the extensively sampled fields, considering each individual field, and for a generalized model where the field factor was a fixed effect. Geospatial statistics were used to determine significant spatial patterns for the different populations of both pathogen groups and fungal species determined through qPCR and plating. All fields (with or without FCR and CRR pathogens) were included in the analysis, and tests for spatial autocorrelation were undertaken using Moran's I values and transforming them to z scores. This statistic provides an evaluation of whether the observed distribution pattern for an exhibited fungal species is clustered, dispersed, or random. All latitudinal and longitudinal coordinates for the field locations were corrected to a decimal degree and tests for each fungal species were implemented within the spatial statistics component from the PROC VARIOGRAM of SAS. Relationships between the extracted environmental variables from the GIS database and population of both pathogen groups obtained by qPCR or percentage of infection for each pathogen of interest were analyzed using PROC COR. Spearman's

coefficient of rank correlation was used to avoid variance differences and distribution effect of analyzed variables. In addition, Kruskal-Wallis rank sum tests and nonparametric multiple comparisons for the differences in ranks with a Wilcoxon rank-sum tests and Benjamini- Hochberg correction method were conducted to determine differences in level of plant infection among the general soil type classifications for Montana by using the packages "Rcmdr" and "PairwiseCI" of the R-Software (www.r-project.org). Associations between crop production factors and values obtained from the qPCR quantification were analyzed using a bivariate distance vector in a permutation multivariable analysis of variance (PerMANOVA) through the adonis function from the package "vegan" of the R-software. This function permits the analysis of univariate or multivariate data using any distance measure and linear model. The calculated statistic (*pseudo-F*) is calculated, like a traditional *F* statistic, as the sum of the squared distances among groups divided by the sum of the squared distances within groups relative to the overall sum of the pairwise distances (1,36). This statistical method uses Euclidean distance permutations to generate the MANOVA by assuming the same distribution for all the groups or variables analyzed. Nonparametric Wilcoxon-Mann-Whitney or Kruskal-Wallis tests were considered to determine significant differences between two or more groups in the crop factor variables by using the Package "Rcmdr" of R-Software

KLIMISCH EVALUATION

1. Relevance of study:		**Relevant with restrictions**
	Comment:	The influence of glyphosate on plant diseases has already been addressed in the current RAR as part of the efficacy evaluation for glyphosate.
2. Reliability of study:		**Not reliable**
	Comment:	Glyphosate was not used as a test item in the described experiments. No information is given about glyphosate application (time of application, type and dosage of used glyphosate formulation, application frequency) in the fields sampled during the survey.
3. Klimisch code:		**Klimisch rating of 3**

Pasaribu et al., 2013

Pasaribu, A. Mohamad, R.B. Hashim,A. Rahman, Z.A. Omar, D. Morshed, M.M.	2013	Effect of herbicide on sporulation and infectivity of versicular arbuscular mycorrhizal (*Glomus mosseae)* symbiosis with peanut plant The Journal of Animal & Plant Sciences Volume: 23 Issue: 6 Pages: 1671-1678 DOI: Not stated

Abstract
Arbuscular mycorrhizal fungi are of interest for their reported roles in preserving soil fertility in agroecosystem, which form mutualistic symbiosis with the roots of most agricultural plants. In the past, most research on VA mycorrhizae has been focused on possible responses to fungicides, rather than on the potential effects on mycorrhizal symbiosis following herbicide application. We studied the effect of three application rates of two widely used herbicides (1.8, 3.6 and 5.4 µg a.i. g^{-1} for alachlor and 1.08, 2.16 and 3.24 µg a.i. g^{-1} for glyphosate) on sporulation and infection of peanut plant by *G mosseae* under greenhouse conditions. The result of the study showed that the symbiont fungus *G mosseae* responds in a differential way to two different herbicides tested, and the fungus sensitivity to alachlor proved significantly higher than the glyphosate. However, none of the herbicide treatments affected the external hyphal length and SDH (Succinate dehydrogenase) activity. But the spore number, total and active infection intensity of internal hyphae was significantly reduced with the increasing rates of alachlor application, while glyphosate had no significant effects at all application rates. Consequently, P (phosphorus) inflow through mycorrhizal hyphae was significantly increased with the application rates of glyphosate, with the highest value (41.48 and 479.72 x10-13 mol P m^{-1} s^{-1} hyphal inflow and hyphal uptake, respectively) obtained at field recommended glyphosate rate (2.16 µg a.i. g^{-1}). Therefore, symbiotic functions of *G. mosseae* with host plant could be affected by the depressive effects of herbicides that are apparently related to the types of herbicide and their rates of application.

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup
Active substance(s):	Glyphosate
Surfactant:	Not stated
Source of test substance:	Monsanto
Lot/Batch #:	Not stated
Purity:	Not stated

2. Vehicle and/or positive control: Not stated

3. Test organism:

Species:	*G. mosseae*
Cultivar:	Not stated
Source:	Inoculums of *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe UK 118 obtained from INVAM (International Culture Collection of VA Mycorrhizal Fungi), which consisted of spores, external hyphae and infected root fragment.

Crop growth stage at treatment:	Not stated
Holding conditions prior to test:	The inoculums were then propagated on *Sorghum bicolor* for 4 months in the glasshouse pot cultures using the method of Feldmann and Idczak (1991). Enumeration of the infective VAM propagules of fungi was determined by using most probable number (MPN) method as described by Sieverding (1991), resulting in 88.32 infective propagules/100 g inoculum. The spores of *G. mosseae* from above inoculums were isolated, and the number was 5.6 spores/g inoculum, as determined by the wet sieving method. The mycorrhizal inoculum was inoculated at 10% by weight per pot soil, before sowing of peanut in the surface soil layer of the pot, and then covered with a 5.0 cm soil.

4. Test system:

Study type:	Greenhouse experiment
Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	Not stated
Treatments:	The herbicide treatments were applied as soil drench of 20 mL solution, before sowing of peanut. The control was treated with the same amount of sterile distilled water.
Replicates:	Treatments were arranged in a completely randomized design (CRD) with three replicates.
Test concentrations:	Three concentrations (rates) of each herbicide: 1.8, 3.6 and 5.4 µg a.i. g^{-1} dry soil for alachlor; and 1.08, 2.16 and 3.24 µg a.i. g^{-1} dry soil for glyphosate, were applied. These application rates represented 0.5, 1 and 1.5 times (x) their recommended field application rates (Alachlor 400 g a.i./ha, and glyphosate, 800 g a.i./ha).
Application devices:	Soil drench
Application verification:	Not stated

5. Environmental conditions:

Study site:	Not stated
Weather conditions / Temperature / relative humidity:	Not stated
Test medium / Soil at study site	Sterilized (121°C for 1 hour) soil:sand mixture (1:3). The soil was bungor series soil (Classification: subactive typic paleudult) consisted of 34.9% sand, 16.4% silt, and 48.7% clay.
Light intensity	Not stated
pH:	Not stated
Organic matter (Corg):	Not stated

Fertilizer:

6. Observations/analyses:

Measurements: Plants were harvested destructively at 4 and 6 weeks after planting (WAP).

Measurement of spore number in soil

Effect of herbicides on spore number of *G. mosseae* in the soil was determined by the wet sieving and decanting method as described by Gerdemann and Nicolson (1963). At each harvest, 20 g soil sample from each replicate was collected to a depth of 0-10 cm mycorrhizosphere soils. The soil sample was then wet sieved, and rinsed with 200 mL distilled water in a 500 mL beaker. The suspension was then poured through a series of sieves arranged in a decreasing mesh size from 300, 250, 106, and 45 µm. The trapped particles were then rinsed three times with 200 mL distilled water. The particles retained on the 106 and 45 µm sieves were transferred to a Petri dish, which was marked with parallel lines (±7 mm apart) to separate microscope fields for spore counting. Number of spores was counted by moving the dish systematically through all the lines under microscope.

Measurement of external hyphal length

The extraction and measurement of external hyphae were carried out using the method of Abbott and Robson (1995). At harvest time of peanut plant, five cores of moist soil were taken with a cork borer from each pot, and mixed well in a plastic bag. A 10 g subsamples of soil were agitated with 400 mL distilled water for 1 min and decanted through 250 and 53 µm sieves. These were then mixed together and agitated again with distilled water followed by vigorous steering on a magnetic stirrer for 5 min. A 50 mL aliquot was taken and passed through 1.2 µm Millipore filter. Estimation of the total hyphal length was done by staining the retained material on filter in trypanblue (TB) in acidic glycerol. It (TB in acidic glycerol) was prepared by mixing 500 mL glycerol, 450 mL H2O, 50 mL 1% HCL, and 0.05 % trypen blue (Koske and Gemma, 1989). Total hyphal length was finally measured by using gridline-intersect method under a dissecting microscope at 250x magnification bearing a grid (10 x 10 -mm squares) in eyepiece micrometer as described by Newman (1966) and Tennant (1975). Hyphal length was calculated using the following formula:

Hyphal length/grid (cm/g soil) = c x n x g x a/b x 1/s

Where, c = constant (11/14)

n = no. of intersections

g = grid unit
a = area of filter covered by sample (mm2)
b = area of grid (mm2)
s = soil on filter (g)

Measurement of internal hyphal infectivity

A combined method of Phillips and Hayman (1970), and
Koske and Gemma (1989) were used for observing the activity of internal hyphae of VAM fungi. Total infection intensity of the internal hyphae of *G. mosseae* was determined by staining the trypan blue (TB). At each harvest, roots were sampled and washed thoroughly on tap water, then placed into 50 mL McCartney bottles and kept overnight in 50% ethanol for fixing. The roots *ca* 3g (f. wt.) were fixed in 10% KOH, and heated in a water bath at 90°C for 1 hour. The KOH solution was poured
off and the roots was rinsed in two changes of 200 mL tap water, and finally acidified by soaking in 1% HCL for 5 min. The acidified roots were stained in 0.05% trypan blue mixed with an acidic glycerol solution. The stained roots were heated in a 90°C water bath for 60 min. TB solution was then poured off, and the roots were distained in acidic glycerol. The distained roots were cut into 1 cm sections, and 10 cuttings among them were mounted in glycerol onto a glass slide under stereomicroscope to examine the presence or absence of VAM arbuscules, vesicles and/or internal hyphae. The total and active infection intensity of the internal hyphae was calculated using the formulae given by Trouvelot *et al*. (1986):

$$M\% = (95n5 + 70n4 + 30n3 + 5n2 + 1n1)/ N$$

Where, M= Intensity of infection
n5, n4, n3… n1 respectively designated number of fragments notes 5, 4,..,1
(0 = 0%, 1 = 1-5%, 2 = 6-10%, 3 = 11-50%, 4 = 51-90%, 5 = >90%)
N = Number of observed fragments

The active infection intensity of internal hyphae, however, was determined using the fungal SDH (Succinate dehydrogenase) staining for metabolically active fungus. The SDH activity was determined histochemically by the deposition of purple formazan following reduction of nitroblue tetrazolium (NBT in the presence of succinate (Smith and Gianinazzi-Pearson, 1990). Roots were cut into 0.5 cm length and incubated overnight in the reaction medium containing 50 mM Tris- HCL (pH 7.4), 0.5 mM MgCl2, 1 mg mL^{-1} NBT, 0.25 M Na_2 Succinate. The stained roots were the rinsed with distilled water, and boiled in 20% choral hydrate for 10-15 min. The

distained root cuttings were mounted in glycerol onto a glass slide under stereomicroscope to examine the presence of dark purple stain in the root fragments. The proportion of SDH activity of the internal hyphae was calculated by using the formulae as below:

Active infection intensity of internal hyphae revealed by SDH stain

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--------- *X 100*

Total infection intensity of internal hyphae revealed by TB staining

Measurement of P (phosphorus) inflow into peanut

The effect of herbicides on P inflow was measured by calculating the P inflow into plant root at 0-28 and 28-42 days after planting (DAP) of peanut. The P inflow into plant roots were calculated using the formula of Brewster and Tinker (1972) as below:

$I = (p_2\text{-}p_1) \times In\ (L_2L_1)\ [t_2\text{-}_{t1}]^{-1}$

Where, I = P inflow to roots (mol P m-1 root length s^{-1})

P = concentration of plant phosphorus, p1 = first period,

p2 = second period

L = root length, L1 = first period, L2 = last period

T = plant age, t1 = first period, t2 = last period

P concentration in plant shoot was determined using the wet digestion method of concentrated sulfuric acid (H2SO4) and perchloric acid (Abdulhamid and Dynoodt, 1981). However, the contribution of the mycorrhizal hyphae to the root inflow (hyphal inflow) was calculated by subtracting the inflow in the control non-mycorrhizal plants (Inm) from that of the mycorrhizal plants (Im). Hyphal uptake was determined by the rate of P uptake by the external hyphae (mol P m^{-1} external hyphae length s^{-1}).

Parameters measured: spore number in soil , external hyphal length, internal hyphal infectivity, P inflow into peanuts

Statistics: Data were analyzed by using the analysis of variance (ANOVA) and mean separation was done by Least Significance Difference (LSD) at 5% level. Some data were also analyzed by the contrast test.

KLIMISCH EVALUATION

1. Relevance of study: **Relevant with restrictions**

	Comment:	The influence of glyphosate on soil microogranismen has already been addressed in the current RAR as part of the efficacy evaluation for glyphosate.
2. Reliability of study:		**Not reliable**
	Comment:	Not all relevant details on the investigation are reported in the present publication. This concerns the following aspects: - No information on the preparation of the test solutions of the test item is given. - No information is given about the used formulation of glyphosate neither the sources. - The application via soil drench is not comparable to field application - No validation of concentrations of glyphosate in the soil/rhizosphere. -
3. Klimisch code:		**Klimisch rating of 3**

Samac & Foster-Hartnett, 2012

Samac, D. A. Foster-Hartnett, D	2012	Effect of Glyphosate Application on Foliar Diseases in Glyphosate-Tolerant Alfalfa Plant Diseases Volume: 96 Pages: 1104- 1110 DOI: 10.1094/PDIS-08-11-0715-RE

Abstract
Glyphosate, the active ingredient in Roundup herbicide, inhibits 5- enol-pyruvyl shikimate 3-phophate synthase (EPSPS), an enzyme found in plants, fungi, and bacteria. Plants engineered for glyphosate tolerance with a glyphosate-insensitive EPSPS take up and translocate the herbicide throughout the plant. In greenhouse experiments, we found that application of glyphosate at the recommended field application rate completely controlled alfalfa rust (*Uromyces striatus*) on 4- week-old plants inoculated with the fungus 3 days after glyphosate treatment. Control was effective in all seven cultivars tested. The level of protection declined with time after application, indicating that control is transitory and protection declined with time after inoculation, suggesting that protective treatments have fungistatic activity. Complete control of rust was obtained when glyphosate was applied up to 10 days after inoculation with rust spores, indicating that the herbicide also has curative activity. Treatment increased protection from anthracnose, caused by *Colletotrichum trifolii*, a hemibiotrophic pathogen, and reduced symptom severity for spring black stem and leaf spot, caused by *Phoma medicaginis*, a necrotrophic pathogen. These results indicate that glyphosate could be used to help manage foliar diseases in glyphosate-tolerant alfalfa.

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup WeatherMax
Active substance(s):	Glyphosate
Surfactant:	Not stated
Source of test substance:	Monsanto
Lot/Batch #:	Not stated
Purity:	Not stated
2. Vehicle and/or positive control:	Not stated
3. Test organism:	
Species:	Alfalfa (*Medicago sativa*)**,** *Uromyces striatus*, *Colletotrichum trifolii* and *Phoma medicaginis*
Cultivar:	Glyphosate- tolerant varieties of alfalfa: 'DKA34-17RR' (fall dormancy 3), 'Liberator RR' (fall dormancy 4), 'Consistency RR' (fall dormancy 4), 'Revolution RR' (fall dormancy 8), 'DKA84-10RR' (fall dormancy 8), and 'WL660RR' (fall dormancy 9). Standard check alfalfa varieties: 'MSA-CW3An3' (rust resistant), 'Moapa 69' (rust susceptible), 'Saranac AR' (anthracnose resistant), 'Saranac' (anthracnose susceptible), 'Ramsey' (spring black stem and leaf spot resistant), and 'Ranger' (spring black stem and leaf spot susceptible).
Source:	Glyphosate- tolerant varieties of alfalfa were obtained from Peter Reisen, Forage Genetics International, Nampa, ID. Seed of 'DKA41-18RR' (fall dormancy 4) was provided by Paulette Pierson, Monsanto Company, St. Louis. Standard check alfalfa varieties were obtained from JoAnn Lamb, United States Department of Agriculture– Agricultural Research Service (USDA-ARS), Saint Paul, MN. Urediniospores of *U. striatus* were collected from field-grown plants at the Sand Plain Experimental Research Farm, Becker, MN and propagated on alfalfa plants Saranac or DKA41-18RR in the greenhouse. *C. trifolii* race 2 was obtained from Nichole O'Neill, USDAARS, Beltsville, MD. *P. medicaginis* strain 24.2M1a was obtained from Claudia Castell-Miller, University of Minnesota, Saint Paul.
Crop growth stage at treatment:	Not stated
Holding conditions prior to test:	Seed were planted in five rows in Nalgene tubs (38 cm wide by 51 cm long by 13 cm deep). After 2 weeks, plants were thinned to 15 or 25 plants/row, and treated with Marathon (1% granular imidacloprid; Olympic Horticultural Products, Mainland, PA) to control thrips.

4. Test system:

Study type:	Greenhouse experiment
Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	Not stated
Treatments:	Spores of ***U. striatus*** were stored at 4°C for up to 8 weeks before use. Urediniospores were applied to 4-week-old plants at approximately 0.03 mg/plant at a concentration of 3 mg/ml in Soltrol 170 oil (Phillips Petroleum, Bartlesville, OK) using an artist's airbrush sprayer (Paasche Airbrush Company, Harwood Heights, IL) pressurized by an air pump (138 kPa). All experiments used plants of DKA41-18RR, unless noted otherwise. Inoculated plants were incubated in a dark mist chamber at 25°C for 24 h. After foliage had dried, plants were transferred back into the greenhouse. Plants were inoculated 3 days after glyphosate treatment for experiments to test the protective activity of glyphosate. Inoculations were done 10 or 17 days after glyphosate treatment to evaluate the duration of protection. In experiments testing the curative effect of glyphosate, plants were inoculated and incubated for 24 h in the mist chamber; then, glyphosate was applied once at 1× the recommended field application rate at 2, 4, 6, 8, 10, and 12 days post-inoculation (dpi). ***C. trifolii*** race 2 was grown on potato dextrose agar (PDA) plates at room temperature. Conidia were harvested in sterile distilled water with 0.01% Tween20 and adjusted to 2 × 106 conidia/ml. Six-week-old DKA44-18RR plants were treated with glyphosate at 1×, 2×, or 4× the recommended field application rate as described above and, 3 days later, inoculated with *C. trifolii* spores sprayed to run-off with a hand-held sprayer. Plants were incubated in a dark mist chamber for 48 h and then moved back into the greenhouse once leaves had dried. Conidiospores of ***P. medicaginis*** were produced on PDA plates incubated at room temperature. The inoculum was adjusted to 1 × 106 spores/ml in sterile water with 0.01% Tween20 and applied to 6-week-old plants of DKA44-18RR to runoff using a hand-held sprayer. Plants were placed in a dark mist chamber for 48 h and then transferred back to the greenhouse after leaves had dried. Glyphosate was applied at in a volume of approximately 187 liters/ha. Plants were not watered for 24 h post application. Standard check varieties included in experiments as inoculation controls were

	not treated with glyphosate.
Replicates:	There were three replicates arranged in a completely randomized design in each experiment and each experiment was done two to four times.
Test concentrations:	0.25×, 0.5×, 1×, 2×, or 4× the recommended field application rate for glyphosate-tolerant plants. The 1× rate treatment contains glyphosate at 1.7 kg acid equivalent/ha
Application devices:	Overhead sprayer
Application verification:	Not stated

5. Environmental conditions:

Study site:	Not stated
Weather conditions / Temperature / relative humidity:	25°C
Test medium / Soil at study site	Nalgene tubs containing steamed soil/MetroMix 200 (2:1, vol/vol; Scotts-Sierra Horticultural Products, Marysville, OH).
Light intensity	16-h photoperiod
pH:	Not stated
Organic matter (Corg):	Not stated
Fertilizer:	

6. Observations/analyses:

Measurements:	Disease was scored on 12 to 16 plants grown in the three middle rows in each replicate. Rust symptoms of ***U. striatus*** on the most highly infected leaf on each plant were scored 14 dpi on a 0-to-3 scale in which 0 = no symptoms; 1 = 1 to 9 small closed pustules; 2 = 10 to 20 open pustules, no halos; and 3 = more than 20 open of pustules with necrotic or pronounced chlorotic halos. A score of 0 or 1 was considered protected from disease. In some experiments, disease scoring was done using the same rating scale at 14, 21, and 28 dpi. Disease symptoms of ***C. trifolii*** were scored 15 days dpi on a 0-to-4 scale, in which 0 = no symptoms, 1 = 1 to 10 stem lesions, 2 = more than 10 stem lesions, 3 = more than 10 stem lesions with apical necrosis, and 4 = dead plant. A score of 0 or 1 was considered protected from disease. DKA44- 18RR is resistant to race 1 of *C. trifolii* but susceptible to race 2. Symptoms of ***P. medicaginis*** were scored 14 dpi on a 0-to-4 scale, in which 0 = no symptoms; 1 = lesions less than 2 mm; 2 lesions greater than 2 mm, with necrotic centers; 3 = lesions greater than 3 mm; and 4 = lesions greater than 3 mm, with defoliation or dead plant. A score of 0 or 1 was considered protected from disease.
Parameters measured:	Disease symptoms of *U. striatus*, *C. trifolii* and *P. medicaginis*

Statistics:	Statistical analysis was done using SAS software (version 9.2; SAS Institute Inc., Cary, NC). The x^2 analysis and Fisher's exact test for categorical data were used. A *P* value of less than 0.05 was considered statistically significant.

KLIMISCH EVALUATION

1. Relevance of study:		**Relevant with restrictions**
	Comment:	The influence of glyphosate on plant diseases has already been addressed in the current RAR as part of the efficacy evaluation for glyphosate.
2. Reliability of study:		**Reliable with restrictions**
	Comment:	Not all relevant details on the investigation are reported in the present publication. This concerns the following aspects: - It is not clear if the glyphosate formulation tested incl. co-formulants and application rates are relevant for uses in the EU. -
3. Klimisch code:		**Klimisch rating of 2**

Druille et al., 2013

Druille, M. Cabello, M.N. Omacini, M. Golluscio, R.A.	2013	Glyphosate reduces spore viability and root colonization of arbuscular mycorrhizal fungi Applied Soil Ecology Volume: 64 Pages: 99– 103 DOI: 10.1016/j.apsoil.2012.10.007

Abstract
Glyphosate is the most widely used herbicide in the world, but its effects on non-target organisms, such as arbuscular mycorrhizal fungi (AMF), are unclear. No studies have been found that made reference to effects of glyphosate on AMF spore viability despite its importance as a source of propagules for the perpetuation and spread of AMF in the system. The objective of this study was to evaluate the effect of glyphosate application on AMF spore viability, and their ability to colonize roots. Soil samples were collected from a grassland area located in the Flooding Pampa region (Argentina). We evaluated three herbicide rates: 0, 0.26 and 1× recommended field rate, 10 and 30 days after application. Part of the soil from each tray was used to estimate the spore viability, and the remainder was used as substrate for growing *Lolium multiflorum* Lam. One month after sowing, total root colonization and percentage of arbuscules and vesicles were determined. The spore viability in herbicide untreated soils was between 5.8- and 7.7-fold higher than in treated soils. This reduction was detected even when the lower rate was applied. Root colonization was significantly lower in plants grown in glyphosate treated soil than in untreated ones. A decrease in arbuscular

colonization (but not in vesicles) was found in plants grown in soils treated with the highest herbicide rate. That would indicate that symbiosis functionality was affected, given that arbuscules are the main site for host–fungus nutrient exchange. The results indicate that soil residence time of glyphosate and/or its degradation products was enough to reduce AMF spore viability and their ability to colonize roots. This decrease in propagules viability may affect plant diversity, taking into account the different degrees of mycorrhizal dependency between plant species that may coexist in grassland communities.

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup WeatherMax
Active substance(s):	Glyphosate
Surfactant:	Not stated
Source of test substance:	Monsanto
Lot/Batch #:	Not stated
Purity:	Not stated

2. Vehicle and/or positive control: Not stated

3. Test organism:

Species:	*Lolium multiflorum*, Arbuscular mycorrhizal fungi (AMF)
Cultivar:	Not stated
Source:	Soil samples were collected in spring 2010 from a grazed grass-land located near Azul, in the center of the Flooding Pampa region (36°40'S, 59°32'W, 80 m above sea level). The average annual temperature in the region is 14.6 °C, and annual precipitation is 895 mm yr^{-1}. The grass-land was dominated by a humid mesophytic meadow community, and dominant species include *L. multiflorum* Lam., *Paspalum dilatatum* Poir., *Bothriochloa laguroides* (D.C.) Herter, *Sporobolus indicus* (L.) R. Br., *Panicum milioides* Nees ex Trin., *Nassella neesiana* (Trin. & Rupr.) Barkworth, *Briza subaristata* Lam., *Piptochaetium montevidensis* (Spreng.) Parodi, and *Danthonia mon-tevidensis* Hack. & Arechav. In this type of grasslands, glyphosate is applied in late summer to promote the growth of winter annuals, but this field had no history of herbicide treatment.
Crop growth stage at treatment:	Not stated
Holding conditions prior to test:	Composite soil samples from the top 10 cm were taken, packed, labeled and transported to the laboratory. Samples were mixed and sieved using a 2 mm mesh to remove plant tissues, and the equivalent of 160 g dry soil was placed in 21 × 16 cm trays, 2 cm deep.

4. Test system:

Study type:	Greenhouse/Laboratory experiment
Guideline:	Non
GLP:	No

Guideline deviations:	Not applicable
Duration of study:	Not stated
Treatments:	Treatment factors were herbicide rate and sampling date. Single trays were the experimental units. Sampling date had two levels: 10 and 30 days after application.
Replicates:	A completely randomized design with a 3 × 2 factorial array of treatments and five replications was used.
Test concentrations:	0, 0.8 and 3 l glyphosate ha^{-1} (0, 0.26 and 1× recommended field rate, respectively).
Application devices:	The herbicide was applied using a stationary spray, with a flat fan nozzle (Bertolotti 110SF04), delivering a volume of 120 L ha^{-1} at a speed of 4.6 km h^{-1} and a pressure of 3 bar.
Application verification:	Not stated

5. Environmental conditions:

Study site:	Not stated
Weather conditions / Temperature / relative humidity:	25–35 °C in greenhouse. Soil was kept at field capacity during all the experiment.
Test medium / Soil at study site	The soil was classified as a typical Natraquoll/US Soil Taxonomy (Mollic Gleyic Solonetz/FAO Soil Taxonomy), with 3.5% organic matter and 5.6 ppm P.
Light intensity	Not stated
pH:	Not stated
Organic matter (Corg):	Not stated
Fertilizer:	

6. Observations/analyses:

Measurements:	In addition to determining effects of glyphosate on spore viability we also assessed plant mortality for each herbicide rate. To do this we set up 15 additional trays in which we grew *L. multiflorum* (15 plants per tray) for 1 month prior to treatment. We included five of these trays at each herbicide rate during glyphosate application. Seven days later, we evaluated the survival rate (as the percentage, out of 15, of plants alive within a tray) and chlorophyll content in leaves using a Minolta chlorophyll meter SPAD 502. The latter was measured to assess the vigor of the plants after herbicide application. Control trays only received water. After application, the trays were irrigated with 200 cm3 of water to allow the incorporation of the herbicide in depth. Part of the soil of each tray was used to estimate the spore viability, and the remainder was used as substrate for growing L. multiflorum. Both the assessment of spore viability and the sowing of *L. multiflorum* were carried out in the two sampling dates proposed (10

	and 30 days after application). This species was used as a host plant to assess AMF spores ability to colonize roots. Following glyphosate application, the trays were kept in a greenhouse until harvest of L. multiflorum. **AMF spore isolation and viability assessment** Spores were extracted from 50 g sub-samples of air-dried soil for each sample. They were wet-sieved and decanted and the supernatant was centrifuged in a sucrose gradient. An and Hendrix (1988) procedure was used to determine viable spores, developing a red color with the tetrazolium bromide vital stain MTT [3-(4,5- dimethylthiazol-yl)-2,5-diphenyl-2H-tetrazolium bromide]. Spore suspensions were diluted 1:1 with a solution of 0.5 mg MTT ml^{-1} and incubated for 40 h. **Percentage of root colonization** Plants were harvested 33 days after sowing. Roots were washed in tap water and cleared with 10% KOH for 15 min at 90 °C, placed in 1% HCl for 10 min and then stained with 0.05% lactic–glycerol–Trypan Blue for 5 min at 100 ◦C. A total of 20 root fragments (ca. 1 cm long) from each plant were mounted on slides in a polyvinyl alcohol–lactic acid–glycerol solution and examined under microscope at ×200 magnification. Root colonization was assessed with the method of McGonigle et al. (1990); the total colonized roots, and the fraction of root length containing arbuscules and vesicles were determined.
Parameters measured:	AMF spore viability, *L. multiflorum* survival, chlorophyll content in *L. multiflorum* leaves, total root colonization, percentages of AMF arbuscules and vesicles
Statistics:	Analysis of variance (ANOVA) was performed to determine principal effects of herbicide rate on survival rate and chlorophyll content in leaves, and spore viability. Fungal traits (total root colonization, and percentages of arbuscules and vesicles) were analyzed in a three-way MANOVA. When MANOVA showed significant results, we used univariate ANOVA analysis to determine which of the response variables were most affected by treatments. Percent data was arcsine square-root transformed ($y = \arcsin\sqrt{x}$) before carrying out each analysis to obtain homoge-nous variances. The significance level was set at $\alpha = 0.05$. Treatment means were compared using Tukey test when significant F values were found.

KLIMISCH EVALUATION

1. Relevance of study:		**Relevant with restrictions**
	Comment:	The influence of glyphosate on soil micorrogranism such as arbuscular mycorrhizal fungi has already been addressed in the current RAR as part of the efficacy evaluation for glyphosate.
2. Reliability of study:		**Not reliable**
	Comment:	Not all relevant details on the investigation are reported in the present publication. This concerns the following aspects: - No information is given about the used formulation of glyphosate neither the sources. - It is not clear if the glyphosate formulation tested includes co-formulants and if application rates are relevant for uses in the EU. -
3. Klimisch code:		**Klimisch rating of 3**

Druille et al., 2013

Druille, M. Omacini, M. Golluscio, R.A. Cabello, M.N.	2013	Arbuscular mycorrhizal fungi are directly and indirectly affected by glyphosate application Applied Soil Ecology Volume: 72 Pages: 143-149 DOI: 10.1016/j.apsoil.2013.06.011

Abstract
Glyphosate is a systemic non-selective herbicide, the most widely used in the world. Alongside with its use in agricultural and forestry systems, this herbicide is used in grasslands in late summer with the aim of promoting winter species with the consequent increase in stocking rate. However, its effects on non-target organisms, such as arbuscular mycorrhizal fungi (AMF), are unclear. Arbuscular mycorrhizal fungi (AMF) colonize the root of more than 80% of terrestrial plants, improving their growth and survival, and therefore playing a key role in ecosystem structure and function. The aim of this work was to investigate the possible pathways through which glyphosate application affects AMF spores viability and root colonization in grassland communities. Our hypothesis is that glyphosate application can damage AMF directly (through contact with spores and external hyphae) or indirectly through the changes it generates on host plants. The experiment had a factorial array with three factors: (1) plant species, at two levels (*Paspalum dilatatum* and *Lotus tenuis*), (2) doses of glyphosate, at three levels (0 l ha^{-1}, 0.8 l ha^{-1} and 3 l ha^{-1}), and (3) application site, at two levels: soil (direct pathway) and plant foliage (indirect pathway). Spore viability was reduced even under the lowest glyphosate rate, but only when it was applied on the soil. Total root colonization for both species was similarly decreased when glyphosate was applied to plant foliage or on soil, with no difference between 0.8 and 3 l ha^{-1}. The number of arbuscules was

20% lower when glyphosate was applied on plant foliage, than when it was applied on the soil. Our findings illustrate that glyphosate application negatively affects AMF functionality in grasslands, due to different causes depending on the herbicide application site. While, under field conditions, the occurrence of direct and/or indirect pathways will depend on the plant cover at the time of glyphosate application, the consequences of this practice on the plant community structure will vary with the mycorrhizal dependence of the species composition regardless of the pathway involved.

MATERIALS AND METHODS

1. Test material:

Test item:	Not stated
Active substance(s):	Glyphosate
Surfactant:	Not stated
Source of test substance:	Monsanto
Lot/Batch #:	Not stated
Purity:	Not stated

2. Vehicle and/or positive control: Not stated

3. Test organism:

Species:	*Paspalum dilatatum* and *Lotus tenuis* and associated arbuscular mycorrhizal fungi (AMF)
Cultivar:	Not stated
Source:	Plants of *P. dilatatum* and *L. tenuis* were collected in summer 2011 from a grazed grassland located near Azul, in the center of the Flooding Pampa region (36°40'S, 59°32'W, 80 m above sea level). The average annual temperature in the region is 14.6 °C, and annual precipitation is 895 mm yr^{-1}. Although in this type of grasslands, glyphosate is applied in late summer to promote the growth of winter annuals, the study site had no history of herbicide treatment.
Crop growth stage at treatment:	Glyphosate application was performed 10 days after transplantation to ensure plant establishment.
Holding conditions prior to test:	In mid-February, plants of the same age and size were collected in the field, and placed individually in 1 l plastic pots with their associated soil. The soil was handled with caution, in order to maintain its structure (mainly its porosity). Plants were kept in a greenhouse at 25–35 ◦C until harvest, and watered to maintain the soil at field capacity, without addition of fertilizers. These were distributed following a completely random design and rotated weekly.

4. Test system:

Study type:	Greenhouse/Laboratory experiment
Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	Not stated
Treatments:	The experiment had a completely randomized design

	with a factorial array with three factors: (1) plant species, at two levels (*P. dilatatum* and *L. tenuis*), (2) doses of glyphosate, at three levels (0 l ha^{-1}, 0.8 l ha^{-1} and 3 l ha^{-1}), and (3) application site, at two levels: soil (direct pathway) and plant foliage (indirect pathway). In the first case, glyphosate was applied to the pots once dissolved in 200 cm^3 of water, equivalent to a concentration of 3.8 and 14.5 mg l^{-1} (0.79 and 3 mg kg^{-1}), for the treatment of 0.8–3 l ha^{-1}, respectively. The volume of glyphosate solution applied allowed carrying the moisture content of the soils at field capacity (approximately 20%). This application was made taking care that the solution was not in contact with plant foliage. In the second case, the herbicide was applied to plant foliage using a brush to carefully cover the leaf surface, without coming into contact with soil. Plants for this treatment were watered with 200 cm^3 of water, to equalize the water supplied when glyphosate was applied on the soil.
Replicates:	Five
Test concentrations:	0 l ha^{-1}, 0.8 l ha^{-1} and 3 l ha^{-1}
Application devices:	Soil application: dissolved in 200 cm^3 of water Plant foliage application: brush
Application verification:	Not stated
5. Environmental conditions:	
Study site:	Not stated
Weather conditions / Temperature / relative humidity:	25–35 °C in greenhouse. Soil was kept at field capacity during all the experiment.
Test medium / Soil at study site	The soil was classified as a clay loam typical Natraquoll/US Soil Taxonomy (Mollic Gleyic Solonetz/FAO Soil Taxonomy) with 5.6 ppm P.
Light intensity	Not stated
pH:	6.3
Organic matter (Corg):	3.5%
Fertilizer:	No addition of fertilizers.
6. Observations/analyses:	
Measurements:	**AMF spore isolation and viability assessment** Spores were extracted from 50 g sub-samples of air-dried soil for each sample. They were wet-sieved and decanted and the supernatant was centrifuged in a sucrose gradient. The experimental soil had an average density of 160 spores per 50 g of dry soil. An and Hendrix (1988) procedure was used to determine viable spores, developing a red color with the tetrazolium bromide vital stain MTT [3-(4,5-dimethylthiazol-yl)-2,5-diphenyl-2H-tetrazolium bromide]. Spore suspensions were diluted 1:1 with a solution of 0.5 mg MTT ml^{-1} and incubated for 40 h.

	AMF root colonization Plants were harvested 15 days after glyphosate application. Roots were washed in tap water and cleared with 10% KOH for 15 min at 90 ◦C, placed in 1% HCl for 10 min and then stained with 0.05% lactic–glycerol–Trypan Blue for 5 min at 100 ◦C. A total of 20 root fragments (ca. 1-cm long) from each plant were mounted on slides in a polyvinyl alcohol–lactic acid–glycerol solution and examined under microscope at 200× magnification. Root colonization was assessed with the method proposed by McGonigle et al. (1990); the total colonized roots and the fraction of root length containing arbuscules and vesicles were determined. **Chlorophyll concentration and green biomass** To estimate plant vigor, chlorophyll concentration was nondestructively measured using a chlorophyll meter (SPAD-502, Minolta) 15 days after application. Measurements consisted of 10 readings, five on each side of the midrib. Plants were then harvested from the surface of the soil, and green biomass was separated from dead biomass. Dry weight was determined after oven drying for 72 h at 70 ◦C.
Parameters measured:	AMF spore viability, chlorophyll content in plants, green biomas, total colonized roots and the fraction of root length containing arbuscules and vesicles
Statistics:	Spore viability, chlorophyll content and green biomass data were analyzed using Analysis of Variance (ANOVA). Fungal traits (total root colonization, and percentages of arbuscules and vesicles) were analyzed in a three-way MANOVA. This analysis allows considering fungal traits as a collective measure of AMF response. Pillai's trace was used as the multivariate criterion. When MANOVA showed significant results, we used protected univariate ANOVA analysis to determine which of the response variables was the most affected by treatments. To obtain homogenous variances, percent data were arcsine square-root transformed ($y = \arcsin \sqrt{x}$) before carrying out each analysis. The significance level was set at $\alpha = 0.05$. Treatment means were compared using Tukey test when significant F values were found. Although field collected plants were of similar size, green biomass was used as a covariate in the analysis of variance (MANOVA and ANOVA) when evaluating the effect of glyphosate on root colonization.

KLIMISCH EVALUATION

1. Relevance of study:		**Relevant with restrictions**
	Comment:	The influence of glyphosate on soil micorrogranism such as arbuscular mycorrhizal fungi has already been addressed in the current RAR as part of the efficacy evaluation for glyphosate.
2. Reliability of study:		**Not reliable**
	Comment:	The (commercial) formulation and source of glyphosate is not stated. The plant foliage application of glyphosate with a brush does not allow for an accurate application of the target dose.
3. Klimisch code:		**Klimisch rating of 3**

Renewal Assessment Report

18 December 2013

Revised
29 January 2015

Glyphosate

Volume 3

Annex B.4

Proposals for the classification and labelling

RMS: Germany **Co-RMS: Slovakia**

Version history (Volume 3, chapter B.4)

Date	**Reason for revision**
19 December 2013	Initial Renewal Assessment Report
29 January 2015	Updated following RAR peer review, taking into account the reporting table, the evaluation table and additional data

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B.4 Proposals for the classification and labelling

B.4.1 Proposals for the classification and labelling of the active substance (Annex IIA 9)

In accordance with Directives 67/548/EEC for glyphosate (acid) the following classification / labelling is necessary:

Xi - Irritant
R41 - Risk of serious damage to eyes

According to the criteria given in Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008, the following classification / labelling for glyphosate (acid) is necessary:

Serious eye damage, cat. 1
H318 - Causes serious eye damage
H411 - Toxic to aquatic life with long lasting effects
Aquatic chronic 2
GHS09
P273
P391
P501

B.4.2 Proposals for the classification and labelling of preparations (Annex IIIA 11.3 and 11.4)

For the preparation MON 52276 the following classification / labelling is proposed in accordance with Directives 67/548/EEC and 1999/45/EC:

None

According to the criteria given in Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008, the following classification / labelling for toxicological hazards of the preparation is proposed:

~~None~~
H411 - Toxic to aquatic life with long lasting effects
Aquatic chronic 2
GHS09
P273
P391
P501

‘15.8 percent of the mixture consist of ingredients of unknown inhalation toxicity.’

B.4.3 References relied on

No references submitted.

Renewal Assessment Report

18 December 2013

Revised
29 January 2015

Glyphosate

Volume 3

Annex B.5

Methods of analysis

RMS: Germany **Co-RMS: Slovakia**

Version history (Volume 3, chapter B.5)

Date	**Reason for revision**
19 December 2013	Initial Renewal Assessment Report
29 January 2015	Updated following RAR peer review, taking into account the reporting table, the evaluation table and additional data

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B.5 Methods of analysis

B.5.1 Analytical methods for formulation analysis (Annex IIA 4.2; Annex IIIA 5.2)

B.5.1.1 Methods for the determination of pure active substance in the active substance as manufactured (Annex IIA 4.2.1)

The applicability of the CIPAC method was demonstrated by some of the applicants. However, the CIPAC method was the basis for most of the submitted analytical methods. Amendments were made where appropriate.

B.5.1.1.1 Industrias Afrasa, S.A.

Reference:
Norris (2012), Validation of the method of determination of active substance and specified impurities in glyphosate technical material, report no. DNA1301, INA (BVL no 2317380)

Principle of the method:
After homogenisation, the technical material is dissolved in deionised water. Glyphosate is determined by reverse phase high performance liquid chromatography (HPLC). Quantification is made by comparison of the peak area with an external standard.

Column: Phenomenex SAX, 250 mm x 4.6 mm
Mobile phase: 0.8437 g of monopotassium phosphate in 960 mL water + 40 mL methanol pH adjusted to 2.0 with phosphoric acid
Detector wavelength: 195 nm

Findings:

Table B.5.1-1: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 6)**	**interference**
Glyphosate (HPLC)	0.0005 mg/mL – 2.0 mg/mL 0.9999	0.05	None

The specificity of the method was demonstrated by retention time match and by comparison of the UV-spectra with reference standard.

Conclusion:
The method is acceptably validated and allows the determination of glyphosate in the technical material.

B.5.1.1.2 Agria SA

Reference:
Ravi (2007), Studies on the chemical composition of five batches of glyphosate technical, report no. 05197, IMA (BVL no 2317477)

Principle of the method:
After homogenisation, the technical material is dissolved using mobile phase. Glyphosate is determined by reverse phase HPLC/UV. Quantification is made by external standard calibration.

Column: Stainless steel Whatman Partisil 10 SAX, 250 mm x 4.6 mm
Mobile phase: 0.8437 g of KH_2PO_4 in 960 mL water + 40 mL methanol pH adjusted to 1.9 with orthophosphoric acid
Detector wavelength: 195 nm

Findings:

Table B.5.1-2: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 6)**	**interference**
Glyphosate (HPLC)	3.2 mg/L – 4.8 mg/L 0.9957	0.080	None

The specificity of the method was demonstrated by retention time match and by comparison of the UV spectra with reference standard.

Conclusion:
The method is acceptably validated and allows the determination of glyphosate in the technical material.

B.5.1.1.3 Agrichem B.V.

References:
Norris (2012), Validation of the Methods of Analysis used for Glyphosate and Specified Impurities, report no. DNA0686, PUJ (BVL no 2315362)

Norris (2012), Validation of the Methods of Analysis used for Glyphosate and Specified Impurities (1st addendum), report no. DNA0686, PUJ (BVL no 2315364)

Principle of the method:
After homogenisation, the technical material is dissolved in water. Glyphosate is determined by reverse phase HPLC/UV. Quantification is made by external standard calibration.

Column: Phenomenex Sphere Clone 5µm, 250 mm x 4.6 mm
Mobile phase: 96 : 4 (v/v) 0.005 M KH_2PO_4 in water/ methanol, adjusted to pH 2.3
Detector wavelength: 195 nm

Findings:

Table B.5.1-3: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 6)**	**interference**
Glyphosate (HPLC)	0.1 mg/mL – 2.0 mg/mL 0.9999	0.3	None

The specificity of the method was demonstrated by retention time match and by comparison of the UV spectra with reference standard.

Conclusion:
The method is acceptably validated and allows the determination of glyphosate in the technical material.

CIPAC method:
Reference:
Russchen (2007), Validation of the HPLC-method to determine the content of glyphosate in glyphosate technical, report no. 070201.00, AGC (BVL no 2315366)

The method to determine glyphosate is based on CIPAC HANDBOOK, Analysis of Technical and Formulated Pesticides, volume H (1998) p. 182, 284/SL(M)/-.
No interferences were observed and the applicability of the method was demonstrated. Additional validation data are not required.

B.5.1.1.4 AGRO TRADE GmbH

Reference:
Yusuf Vohra (2009), Preliminary analyses of five representative production batches of glyphosate acid technical grade active ingredient (TGAI) to determine % glyphosate and to quantify its associated impurities (Volume I and II), report no. 8880, AGT (BVL no 2317346)

Principle of the method:
After homogenisation, the technical material is dissolved using mobile phase. Glyphosate is determined by reverse phase HPLC/UV. Quantification is made by external standard calibration.

Column: Inertsil, C-8, 250 mm x 4.6 mm, 5 µm
Mobile phase: 0.1 % H_3PO_4 in water
Detector wavelength: 195 nm

Findings:

Table B.5.1-4: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 5)**	**interference**
Glyphosate (HPLC)	50.8 mg/L – 152.5 mg/L 0.999	0.03	None

Conclusion:
The method is acceptably validated and allows the determination of glyphosate in the technical material.

B.5.1.1.5 Albaugh UK Ltd.

References:
Diehl (2008), Glyphosate TC: Method Validation for the Analysis of Manufacturing Process Related Impurities, report no. SSL08807, ALB (BVL no 2315403)

Principle of the method:
The technical material is dissolved and adjusted to pH 9.5 using aqueous isopropylamine solution of 2 mol/L and D_2O for NMR-lock. Glyphosate is quantified by ^{31}P-NMR using phosphonoacetic acid as internal standard.

Findings:

Table B.5.1-5: Validation data for the determination of glyphosate in the technical material

	linearity (linear between) *, Corr. Coeff.	**precision - repeatability (%RSD) (n = 5)**	**interference**
Glyphosate	0.64 – 1.88 0.9993	0.30	None

* The quotient of the integrated signals of the analyte and the internal standard was plotted against the quotient of the mass ratio of the analyte and the internal standard. The linearity ranges refers to the mass ratio between analyte and internal standard. Approximately 80 mg of the internal standard was used for the linearity test.

The specificity of the method was established by chemical shift at pH 9.5.

Conclusion:
The method is acceptably validated and allows the determination of glyphosate in the technical material.

B.5.1.1.6 Arysta LifeScience

Reference:
Hubbard (2009), Analysis of 5 batches of technical glyphosate for the active ingredient and relevant impurities with associated validation, report no. OA01682, AAP (BVL no 2317612)

Principle of the method:
The glyphosate technical was analysed according to the AOAC-CIPAC Provisional Method 1983, HPLC/UV method. After homogenisation, the technical material is dissolved using mobile phase. Glyphosate is determined by HPLC/UV using external standard calibration.

Column: SAX, 250 mm x 4.6 mm
Mobile phase: 96 : 4 (v/v) 6.2 mM phosphate buffer/ methanol, adjusted with phosphoric acid (~ pH 1.9)
Detector wavelength: 195 nm

The specificity of the method was demonstrated, interferences with impurities were not observed. As CIPAC methods are ring validated no validation studies have been conducted.

Conclusion:
The method allows the determination of glyphosate in the technical material.

B.5.1.1.7 Barclay Chemicals (R&D) Ltd.)

Source 1: (for details see Volume 4)
Reference:
Norris (2009), Validation of the method of analysis used for glyphosate and specified impurities, report no. DNA 0775, JGH (BVL no 2315439)

Principle of the method:
After homogenisation, the technical material is dissolved in methanol. Glyphosate is determined by HPLC-DAD using external standard calibration.

Column: Capital Analytical SAX-SL-5-3633, 250 mm x 4.6 mm, 5 µm
Mobile phase: 95 : 5 (v/v) 0.005 M KH_2PO_4 in water/ methanol, adjusted pH 2.5
Detector wavelength: 195 nm

Findings:

Table B.5.1-6: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 6)**	**interference**
Glyphosate (HPLC)	0.2 mg/mL – 2.0 mg/mL 0.9999	0.191	None

The specificity of the method was demonstrated by retention time match and by comparison with reference standard.

Conclusion:

The method is acceptably validated and allows the determination of glyphosate in the technical material.

Source 2: (for details see Volume 4)

References:

Fox (2005), Validation of a HPLC method for the Quantitative analysis of impurities in glyphosate technical acid and 5-batch analysis of glyphosate technical acid for active ingredient, material insoluble in NaOH and moisture content, report no. BAR-001, BCL (BVL no 2315440)

Norris (2006), Analysis of a sample of glyphosate technical material, to show the method specificity, report no. DNA0055, BCL (BVL no 2315446)

Principle of the method:

CIPAC method 284/TC/(M) is used for the determination of glyphosate. The technical material is dissolved in phosphate buffer mobile phase. Glyphosate is determined by HPLC-DAD using external standard calibration.

Column:	Partisil 10 SAX, 250 mm x 4.6 mm
Mobile phase:	0.8437 g of monopotassium phosphate in 960 mL water + 40 mL methanol pH adjusted to 1.9 with 85 % phosphoric acid
Detector wavelength:	195 nm

As CIPAC methods are ring validated no validation studies have been conducted. The specifity of the method was demonstrated.

Conclusion:

The method is acceptably validated and allows the determination of glyphosate in the technical material.

B.5.1.1.8 Brokden S.L.

Reference:

Wasser (2008), Purity profile for 5 batches of glyphosate technical, report no. A7190, BRK (BVL no 2317738)

Principle of the method:

The method used is based on CIPAC method 284/TC/M/3.

After homogenisation, the technical material is dissolved using mobile phase. Glyphosate is determined by reverse phase HPLC/UV. Quantification is made by external standard calibration.

Column: Hamilton PRPX-100, 10 µm, 250 mm x 4.1 mm
Mobile phase: 0.8437 g of KH_2PO_4 in 960 mL purified water + 40 mL methanol, adjusted to pH 1.9 with 85 % phosphoric acid
Detector wavelength: 195 nm

Findings:

Table B.5.1-7: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 5)**	**interference**
Glyphosate (HPLC)	3.3 g/L – 5.0 g/L 0.99873	0.07	None

The specificity of the method was demonstrated by retention time match and by comparison of the UV spectra with reference standard.

Conclusion:
The method is acceptably validated and allows the determination of glyphosate in the technical material.

B.5.1.1.9 Bros Spolka Jawna B.P. Miranowscy

Reference:
Hongxia Li (2010), Preliminary analysis and enforcement analytical method of glyphosate TGAI, report no. NC-2009-019, [redacted] (BVL no 2317822)

Principle of the method:
After homogenisation, the technical material is dissolved in water. Glyphosate is determined by reverse phase HPLC-DAD. Quantification is made by external standard calibration.

Column: Agilent Zorbax SAX, 5 µm, 250 mm x 4.6 mm
Mobile phase: water, pH adjusted to 1.5 – 1.8 by H_3PO_4
Detector wavelength: 195 nm

Findings:

Table B.5.1-8: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 6)**	**interference**
Glyphosate (HPLC)	3.8 g/L – 6.1 g/L 0.9998	1.0	None

The active substance was identified using HPLC-DAD, HPLC-MS and NMR against the glyphosate analytical standard.

Conclusion:
The method is acceptably validated and allows the determination of glyphosate in the technical material.

B.5.1.1.10 Cheminova A/S

References:
Pedersen (2002), Validation of Analytical Method VAM 002-04 for determination of Glyphosate (CAS No. 1071-83-6) in Glyphosate Technical, Glyphosate Concentrate and Glyphosate Formulations, report no. VAL 002-04 , CHE (BVL no 2315500)

Pedersen (2004), Analytical Method VAM 002-04: Determination of Glyphosate (CAS No. 1071-83-6) in Glyphosate Technical, Glyphosate Concentrate and Glyphosate SL and SG Formulations, report no. VAM 002-04 version 4, CHE (BVL no 2315498)

Hinz (2009), Analytical Method VAM 002-04: Determination of Glyphosate (CAS No. 1071-83-6) in Glyphosate Technical, Glyphosate Concentrate and Glyphosate SL and SG Formulations, report no. VAM 002-04 version 5, CHE (BVL no 2315506)

Principle of the method VAM 002-04:
After homogenisation, the technical material is dissolved in Milli-Q water. Glyphosate is determined by HPLC-UV using external standard calibration.

Column: Hamilton PRP-X100, 10 µm, 150 mm x 4.1 mm
Mobile phase: Gradient:
4 mL H_3PO_4 + 40 mL methanol, diluted to 1L with Milli-Q water /
50 mL H_3PO_4 + 250 mL methanol, diluted to 1L with Milli-Q water
Detector wavelength: 195 nm

Findings:

Table B.5.1-9: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD)**	**interference**
Glyphosate (HPLC)	0.26 % - 0.74 % 0.9999	0.3 – 1.1 (5 batches, each n = 5)	None

The specificity of the method was demonstrated by retention time match and by comparison of the UV-spectra with reference standard.

Conclusion:
The method is acceptably validated and allows the determination of glyphosate in the technical material.

Studies which did not meet the requirements or are not relevant for evaluation:
Pedersen (2004), Amendment 1 to : Validation of Analytical Method VAM 002-04 for Determination of Glyphosate (CAS No. 1071-83-6) in Glyphosate Technical, Glyphosate Concentrate and Glyphosate Formulations, report no. VAL 002-04 Amdt No. 1, CHE (BVL no 2315502)
Justification: The amendment is related to formulations.

Pedersen (2004), Amendment 2 to : Validation of Analytical Method VAM 002-04 for Determination of Glyphosate (CAS No. 1071-83-6) in Glyphosate Technical, Glyphosate Concentrate and Glyphosate Formulations, report no. VAL 002-04 Amdt No. 2, CHE (BVL no 2315504)
Justification: The amendment is related to formulations.

B.5.1.1.11 Dow Agro Sciences

References:
Nelson (2008), Analysis of product samples for active ingredient and impurities in technical grade of glyphosate, report no. FOR-07-044, DOW (BVL no. 2431693)

Nelson (2006), Analysis of product samples for active ingredient and impurities in technical grade of glyphosate (GF-1548), report no. FOR04019, DOW (BVL no. 2431695)

Nelson (2008), Analysis of product samples for active ingredient and impurities in technical grade of glyphosate, report no. FOR-07-062, DOW (BVL no. 2431697)

Principle of the method:
AOAC method 983.10 is used for the determination of glyphosate in the technical material. After homogenisation, the technical material is dissolved in mobile phase. Glyphosate is determined by reverse phase HPLC/UV using external standard calibration.

Column: Whatman Partisil SAX, 10 µm, 250 mm x 4.6 mm
Mobile phase: 4 : 96 (v/v) methanol/ phosphate buffer, pH ~ 1.9
Detector wavelength: 195 nm

Conclusion:
The specificity of the method was demonstrated. No further validation data are required for the AOAC method. The method is acceptably validated and allows the determination of glyphosate in the technical material.

B.5.1.1.12 Excel Crop Care (Europe) N.V

Reference:
Comb (2005), Glyphosate technical - Five-batch analysis - Method validation, report no. NUF/083, NUF (BVL no 2315594)

Principle of the method:
Glyphosat was assayed by CIPAC validated method 284/TC/(M).
After homogenisation, the technical material is dissolved using mobile phase. Glyphosate is determined by reverse phase HPLC/UV. Quantification is made by external standard calibration.

Column: Partisil 10 SAX, 250 mm x 4.6 mm
Mobile phase: 0.84 g of KH_2PO_4 in 960 mL water + 40 mL CH_3OH
pH adjusted to 1.9 with H_3PO_4
Detector wavelength: 195 nm

Findings:

Table B.5.1-10: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 10)**	**interference**
Glyphosate (HPLC)	1.2 g/L – 6 g/L 1.0000	0.36	None

The specificity of the method was demonstrated by retention time match and by comparison of the UV spectra with reference standard.

Conclusion:
The applicability of the CIPAC method was demonstrated. No further validation data are required. The method allows the determination of glyphosate in the technical material.

B.5.1.1.13 Feinchemie Schwebda GmbH

Reference:
Denny (2010), Purity profile of 5 batches of glyphosate technical, report no. A9033, MAH (BVL no. 2320229)

Principle of the method (CIPAC Method 284/TC/M):
After homogenisation, the technical material is dissolved using mobile phase. Glyphosate is determined by reverse phase HPLC/UV. Quantification is made by external standard calibration.

Column: Hamilton PRPX-100, 10 µm, 250 mm x 4.6 mm
Mobile phase: 0.8437 g of KH_2PO_4 in 960 mL H_2O + 40 mL CH_3OH adjusted to pH 1.9 with 85 % H_3PO_4
Detector wavelength: 195 nm

Findings:

Table B.5.1-11: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 5)**	**interference**
Glyphosate (HPLC)	3.1 g/L – 4.7 g/L 0.9987	0.15	None

Conclusion:
The method is acceptably validated and allows the determination of glyphosate in the technical material.

B.5.1.1.14 Helm AG

<u>Source 1: (for details see Volume 4)</u>
Reference:
Norris (2009), Validation of the methods of analysis used for glyphosate and specified impurities, in compliance with Good Laboratory Practice, report no. DNA0775, HEL (BVL no 2315649)

Principle of the method:
After homogenisation, the technical material is dissolved in methanol. Glyphosate is determined by reverse phase HPLC/UV. Quantification is made by external standard calibration.

Column: Capital Analytical SAX-SL-5-36333, 5 µm, 250 mm x 4.6 mm
Mobile phase: 5 : 95 (v/v) CH_3OH / 0.005 M KH_2PO_4 in H_2O, adjusted to pH 2.5
Detector wavelength: 195 nm

Findings:

Table B.5.1-12: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 6)**	**interference**
Glyphosate (HPLC)	0.25 mg/mL – 2.0 mg/mL 0.9999	0.2	None

The specificity of the method was demonstrated by retention time match and by comparison of the UV spectra with reference standard.

Conclusion:
The method is acceptably validated and allows the determination of glyphosate in the technical material.

Source 2: (for details see Volume 4)
Reference:
Clipston (2010), 5-Batch analysis of Glyphosate acid TGAI in accordance with EC Commission Directive 94/37/EC Article 1.9 to 1.11 (amending EEC Council Directive 91/414/EEC), report no. EPP00009, HEL (BVL no 2320514)

Principle of the method:
CIPAC method 284 is used for the determination of glyphosate. The technical material is dissolved in the mobile phase and analysed by HPLC/UV with external standard calibration.

Column: Hichrom Partisil 5 SAX, 250 mm x 4.6 mm, 5 µm
Mobile phase: 96:4 (v/v) HPLC grade H_2O with KH_2PO_4 (0.879 g/L) / CH_3OH, adjusted to pH 2.1 using H_3PO_4
Detector wavelength: 195 nm

Findings:

Table B.5.1-13: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 6)**	**interference**
Glyphosate (HPLC)	Not determined	0.7	None

Conclusion:
As there is no interference, no validation data are required for CIPAC method 284. The method is acceptably validated and allows the determination of glyphosate in the technical material.

Source 3: (for details see Volume 4)
Reference:
Sardinha (2008), Qualitative and Quantitative Profile of the test substance GLYPHOSATE TECHNICAL (Five Batch Analysis), report no. 3996.030.288.07, HEL (BVL no 2320537)

Principle of the method:
The technical material is dissolved in water and analysed by HPLC-UV with external standard calibration.

Column: PRP – X 100, 10 µm, 250 mm x 4.1 mm
Mobile phase: 960 : 40 : 0.844 (v/v/w) H_2O / CH_3OH / KH_2PO_4, adjusted to pH 2.1 with H_3PO_4
Detector wavelength: 195 nm

Findings:

Table B.5.1-14: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 10)**	**interference**
Glyphosate (HPLC)	1 g/L – 4 g/L 1.0000	0.13	None

Conclusion:
The method is acceptably validated and allows the determination of glyphosate in the technical material.

<u>Source 4: (for details see Volume 4)</u>
Reference:
Zepperitz (2011), Glyphosate 95 % TC: Quantification and Identification of the Active Ingredient and Impurities in Five Batches by Validated Methods, report no. C86955, HEL (BVL no 2320584)

Principle of the method:
After homogenisation, the technical material is dissolved using mobile phase. Glyphosate is determined by reverse phase HPLC/UV with an external standard calibration.

Column: Exsil 80 NH2, 5 µm, 250 mm x 4.6 mm
Mobile phase: Phosphate buffer (5.04 g KH_2PO_4 + 3.84 g H_3PO_4 / 2 L water, pH 1.91)
Detector wavelength: 196 nm

Findings:

Table B.5.1-15: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 10)**	**interference**
Glyphosate (HPLC)	0.5 g/L – 1.4 g/L 0.9996	0.92	None

Conclusion:
The method is acceptably validated and allows the determination of glyphosate in the technical material.

Source 5: (for details see Volume 4)

Reference:

Skopec (2009), Determination of Active Content and Impurity Profile of Glyphosate, report no. RS-001, HEL (BVL no 2320625)

Principle of the method:

The technical material is dissolved in a phosphate buffer solution (HPLC mobile phase) and analysed by HPLC/UV with external standard calibration.

Column: Exsil™ Amino column, 250 mm x 4.6 mm, 5 µm
Mobile phase: Phosphate buffer, KH_2PO_4 4.0 g/L / H_3PO_4 3.0 g/L
Detector wavelength: 196 nm

Findings:

Table B.5.1-16: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 7)**	**interference**
Glyphosate (HPLC)	1.4 g/L – 2.6 g/L 0.9996	0.20	None

Conclusion:

The method is acceptably validated and allows the determination of glyphosate in the technical material.

Source 6: (for details see Volume 4)

Reference:

Skopec (2009), Determination of Active Content and Impurity Profile of Glyphosate, report no. OS-012, HEL (BVL no 2320658)

Principle of the method:

The technical material is dissolved in the mobile phase and analysed by HPLC/UV with external standard calibration.

Column: Exsil™ Amino column, 250 mm x 4.6 mm, 5 µm
Mobile phase: Phosphate buffer, KH_2PO_4 4.0 g/L / H_3PO_4 3.0 g/L
Detector wavelength: 196 nm

Findings:

Table B.5.1-17: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 7)**	**interference**
Glyphosate (HPLC)	1.4 g/L – 2.6 g/L 0.9991	0.2	None

Conclusion:
The method is acceptably validated and allows the determination of glyphosate in the technical material.

<u>Source 7: (for details see Volume 4)</u>
Reference:
Bockholt (2010), Glyphosate TC-Analytical Profile of 5 Batches, report no. SSL04409, HEL (BVL no 2434971)

Principle of the method:
CIPAC method 284 is used for the determination of glyphosate. The technical material is dissolved in the mobile phase and analysed by HPLC/UV with external standard calibration.

Column: Reprosil 80, 10 µm, 250 mm x 4.6 mm
Mobile phase: 0.8437 g of KH_2PO_4 in 960 mL H_2O + 40 mL CH_3OH, adjusted to pH 1.9 with H_3PO_4
Detector wavelength: 195 nm

Conclusion:
As there is no interference, no validation data are required for CIPAC method 284. The method is acceptably validated and allows the determination of glyphosate in the technical material.

Reference:
Diehl (2010), Glyphosate TC - Method Validation for the Analysis of Manufacturing Process Related Impurities, report no. SSL04309, HEL (BVL no 2315697)

Principle of the method:
The technical material and phosphonoacetic acid as internal standard are dissolved in D_2O and adjusted to pH 9.0 - 9.1 with 4 mol/L aqueous isopropylamine solution. Glyphosate is quantified by ^{31}P-NMR.

Findings:

Table B.5.1-18: Validation data for the determination of glyphosate in the technical material

	linearity (linear between)*, Corr. Coeff.	**precision - repeatability (%RSD) (n = 5)**	**interference**
Glyphosate	0.62 – 1.87 1.0000	0.13	None

* The quotient of the integrated signals of the analyte and the internal standard was plotted against the quotient of the mass ratio of the analyte and the internal standard. The linearity range refers to the mass ratio between analyte and internal standard. Approximately 80 mg of the internal standard was used for the linearity test.

The specificity of the method was established by chemical shift at pH 9.1.

Conclusion:
The method is acceptably validated and allows the determination of glyphosate in the technical material.

B.5.1.1.15 Monsanto

A CIPAC method for the determination of glyphosate in technical material is available. The applicability has been demonstrated in the study of Overton and Pelezo, 1987 (CHE9600567).

B.5.1.1.16 Nufarm GmbH & Co. KG

Reference:
Diehl (2010), Glyphosate TC - Method Validation for the Analysis of Manufacturing Process Related Impurities, report no. SSL04309, NUF (BVL no 2315730)

Principle of the method:
The technical material and phosphonoacetic acid as internal standard are dissolved in D_2O and adjusted to pH 9.0 - 9.1 with 4 mol/L aqueous isopropylamine solution. Glyphosate is quantified by ^{31}P-NMR.

Findings:

Table B.5.1-19: Validation data for the determination of glyphosate in the technical material

	linearity (linear between)*, Corr. Coeff.	**precision - repeatability (%RSD) (n = 5)**	**interference**
Glyphosate	0.622 – 1.874 1.0000	0.13	None

* The quotient of the integrated signals of the analyte and the internal standard was plotted against the quotient of the mass ratio of the analyte and the internal standard. The linearity ranges refer to the mass ratio between analyte and internal standard. Approximately 80 mg of the internal standard was used for the linearity test.

The specificity of the method was established by chemical shift at pH 9.1.

Conclusion:
The method is acceptably validated and allows the determination of glyphosate in the technical material.

B.5.1.1.17 Rotam Agrochemical Europe Ltd.

Reference:
Desai, H (2008) Preliminary Analysis of Five Representative Production Batches of Glyphosate Technical Grade Active Ingredient (TGAI) to Determine % Glyphosate and to Quantify Its Associated Impurities, report no. 8247, RTA (BVL no 2435558)

Principle of the method:
After homogenisation, the technical material is dissolved using mobile phase. Glyphosate is determined by HPLC-UV using external standard calibration.

Column: Inertsil C-8, 5 µm, 250 mm x 4.6 mm
Mobile phase: 0.1 % H_3PO_4
Detector wavelength: 195 nm

Findings:

Table B.5.1-20: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 10)**	**interference**
Glyphosate (HPLC)	0.05 mg/mL – 0.15 mg/mL 0.9977	0.03	None

Conclusion:
The method is acceptably validated and allows the determination of glyphosate in the technical material.

B.5.1.1.18 Sabero Europe B.V.

Reference:
Mcdonald and Craig (2002), Glyphosate TGAI/MP: Analysis and certification of Limits for Glyphosate TGAI/MP: Selected studies to fulfill OPPTS Guidelines 830.1700, 830.1750 and 830.1600 in Accordance with 40 CFR part 158 and EEC Directive 94/37/EEC, report no. 20733, SAO (BVL no 2321334)

Principle of the method LCG01 (CIPAC Method 284/TC/M):
CIPAC method 284 is used for the determination of glyphosate. The technical material is dissolved in the mobile phase and analysed by HPLC/UV with external standard calibration.

Column: Partisil 10 SAX, 250 mm x 4.6 mm
Mobile phase: 0.8437 g KH_2PO_4 in 960 mL H_2O + 40 mL methanol, adjusted to pH 1.9 with 85 % H_3PO_4
Detector wavelength: 195 nm

Findings:

Table B.5.1-21: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 6)**	**interference**
Glyphosate (HPLC)	2 mg/mL – 6 mg/mL 0.9999	0.2	None

Conclusion:
As there is no interference, no validation data are required for CIPAC method 284. The method is acceptably validated and allows the determination of glyphosate in the technical material.

B.5.1.1.19 SAPEC Agro S.A.

References:
Silva (2011), Analytical profile of glyphosate, report no. AI/61/10 Final Rep., SAP (BVL no 2326020)

Silva (2011), Analytical profile of glyphosate (Annexes), report no. AI/61/10 Final Rep., SAP (BVL no 2326001)

Pinto (2008), Glyphosate Analytical Profile, report no. AI/21/08 Final report, SAP (BVL no 2321404)

Principle of the method (CIPAC Method 284):
Glyphosat is analysed using the CIPAC method 284/TC/(M).
After homogenisation, the technical material is dissolved using mobile phase. Glyphosate is determined by reverse phase HPLC/UV using external standard calibration.

Column: Whatman Partisil 10 SAX, 250 mm x 4.6 mm
Mobile phase: 0.8437 g of KH_2PO_4 in 960 mL water + 40 mL CH_3OH adjusted to pH 1.9 with 85 % H_3PO_4
Detector wavelength: 195 nm

Conclusion:
The method for the determination of glyphosate in the technical material is based on CIPAC HANDBOOK, Analysis of Technical and Formulated Pesticides, volume H (1998) p. 182, 284/SL(M)/-.
No interferences were observed and the applicability of the method was demonstrated. Additional validation data are not required.

B.5.1.1.20 Société Financière de Pontarlier

References:
Shen Lu (2007), Preliminary analysis and enforcement analytical method of glyphosate TGAI - Validation of analytical methodology for the assay of active ingredient and related significant impurities and subsequent 5-Batch analysis of glyphosate TGAI, report no. NC-2007-012, SFP (BVL no 2322253)

Huazhen Wen (2009), Spectroscopy complementary data regarding the methods of analysis used in the study NC-2007-012, report no. NC-2007-012, SFP (BVL no 2315820)

Principle of the method NC2007012A:
After homogenisation, the technical material is dissolved in water. Glyphosate is determined by reverse phase HPLC/UV. Quantification is made by external standard calibration.

Column: Agilent Zorbax SAX, 5 µm, 250 mm x 4.6 mm
Mobile phase: H_2O, adjusted to pH 1.8 with H_3PO_4
Detector wavelength: 195 nm

Findings:

Table B.5.1-22: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 6)**	**interference**
Glyphosate (HPLC)	3.4 g/L – 5.9 g/L 1.0000	0.6	None

The specificity of the method was demonstrated by retention time match and by comparison of UV and ^{1}H-NMR spectra with reference standard.

Conclusion:
The method is acceptably validated and allows the determination of glyphosate in the technical material.

B.5.1.1.21 Sinon Corporation

Reference:
~~Wang (2004), Five Batches Analysis of Technical Grade Active Ingredient (TGAI) Glyphosate Acid, CB01-01, SNN (BVL no 2322339)~~
Shyh-Shyan Jwo (2014), The five batches analysis of technical grade active ingredient (TGAI) glyphosate acid, M302, SNN (BVL no 2717921)

Principle of the method LCG01 (CIPAC Method 284):
CIPAC method 284 is used for the determination of glyphosate. The technical material is dissolved in the mobile phase and analysed by HPLC/UV with external standard calibration.

Column: 10 SB (SAX), 250 mm x 4.6 mm
Mobile phase: 0.8437 g of KH_2PO_4 in 960 mL H_2O + 40 mL CH_3OH adjusted to pH 1.9 with H_3PO_4
Detector wavelength: 195 nm

Findings:

Table B.5.1-23: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 6)**	**interference**
Glyphosate (HPLC)	202 mg/L – 1009 mg/L 1.0000 ~~1.0 g/L – 4.8 g/L~~ ~~1.0000~~	0.31 ~~0.3~~ ~~only determined by repeated injection of a standard solution~~	None

Conclusion:
As there is no interference, no validation data are required for CIPAC method 284. The method is acceptably validated and allows the determination of glyphosate in the technical material.

B.5.1.1.22 Syngenta

References:
Clarke, Massey (2002), AMP10089-03B - The Determination of Glyphosate and associated Impurities in technical Material by Ion Chromatography, report no. R61837/0272, SYD (BVL no 2315873)

Clarke (2002), Method Validation: AMP10089-03B 'The Determination of Glyphosate and Associated Impurities in Technical Material by Ion Chromatography', report no. R61837/0273/ RJ3310B, SYD (BVL no 2415613)

Principle of the method AMP10089-03B:
After homogenisation, the technical material is dissolved in ASTM Type 1 water. Glyphosate is determined by ion exchange chromatography with conductivity detection. Quantification is made by external standard calibration.

Column: IonPac AS11-HC, 250 mm x 4.0 mm (analytical column)
Mobile phase: Sodium hydroxide gradient (i.e. eluents of 100 mM sodium hydroxide and ASTM Type 1 water)

Findings:

Table B.5.1-24: Validation data for the determination of glyphosate in the technical material

	linearity (linear between), Corr. Coeff.	**precision - repeatability (%RSD) (n = 14)**	**interference**
Glyphosate	0.62 mg/mL – 0.78 mg/mL 0.9999	0.60 0.39	None

The specificity of the method was demonstrated by comparison of MS-spectra with reference standard.

Conclusion:
Clarification concerning the linearity data is needed. It seems that the given data and the presented graph do not correspond. Furthermore, it seems that the number of used levels is not in compliance with SANCO/3030/2003.
Without this clarification a comprehensive assessment of the method is not possible.

B.5.1.1.23 United Phosphorus Ltd./Cerexagri s.a.s

Reference:
Doyle (2009), 5 Batch Analysis of Glyphosate Technical Material for significant impurities, known impurities and materials insoluble in NaOH, including amendment, report no. UPL-007, UPL (BVL no 2325565)

Principle of the method (CIPAC 284 Handbook C, pp. 2132):
After homogenisation, the technical material is dissolved using mobile phase. Glyphosate is determined by reverse phase HPLC/UV. Quantification is made by external standard calibration.

Column: Stainless steel packed with a strong ion exchange resin e.g. Partisil 10 SAX as supplied by Whatman Inc. or equivalent, 250 mm x 4.6 mm
Mobile phase: 0.8437 g of KH_2PO_4 in 960 mL water + 40 mL methanol pH adjusted to 1.9 with phosphoric acid
Detector wavelength: 195 nm

As CIPAC methods are ring validated no validation studies have been conducted.

Conclusion:
The applicability of the CIPAC method has been demonstrated by providing chromatograms.

B.5.1.1.24 Wynca UK Limited

Reference:
Bockholt (2010), Glyphosate TC-Analytical Profile of 5 Batches, SSL04409, ZXC (BVL no 2404947)

Principle of the method:
CIPAC method 284 is used for the determination of glyphosate. The technical material is dissolved in the mobile phase and analysed by HPLC/UV with external standard calibration.

Column: Reprosil 80 10 µm, 250 mm x 4.6 mm
Mobile phase: 0.8437 g of KH_2PO_4 in 960 mL water + 40 mL methanol pH adjusted to 1.9 with phosphoric acid
Detector wavelength: 195 nm

Conclusion:
As there is no interference, no validation data are required for CIPAC method 284. The method is acceptably validated and allows the determination of glyphosate in the technical material.

Reference:
Diehl (2010), Glyphosate TC: Method Validation for the Analysis of Manufacturing Process Related Impurities, SSL04309, ZXC (BVL no 2315935)

Principle of the method:
The technical material and phosphonoacetic acid as internal standard are dissolved in D_2O and adjusted to pH 9.0 - 9.1 with 4 mol/L aqueous isopropylamine solution. Glyphosate is quantified by ^{31}P-NMR.

Findings:

Table B.5.1-25: Validation data for the determination of glyphosate in the technical material

	linearity (linear between)*, Corr. Coeff.	**precision - repeatability (%RSD) (n = 5)**	**interference**
Glyphosate	0.62 – 1.87 1.0000	0.13	None

* The quotient of the integrated signals of the analyte and the internal standard was plotted against the quotient of the mass ratio of the analyte and the internal standard. The linearity ranges refers to the mass ratio between analyte and internal standard. Approximately 80 mg of the internal standard was used for the linearity test.

The specificity of the method was established by chemical shift at pH 9.1.

Conclusion:
The method is acceptably validated and allows the determination of glyphosate in the technical material.

B.5.1.2 Methods of analysis for the determination of relevant impurities (Annex IIA 4.2.3)

It should be noted that the current implementing regulation (EU) No 540/2011 stipulate none of the impurity as relevant impurity. However, the FAO specification 284/TC (2000/2001), applicable only for certain manufactures, mentions formaldehyde (maximum content of 1.3 g/kg of the glyphosate content) and *N*-nitrosoglyphosate (max. content 1 mg/kg).

The outcome of the assessment of the RMS in the context of the renewal procedure revealed that the impurities formaldehyde and *N*-nitrosoglyphosate has to be regarded as relevant impurities. The maximum contents are < 1 g/kg and < 1 mg/kg, respectively.
~~Furthermore, it should be noted that the assessment concerning the possible relevance of certain impurities could not be finalised due to the fact that some toxicological data are missing (see Vol. 4).~~

Reference:
Probst (2012), NNG and formaldehyde method validations in MON 52276 and MON 77973, report no. MSL0024115, EGT (BVL no 2309030)

Determination of *N*-nitrosoglyphosate (NNG)
Principle of the method PC-ME-0766-02:
A cation exchange column is used to separate NNG from some other components present in the sample. Before NNG elutes from the clean-up column, an electric valve switches and a cut containing NNG is eluted onto the anion exchange analytical column. After NNG elutes, the valve then switches back and the clean-up column effluent goes to waste. The analytical column separates NNG from other components and the effluent is air segmented prior to addition of *N*-(1-naphthyl)ethylenediamine/ HBr and sulfanilamide reagents. The azo dye formed is detected using a colorimeter set at 550 nm and quantified by external standards.

Column: AX-300 Aquapore, 250 mm x 4 mm, 7 µm
Mobile phase: KH_2PO_4 in HPLC grade water/methanol, adjusted to pH 2.3 with 85 % H_3PO_4
Detector wavelength: 550 nm

Findings:

Table B.5.1-26: Validation data for the determination of the impurity NNG in technical glyphosate

Impurity		Specificity/ interferences	Linearity (R^2) (conc. range)	Accuracy fortification level (ppm)	recovery (%)	Repeatability (% RSD) (n = 15)
20	NNG	demonstrated[1]; no interferences	0.9985 (0.007 ppm - 0.117 ppm)	0.50 0.75	93 96	3.0

[1] The method is specific for nitroso compounds and nitrite. Nitroso groups can be cleaved via UV degradation. If the signal disappears, there are no interferences. The applicant stated that for technical material MON 77973 and the formulation MON 52276 no interferences were observed.

Conclusion:
The method is acceptably validated and allows the determination of the impurity *N*-nitroso-glyphosate (NNG) in the technical material.
The LOQ was determined to be 0.125 ppm.

Determination of formaldehyde
Principle of the method PC-ME-1137-02:
Analytes are separated on an ion exclusion column. Formaldehyde is determined by a post-column Hantzsch reaction. The column effluent is mixed with the post column reagent (PCR) containing ammonium acetate and acetyl acetone. Formaldehyde reacts to produce 3,5-diacetyl-1,4-dihydrolutidine which is then determined by UV/VIS detection at a wave-length of 420 nm.

Column: BioRad Fast Acid Analysis, 100 mm x 7.8 mm, 9 µm
Mobile phase: HPLC grade water
Detector wavelength: 420 nm

Findings:

Table B.5.1-27: Validation data for the determination of the impurity formaldehyde in technical glyphosate

Impurity		Specificity/ interferences	Linearity (R^2) (conc. range)	Accuracy fortification level (ppm)	recovery (%)	Repeatability (% RSD) (n = 15)
21	Formal-dehyde	demonstrated*; no interferences	1.0000 1.883 ppm - 173.6 ppm	52.96 53.34 61.85 58.73	110.9 109.0 110.2 114.2	2.5

* Any compound that is not separated from formaldehyde on the column and reacts with the PCR will cause a positive bias for formaldehyde. However, the post-column reaction used in this method is so selective for formaldehyde that other expected components will not affect this determination.

While not typically found at significant levels in glyphosate technical materials, methanol are known possible interferences. While not a chromatographic interference, methanol at concentrations greater than 1 % interfere with the post-column reaction in a way that adversely affects peak shape. Therefore it is important to ensure that any samples analysed contain less than 1 % methanol.

Conclusion:
The method is acceptably validated and allows the determination of the impurity formaldehyde in the technical material.
The LOQ was determined to be 20 ppm.

It should be noted that in addition to the study of Probst (2012, BVL no 2309030), submitted by the Task Force, the individual manufactures have submitted analytical methods for the determiantion of NNG and formaldehyde. These are described and assessed in Volume 4.

B.5.1.3 Analytical methods for the determination of the active substance in plant protection products (Annex IIIA 5.2.1)

Reference:
Bates (2001), Determination of glyphosate content in formulations MON 78043, MON 78044 and MON 2139 (glyphosate 360 g/L) SL by HPLC: validation of the analytical method, MSL-17401, EGT (BVL no 2315973)

~~It should be noted that the applicant has confirmed in the reporting table that the composition of the formulations MON 78043 and MON 78044 could be submitted to demonstrate the comparisation of the formulations. This would support the applicability of the analytical method.~~
The analytical method is considered as applicable to determine the active substance in MON 78043, MON 78044 and MON 52276 as the composition of the formulations is equivalent. The detailed composition of the formulations is given in Volume 4 (Monsanto).

Principle of the method (AOAC-CIPAC method 284/SL/(M)/3):
After homogenisation, the technical material is dissolved using mobile phase. Glyphosate is determined by high performance liquid chromatography (HPLC) using ion-exchange chromatography with UV detection. Quantification is made by comparison of the peak area with an external standard.

Column: Partisil 10 SAX, 250 mm x 4.6 mm
Mobile phase: water - methanol (96 - 4 v/v) containing about 0.84 g/L of KH_2PO_4 and adjusted with phosphoric acid to pH 1.9.
Detector wavelength: 195 nm

Findings:

Table B.5.1-28: Validation data for the determination of glyphosate in the plant protection product MON 52276

Specificity/ interferences	Linearity (R^2)	Accuracy (n = 4)		Repeatability (% RSD) (n = 5)
		fortification level (%)	mean recovery (%)	
demonstrated; no interferences	1.0000	30.53	100.1	0.12

If MON78043 is not identical with MON 52276 further validation data and the applicablity of the CIPAC method are necessary (see above).

The specificity of the method was demonstrated by retention time match with reference standard.

Conclusion:
The method is acceptably validated and allows the determination of glyphosate in the plant protection product.

CIPAC method:

CIPAC method 284/SL/(M)/- is suitable for the determination of glyphosate in SL formulations. Depends on the outstanding confirmation concerning the plant protection product comparability.

B.5.1.4 Analytical methods for the determination of relevant impurities and formulants in plant protection products (Annex IIIA 5.2.4)

It should be noted that the current implementing regulation (EU) No 540/2011 stipulate none of the impurity as relevant impurity. However, the FAO specification 284/TC (2000/2001), applicable only for certain manufactures, mentions formaldehyde (maximum content of 1.3 g/kg of the glyphosate content) and *N*-nitrosoglyphosate (max. content 1 mg/kg).

The outcome of the assessment of the RMS in the context of the renewal procedure revealed that the impurities formaldehyde and *N*-nitrosoglyphosate has to be regarded as relevant impurities. The maximum contents are < 1 g/kg and < 1 mg/kg, respectively.
~~Furthermore, it should be noted that the assessment concerning the possible relevance of certain impurities could not be finalised due to the fact that some toxicological data are missing (see Vol. 4).~~

Reference:
Probst (2012), NNG and formaldehyde method validations in MON 52276 and MON 77973, MSL0024115, EGT (BVL no 2315974)

Determination of *N*-nitrosoglyphosate (NNG)
Principle of the method PC-ME-0766-02:
A cation exchange column is used to separate NNG from some other components present in the sample. Before NNG elutes from the clean-up column, an electric valve switches and a cut containing NNG is eluted onto the anion exchange analytical column. After NNG elutes, the valve then switches back and the clean-up column effluent goes to waste. The analytical column separates NNG from other components and the effluent is air segmented prior to addition of *N*-(1-naphthyl)ethylenediamine/ HBr and sulfanilamide reagents. The azo dye formed is detected using a colorimeter set at 550 nm and quantified by external standards.

Column: AX-300 Aquapore, 250 mm x 4 mm, 7 µm
Mobile phase: KH_2PO_4 in HPLC grade water/methanol, adjusted to pH 2.3 with 85 % H_3PO_4
Detector wavelength: 550 nm

Findings:

Table B.5.1-29: Validation data for the determination of the impurity NNG in MON 52276

Impurity		Specificity/ interferences	Linearity (R^2) (conc. range)	Accuracy fortification level (ppm)	recovery (%)	Repeatability (% RSD) (n = 15)
20	NNG	demonstrated[1]; no interferences	0.9991 (0.007 ppm - 0.117 ppm)	0.43 0.61	95.1 93.9	1.5

[1] The method is specific for nitroso compounds and nitrite. Nitroso groups can be cleaved via UV degradation. If the signal disappears, there are no interferences. The applicant stated that for technical material MON 77973 and the formulation MON 52276 no interferences were observed.

The LOQ was determined to be 0.43 ppm.

Conclusion:
The method is acceptably validated and allows the determination of the impurity *N*-Nitroso-glyphosate (NNG) in the representative ppp.

Determination of formaldehyde
Principle of the method PC-ME-1137-02:
Analytes are separated on an ion exclusion column. Formaldehyde is determined by a post-column Hantzsch reaction. The column effluent is mixed with the post column reagent (PCR) containing ammonium acetate and acetyl acetone. Formaldehyde reacts to produce 3,5-diacetyl-1,4-dihydrolutidine which is then determined by UV/Vis detection at a wavelength of 420 nm.

Column: BioRad Fast Acid Analysis, 100 mm x 7.8 mm, 9 µm
Mobile phase: HPLC grade water
Detector wavelength: 420 nm

Findings:

Table B.5.1-30: Validation data for the determination of the impurity formaldehyde in MON 52276

Impurity		Specificity/ interferences	Linearity (R^2) (conc. range)	Accuracy fortification level (ppm)	recovery (%)	Repeatability (% RSD) (n = 15)
21	Form-aldehyde	demonstrated*; no interferences	1.0000 1.883 ppm - 173.6 ppm	155.508 156.702 307.429 314.656	84.6 88.0 90.3 90.1	2.88

* Any compound that is not separated from formaldehyde on the column and reacts with the PCR will cause a positive bias for formaldehyde. However, the post-column reaction used in this method is so selective for formaldehyde that other expected components will not affect this determination.

While not typically found at significant levels in glyphosate technical materials, methanol are known possible interferences. While not a chromatographic interference, methanol at concentrations greater than 1 % interfere with the post-column reaction in a way that adversely affects peak shape. Therefore it is important to ensure that any samples analysed contain less than 1 % methanol.

The LOQ was determined to be 20 ppm.

Conclusion:
The method is acceptably validated and allows the determination of the impurity formaldehyde in the technical material.

Determination of 1,4-dioxane

In addition to the determiantion of the relevant impurities in the ppp, an anlytical method for the 1,4-dioxane has been submitted.

Reference:

Schuette, Nord (1994), Analytical method for the determination of 1,4-dioxane in MON-0818 surfactant, other ethoxylated surfactants and glyphosate formulations, AQC-931-94, MOD (CHE9700031)

For the determination of 1,4-dioxane in MON 0818 surfactant, other ethoxylated surfactants and glyphosate formulations the principle is head space gaschromatography and detection with a flame ionisation detector (see FAO Specification, 1993).

Method validation data:

Linearity:	not submitted
Accuracy:	not submitted
RSD:	5.2 %

B.5.2 Analytical methods (residue) for plants, plant products, foodstuffs of plant and animal origin, feedingstuffs (Annex IIA 4.3; Annex IIIA 5.3.1)

B.5.2.1 Plant matrices

B.5.2.1.1 Acceptable methods/reports

B.5.2.1.1.1 Method 1

Note: Method 1 is not presented by Monsanto on behalf of the Glyphosate Task Force but required for Annex 1 inclusion.

Reference:	no (study was not provided with the dossier)
Report:	Pentz, A.M. and Bramble, F.Q. (2007) Analytical method for the determination of glyphosate and degradate residues in various crop matrices using LC/MS/MS E. I. du Pont de Nemours and company, Wilmington Delaware, USA Laboratory Project ID: DuPont-15444 Revision-1 Date 13.11.2007, not published, ASB2008-2635
Guidelines:	EPA OPPTS 860.1340, SANCO/825/00 Rev. 7
Deviations:	No
GLP:	No
Acceptability:	The study is considered to be acceptable.

Materials and Methods:

Test material: commodities with high water content (plum, corn grain, corn and soybean forage), high oil content (soybean seed, corn oil, soya oil), dry crops (soybean hay; flour, meal and starch of corn) and one acidic fruit (lime)

Fortified analyte(s): glyphosate, N-acetyl-glyphosate (sodium salt), AMPA, N-acetyl AMPA

Analyte(s) determined as:
glyphosate, N-acetyl-glyphosate, AMPA, N-acetyl AMPA

Principle of the method:

Extraction and clean-up of oils:
The samples are extracted twice by shaking with a mixture of 0.02 M phosphoric acid and dichloromethane (1:1, v/v). After centrifugation, the aqueous phase is separated and internal standards are added. This is the final extract. The sample concentration in final extracts is 0.04 g/mL.
Extraction and C18 SPE of corn flour and corn meal:
The test portion is homogenized in 0.025 N HCl/methanol (96:4, v/v). After decantation of the supernatant the extraction is repeated twice with 0.1 % aqueous formic acid/methanol (96:4, v/v). The aqueous extract is washed with dichloromethane and filtered. After addition of internal standards, the clear extract is filtered through a C18 SPE column.

Extraction and C18 SPE of all other matrices:
The test portion is homogenized in 0.1 % aqueous formic acid/methanol (96:4, v/v). After decantation of the supernatant the extraction is repeated twice. The aqueous extract is washed with dichloromethane and filtered. (Extracts of soybean seed and soybean meal have to be heated before filtration to precipitate proteins.) After addition of internal standards, the clear extract is filtered through a C18 SPE column.

Further cleanup for glyphosate, N-acetyl-glyphosate, N-acetyl-AMPA (not for oils):
An aliquot of the C18 eluate is adjusted to basic pH with concentrated ammonium hydroxide solution (or triethylamine for extracts of acidic crops, respectively) and diluted with water or methanol. The alkaline, methanolic extract is added to a strong anion exchange SPE column (Oasis MAX). After washing with three different solvent mixtures, residues of glyphosate, N-acetyl-glyphosate and N-acetyl-AMPA are eluted with 1 % trifluoroacetic acid in methanol/water (90:10, v/v). These eluates are evaporated to dryness and reconstituted in 0.02 M phosphoric acid. The sample concentration in final extracts is 0.02 g/mL.

Further cleanup for AMPA (not for oils):
An aliquot of the C18 eluate is diluted with methanol. The methanolic extract is added to a strong cation exchange SPE column (Oasis MCX). Residues of AMPA are subsequently eluted with water and methanol. The eluate is evaporated to the aqueous reminder and acidified with phosphoric acid. The sample concentration in final extracts is 0.04 g/mL.

The residues are determined by LC-MS/MS with positive electrospray ionisation (ESI+) in the selected reaction mode using a Luna Phenyl-Hexyl column. The reported results are based on calibration with an internal standard for glyphosate and AMPA. The following transitions have been used:
Glyphosate: m/z 170 → 87.7
N-acetyl-glyphosate: m/z 212 → 87.9
AMPA: m/z 111.8 → 30
N-acetyl-AMPA: m/z 154 → 30
internal standards:
1,2 $^{13}C_2$ ^{15}N-glyphosate: m/z: 173 → 90.7
$^{13}C^{15}N$-AMPA: m/z: 113.8 → 32

Findings:
The method allows the simultaneous determination of both components of the proposed residue definition for enforcement purposes in ~~lentils~~ sweet corn, oilseeds rape, soya beans and maize (glyphosate and N-acetyl-glyphosate). Additionally, it allows the determination of residues of AMPA and N-acetyl AMPA. The validated limit of quantification in all matrices (high water content, high oil content, dry and fruits with high acid content) is 0.05 mg/kg for each compound. Individual blank values are reported as raw data and were below 30 % of LOQ. This is confirmed by chromatograms of control samples. Acceptable chromatograms of calibration standards, samples and blank materials, adequate recovery data and information on the precision of the method are presented. Linear calibration is performed for all analytes in the range 0.5 – 50 ng/mL using standards in solvent at seven levels. The calibrated range corresponds to 0.025 – 2.50 mg/kg (AMPA in oils: 0.012 – 1.25 mg/kg). This is acceptable, except for highest validation levels for N-acetyl-AMPA in corn forage and stover, which are outside the calibrated range. The correlation coefficients of calibration graphs are > 0.999. Information on calibration is reported in Table B.5.2-1. For validation data see Tables B.5.2-2 to B.5.2-5.
Additional MS/MS transitions for the confirmation of results are proposed but not validated in this study.

Conclusion:
The study of Pentz and Bramble (2007, ASB2008-2635) describes an adequately validated and sensitive method for the determination of residues of glyphosate and N-acetyl-glyphosate (also applicable for AMPA and N-acetyl AMPA) in commodities with high water content, commodities with high oil content, dry crops and fruits of high acid content.

Table B.5.2-1: Calibration data of the DuPont method for residues of glyphosate, N-acetyl-glyphosate, N-acetyl AMPA and AMPA in commodities of plant origin (Pentz and Bramble, 2007, ASB2008-2635)

Calibration parameter	Results of evaluation
Accepted calibration range in concentration units	0.5 – 50 ng/mL
Accepted calibration range in mass fraction units	0.012 – 1.25 mg/kg (AMPA in oils) 0.025 – 2.50 mg/kg (all other analytes/matrices)
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)	yes
Where matrix effects investigated?	no
The absence of interference >30 % of LOQ in blank samples was demonstrated?	yes
Chromatogram of sample provided spiked at LOQ demonstrating sufficient sensitivity and signal-to-noise ratio?	yes

Table B.5.2-2: Validation of the DuPont method for glyphosate residues in commodities of plant origin (Pentz and Bramble, 2007, ASB2008-2635)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Pentz and Bramble, 2007a (ASB2008-2635)	LC-MS/MS, Phenyl-Hexyl column, m/z 170 → 87.7	plums	0.05 0.5	95 91	4 4	5 5
		limes	0.05 0.5	100 99	8 6	5 5
		corn forage	0.05 0.5	83 80	10 10	13 12
		corn grain	0.05 0.5	77 79	7 10	12 12
		corn stover	0.05 0.5	82 83	7 4	10 10
		corn oil	0.05 0.5	99 99	4 2	10 10
		corn flour	0.05 0.5	91 79	8 12	5 5
		corn grits	0.05 0.5	86 82	5 10	5 5
		corn starch	0.05 0.5	78 80	4 7	5 5
		corn meal	0.05 0.5	99 92	12 7	5 5
		soybean forage	0.05 0.5	98 94	15 5	8 7
		soybean seed	0.05 0.5	85 78	6 5	5 5
		soybean hay	0.05 0.5	94 80	9 4	5 5
		soybean oil	0.05 0.5	99 93	6 9	5 5
		soybean meal	0.05 0.5	93 79	6 2	5 5
		soybean hulls	0.05 0.5	84 75	10 3	5 5

Table B.5.2-3: Validation of the DuPont method for N-acetyl-glyphosate residues in commodities of plant origin (Pentz and Bramble, 2007, ASB2008-2635)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Pentz and Bramble, 2007a (ASB2008-2635)	LC-MS/MS, Phenyl-Hexyl column, m/z 212 → 87.9	plums	0.05 0.5	102 93	9 8	5 5
		limes	0.05 0.5	86 91	9 6	5 5
		corn forage	0.05 0.5	90 86	6 7	13 12
		corn grain	0.05 0.5	87 89	6 5	12 12
		corn stover	0.05 0.5	91 90	5 5	10 10
		corn oil	0.05 0.5	99 100	2 4	10 10
		corn flour	0.05 0.5	85 83	7 7	5 5
		corn grits	0.05 0.5	81 85	2 13	5 5
		corn starch	0.05 0.5	95 94	3 1	5 5
		corn meal	0.05 0.5	80 81	11 3	5 5
		soybean forage	0.05 0.5	91 93	8 9	8 7
		soybean seed	0.05 0.5	97 95	2 3	5 5
		soybean hay	0.05 0.5	94 86	8 2	5 5
		soybean oil	0.05 0.5	94 98	2 2	5 5
		soybean meal	0.05 0.5	89 93	8 5	5 5
		soybean hulls	0.05 0.5	99 100	5 3	5 5

Table B.5.2-4: Validation of the DuPont method for residues of AMPA in commodities of plant origin (Pentz and Bramble, 2007, ASB2008-2635)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Pentz and Bramble, 2007a (ASB2008-2635)	LC-MS/MS, Phenyl-Hexyl column, m/z 111.8 → 30	plums	0.05 0.5	95 100	8 9	5 5
		limes	0.05 0.5	95 98	7 5	5 5
		corn forage	0.05 0.5	98 91	10 4	13 12
		corn grain	0.05 0.5	109 97	9 8	12 12
		corn stover	0.05 0.5	97 90	6 9	10 10
		corn oil	0.05 0.5	102 90	19 7	10 10
		corn flour	0.05 0.5	87 76	9 4	5 5
		corn grits	0.05 0.5	90 80	3 3	5 5
		corn starch	0.05 0.5	98 92	4 2	5 5
		corn meal	0.05 0.5	99 81	15 8	5 5
		soybean forage	0.05 0.5	90 85	9 11	8 7
		soybean seed	0.05 0.5	94 78	12 5	5 5
		soybean hay	0.05 0.5	99 79	8 5	5 5
		soybean oil	0.05 0.5	107 95	9 1	5 5
		soybean meal	0.05 0.5	84 74	5 2	5 5
		soybean hulls	0.05 0.5	87 80	5 5	5 5

Table B.5.2-5: Validation of the DuPont method for residues of N-acetyl AMPA in commodities of plant origin (Pentz and Bramble, 2007, ASB2008-2635)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Pentz and Bramble, 2007a (ASB2008-2635)	LC-MS/MS, Phenyl-Hexyl column, m/z 154 → 30	plums	0.05 0.5	102 100	5 8	5 5
		limes	0.05 0.5	94 107	12 9	5 5
		corn forage	0.05 5.0	87 91	13 9	22 21
		corn grain	0.05 0.5	87 85	8 7	11 10
		corn stover	0.05 10.0	83 90	8 6	24 17
		corn oil	0.05 0.5	101 101	6 3	6 6
		soybean forage	0.05 0.5	86 85	7 6	4 10
		soybean seed	0.05 0.5	89 85	13 16	17 10
		soybean hay	0.05 0.5	75 78	7 5	23 6

B.5.2.1.1.2 Independent laboratory validation of method 1

Reference: no (study was not provided with the dossier)
Report: Seal, S., Dillon, R. (2007)
Independent laboratory validation of DuPont-15444, "Analytical method for the determination of glyphosate and relevant metabolite residues in various crop matrices using LC/MS/MS"
Pyxant Labs Inc., Colorado Springs, USA
DuPont Project ID: DuPont-21313
Date 24.04.2007, not published, ASB2008-2637
Guidelines: EPA OPPTS 860.1340, SANCO/825/00 Rev. 7
Deviations: No
GLP: Yes
Acceptability: The study is considered to be acceptable.

Materials and Methods:
Test material: commodities with high water content (grapes), high oil content (soybean seed)
Note: In SANCO/825/00 Rev. 7 grapes were considered as commodity with high water content.

Fortified analyte(s): glyphosate, N-acetyl-glyphosate (sodium salt), AMPA, N-acetyl-AMPA

Analyte(s) determined as:
Glyphosate, N-acetyl-glyphosate, AMPA, N-acetyl-AMPA

Principle of the method:

With one exception the method was identical to the method described by Pentz and Bramble (2007). The only difference is the use of the eluates obtained by the AMPA cleanup (with strong cation exchange SPE) for the determination of AMPA and N-acetyl AMPA.

The residues are determined by LC-MS/MS with positive electrospray ionization (ESI+) in the selected reaction mode using a Luna Phenyl-Hexyl column. The reported results are based on calibration with an internal standard for glyphosate and AMPA. The following transitions have been used:
Glyphosate: m/z 170 → 87.3
N-acetyl-glyphosate: m/z 212 → 170.2
AMPA: m/z 112.1 → 30.2
N-acetyl-AMPA: m/z 154 → 112.1
Internal standards:
1,2 $^{13}C_2$ ^{15}N-glyphosate: m/z: 173.1 → 91.0
$^{13}C^{15}N$-AMPA: m/z: 113.1 → 30.2

Findings:
The study describes a successful validation of the DuPont method. The deviation of the clean-up procedure for N-acetyl-AMPA is not relevant, because it refers to an analyte, which is not included in the proposed residue definition. Another deviation is the use of MS/MS transitions originally proposed for confirmatory purposes. Also this deviation is considered as less relevant.
The validated limit of quantification in both matrices (high water content and high oil content) is 0.05 mg/kg for each compound. Individual blank values were below 30 % of LOQ. Acceptable chromatograms of calibration standards, samples and blank materials, adequate recovery data and information on the precision of the method are presented. Linear calibration is performed for all matrices in the range 0.5 – 100 ng/mL (grapes) and 0.5 – 20 ng/mL (soybean seed) using standards in solvent at seven levels. The calibrated range corresponds to 0.025 – 5.0 mg/kg (grapes) and 0.025 – 1 mg/kg (soybean seed), respectively. Soy bean seed samples of the 20 mg/kg fortification level were 100 fold diluted. The correlation coefficients of reproduced calibration graphs are > 0.998. Information on calibration is reported in Table B.5.2-6. For validation data see Tables B.5.2-7 to B.5.2-10.
Additional MS/MS transitions for the confirmation of results are proposed but not validated in this study.

Conclusion:
The independent laboratory validation of the DuPont method conducted by Seal and Dillon (2007, ASB2008-2637) confirms the applicability of the DuPont method for the simultaneous determination of both components of the proposed residue definition for monitoring (glyphosate and N-acetyl-glyphosate) in plant materials.

Table B.5.2-6: Calibration data of the independent validation of the DuPont method for residues of glyphosate, N-acetyl-glyphosate, N-acetyl AMPA and AMPA in commodities of plant origin (Seal and Dillon, 2007, ASB2008-2637)

Calibration parameter	Results of evaluation
Accepted calibration range in concentration units	0.5 – 100 ng/mL (grapes) 0.5 – 20 ng/mL (soybean seed)
Accepted calibration range in mass fraction units	0.025 –5 mg/kg (grapes) 0.025 – 1 mg/kg (soybean seed)
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)	yes
Where matrix effects investigated?	no
The absence of interference >30 % of LOQ in blank samples was demonstrated?	yes
Chromatogram of sample provided spiked at LOQ demonstrating sufficient sensitivity and signal-to-noise ratio?	yes

Table B.5.2-7: Independent validation of the DuPont method for glyphosate residues in commodities of plant origin (Seal and Dillon, 2007, ASB2008-2637)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Seal and Dillon, 2007 (ASB2008-2637)	LC-MS/MS, Phenyl-Hexyl column, m/z 170 → 87.3	grapes	0.05 0.2	81 80	8 7	5 5
		soybean seed	0.05 20	96 85	13 7	5 5

Table B.5.2-8: Independent validation of the DuPont method for N-acetyl-glyphosate residues in commodities of plant origin (Seal and Dillon, 2007, ASB2008-2637)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Seal and Dillon, 2007 (ASB2008-2637)	LC-MS/MS, Phenyl-Hexyl column, m/z 212 → 170.2	grapes	0.05 0.2	95 86	4 4	5 5
		soybean seed	0.05 20	84 90	4 4	5 5

Table B.5.2-9: Independent validation of the DuPont method for residues of AMPA in commodities of plant origin (Seal and Dillon, 2007, ASB2008-2637)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Seal and Dillon, 2007 (ASB2008-2637)	LC-MS/MS, Phenyl-Hexyl column, m/z 112.1 → 30.2	grapes	0.05 0.2	81 73	3 3	5 5
		soybean seed	0.05 20	91 89	10 10	5 5

Table B.5.2-10: Independent validation of the DuPont method for residues of N-acetyl AMPA in commodities of plant origin (Seal and Dillon, 2007, ASB2008-2637)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Seal and Dillon, 2007 (ASB2008-2637)	LC-MS/MS, Phenyl-Hexyl column, m/z 154 → 112.1	grapes	0.05 0.2	93 82	9 5	5 5
		soybean seed	0.05 20	96 111	5 16	5 5

~~**B.5.2.1.1.3 Method 2**~~

~~**Reference:**~~ ~~IIA, 4.3/02~~
~~**Report:**~~ ~~Anderson, L. and Ely, S.V. (2001)~~
~~N-(phosphonomethyl) glycine (PMG) and aminomethyl phosphonic acid (AMPA) Validation of a residue analytical method for the determination of the residues in various crops.~~
~~Syngenta, Jealott's Hill International, Research Centre, Bracknell, UK~~
~~Report No.: RJ3119B. (Syngenta File No. ASF71/0238)~~
~~Date 27.03.2001, not published, ASB2012-12364~~
~~**Guidelines:**~~ ~~EPA OPPTS 860.1340, SANCO/3029/99 Rev. 4, SANCO/825/00 Rev. 7~~
~~**Deviations:**~~ ~~No~~
~~**GLP:**~~ ~~Yes~~
~~**Acceptability:**~~ ~~The study is considered to be acceptable.~~

~~**Materials and Methods:**~~
~~Test material: commodities with high water content (cabbage head, melon), high oil content (flax), dry crops (oat grain, rye straw) and one matrix which is difficult to analyse (coffee)~~

~~Fortified analyte(s): glyphosate (N-(phosphonomethyl) glycine) and AMPA (aminomethylphosphonic acid)~~

~~Analyte(s) determined as:~~
~~Glyphosate as N-([Bis-(2,2,3,3,4,4,4-heptafluorobutoxy)-phosphinyl]methyl)-N-(trifluoracetyl)-glycine,2,2,3,3,4,4,4-heptafluorobutotyl; $C_{17}H_{10}F_{24}O_6NP$, molar mass = 811 g/mol~~
~~AMPA as trifluoroacetylamino-methylphosphonic acid, bis(2,2,3,3,4,4,4-heptafluorobutyl ester; $C_{11}H_7F_{14}NO_4P$ $C_{11}H_7F_{17}NO_4P$, molar mass = 571 g/mol~~

~~Principle of the method: Samples are homogenised with a mixture of water and dichloromethane followed by centrifugation. The dichloromethane phase is discarded.~~

~~Oily or mucilaginous crops:~~
~~To remove the fat, a methanol precipitation procedure is used. This involves addition of methanol to the initial aqueous extract to precipitate co-extractives at reduced temperature followed by centrifugation. After additional filtration, the supernatant is used in the derivatisation procedure.~~

~~Other crops:~~
~~An acidified aliquot of the aqueous extract (pH ca. 2) is loaded on a cation exchange column (Bio-Rad Poly-Prep AG 50W-8X, H^+-Form). The column is washed with a mixture of water/methanol/concentrated HCl (160+40+2.7, V/V/V). The washing solution is discarded. Residues of glyphosate and AMPA are eluted with a larger volume of the same mixture of water/methanol/concentrated HCl. The eluate is evaporated to dryness, reconstituted in a small volume of the eluent and used in the derivatisation procedure.~~

~~Glyphosate and AMPA in the purified extracts are derivatised starting at – 50 °C in a mixture of trifluoroacetic anhydride and heptafluorobutanol. The derivatisation mixture is slowly heated to about 95 °C and derivatisation is finalised within one hour. After derivatisation, excess reagents and volatile by-products are evaporated and the residuum is dissolved in ethyl acetate. The residues are determined by gas liquid chromatography with mass selective detection (GC-MSD) in the select ion monitoring (SIM) mode using a BPX-5 column (5 % phenyl polydimethylsiloxane). The reported results are based on m/z 612 for glyphosate and m/z 446 for AMPA. Fragment ions m/z 611 and 584 are reported as qualifier ions for glyphosate and m/z 372 and 502 are proposed as qualifiers for AMPA.~~

~~**Findings:**~~
~~The method is very time consuming (10 samples over the course of two days) and requires the preparation of analytical standards by derivatisation.~~
~~The validated limit of quantification in all matrices (high water content, high oil content, dry and difficult) is 0.05 mg/kg. Individual blank values are not reported, but it is mentioned in the report that these values were below 30 % of LOQ. Except for glyphosate and AMPA in rye straw this is confirmed by chromatograms of control samples. Acceptable chromatograms from derivatised standard solutions, samples and blank materials, adequate recovery data and information on the precision of the method are presented. Linear calibration is performed for both analytes in the range 0.625 – 62.5 ng/mL using standards in solvent at four levels. The calibrated range corresponds to 0.05 – 5.00 mg/kg. The correlation coefficients are > 0.994. Calibration graphs are reported. Information on calibration is reported in Table B.5.2-11. For validation data see Tables B.5.2-12 and B.5.2-13.~~
~~Limited examples of peak ratios between quantifier and qualifier ions are reported which do not allow to accept this procedure as confirmatory method for all matrices at all levels.~~

~~Conclusion:~~

~~The study of Anderson and Ely (2001, ASB2012-12364) describes an adequately validated and sensitive method for the determination of glyphosate in commodities with high water content, commodities with high oil content, dry crops and coffee. However, the method does not allow the determination of the second component (N-acetyl-glyphosate) of the proposed residue definition for enforcement purposes in lentils sweet corn, oilseeds rape, soya beans and maize.~~

~~Because of two cleanup steps (partition and SPE), two evaporations to dryness and a difficult derivatisation (starting at – 50 °C) the practical relevance of this monitoring method will be limited; however from formal reasons (requirements of guidance document SANCO/825/00 rev 8.1) the method has to be accepted. The limitations are the single point calibration based on four instead of five levels. The use of standards in solvents was found inappropriate in a further study of this method (Gemrot, 2010, ASB2012-12427).~~

~~Table B.5.2-11: Calibration data of the analytical method RAM 328/01 for residues of glyphosate and AMPA in commodities of plant origin (Anderson and Ely, 2001, ASB2012-12364)~~

~~Calibration parameter~~	~~Results of evaluation~~
~~Accepted calibration range in concentration units~~	~~0.625 – 62.5 ng/mL~~
~~Accepted calibration range in mass fraction units~~	~~0.05 – 5.00 mg/kg~~
~~Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)~~	~~no (single points, four levels)~~
~~Where matrix effects investigated?~~	~~no~~
~~The absence of interference >30 % of LOQ in blank samples was demonstrated?~~	~~yes (in most cases)~~
~~Chromatogram of sample provided spiked at LOQ demonstrating sufficient sensitivity and signal-to-noise ratio?~~	~~yes~~

~~Table B.5.2-12: Validation of method RAM 328/01 for glyphosate residues in commodities of plant origin (Anderson and Ely, 2001, ASB2012-12364)~~

~~Reference~~	~~Detection method~~	~~Matrix~~	~~Fortification level [mg/kg]~~	~~Average recovery [%]~~	~~RSD [%]~~	~~No. of analyses~~
~~Anderson and Ely, 2001 (ASB2012-12364)~~	~~GC-MS, BPX-5 column, m/z 612~~	~~Flax~~	~~0.05~~ ~~0.5~~ ~~5~~	~~110~~ ~~91~~ ~~88~~	~~10~~ ~~9~~ ~~9~~	~~5~~ ~~5~~ ~~5~~
		~~Cabbage~~	~~0.05~~ ~~0.5~~ ~~5~~	~~87~~ ~~94~~ ~~87~~	~~10~~ ~~6~~ ~~7~~	~~6~~ ~~5~~ ~~5~~
		~~Melon~~	~~0.05~~ ~~0.5~~ ~~5~~	~~90~~ ~~99~~ ~~90~~	~~4~~ ~~9~~ ~~4~~	~~6~~ ~~5~~ ~~5~~
		~~Oat grain~~	~~0.05~~ ~~0.5~~ ~~5~~	~~99~~ ~~94~~ ~~92~~	~~13~~ ~~3~~ ~~6~~	~~6~~ ~~5~~ ~~5~~
		~~Rye straw~~	~~0.05~~ ~~0.5~~ ~~5~~	~~101~~ ~~100~~ ~~92~~	~~15~~ ~~7~~ ~~2~~	~~6~~ ~~5~~ ~~5~~
		~~Coffee~~	~~0.05~~ ~~0.5~~ ~~5~~	~~108~~ ~~108~~ ~~106~~	~~5~~ ~~8~~ ~~10~~	~~6~~ ~~5~~ ~~5~~

Table B,5.2-13: Validation of method RAM 328/01 for residues of AMPA in commodities of plant origin (Anderson and Ely, 2001, ASB2012-12364)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Anderson and Ely, 2001 (ASB2012-12364)	GC-MS, BPX-5 column, m/z 446	Flax	0.05 0.5 5	105 94 89	5 8 10	5 5 5
		Cabbage	0.05 0.5 5	74 86 70	15 12 16	6 5 5
		Melon	0.05 0.5 5	83 98 89	17 14 4	6 5 5
		Oat grain	0.05 0.5 5	81 76 85	7 8 11	6 5 5
		Rye straw	0.05 0.5 5	76 99 72	8 6 7	6 5 5
		Coffee	0.05 0.5 5	104 84 94	15 5 10	6 5 5

B.5.2.1.1.4 Independent laboratory validation of method 2

Reference: IIA 4.3/04
Report: Crook, S.J. (2007)
Glyphosate: Provision of independent laboratory validation data to support analytical methods RAM 328/01 and RAM 308/01. Syngenta, Jealott's Hill International, Research Centre, Bracknell, UK
Technical letter reference J6696/01. (Syngenta File No. ICI224/0939)
Date 29.01.2007, not published, ASB2012-12387
Guidelines: no
Deviations:
GLP: no
Acceptability: The study is considered to be acceptable.

Findings:
In contrast to the title of the document, only the reproducibility of the method RAM 328/01 is addressed in the technical letter by Crook (2007, ASB2012-12387). Validation data for method RAM 308/01 (applicable for matrices of animal origin) are not summarised. The presented data are taken from a publication of Alferness and Wiebe, 2001 (ASB2012-12387).

Conclusion:
See below the description of the study of Alferness and Wiebe, 2001 (ASB2012-12387).

~~**Reference:**~~	~~no (study was not provided with the dossier)~~
~~**Report:**~~	~~Alferness, P.L. and Wiebe, L.A. (2001), Determination of Glyphosate and Aminomethylphosphonic Acid in Crops by Capillary Gas Chromatography with Mass-Selective Detection: Collaborative Study, J AOAC International 84 (2001) 823–846, ASB2012-12387~~
~~**Guidelines:**~~	~~no~~
~~**Deviations:**~~	~~not applicable~~
~~**GLP:**~~	~~no~~
~~**Acceptability:**~~	~~The study is considered to be acceptable.~~

~~**Materials and Methods:**~~

~~Test material: commodities with high water content (soya forage), high oil content (walnut nutmeat), and dry crops (corn grain)~~

~~Fortified analyte(s): glyphosate (N-(phosphonomethyl) glycine) and AMPA (aminomethylphosphonic acid)~~

~~Analyte(s) determined as:~~
~~Glyphosate as N-([Bis-(2,2,3,3,4,4,4-heptafluorobutoxy)-phosphinyl]methyl)-N-(trifluoracetyl)-glycine,2,2,3,3,4,4,4-heptafluorobutotyl; $C_{17}H_{10}F_{24}O_6NP$, molar mass = 811 g/mol~~
~~AMPA as trifluoroacetylamino-methylphosphonic acid, bis(2,2,3,3,4,4,4-heptafluorobutyl ester; $C_{11}H_7F_{14}NO_4P$ $C_{11}H_7F_{17}NO_4P$, molar mass = 571 g/mol~~

~~Principle of the method: Samples are homogenised in water and solids are separated by centrifugation. An aliquot of the supernatant is washed with dichloromethane followed by centrifugation. If high protein crops (soya forage) are extracted, the aliquot of the supernatant is acidified with HCl before the partition step (washing) with dichloromethane.~~
~~The dichloromethane phase is discarded. For oily crops (nutmeat), the partition step (washing) with dichloromethane is repeated.~~
~~The cleanup of an acidified aliquot of the aqueous extract and the derivatisation of glyphosate and AMPA in the purified extracts are conducted as described in the study of Anderson and Ely (2001, ASB2012-12364). After derivatisation, the residuum is dissolved in a solution of 0.2 % citral in ethyl acetate. (Citral is used as analyte protectant.)~~
~~The residues are determined by gas liquid chromatography with mass selective detection (GC-MSD) in the select ion monitoring (SIM) mode using a capillary column with cross-linked, 95 % methyl–5 % phenyl silicone phase (DB-5 or equivalent). The reported results are based on m/z 611.5 for glyphosate and m/z 446 for AMPA.~~

~~**Findings:**~~

~~The validated limit of quantification in all matrices (high water content, high oil content and dry) is 0.05 mg/kg. The average of blank values was 0.004 mg/kg glyphosate and 0.0045 mg/kg AMPA. 2 out of 34 control samples had glyphosate residues > 30 % LOQ.~~
~~Acceptable chromatograms from derivatised standard solutions, samples and blank materials, adequate recovery data and information on the precision of the method are presented.~~
~~Calibration is performed for both analytes (glyphosate and AMPA) in the range 1 – 50 ng/mL using standards in solvent at three levels. The calibrated range corresponds to 0.05 – 2.50 mg/kg (soya forage and walnut nutmeat) or to 0.066 – 3.3 mg/kg dry crops (corn grain), i.e. calibration started at too high concentrations. Correlation coefficients or calibration graphs~~

~~are not reported. Information on calibration is reported in Table B.5.2-14. For validation data see Tables B.5.2-15 and B.5.2-16.~~

~~Conclusion:~~

~~The study of Alferness and Wiebe (2001, ASB2012-12387) describes the successful independent laboratory validation of the method RAM 328/01 (AOAC official method 2000.05) for the determination of residues of glyphosate and AMPA in commodities with high water content, commodities with high oil content, and dry crops. However, the method does not allow the determination of the second component (N-acetylglyphosate) of the proposed residue definition for enforcement purposes in lentils sweet corn, oilseeds rape, soya beans and maize.~~

~~There are only minor modifications in the extraction (high protein crops) and cleanup (oily crops) compared to the study of Anderson and Ely (2001, ASB2012-12364). Another minor modification is the use of an analyte protectant.~~

~~Because of two cleanup steps (partition and SPE), two evaporations to dryness and a difficult derivatisation (starting at – 50 °C) the practical relevance of this proposed monitoring method will be limited, however from formal reasons (requirements of guidance document SANCO/825/00 rev 8.1) the method has to be accepted.~~

~~Table B.5.2-14: Calibration data of the AOAC method 2000.05 (similar to RAM 328/01) for residues of glyphosate and AMPA in commodities of plant origin (Alferness and Wiebe, 2001, ASB2012-12387)~~

~~Calibration parameter~~	~~Results of evaluation~~
~~Accepted calibration range in concentration units~~	~~1 – 50 ng/mL~~
~~Accepted calibration range in mass fraction units~~	~~0.066 – 3.3 mg/kg (nutmeat)~~ ~~0.05 – 2.5 mg/kg (other matrices)~~
~~Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)~~	~~no (single points, three levels)~~
~~Where matrix effects investigated?~~	~~no~~
~~The absence of interference >30 % of LOQ in blank samples was demonstrated?~~	~~yes (in most cases)~~
~~Chromatogram of sample provided spiked at LOQ demonstrating sufficient sensitivity and signal-to-noise ratio?~~	~~yes~~

~~Table B.5.2-15: Validation of AOAC method 2000.05 (similar to RAM 328/01) for glyphosate residues in commodities of plant origin (Alferness and Wiebe, 2001, ASB2012-12387)~~

~~Reference~~	~~Detection method~~	~~Matrix~~	~~Fortification level [mg/kg]~~	~~Average recovery [%]~~	~~RSD [%]~~	~~No. of analyses~~
~~Alferness and Wiebe , 2001 (ASB2012-12387)~~	~~GC-MS, m/z 611.5~~	~~Corn grain~~	~~0.05~~ ~~0.4~~ ~~1.8~~	~~85~~ ~~88~~ ~~90~~	~~15.0~~ ~~18.5~~ ~~21.8~~	~~24~~ ~~24~~ ~~24~~
		~~Soya forage~~	~~0.05~~ ~~0.4~~ ~~1.8~~	~~88~~ ~~97~~ ~~93~~	~~19.7~~ ~~15.2~~ ~~16.4~~	~~24~~ ~~24~~ ~~24~~
		~~Nutmeat~~	~~0.05~~ ~~0.4~~ ~~1.8~~	~~97~~ ~~92~~ ~~93~~	~~9.0~~ ~~11.5~~ ~~19.9~~	~~22~~ ~~22~~ ~~18~~

~~**Table B.5.2-16: Validation of AOAC method 2000.05 (similar to RAM 328/01) for AMPA residues in commodities of plant origin (Alferness and Wiebe, 2001, ASB2012-12387)**~~

~~Reference~~	~~Detection method~~	~~Matrix~~	~~Fortification level [mg/kg]~~	~~Average recovery [%]~~	~~RSD [%]~~	~~No. of analyses~~
~~Alferness and Wiebe , 2001 (ASB2012-12387)~~	~~GC-MS, m/z 446~~	~~Corn grain~~	~~0.05~~ ~~0.4~~ ~~1.8~~	~~88~~ ~~89~~ ~~89~~	~~24.1~~ ~~23.4~~ ~~24.8~~	~~22~~ ~~22~~ ~~22~~
		~~Soya forage~~	~~0.05~~ ~~0.4~~ ~~1.8~~	~~78~~ ~~87~~ ~~87~~	~~42.9~~ ~~24.0~~ ~~20.3~~	~~24~~ ~~24~~ ~~24~~
		~~Nutmeat~~	~~0.05~~ ~~0.4~~ ~~1.8~~	~~92~~ ~~84~~ ~~88~~	~~27.9~~ ~~15.3~~ ~~23.2~~	~~22~~ ~~22~~ ~~18~~

B.5.2.1.2 Confirmatory methods for the determination of glyphosate and N-acetylglyphosate in plant materials

Confirmatory method 1

Reference: IIA, 4.3/05; IIA, 4.3/06
Report: Klimmek, S. (2007)
Validation of the analytical method DFG Method 405 for the determination of glyphosate and its metabolite AMPA in various plant materials
Eurofins Analytik GmbH, Dr. Specht Laboratorien, Hamburg, Germany
Report No.: FCS-0703V
Date: 22.08.2007, not published, ASB2008-5606
and
Klimmek, S., Weber, H. (2008)
Validation of the analytical method DFG Method 405 for the determination of glyphosate and its metabolite AMPA in various plant materials. 1st Amendment to final report
Eurofins Analytik GmbH, Dr. Specht Laboratorien, Hamburg, Germany
Report No.: FCS-0703V
Date: 07.01.2008, not published, ASB2008-5607
Guidelines: SANCO/825/00 Rev. 7
Deviations: No
GLP: yes
Acceptability: The study is considered to be acceptable.

Materials and Methods:
Test material: commodities with high water content (maize green plant), high oil content (oilseed rape), dry crops (maize corn, barley grain and straw, sugar beet root) and acidic fruits (citrus)

Fortified analyte(s): glyphosate (N-(phosphonomethyl) glycine) and AMPA (amino-methylphosphonic acid)

Analyte(s) determined as:
Glyphosate after oxidation to glycine as fluorescent isoindole derivative prepared postcolumn after reaction of glycine with ortho-phthaldialdehyde and 2-mercaptoethanol
AMPA as fluorescent isoindole derivative prepared postcolumn after reaction with ortho-phthalaldehyde and 2-mercaptoethanol

Principle of the method: Samples are homogenized in a mixture of 0.1 N HCl (straw: 6 N HCl) and dichloromethane. After centrifugation an aliquot of the aqueous phase is diluted with water and loaded on a column containing a ligand exchange resin (Chelex 100, pre-conditioned with Fe (III) ions). After washing steps with water and 0.2 N HCl, residues of glyphosate and AMPA are eluted from the column with 6 N HCl. Co-eluted Fe (III) ions are separated by filtration through a column containing an anion exchange resin (AG 1-X8). The filtrate is evaporated to dryness to remove hydrochloric acid and reconstituted with water (pH must be > 1.5). Sample concentration in final extracts is 1.76 g/mL (straw) and 3.6 g/mL (other matrices), respectively.
Glyphosate and AMPA were quantified individually by means of HPLC equipped with an ion exclusion column (Rezex RKP Kalium), post derivatisation unit and a fluorescence detector (excitation 325 nm, emission 530 nm). Determination involves post-column conversion of glyphosate to glycine and reaction of glycine and AMPA with ortho-phthaldialdehyde and mercaptoethanol to produce fluorescent derivatives (325 nm excitation and 530 nm emission).

Findings:
The validated limit of quantification in all matrices (high water content, high oil content, acidic and dry) is 0.05 mg/kg. In all control samples the intensity of signals at the retention time of glyphosate and AMPA was below 30 % LOQ.
Acceptable chromatograms from postcolumn derivatised standard solutions, samples and blank materials, as well as adequate recovery data and information on the precision of the method are presented.
Calibration is performed for both analytes (glyphosate and AMPA) in the range 16 – 2500 ng/mL using standards in solvent at 10 levels. Correlation coefficients of reported calibration graphs are > 0.999. Information on calibration is reported in Table B.5.2-17. For validation data see Tables B.5.2-18 and B.5.2-19.

Conclusion:
The two studies of Klimmek (2007 and 2008, ASB2008-5606 and ASB2008-5607) describe a fully validated confirmatory method for the determination of residues of glyphosate and AMPA in samples of plant origin. However, the method does not allow the determination of the second component (N-acetyl-glyphosate) of the proposed residue definition for enforcement purposes in ~~lentils~~ sweet corn, oilseeds rape, soya beans and maize.

Table B.5.2-17: Calibration data of the DFG method 405 for residues of glyphosate and AMPA in commodities of plant origin (Klimmek, 2007 and 2008, ASB2008-5606 and ASB2008-5607)

Calibration parameter	Results of evaluation
Accepted calibration range in concentration units	16 – 2500 ng/mL
Accepted calibration range in mass fraction units	0.010 – 1.400 mg/kg (straw) 0.005 – 0.680 mg/kg (other matrices)
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)	yes (10 levels)
Where matrix effects investigated?	no
The absence of interference >30 % of LOQ in blank samples was demonstrated?	yes
Chromatogram of sample provided spiked at LOQ demonstrating sufficient sensitivity and signal-to-noise ratio?	yes

Table B.5.2-18: Validation of DFG method 405 for glyphosate residues in commodities of plant origin (Klimmek, 2007 and 2008, ASB2008-5606 and ASB2008-5607)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Klimmek , 2007 and Klimmek, 2008 (ASB2008-5606, ASB2008-5607))	HPLC with postcolumn derivatisation and fluorescence detection	Barley grain	0.05 0.5	85 82	4.9 5.7	5 5
		Barley straw	0.05 0.5	84 72	16 5.6	5 5
		Maize green plant	0.05 0.5	104 105	3.8 1.2	5 5
		Maize corn	0.05 0.5	72 79	8.9 5.6	5 5
		Sugar beet root	0.05 0.5	109 99	3.0 7.5	5 5
		Oil seed rape	0.05 0.5	74 75	4.9 4.9	5 5
		Citrus fruit	0.05 0.5	73 77	4.4 9.6	5 5

Table B.5.2-19: Validation of DFG method 405 for AMPA residues in commodities of plant origin (Klimmek, 2007 and 2008, ASB2008-5606 and ASB2008-5607)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Klimmek , 2007 and Klimmek, 2008 (ASB2008-5606, ASB2008-5607))	HPLC with postcolumn derivatisation and fluorescence detection	Barley grain	0.05 0.5	98 98	3.7 4.7	5 5
		Barley straw	0.05 0.5	98 98	3.8 7.1	5 5
		Maize green plant	0.05 0.5	71 102	3.4 2.4	5 5
		Maize corn	0.05 0.5	91 87	4.4 6.4	5 5
		Sugar beet root	0.05 0.5	100 95	3.6 9.5	5 5
		Oil seed rape	0.05 0.5	75 73	11 4.5	5 5
		Citrus fruit	0.05 0.5	78 80	4.2 14	5 5

Confirmatory method 2

Reference: IIA, 4.3/07
Report: Weber, H. (2012)
Validation of an analytical method for the determination of glyphosate and AMPA in Raw Agricultural Commodities using LC/MS/MS.
Eurofins Agroscience Services Chem GmbH, Hamburg, Germany
Report No.: S11-03331
Date: 08.02.2012, not published, ASB2012-12489
Guidelines: SANCO/825/00 Rev. 8.1
Deviations: No
GLP: yes
Acceptability: The study is considered to be acceptable.

Materials and Methods:
Test material: several commodities with high water content

Fortified analyte(s): glyphosate (N-(phosphonomethyl) glycine) and AMPA (aminomethylphosphonic acid)

Analyte(s) determined as: glyphosate (N-(phosphonomethyl) glycine) and AMPA (aminomethylphosphonic acid)

Principle of the method: Samples are homogenised in a mixture of 0.1 % formic acid and dichloromethane. Following centrifugation, to an aliquot of the aqueous phase internal standards (isotopic labelled glyphosate and AMPA) are added. For final cleanup, the extract is filtered through a polymeric reverse phase SPE column (Strata-X). Sample concentration in final extracts is 0.09 g/mL.

Glyphosate and AMPA residues are determined by liquid chromatography with tandem mass spectrometer (LC-MS/MS) in negative multiple reaction monitoring (MRM) mode using a Bio-Rad Fast Acid HPLC column. For each analyte two transitions are monitored (glyphosate: quantifier: 168→68, qualifier: 168→79; AMPA: quantifier: 110→63, qualifier: 110→79).

Findings:
The validated limit of quantification is 0.05 mg/kg. In all control samples the intensity of signals at the retention time of glyphosate and AMPA was below 30 % LOQ.
Acceptable chromatograms from standard solutions, samples and blank materials, as well as adequate recovery data and information on the precision of the method are presented.
Calibration is performed for both analytes (glyphosate and AMPA) in the range 1.25 – 250 ng/mL using standards in solvent at eight levels. Correlation coefficients of reported calibration graphs are > 0.998. Information on calibration is reported in Table B.5.2-20. For validation data see Tables B.5.2-21 and B.5.2-22.

Conclusion:
The study of Weber (2012, ASB2012-12489) describes a simple, fully validated confirmatory method for the determination of residues of glyphosate and AMPA in samples of plant origin. Unfortunately, validation was limited to commodities with high water content. However, the method does not allow the determination of the second component (N-acetyl-glyphosate) of the proposed residue definition for enforcement purposes in ~~lentils~~ sweet corn, oilseeds rape, soya beans and maize.

Table B.5.2-20: Calibration data of the confirmatory LC-MS/MS method for residues of glyphosate and AMPA in commodities of plant origin (Weber, 2012, ASB2012-12489)

Calibration parameter	Results of evaluation
Accepted calibration range in concentration units	1.25 – 250 ng/mL
Accepted calibration range in mass fraction units	0.014 – 2.80 mg/kg
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)	yes (8 levels)
Where matrix effects investigated?	no (use of labeled internal standards)
The absence of interference >30 % of LOQ in blank samples was demonstrated?	yes
Chromatogram of sample provided spiked at LOQ demonstrating sufficient sensitivity and signal-to-noise ratio?	yes

Table B.5.2-21: Validation of the confirmatory LC-MS/MS method for glyphosate residues in commodities of plant origin (Weber, 2012, ASB2012-12489)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Weber (2012), ASB2012-12489	LC-MS/MS, Bio-Rad Fast Acid HPLC column , m/z 168→68	potato	0.05 0.5	91 87	1.4 3.1	5 5
		carrot	0.05 0.5	90 91	3.4 1.4	5 5
		onion	0.05 0.5	88 85	2.1 1.3	5 5
		cucumber	0.05 0.5	86 89	1.0 1.0	5 5
		cabbage	0.05 0.5	88 91	1.7 1.3	5 5
		cauliflower	0.05 0.5	88 93	0.6 1.8	5 5
		lettuce	0.05 0.5	94 90	4.6 1.6	5 5
		leek	0.05 0.5	87 90	1.5 1.3	5 5
		tomato	0.05 0.5	87 89	3.0 4.7	5 5
	LC-MS/MS, Bio-Rad Fast Acid HPLC column , m/z 168→79	potato	0.05 0.5	93 88	3.5 2.0	5 5
		carrot	0.05 0.5	90 90	3.3 0.6	5 5
		onion	0.05 0.5	91 85	1.6 3.0	5 5
		cucumber	0.05 0.5	90 91	1.8 0.6	5 5
		cabbage	0.05 0.5	90 91	2.4 1.4	5 5
		cauliflower	0.05 0.5	87 90	4.3 2.0	5 5
		lettuce	0.05 0.5	96 90	5.0 1.7	5 5
		leek	0.05 0.5	91 91	2.1 2.1	5 5
		tomato	0.05 0.5	86 87	2.8 4.8	5 5

Table B.5.2-22: Validation of the confirmatory LC-MS/MS method for AMPA residues in commodities of plant origin (Weber, 2012, ASB2012-12489)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Weber (2012), ASB2012-12489	LC-MS/MS, Bio-Rad Fast Acid HPLC column , m/z 110→63	potato	0.05 0.5	88 89	1.7 1.1	5 5
		carrot	0.05 0.5	86 88	2.4 1.7	5 5
		onion	0.05 0.5	84 85	2.2 1.5	5 5
		cucumber	0.05 0.5	84 93	2.6 0.6	5 5
		cabbage	0.05 0.5	85 92	2.1 1.9	5 5
		cauliflower	0.05 0.5	85 92	1.7 2.5	5 5
		lettuce	0.05 0.5	88 93	5.0 2.2	5 5
		leek	0.05 0.5	85 92	1.0 0.9	5 5
		tomato	0.05 0.5	86 89	3.5 3.5	5 5
	LC-MS/MS, Bio-Rad Fast Acid HPLC column , m/z 110→79	potato	0.05 0.5	87 93	5.0 1.6	5 5
		carrot	0.05 0.5	84 91	3.4 1.2	5 5
		onion	0.05 0.5	82 86	3.6 2.1	5 5
		cucumber	0.05 0.5	82 91	3.4 2.8	5 5
		cabbage	0.05 0.5	84 94	2.2 1.9	5 5
		cauliflower	0.05 0.5	84 92	2.2 2.1	5 5
		lettuce	0.05 0.5	92 94	3.0 2.5	5 5
		leek	0.05 0.5	83 92	2.5 1.6	5 5
		tomato	0.05 0.5	84 90	4.4 3.1	5 5

B.5.2.1.3 Additional studies/reports

~~The method used in the study of Anderson and Ely (2001, ASB2012-12364) is fully described in an attached standard operation procedure RAM 328/01. The identical SOP is also described in a separate report by Ely (2001, ASB2012-12418), Report No.: SOP RAM 328/01 (Syngenta File No. ASF71/0237), Reference IIA, 4.3/01.~~

The applicability of the multi residue method S19 for the determination of glyphosate-trimesium in plant and animal matrices is considered in the study of Runnels (1993, ASB2009-5598). Based on the physico-chemical properties of glyphosate-trimesium it could be clearly shown that the multiresidue method DFG S-19 cannot be used for the quantification of glyphosate residues.

B.5.2.1.4 Studies which do not fulfill the requirements

Not accepted study 1:

Reference:	IIA, 4.3/02
Report:	Anderson, L. and Ely, S.V. (2001) N-(phosphonomethyl) glycine (PMG) and aminomethyl phosphonic acid (AMPA) Validation of a residue analytical method for the determination of the residues in various crops. Syngenta, Jealott's Hill International, Research Centre, Bracknell, UK Report No.: RJ3119B. (Syngenta File No. ASF71/0238) Date 27.03.2001, not published, ASB2012-12364
Guidelines:	EPA OPPTS 860.1340, SANCO/3029/99 Rev. 4, SANCO/825/00 Rev. 7
Deviations:	yes
GLP:	Yes
Acceptability:	The study is considered to be not acceptable.

Findings and conclusion:
This study uses a modified Alferness method without stable isotope labelled internal standards. Glyphosate and AMPA in purified extracts are derivatised starting at – 60 °C in a mixture of trifluoroacetic anhydride and heptafluorobutanol. The amount of residues is determined by gas liquid chromatography with mass selective detection.
Limited examples of peak ratios between quantifier and qualifier ions are reported which do not allow to accept this procedure as confirmatory method. The method does not allow the determination of the second component (N-acetyl-glyphosate) of the proposed residue definition for enforcement. Finally, the efficiency of the derivatisation step was not demonstrated as required in accordance to SANCO/825/00 rev. 8.1. Therefore, the extent of validation of the method is not sufficient.

Not accepted study 2:

Reference:	IIA, 4.3/01
Report:	Ely, S. V. (2001) Residue analytical method for the analysis of N-(Phosphonomethyl)Glycine (PMG) and Aminomethylphosphonic acid (AMPA) in crops Syngenta, Jealott's Hill Research Centre, Bracknell, UK Report No. RAM 328/01, (Syngenta File No. ASF71/0237), ASB2012-12418
Guidelines:	no
Deviations:	
GLP:	
Acceptability:	The study is not considered to be acceptable.

Findings and conclusion:
The method used in "not accepted study 1" is fully described in an attached standard operation procedure RAM 328/01. In addition, the plain SOP (RAM 328/01) is provided by the applicants in study 2. Conclusions see above.

Not accepted study 3:

Reference:	no (study was not provided with the dossier)
Report:	Alferness, P.L. and Wiebe, L.A. (2001), Determination of Glyphosate and Aminomethylphosphonic Acid in Crops by Capillary Gas Chromatography with Mass-Selective Detection: Collaborative Study, J AOAC International 84 (2001) 823 – 846, ASB2012-12387
Guidelines:	no
Deviations:	not applicable
GLP:	no
Acceptability:	The study is considered to be not acceptable.

Findings and conclusion:
This study uses a modified Alferness method without stable isotope labelled internal standards. Glyphosate and AMPA in purified extracts are derivatised starting at – 60 °C in a mixture of trifluoroacetic anhydride and heptafluorobutanol. The amount of residues is determined by gas liquid chromatography with mass selective detection.
The method does not allow the determination of the second component (N-acetyl-glyphosate) of the proposed residue definition for enforcement. Finally, the efficiency of the derivatisation step was not demonstrated as required in accordance to SANCO/825/00 rev. 8.1. Therefore, the extent of validation of the method is not sufficient.

Not accepted study 4:

Reference:	IIA 4.3/04
Report:	Crook, S.J. (2007) Glyphosate: Provision of independent laboratory validation data to support analytical methods RAM 328/01 and RAM 308/01. Syngenta, Jealott's Hill International, Research Centre, Bracknell, UK Technical letter reference J6696/01. (Syngenta File No. ICI224/0939) Date 29.01.2007, not published, ASB2012-12387
Guidelines:	no
Deviations:	
GLP:	no
Acceptability:	The study is considered to be not acceptable.

Findings and conclusion:
In contrast to the title of the document, only the reproducibility of the method RAM 328/01 is addressed in study 4. The presented data are taken from a publication of Alferness and Wiebe, 2001 (see "not accepted study 3").

Not accepted study 5:

Reference:	IIA, 4.3/03
Report:	Gemrot, F. (2010) Validation of analytical methods RAM 328/01 for the determination of glyphosate and AMPA in orange and sunflower seeds. Syngenta, Jealott's Hill International, Research Centre, Bracknell, UK Report No.: TK0012393-REG. (Syngenta File No. S10-02992) Date 13.09.2010, not published, ASB2012-12427
Guidelines:	EPA OPPTS 860.1340, EPA OPPTS 850.7100, SANCO/3029/99 Rev. 4, SANCO/825/00 Rev. 7 OECD Guidance Document ENV/JM/MONO(2007)17
Deviations:	No
GLP:	yes
Acceptability:	The study is considered to be not acceptable ~~because of inappropriate validation levels~~.

Findings and conclusion:
This study uses a modified Alferness method without stable isotope labelled internal standards. Glyphosate and AMPA in purified extracts are derivatised starting at – 60 °C in a mixture of trifluoroacetic anhydride and heptafluorobutanol. The amount of residues is determined by gas liquid chromatography with mass selective detection.
The validated limit of quantification in orange and sunflower seed (0.5 and 20 mg/kg, respectively) is not sufficient to check MRLs for acidic matrices and those of high oil content. The extent of validation (only one level) was not sufficient. The method does not allow the determination of the second component (N-acetyl-glyphosate) of the proposed residue definition for enforcement. Finally, the efficiency of the derivatisation step was not demonstrated as required in accordance to SANCO/825/00 rev. 8.1. Therefore, the extent of validation of the method is not sufficient.

~~**Materials and Methods:**~~
~~Test material: acidic commodities (oranges) and those with high oil content (sunflower seeds)~~

~~Fortified analyte(s): glyphosate (N-(phosphonomethyl) glycine) and AMPA (aminomethylphosphonic acid)~~

~~Analyte(s) determined as:~~
~~Glyphosate as N-([Bis-(2,2,3,3,4,4,4-heptafluorobutoxy)-phosphinyl]methyl)-N-(trifluoracetyl)-glycine,2,2,3,3,4,4,4-heptafluorobutotyl; $C_{17}H_{10}F_{24}O_6NP$, molar mass = 811 g/mol~~
~~AMPA as trifluoroacetylamino-methylphosphonic acid, bis(2,2,3,3,4,4,4-heptafluorobutyl ester; $C_{11}H_7F_{14}NO_4P$ $C_{11}H_7F_{17}NO_4P$, molar mass = 571 g/mol~~

~~Principle of the method: Samples are homogenised with a mixture of water and dichloromethane followed by centrifugation. The dichloromethane phase is discarded.~~

~~Sunflower seeds:~~

~~After addition of methanol extracts are stored between 0 – 9 °C for 1 – 12 h to remove the fat. The supernatant is filtrated through a 0.65 µm cellulose membrane.~~

~~Oranges:~~
~~An aliquot of the aqueous extract is reduced to dryness and reconstituted in water/methanol/conc. HCl (160+40+2.7; V/V/V).~~

~~Glyphosate and AMPA in the raw extracts (no clean-up on a cation exchange column) are derivatised starting at – 50 °C in a mixture of trifluoroacetic anhydride and heptafluorobutanol. The derivatisation mixture is slowly heated to about 95 °C and finalised within one hour. After derivatisation, excess reagents and volatile by-products are evaporated and the residuum is dissolved in ethyl acetate.~~
~~The residues are determined by gas liquid chromatography with mass selective detection (GC-MSD) in the select ion monitoring (SIM) mode using m/z 612, 611 and 584 for glyphosate and m/z 446, 372 and 502 for AMPA.~~

~~**Findings:**~~
~~The method is not identical to the method used by Anderson L and Ely SV (2001), because no clean-up on a cation exchange column was used. Validation was not conducted at two levels for each matrix. A chromatogram of an orange extract spiked at 0.5 mg/kg is not presented.~~
~~The validated limit of quantification in orange and sunflower seed is 0.5 and 20 mg/kg, respectively, which is not sufficient to control most regulated MRLs. Blank values are not reported but chromatograms of control samples demonstrate that the blanks are distinctly below 30 % of LOQ. Linear calibration is performed for both analytes (glyphosate and AMPA) in the range 0.3 – 20 ng/mL using standards in matrix at 6 – 7 levels. The calibrated range corresponds to 0.024 – 1.60 mg/kg. Confirmatory transitions are validated at the same high level. For validation data see Tables B.5.2-23 to B.5.2-26.~~

~~**Conclusion:**~~
~~The validated limit of quantification in orange and sunflower seed (0.5 and 20 mg/kg, respectively) is not sufficient to check MRLs for acidic matrices and those of high oil content. The extent of validation (only one level) was not sufficient.~~
~~**The efficiency of the derivatisation step was not demonstrated as required in accordance to SANCO/825/00 rev. 8.1. Therefore, the extent of validation of the method is not sufficient.**~~

~~**Table B.5.2-23: Validation of modified method RAM 328/01 for residues of glyphosate in commodities of plant origin (Gemrot, 2010, ASB2012-12427)**~~

~~**Reference**~~	~~**Detection method**~~	~~**Matrix**~~	~~**Fortification level [mg/kg]**~~	~~**Average recovery [%]**~~	~~**RSD [%]**~~	~~**No. of analyses**~~
~~Gemrot, 2010 (ASB2012-12427)~~	~~GC-MS, BPX-5 column, m/z 612~~	~~orange~~	~~0.50~~	~~83~~	~~7~~	~~5~~
		~~Sunflower seed~~	~~20~~	~~82~~	~~6~~	~~5~~

~~Table B.5.2-24: Validation of modified method RAM 328/01 for residues of AMPA in commodities of plant origin (Gemrot, 2010, ASB2012-12427)~~

~~Reference~~	~~Detection method~~	~~Matrix~~	~~Fortification level [mg/kg]~~	~~Average recovery [%]~~	~~RSD [%]~~	~~No. of analyses~~
~~Gemrot, 2010 (ASB2012-12427)~~	~~GC-MS, BPX-5 column, m/z 446~~	~~orange~~	~~0.50~~	~~87~~	~~8~~	~~5~~
		~~Sunflower seed~~	~~20~~	~~80~~	~~6~~	~~5~~

~~Table B.5.2-25: Validation of confirmatory transitions of modified method RAM 328/01 for residues of glyphosate in commodities of plant origin (Gemrot, 2010, ASB2012-12427)~~

~~Reference~~	~~Detection method~~	~~Matrix~~	~~Fortification level [mg/kg]~~	~~Average recovery [%]~~	~~RSD [%]~~	~~No. of analyses~~
~~Gemrot, 2010 (ASB2012-12427)~~	~~m/z 611~~	~~orange~~	~~0.50~~	~~92~~	~~6~~	~~5~~
	~~m/z 584~~	~~orange~~	~~0.50~~	~~90~~	~~9~~	~~5~~
	~~m/z 611~~	~~Sunflower seed~~	~~20~~	~~83~~	~~6~~	~~5~~
	~~m/z 584~~	~~Sunflower seed~~	~~20~~	~~81~~	~~4~~	~~5~~

~~Table B.5.2-26: Validation of confirmatory transitions of modified method RAM 328/01 for residues of AMPA in commodities of plant origin (Gemrot, 2010, ASB2012-12427)~~

~~Reference~~	~~Detection method~~	~~Matrix~~	~~Fortification level [mg/kg]~~	~~Average recovery [%]~~	~~RSD [%]~~	~~No. of analyses~~
~~Gemrot, 2010 (ASB2012-12427)~~	~~m/z 372~~	~~orange~~	~~0.50~~	~~87~~	~~7~~	~~5~~
	~~m/z 502~~	~~orange~~	~~0.50~~	~~87~~	~~8~~	~~5~~
	~~m/z 372~~	~~Sunflower seed~~	~~20~~	~~79~~	~~5~~	~~5~~
	~~m/z 502~~	~~Sunflower seed~~	~~20~~	~~80~~	~~7~~	~~5~~

Not accepted study 6:

Reference: IIA, 4.3
Report: Anon. (????)
EFSA inquiry at the bottom of first page, reporting table 1 (29) - Analytical method
ASB2014-9564
Acceptability: The study is considered to be not acceptable.

Findings and conclusion:
The Glyphosate Task Force argues in a very short statement that it is impossible to prepare the pure derivatives of glyphosate and AMPA, which are used in the Alferness method. Such

pure derivatives are required to fulfill the requirement of SANCO/825/00 rev. 8.1. The main argument is the low stability of these derivatives. On the other hand, in the study of Garrigue (2014, ASB2014-9489) a stability of derivatives for at least 8 days was demonstrated. Therefore, RMS does not accept the statement.

The Glyphosate Task Force further argues that N-acetyl-glyphosate should not be included into the residue definition. Also in this case RMS did not follow the arguments of the Glyphosate Task Force.

B.5.2.2 Animal matrices

B.5.2.2.1 Acceptable methods/reports

B.5.2.2.1.1 Method 1

Note: Method 1 is not presented by Monsanto on behalf of the Glyphosate Task Force but required for Annex 1 inclusion.

Reference:	no (study was not provided with the dossier)
Report:	Pentz AM and Bramble FQ (2007) Analytical method for the determination of N-acetylglyphosate and other analytes in various animal matrices using LC/MS/MS E. I. du Pont de Nemours and company, Wilmington Delaware, USA Project Identification Number: DuPont-20009 Date 20.11.2007, not published, ASB2008-2636
Guidelines:	EPA OPPTS 860.1340, SANCO/825/00 Rev. 6
Deviations:	No
GLP:	No
Acceptability:	The study is considered to be acceptable.

Materials and Methods:
Test material: Milk, egg, muscle, fat, liver, kidney

Fortified analyte(s): glyphosate, N-acetyl-glyphosate (sodium salt), AMPA, N-acetyl-AMPA

Analyte(s) determined as:
Glyphosate, N-acetyl-glyphosate, AMPA, N-acetyl-AMPA

Principle of the method:
Extraction and clean-up of milk and egg:
A mixture of 0.1 % aqueous formic acid/methanol (96:4, v/v) is vortex mixed with samples. The acidic solution is defatted with hexane. The hexan phase is discarded after centrifugation. A second washing is conducted with methylene chloride. After centrifugation an aliquot of the upper aqueous phase is filtrated through a C18 SPE column and fortified with internal standards.
A first aliquot of the C18 eluate is diluted with water. The diluted extract is added to a strong anion exchange SPE column (Oasis MAX). After washing with three different solvent mixtures, residues of glyphosate, N-acetyl-glyphosate and N-acetyl-AMPA are eluted with 1 % trifluoroacetic acid in methanol/water (90:10, v/v). This eluate is evaporated to dryness

and reconstituted in 0.02 M phosphoric acid. The sample concentration in final extracts is 0.02 g/mL.
A second aliquot of the C18 eluate (with internal standards) is added to a strong cation exchange SPE column (Oasis MCX). Residues of N-acetyl-glyphosate, AMPA and N-acetyl-AMPA are eluted with methanol. The eluate is evaporated to the aqueous reminder and acidifid with phosphoric acid. The sample concentration in final extracts is 0.03 g/mL.
The MAX clean-up was generally used for glyphosate and for N-acetyl-glyphosate and N-acetyl-AMPA in eggs and skim milk. The MCX clean-up was generally used for AMPA and for N-acetyl-glyphosate and N-acetyl-AMPA in whole milk and cream.

Extraction and clean-up of muscle, fat, liver and kidney:
Samples are mixed with the double amount of C18 sorbent (solid phase dispersion). The solid material is extracted with 0.1N HCl/methanol (96:4, v/v) by shaking. After centrifugation the remaining pellet is reextracted with water.
A first aliquot of the combined extract is adjusted to basic pH with triethylamine and diluted with acetonitrile and methanol to precipitate unsoluble matrix. After centrifugation, the supernatant is fortified with internal standards and added to a strong anion exchange SPE column (Oasis MAX). After washing with three different solvent mixtures, residues of glyphosate, N-acetyl-glyphosate and N-acetyl-AMPA are eluted with 1 % trifluoroacetic acid in methanol/water (90:10, v/v). These eluates are evaporated to dryness and reconstituted in 0.02 M phosphoric acid. The sample concentration in final extracts is 0.02 g/mL for fat, kidney and liver and 0.04 g/mL for muscle, respectively.
A second aliquot of the extract is fortified with internal standards and diluted with acetonitrile and methanol. The diluted aliquot is added to a strong cation exchange SPE column (Oasis MCX). Residues of AMPA are eluted with water and methanol. The eluate is evaporated to the aqueous reminder and acidifid with phosphoric acid. The sample concentration in final extracts is 0.02 g/mL for fat, kidney and liver and 0.04 g/mL for muscle, respectively.

The residues are determined by LC-MS/MS with positive and negative electrospray ionisation (ESI+ and ESI-) in the selected reaction mode using a Luna Phenyl-Hexyl column. The reported results are based on calibration with an internal standard for glyphosate and AMPA. The following transitions have been used:
ESI- (chicken muscle, AMPA in chicken liver)
Glyphosate: m/z 168 → 62.9
N-acetyl-glyphosate: m/z 210 → 79.0
AMPA: m/z 110.0 → 63.1
N-acetyl-AMPA: m/z 152 → 63
internal standards:
1,2 $^{13}C_2$ ^{15}N-glyphosate: m/z: 171 → 62.9
$^{13}C^{15}N$-AMPA: m/z: 112 → 63
ESI+ (other matrices and analytes)
Glyphosate: m/z 170 → 87.7
N-acetyl-glyphosate: m/z 212 → 87.9
AMPA: m/z 111.8 → 30
N-acetyl-AMPA: m/z 154 → 30
internal standards:
1,2 $^{13}C_2$ ^{15}N-glyphosate: m/z: 173 → 90.7
$^{13}C^{15}N$-AMPA: m/z: 113.8 → 32

Findings:
The method allows the simultaneous determination of both components of the proposed residue definition for enforcement. Additionally, it allows the determination of residues of AMPA and N-acetyl-AMPA. The validated limit of quantification in kidney, liver and fat is 0.05 mg/kg for all compounds. In milk, cream, egg, muscle the limit of quantification is 0.025 mg/kg for all compounds. Individual blank values are reported as raw data and were below 30 % of LOQ. This is confirmed by chromatograms of control samples. Acceptable chromatograms of calibration standards, samples and blank materials, adequate recovery data and information on the precision of the method are presented. Linear calibration is performed for AMPA in the range 0.5 – 50 ng/mL using standards in solvent at seven levels. All other analytes are calibrated in the range 0.25 – 20 ng/mL. The calibrated range in mg/kg differs for each matrix. The correlation coefficients of reproduced calibration graphs are > 0.995. Information on calibration is reported in Table B.5.2-27. For validation data see Tables B.5.2-28 to B.5.2-31. Additional MS/MS transitions for the confirmation of results are proposed but not validated in this study.

Conclusion:
The study of Pentz and Bramble (2007, ASB2008-2636) describes an adequately validated and sensitive method for the determination of residues of glyphosate and N-acetyl-glyphosate (also applicable for AMPA and N-acetyl-AMPA) in milk, egg, meat, fat, liver, kidney.

Table B.5.2-27: Calibration data of the DuPont method for residues of glyphosate, N-acetyl-glyphosate, N-acetyl-AMPA and AMPA in commodities of animal origin (Pentz and Bramble, 2007, ASB2008-2636)

Calibration parameter	Results of evaluation
Accepted calibration range in concentration units	0.50 – 50 ng/mL (AMPA) 0.25 – 20 ng/mL (other analytes)
Accepted calibration range in mass fraction units	0.025 – 2.50 mg/kg (AMPA in liver , kidney and fat) 0.016 – 1.60 mg/kg (AMPA in eggs, milk and cream) 0.013 – 1.250 mg/kg (AMPA in meat) 0.006 – 0.5 mg/kg (other analytes in meat) 0.013 – 1.0 mg/kg (other analytes in liver, kidney, fat, eggs, skim milk) 0.013 – 1.0 mg/kg (glyphosate in whole milk and cream) 0.008 – 0.67 mg/kg (N-acetyl-glyphosate and N-acetyl-AMPA in whole milk and cream)
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)	yes
Where matrix effects investigated?	no
The absence of interference >30 % of LOQ in blank samples was demonstrated?	yes
Chromatogram of sample provided spiked at LOQ demonstrating sufficient sensitivity and signal-to-noise ratio?	yes

Table B.5.2-28: Validation of the DuPont method for glyphosate residues in commodities of animal origin (Pentz and Bramble, 2007, ASB2008-2636)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Pentz and Bramble, 2007 (ASB2008-2636)	LC-MS/MS, Phenyl-Hexyl column, m/z 170 → 88 (m/z 168 → 63 for chicken muscle)	whole milk	0.025 0.05 0.5	97 100 80	14 13 7	9 7 7
		skim milk	0.025 0.05 0.5	93 85 85	12 4 7	5 5 5
		cream	0.025 0.05 0.5	99 95 83	13 5 5	5 5 5
		whole egg	0.025 0.05 0.5	88 89 85	6 7 2	5 5 5
		egg yolks	0.025 0.05 0.5	98 90 89	10 8 7	5 5 5
		egg whites	0.025 0.05 0.5	83 88 89	7 3 6	5 5 5
		beef and chicken liver	0.05 0.5	90 82	10 5	11 9
		kidney	0.05 0.5	98 87	15 4	6 7
		fat	0.05 0.5	98 94	11 4	6 6
		beef and chicken muscle	0.025 0.25	92 84	10 4	7 7

Table B.5.2-29: Validation of the DuPont method for N-acetyl-glyphosate residues in commodities of animal origin (Pentz and Bramble, 2007, ASB2008-2636)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Pentz and Bramble, 2007 (ASB2008-2636)	LC-MS/MS, Phenyl-Hexyl column, m/z 212 → 88 (m/z 210 → 79 for chicken muscle)	whole milk	0.025 0.05 0.5	80 78 79	5 3 3	9 7 7
		skim milk	0.025 0.05 0.5	95 93 91	2 8 5	5 5 5
		cream	0.025 0.05 0.5	78 82 82	3 4 3	5 5 5
		whole egg	0.025 0.05 0.5	97 87 87	13 6 4	5 5 5
		egg yolks	0.025 0.05 0.5	88 90 95	6 5 10	5 5 5
		egg whites	0.025 0.05 0.5	103 96 95	9 10 2	5 5 5
		beef and chicken liver	0.05 0.5	90 89	11 10	11 9
		kidney	0.05 0.5	99 83	11 5	6 7
		fat	0.05 0.5	100 90	6 6	6 6
		beef and chicken muscle	0.025 0.25	92 81	13 8	7 7

Table B.5.2-30: Validation of the DuPont method for residues of AMPA in commodities of animal origin (Pentz and Bramble, 2007, ASB2008-2636)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Pentz and Bramble, 2007 (ASB2008-2636)	LC-MS/MS, Phenyl-Hexyl column, m/z 112 → 30 (m/z 110 → 63 for chicken liver and muscle)	whole milk	0.025 0.05 0.5	87 85 80	3 7 6	9 7 7
		skim milk	0.025 0.05 0.5	94 84 76		2 2 2
		cream	0.025 0.05 0.5	93 88 82	5 8 4	5 5 5
		whole egg	0.025 0.05 0.5	105 96 84	7 10 3	5 5 5
		egg yolks	0.025 0.05 0.5	110 90 94		2 2 2
		egg whites	0.025 0.05 0.5	91 94 86	16 5 2	4 5 5
		beef and chicken liver	0.05 0.5	97 94	12 10	10 9
		kidney	0.05 0.5	92 89	15 13	6 7
		fat	0.05 0.5	103 93	6 3	5 5
		beef and chicken muscle	0.025 0.25	94 94	9 6	6 5

Table B.5.2-31: Validation of the DuPont method for residues of N-acetyl-AMPA in commodities of plant origin (Pentz and Bramble, 2007, ASB2008-2636)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Pentz and Bramble, 2007 (ASB2008-2636)	LC-MS/MS, Phenyl-Hexyl column, m/z 154 → 30 (m/z 152 → 63 for chicken muscle)	whole milk	0.025 0.05 0.5	81 79 82	7 4 3	9 7 7
		skim milk	0.025 0.05 0.5	95 99 101	8 7 3	5 5 5
		cream	0.025 0.05 0.5	87 83 93	14 8 5	5 5 5
		whole egg	0.025 0.05 0.5	92 99 97	9 8 6	5 5 5
		egg yolks	0.025 0.05 0.5	93 96 106	5 2 5	5 5 5
		egg whites	0.025 0.05 0.5	90 87 92	4 8 5	5 5 5
		beef and chicken liver	0.05 0.5	83 81	16 12	10 9
		kidney	0.05 0.5	82 79	10 9	6 6
		fat	0.05 0.5	88 87	5 8	6 6
		beef and chicken muscle	0.025 0.25	83 80	10 9	6 5

B.5.2.2.1.2 Independent laboratory validation of method 1

Reference: no (study was not provided with the dossier)
Report: Karnik, S., Dillon, R. (2007)
Independent laboratory validation of DuPont-20009, "Analytical method for the determination of N-acetalglyphosate and other analytes in various animal matrices using LC/MS/MS"
Pyxant Labs Inc., Colorado Springs, USA
Project Identification Numbers: DuPont-21372; Pyxant Labs Inc. ID: 1806, Date 03.12.2007, not published, ASB2008-2634
Guidelines: EPA OPPTS 860.1340, SANCO/825/00 Rev. 7
Deviations: No
GLP: yes
Acceptability: The study is considered to be acceptable.

Materials and Methods:
Test material: Milk, egg, muscle, liver

Fortified analyte(s): glyphosate, N-acetyl-glyphosate (sodium salt), AMPA, N-acetyl-AMPA

Analyte(s) determined as:
Glyphosate, N-acetyl-glyphosate, AMPA, N-acetyl-AMPA

Principle of the method:
During extraction and cleanup no deviations from primary validation (Pentz and Bramble, 2007b) were needed. In all cases, glyphosate and N-acetyl-AMPA are determined in the MAX eluate and AMPA is determined in the MCX eluate. The selection of eluates for determination of N-acetyl-glyphosate was depending on matrix.

The residues are determined by LC-MS/MS with positive and negative electrospray ionisation (ESI+ and ESI-) in the selected reaction mode using a Luna Phenyl-Hexyl column. The reported results are based on calibration with an internal standard for glyphosate and AMPA. The following transitions have been used:
ESI-
Glyphosate: m/z 167.9 → 63.0 (for liver)
AMPA: m/z 109.9 → 63.0 (for all matrices)
internal standards:
1,2 $^{13}C_2$ ^{15}N-glyphosate: m/z: 170.9 → 63.0
13C15N-AMPA: m/z: 111.9 → 63.0
ESI+
Glyphosate: m/z 170 → 88.1 (for milk, eggs and muscle)
N-acetyl-glyphosate: m/z 212.3 → 170.1 (for all matrices)
N-acetyl-AMPA: m/z 154.2 → 112.0 (for liver)
internal standards:
1,2 $^{13}C_2$ ^{15}N-glyphosate: m/z: 173.0 → 91.1

Findings:
The method allows the simultaneous determination of both components of the proposed residue definition for enforcement. Additionally, it allows the determination of residues of AMPA and N-acetyl-AMPA. The validated limit of quantification in milk, egg and muscle is 0.025 mg/kg for all compounds. In liver the limit of quantification is 0.05 mg/kg for all compounds. Blank values were below 30 % of LOQ. This is confirmed by chromatograms of control samples. Acceptable chromatograms of calibration standards, samples and blank materials, adequate recovery data and information on the precision of the method are presented. Linear calibration is performed for AMPA in the range 0.5 – 50 ng/mL using standards in solvent at seven levels. All other analytes are calibrated in the range 0.25 – 20 ng/mL. The calibrated range in mg/kg differs for each matrix. The correlation coefficients of reproduced calibration graphs are > 0.995. Information on calibration is reported in Table B.5.2-32. For validation data see Tables B.5.2-33 to B.5.2-36. Additional MS/MS transitions for the confirmation of results are proposed but not validated in this study.

Conclusion:
The independent laboratory validation of the DuPont method conducted by Karnik and Dillon (2007, ASB2008-2634) confirms the applicability of the DuPont method for the simultaneous determination of both components of the proposed residue definition for monitoring (glyphosate and N-acetyl-glyphosate) in animal materials.

Table B.5.2-32: Calibration data of the DuPont method for residues of glyphosate, N-acetylglyphosate, N-acetyl AMPA and AMPA in commodities of animal origin (Karnik and Dillon, 2007, ASB2008-2634)

Calibration parameter	Results of evaluation
Accepted calibration range in concentration units	0.50 – 50 ng/mL (AMPA in eggs) 0.25 – 20 ng/mL (other)
Accepted calibration range in mass fraction units	0.025 – 2.50 mg/kg (AMPA in liver) 0.008 – 0.67 mg/kg (AMPA in egss and milk) 0.006 – 0.50 mg/kg (all analytes in meat) 0.013 – 1.0 mg/kg (both other analytes in liver and eggs) 0.013 – 1.0 mg/kg (glyphosate in milk) 0.008 – 0.67 mg/kg (N-acetyl-glyphosate in milk)
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)	yes
Where matrix effects investigated?	no
The absence of interference >30 % of LOQ in blank samples was demonstrated?	yes
Chromatogram of sample provided spiked at LOQ demonstrating sufficient sensitivity and signal-to-noise ratio?	yes

Table B.5.2-33: Validation of the DuPont method for glyphosate residues in commodities of animal origin (Karnik and Dillon, 2007, ASB2008-2634)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Karnik and Dillon, 2007 (ASB2008-2634)	LC-MS/MS, Phenyl-Hexyl column, m/z 170 → 88 (m/z 168 → 63 for chicken muscle)	milk	0.025 0.05 0.25	100 102 99	8 9 6	10 10 10
		eggs	0.025 0.05 0.25	100 107 109	8 4 3	5 5 5
		meat	0.025 0.05 0.25	89 87 85	11 8 4	5 5 5
		liver	0.05 0.1 0.5	106 110 102	11 11 10	5 5 5

Table B.5.2-34: Validation of the DuPont method for N-acetyl-glyphosate residues in commodities of animal origin (Karnik and Dillon, 2007, ASB2008-2634)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Karnik and Dillon, 2007 (ASB2008-2634)	LC-MS/MS, Phenyl-Hexyl column, m/z 212 → 170)	milk	0.025 0.05 0.25	96 89 77	10 7 7	5 5 5
		eggs	0.025 0.05 0.25	100 90 81	12 2 5	5 5 5
		meat	0.025 0.05 0.25	70 84 76	6 7 6	5 5 5
		liver	0.05 0.1 0.5	79 80 72	5 7 4	10 10 10

Table B.5.2-35: Validation of the DuPont method for residues of AMPA in commodities of animal origin (Karnik and Dillon, 2007, ASB2008-2634)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Karnik and Dillon, 2007 (ASB2008-2634)	LC-MS/MS, Phenyl-Hexyl column, m/z 110.0 → 63	milk	0.025 0.05 0.25	105 95 91	6 10 5	5 5 5
		eggs	0.025 0.05 0.25	106 97 94	10 7 5	5 5 5
		meat	0.025 0.05 0.25	91 93 87	3 6 3	5 5 5
		liver	0.05 0.1 0.5	108 104 108	9 6 9	10 10 10

Table B.5.2-36: Validation of the DuPont method for residues of N-acetyl-MPA in commodities of plant origin (Karnik and Dillon, 2007, ASB2008-2634)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Karnik and Dillon, 2007 (ASB2008-2634)	LC-MS/MS, Phenyl-Hexyl column, m/z 154 → 30	liver	0.05 0.1 0.5	87 88 85	6 7 6	5 5 5

~~B.5.2.2.1.1 Method 2~~

~~**Reference:**~~	~~IIA, 4.3/09~~
~~**Report:**~~	~~Clarke, D.M. and Robinson, N.J. (1998)~~ ~~Validation of an analytical method for the determination of [N-(phosphonomethyl) glycine] (PMG) and aminomethylphosphonic acid (AMPA) in kidney, milk and eggs by gas chromatography with mass selective detection.~~ ~~Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell, UK~~ ~~Report No. RJ2585B (Syngenta File No. ICI224/0305)~~ ~~Date 29.06.1998, not published, ASB2012-12398~~
~~**Guidelines:**~~	~~EPA OPPTS 860.1340, SANCO/3029/99 Rev. 4, SANCO/825/00 Rev. 7~~
~~**Deviations:**~~	~~No~~
~~**GLP:**~~	~~yes~~
~~**Acceptability:**~~	~~The study is considered to be acceptable.~~

~~**Materials and Methods:**~~
~~Test material: kidney, milk, eggs, muscle, animal fat, and liver~~

~~Fortified analyte(s): glyphosate (N-(phosphonomethyl) glycine) and AMPA (amino-methylphosphonic acid)~~

~~Analyte(s) determined as:~~
~~Glyphosate as N-([Bis-(2,2,3,3,4,4,4-heptafluorobutoxy)-phosphinyl]methyl)-N-(trifluoracetyl)-glycine,2,2,3,3,4,4,4-heptafluorobutotyl; $C_{17}H_{10}F_{24}O_6NP$, molar mass = 811 g/mol~~
~~AMPA as trifluoroacetylamino-methylphosphonic acid, bis(2,2,3,3,4,4,4-heptafluorobutyl ester; $C_{11}H_7F_{14}NO_4P$ $C_{11}H_7F_{17}NO_4P$, molar mass = 571 g/mol~~

~~Principle of the method: Samples are homogenised with a mixture of 0.6 % acetic acid and dichloromethane (milk) or 0.1 N HCl and dichloromethane (other matrices) followed by centrifugation. The dichloromethane phase is discarded. The partition (washing) step with dichloromethane was repeated for samples of animal fat.~~
~~An aliquot of the acidic aqueous phase is loaded on a cation exchange column (Bio-Rad poly-Prep AG 50W-8X, H+ Form). The column is washed with a mixture of water/methanol/concentrated HCl (160+40+2.7, V/V/V). The washing solution is discarded. Residues of glyphosate and AMPA are eluted with a larger volume of the same mixture of water/methanol/concentrated HCl. The eluate is evaporated to dryness, reconstituted in a small volume of the eluent and used in the derivatisation procedure.~~
~~Glyphosate and AMPA in the purified extracts are derivatised starting at – 60 °C in a mixture of trifluoroacetic anhydride and heptafluorobutanol. The derivatisation mixture is slowly heated to about 95 °C and derivatisation is finalized within one hour. After derivatisation, excess reagents and volatile by-products are evaporated and the residuum is dissolved in a solution of 1 % citral (V/V) in ethyl acetate. Citral is used as analyte protectant. Sample concentration in final extracts is 0.03 g/mL for milk and eggs and 0.015 g/mL for other matrices.~~

~~The residues are determined by gas liquid chromatography with mass selective detection (GC-MSD) in the select ion monitoring (SIM) mode using a HP-5MS column (5 % phenyl poly-dimethylsiloxane). The reported results are based on m/z 612 for glyphosate and m/z 446 for AMPA. Fragment ions m/z 611 and 584 are used as qualifier ions for glyphosate and m/z 372 and 502 are used as qualifiers for AMPA.~~

~~**Findings:**~~
~~The validated limit of quantification is 0.01 mg/kg for eggs and 0.2 mg/kg for kidney. In all other cases a LOQ cannot be derived, because the number of replicate measurements is not sufficient (liver, muscle, fat), information on calibration is missing (milk, liver, muscle, fat) or data of control samples are not reported (liver, muscle, fat). Confirmatory transitions are not validated.~~
~~Blank values are not reported, but chromatograms of control samples of milk, egg and kidney demonstrate that the blanks are distinctly below 0.003 mg/kg for eggs or milk, and 0.066 mg/kg for kidney. Information on control samples of liver, muscle and fat is not reported.~~
~~Linear calibration is performed for both analytes (glyphosate and AMPA) in the range 1.5 – 15 ng/mL (kidney) and 0.3 – 3 ng/mL (eggs) using standards in matrix at 6 levels. Calibration graphs for other matrices are not reported. The calibrated range corresponds to 0.01 – 0.10 mg/kg (eggs) and 0.10 – 1.00 mg/kg (other matrices). Information on calibration is reported in Table B.5.2-37. For validation data see Tables B.5.2-38 and B.5.2-39.~~

~~**Conclusion:**~~
~~Except for the calibrated range, which does not cover the range from 30 % of LOQ to 120 % of the highest level, the method RAM 308/01 was sufficiently validated for glyphosate in eggs. By contrast, the validation is not acceptable for kidney (LOQ too high) and liver, muscle and fat (too low number of samples analyzed). In addition, the method does not allow the determination of the second component (N-acetyl-glyphosate) of the proposed residue definition for enforcement.~~
~~Because of two cleanup steps (partition and SPE), two evaporations to dryness and a difficult derivatisation (starting at – 60 °C) the practical relevance of this monitoring method for eggs will be limited; however from formal reasons (requirements of guidance document SANCO/825/00 rev 8.1) the method has to be accepted for eggs.~~

~~**Table B.5.2-37: Calibration data of the analytical method RAM 308/01 for residues of glyphosate and AMPA in commodities of animal origin (Clarke and Robinson, 1998, ASB2012-12398)**~~

~~**Calibration parameter**~~	~~**Results of evaluation**~~
~~Accepted calibration range in concentration units~~	~~0.3 – 3.0 ng/mL (eggs)~~ ~~1.5 – 15 ng/mL (kidney)~~
~~Accepted calibration range in mass fraction units~~	~~0.01 – 0.10 mg/kg (eggs)~~ ~~0.10 – 1.00 mg/kg (kidney)~~
~~Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)~~	~~yes (6 levels, eggs and kidney)~~
~~Where matrix effects investigated?~~	~~no (use of matrix-matched standards)~~
~~The absence of interference >30 % of LOQ in blank samples was demonstrated?~~	~~yes (for milk, egg and kidney)~~ ~~no (for liver, muscle and fat)~~
~~Chromatogram of sample provided spiked at LOQ demonstrating sufficient sensitivity and signal-to-noise ratio?~~	~~yes (for milk, egg and kidney)~~

~~Table B.5.2-38: Validation of the analytical method RAM 308/01 for residues of glyphosate in commodities of animal origin (Clarke and Robinson, 1998, ASB2012-12398)~~

~~Reference~~	~~Detection method~~	~~Matrix~~	~~Fortification level [mg/kg]~~	~~Average recovery [%]~~	~~RSD [%]~~	~~No. of analyses~~
~~Clarke and Robinson, (1998) ASB2012-12398 (with data from method development reported in Appendix 1)~~	~~GC-MS, HP-5MS column, m/z 612~~	~~kidney~~	~~0.10~~	~~93~~	~~6~~	~~4~~
			~~0.20~~	~~91~~	~~-~~	~~1~~
			~~0.40~~	~~88~~	~~-~~	~~1~~
			~~1.00~~	~~90~~	~~15.5~~	~~5~~
			~~2.00~~	~~95~~	~~-~~	~~1~~
		~~milk~~	~~0.01~~	~~91~~	~~7.8~~	~~5~~
			~~0.02~~	~~75~~	~~-~~	~~1~~
			~~0.05~~	~~86~~	~~-~~	~~1~~
			~~0.10~~	~~87~~	~~4.1~~	~~5~~
		~~eggs~~	~~0.01~~	~~89~~	~~16.0~~	~~5~~
			~~0.02~~	~~88~~	~~-~~	~~1~~
			~~0.05~~	~~70~~	~~-~~	~~1~~
			~~0.10~~	~~92~~	~~16.4~~	~~5~~
		~~liver~~	~~0.20~~	~~87~~	~~-~~	~~1~~
			~~0.40~~	~~98~~	~~-~~	~~1~~
			~~1.00~~	~~87~~	~~-~~	~~1~~
			~~2.00~~	~~98~~	~~-~~	~~1~~
		~~muscle~~	~~0.03~~	~~81~~	~~-~~	~~1~~
			~~0.10~~	~~83~~	~~-~~	~~1~~
			~~0.20~~	~~70~~	~~-~~	~~1~~
			~~0.50~~	~~72~~	~~-~~	~~1~~
		~~fat~~	~~0.03~~	~~79~~	~~-~~	~~1~~
			~~0.10~~	~~62~~	~~-~~	~~1~~
			~~0.20~~	~~72~~	~~-~~	~~1~~
			~~0.50~~	~~72~~	~~-~~	~~1~~

~~Table B.5.2-39: Validation of the analytical method RAM 308/01 for residues of AMPA in commodities of animal origin (Clarke and Robinson, 1998, ASB2012-12398)~~

~~Reference~~	~~Detection method~~	~~Matrix~~	~~Fortification level [mg/kg]~~	~~Average recovery [%]~~	~~RSD [%]~~	~~No. of analyses~~
~~Clarke and Robinson, (1998) ASB2012-12398 (with data from method development reported in Appendix 1)~~	~~GC-MS, HP-5MS column, m/z 446~~	~~kidney~~	~~0.10~~	~~112~~	~~9.2~~	~~4~~
			~~0.20~~	~~78~~	-	~~1~~
			~~0.40~~	~~72~~	-	~~1~~
			~~1.00~~	~~102~~	~~19.7~~	~~5~~
			~~2.00~~	~~86~~	-	~~1~~
		~~milk~~	~~0.01~~	~~106~~	~~6.3~~	~~5~~
			~~0.02~~	~~77~~	-	~~1~~
			~~0.05~~	~~80~~	-	~~1~~
			~~0.10~~	~~83~~	~~6.1~~	~~5~~
		~~eggs~~	~~0.01~~	~~101~~	~~17.3~~	~~5~~
			~~0.02~~	~~71~~	-	~~1~~
			~~0.05~~	~~77~~	-	~~1~~
			~~0.10~~	~~87~~	~~6.6~~	~~5~~
		~~liver~~	~~0.20~~	~~76~~	-	~~1~~
			~~0.40~~	~~77~~	-	~~1~~
			~~1.00~~	~~73~~	-	~~1~~
			~~2.00~~	~~76~~	-	~~1~~
		~~muscle~~	~~0.03~~	~~89~~	-	~~1~~
			~~0.10~~	~~88~~	-	~~1~~
			~~0.20~~	~~90~~	-	~~1~~
			~~0.50~~	~~86~~	-	~~1~~
		~~fat~~	~~0.03~~	~~118~~	-	~~1~~
			~~0.10~~	~~81~~	-	~~1~~
			~~0.20~~	~~86~~	-	~~1~~
			~~0.50~~	~~84~~	-	~~1~~

B.5.2.2.1.2 Method 2

Reference: IIA, 4.3/13

Report: Schneider, V. (2001)
Validation of an analytical method for the determination of glyphosate in foodstuff of animal origin (meat, eggs, milk)
UCL GmbH, Cologne, Germany
Report no.: PR01/005
Date 20.07.2001, not published, MET2005-367

Guidelines: SANCO/825/00 Rev. 6

Deviations: No

GLP: yes

Acceptability: The study is considered to be acceptable.

Materials and Methods:
Test material: animal meat, eggs and milk

Fortified analyte(s): glyphosate (N-(phosphonomethyl) glycine) and AMPA (aminomethylphosphonic acid)

Analyte(s) determined as:
Glyphosate as N-([Bis-(2,2,2-trifluoroethoxy)-phosphinyl] methyl)-N-(trifluor¬acetyl)-glycine,2,2,2-trifluoroethotyl; $C_{11}H_{10}F_{12}O_6NP$, molar mass = 511 g/mol
AMPA as trifluoroacetylamino-methylphosphonic acid, bis(2,2,2-trifluoroethyl ester; $C_6H_7F_9NO_4P$, molar mass = 371 g/mol

Principle of the method:
Meat:
After addition of internal standards, the homogenized sample was stirred in about 1 % aqueous NaOH solution (ca. 0.2 N). After centrifugation the supernatant was heated to 100 °C and held at this temperature for 10 min. After cooling the solution is centrifuged again.
Milk:
After addition of internal standards, proteins are precipitated at 37 °C by stirring with an enzyme (Rennin). Solids are separated by centrifugation. To an aliquot sodium hydroxide was added to obtain a NaOH concentration of about 1 % (ca. 0.2 N). After leaving 5 min at room temperature the solution is centrifuged again.
Eggs:
After addition of internal standards, the homogenised sample was stirred in about 1 % aqueous NaOH solution (ca. 0.2 N) for 15 min. Finally, the mixture is centrifuged.

The alkaline aqueous extracts (pH ca. 13) are loaded on an anion exchange column (Dowex AG1X8-100), which was conditioned with 1 % ammonium hydroxide. The column is washed first with 1 % ammonium hydroxide and than with water. The washing solution is discarded. Residues of glyphosate and AMPA are eluted with 1 N sodium hydroxide solution. After addition of charcoal, the eluate is heated to 100 °C. After cooling the solution is centrifuged and the supernatant is evaporated to dryness.
To the dry residue phosphoric acid and trifluoroacetic acid are added. Glyphosate and AMPA in the purified extracts are derivatised with a mixture of trifluoroacetic anhydride and trifluoroethanol (2+1, V/V). The derivatisation mixture is heated to about 70 °C and derivatisation is finalised within one hour. After derivatisation, excess reagents and volatile by-products are removed by rotary evaporation. The concentrated remainder is partitioned between dichloromethane and 5 % aqueous ammonium hydroxide solution. The dichloromethane phase containing the derivatives is dried, filtered and evaporated. The remainder is reconstituted in toluene.
The residues are determined by gas liquid chromatography with mass selective detection (GC-MSD) in the select ion monitoring (SIM) mode using a CP-Sil 19 column (14 % cyanopropylphenyl/ 86 % polydimethylsiloxane). The reported results are based on m/z 411 for glyphosate and m/z 302 for AMPA. For internal standards m/z 412 and m/z 303 are used for ^{13}C-glyphosate and ^{15}N-AMPA, respectively.

Findings:
The validated limit of quantification is 0.05 mg/kg for all matrices. Blank values are not reported, but chromatograms of control samples of milk and eggs demonstrate that the blanks are distinctly below 30 % of LOQ. A chromatogram of a control sample of meat is not reported (because the chromatogram in the figure labeled as control is a spiked sample).
Quadratic calibration is performed for both analytes (glyphosate and AMPA) in all matrices in the range 0.04 – 0.60 mg/kg based on stable isotope labelled standards. Confirmatory transitions are not validated. Information on calibration is reported in Table B.5.2-40. For validation data see Tables B.5.2-41 and B.5.2-42.

Conclusion:
The study of Schneider (2001, MET2005-367) describes a successfully validated method for glyphosate in animal meat, fat and milk. However, the method does not allow the determination of the second component (N-acetyl-glyphosate) of the proposed residue definition for enforcement.
Because of several cleanup steps (saponification, enzymatic protein precipitation, partition, anion exchange chromatography, and adsorption on charcoal), three evaporations to dryness and a derivatisation, the practical relevance of this monitoring method will be limited. However from formal reasons (requirements of guidance document SANCO/825/00 rev 8.1) the method has to be accepted.

Table B.5.2-40: Calibration data of the GC-MS method for residues of glyphosate and AMPA in commodities of animal origin (Schneider, 2001, MET2005-367)

Calibration parameter	Results of evaluation
Accepted calibration range in concentration units	1.99 – 29.8 µg/mL (ISTD: 25 µg/mL)
Accepted calibration range in mass fraction units	0.04 – 0.60 mg/kg
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)	yes
Where matrix effects investigated?	not required (use of stable-isotope labelled standards)
The absence of interference >30 % of LOQ in blank samples was demonstrated?	yes
Chromatogram of sample provided spiked at LOQ demonstrating sufficient sensitivity and signal-to-noise ratio?	yes

Table B.5.2-41: Validation of the GC-MS method for residues of glyphosate in commodities of animal origin (Schneider, 2001, MET2005-367)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Schneider, 2001 (MET2005-367)	GC-MS, m/z 411	Meat	0.05 0.5	100 101	9.6 2.2	5 5
		Eggs	0.05 0.5	93 101	4.3 2.4	5 5
		Milk	0.05 0.5	92 102	4.1 2.7	5 5

Table B.5.2-42: Validation of the GC-MS method for residues of AMPA in commodities of animal origin (Schneider, 2001, MET2005-367)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Schneider, 2001 (MET2005-367)	GC-MS, m/z 411	Meat	0.05 0.5	103 101	5.9 12.2	5 5
		Eggs	0.05 0.5	95 92	9.1 4.0	5 5
		Milk	0.05 0.5	91 103	9.9 2.3	5 5

~~**B.5.2.2.2 Additional studies/reports**~~

~~The method used in the study of Clarke and Robinson (1998, ASB2012-12398) is fully described in an attached standard operation procedure RAM 308/01. The identical SOP is also described in a separate report by Robinson (1998, ASB2012-7836), Report No. RAM 308/01 (Syngenta File No. ICI224/0285, Reference IIA, 4.3/08).~~

~~In the former version of the method RAM 308/01 (i.e. method RR93-104B RES), which is not included in the dossier, the washing of the acidic aqueous extract was done with chloroform. The report of Robinson (1998, ASB2012-12465) describes a first study for the replacement of chloroform by dichloromethane.~~

B.5.2.2.3 Studies which do not fulfill the requirements

Not accepted study 1:

Reference:	IIA, 4.3/09
Report:	Clarke, D.M. and Robinson, N.J. (1998) Validation of an analytical method for the determination of [N-(phosphonomethyl) glycine] (PMG) and aminomethylphosphonic acid (AMPA) in kidney, milk and eggs by gas chromatography with mass selective detection. Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell, UK Report No. RJ2585B (Syngenta File No. ICI224/0305) Date 29.06.1998, not published, ASB2012-12398
Guidelines:	EPA OPPTS 860.1340, SANCO/3029/99 Rev. 4, SANCO/825/00 Rev. 7
Deviations:	yes
GLP:	yes
Acceptability:	The study is considered to be not acceptable.

Findings and conclusion:
This study uses a modified Alferness method without stable isotope labelled internal standards. Glyphosate and AMPA in purified extracts are derivatised starting at – 60 °C in a mixture of trifluoroacetic anhydride and heptafluorobutanol. The amount of residues is determined by gas liquid chromatography with mass selective detection.
The calibrated range does not cover the range from 30 % of LOQ to 120 % of the highest level. An LOQ could not be derived, because the number of replicate measurements is not sufficient (liver, muscle, fat), information on calibration is missing (milk, liver, muscle, fat) or data of control samples are not reported (liver, muscle, fat). The method does not allow the determination of the second component (N-acetyl-glyphosate) of the proposed residue definition for enforcement. Finally, the efficiency of the derivatisation step was not demonstrated as required in accordance to SANCO/825/00 rev. 8.1. Therefore, the extent of validation of the method is not sufficient.

Not accepted study 2:

Reference:	IIA, 4.3

Report:	Robinson, N. J. (1998) Residue analytical method for the determination of [N-(Phosphonomethyl)glycine] (PMG) and Aminomethylphosphonic acid (AMPA) in animal products Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell, UK Report No. RAM 308/01, ASB2012-7836
Guidelines:	no
Deviations:	
GLP:	
Acceptability:	The study is considered to be not acceptable.

Findings and conclusion:
The method used in "not accepted study 1" is fully described in an attached standard operation procedure RAM 308/01. In addition, the plain SOP (RAM 308/01) is provided by the applicants in study 2. Conclusions see above.

Not accepted study 3:

Reference:	IIA, 4.3
Report:	Robinson, N. J. (1998) GLYPHOSATE TRIMESIUM: Replacement of Chloroform in method RR93-104B RES for analysis of N-(Phosphonomethyl)Glycine and Aminomethyl-Phosphonic acid in animal matrices. A method development summary Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell, UK Report No. 36546/01, ASB2012-12465
Guidelines:	no
Deviations:	
GLP:	yes
Acceptability:	The study is considered to be not acceptable.

Findings and conclusion:
The original Alferness method is used in method RR93-104B RES, which is not provided with the dossier. In this original version the washing of the acidic aqueous extracts was done with chloroform instead of dichloromethane. Study 3 describes the consequences of replacement of chloroform by dichloromethane. The replacement of chloroform by dichloromethane resulted in significantly larger recoveries than those obtained with chloroform. Test were performed with milk, eggs, liver, kidney, muscle and fat with an insufficient number of replicates (n = 1).

Not accepted study 4:

Reference:	IIA, 4.3/11
Report:	Solé, C. (2007) Validation of residue method RAM 308.01 for the determination of PMG (ASF71) and AMPA in fat, liver and kidney. ADME Bioanalyses, Vergeze, France Report No. T011644-06 (Syngenta File No. R61837/1121) Date 12.11.2007, not published, ASB2012-7837
Guidelines:	EPA OPPTS 860.1340, SANCO/3029/99 Rev. 4 SANCO/825/00 Rev. 7
Deviations:	yes
GLP:	yes
Acceptability:	The study is considered to be not acceptable ~~because of insufficient sensitivity~~.

Findings and conclusion:
This study uses the modified Alferness method without stable isotope labelled internal standards. Glyphosate and AMPA in purified extracts are derivatised starting at – 60 °C in a mixture of trifluoroacetic anhydride and heptafluorobutanol. The amount of residues is determined by gas liquid chromatography with mass selective detection.
The validated limit of quantification is not sufficient to control regulated MRLs for fat, liver and kidney. In addition, the method does not allow the determination of the second component (N-acetyl-glyphosate) of the proposed residue definition for enforcement.
The efficiency of the derivatisation step was not demonstrated as required in accordance to SANCO/825/00 rev. 8.1. Therefore, the extent of validation of the method is not sufficient.
~~**Materials and Methods:**~~
~~Test material: animal fat, liver and kidney~~

~~Fortified analyte(s): glyphosate (N-(phosphonomethyl) glycine) and AMPA (amino-methylphosphonic acid)~~

~~Analyte(s) determined as:~~
~~Glyphosate as N-([Bis-(2,2,3,3,4,4,4-heptafluorobutoxy)-phosphinyl]methyl)-N-(trifluoracetyl)-glycine,2,2,3,3,4,4,4-heptafluorobutotyl; $C_{17}H_{10}F_{24}O_6NP$, molar mass = 811 g/mol~~
~~AMPA as trifluoroacetylamino-methylphosphonic acid, bis(2,2,3,3,4,4,4-heptafluorobutyl ester; $C_{11}H_7F_{14}NO_4P$, molar mass = 571 g/mol~~

~~Principle of the method: Samples are homogenized with a mixture of 0.1 N HCl and dichloromethane followed by centrifugation. The dichloromethane phase is discarded. The partition (washing) step with dichloromethane was repeated for fat samples.~~
~~An aliquot of the acidic extract (pH ca. 2) is loaded on a cation exchange column (Bio-Rad poly-Prep AG 50W-8X, H+-Form). The column is washed with a mixture of water/methanol/concentrated HCl (160+40+2.7, V/V/V). The washing solution is discarded. Residues of glyphosate and AMPA are eluted with a larger volume of the same mixture of water/methanol/concentrated HCl. The eluate is evaporated to dryness, reconstituted in a small volume of water/methanol/concentrated HCl (160+40+2.7, V/V/V) and used in the derivatization procedure.~~

~~Glyphosate and AMPA in the purified extracts are derivatized starting at – 60 °C in a mixture of trifluoroacetic anhydride and heptafluorobutanol. The derivatization mixture is slowly heated to about 95 °C and derivatization is finalized within one hour. After derivatization, excess reagents and volatile by-products are evaporated and the residuum is dissolved in a solution of 0.2 % citral in ethyl acetate. (Citral is used as analyte protectant.)~~
~~The residues are determined by gas liquid chromatography with mass selective detection (GC-MSD) in the select ion monitoring (SIM) mode using a CP-8 CB column (5 % phenyl poly-dimethylsiloxane). The reported results are based on m/z 612 for glyphosate and m/z 446 for AMPA. Fragment ions m/z 611 and 584 are used as qualifier ions for glyphosate and m/z 372 and 502 are used as qualifiers for AMPA.~~

~~**Findings:**~~
~~The validated limit of quantification is 0.1 mg/kg. Confirmatory transitions are validated with the same LOQ.~~
~~Blank values are not reported but chromatograms of control samples demonstrate that the blanks are distinctly below 30 % of LOQ. Linear calibration is performed for both analytes (glyphosate and AMPA) in the range 0.75 – 30 ng/mL using standards in matrix at 5 – 6 levels. The calibrated range corresponds to 0.050 – 2.00 mg/kg.~~

~~**Conclusion:**~~
~~The validated limit of quantification is not sufficient to control all regulated MRLs for fat, liver and kidney. In addition, the method does not allow the determination of the second component (N-acetylglyphosate) of the proposed residue definition for enforcement.~~

Not accepted study 5:

Reference:	IIA, 4.3/12
Report:	Eversfield, S. (2007), Independent laboratory validation of a method (RAM 308/01) for the determination of glyphosate (ASF 71) and AMPA in milk, liver and egg. CEM Analytical Services Ltd., North Ascot, UK Report No. CEMR-3532 (Syngenta File No. R61837/1112) Date 03.09.2007, not published, ASB2012-12417
Guidelines:	EPA OPPTS 860.1340, SANCO/3029/99 Rev. 4, SANCO/825/00 Rev. 7
Deviations:	~~No~~ yes
GLP:	yes
Acceptability:	The study is considered to be not acceptable. ~~because of deficiencies during calibration.~~

Findings and conclusion:
This study uses the modified Alferness method without stable isotope labelled internal standards. Glyphosate and AMPA in purified extracts are derivatised starting at – 60 °C in a mixture of trifluoroacetic anhydride and heptafluorobutanol. The amount of residues is determined by gas liquid chromatography with mass selective detection.
The reported calibration for glyphosate covers the extreme wide range of three orders of magnitude. The reported intercept of calibration corresponds to 0.013 mg/kg for milk, 0.018 mg/kg for egg and 0.076 mg/kg for liver. Based on this calibration, it was not possible

to accept validation data at 0.01 mg/kg level. In addition, the method does not allow the determination of the second component (N-acetyl-glyphosate) of the proposed residue definition for enforcement. The efficiency of the derivatisation step was not demonstrated as required in accordance to SANCO/825/00 rev. 8.1. Therefore, the extent of validation of the method is not sufficient.

~~**Materials and Methods:**~~
~~Test material: animal fat, liver and kidney~~

~~Fortified analyte(s): glyphosate (N-(phosphonomethyl) glycine) and AMPA (aminomethylphosphonic acid)~~

~~Analyte(s) determined as:~~
~~Glyphosate as N-([Bis-(2,2,3,3,4,4,4-heptafluorobutoxy)-phosphinyl]methyl)-N-(trifluoracetyl)-glycine,2,2,3,3,4,4,4-heptafluorobutotyl; $C_{17}H_{10}F_{24}O_6NP$, molar mass = 811 g/mol~~
~~AMPA as trifluoroacetylamino-methylphosphonic acid, bis(2,2,3,3,4,4,4-heptafluorobutyl ester; $C_{11}H_7F_{14}NO_4P$ $C_{11}H_7F_{17}NO_4P$, molar mass = 571 g/mol~~

~~Principle of the method: See study of Clarke and Robinson, 1998, IIA 4.3/09.~~

~~**Findings:**~~
~~The reported calibration for glyphosate covers the extreme wide range of three orders of magnitude. The reported intercept of calibration corresponds to 0.013 mg/kg for milk, 0.018 mg/kg for egg and – 0.076 mg/kg for liver. Based on such calibration a limit of quantification was not derived.~~

~~**Conclusion:**~~
~~It cannot be excluded that the reported recovery results for all matrices are strongly influenced by invalid calibration. Therefore, the independent laboratory validation of a method (RAM 308/01 by Eversfield (2007, ASB2012-12417) is not acceptable. In addition, the method does not allow the determination of the second component (N-acetyl-glyphosate) of the proposed residue definition for enforcement.~~

Not accepted study 6:

Reference:	IIA, 4.3
Report:	Garrigue, P. (2014) Glyphosate - Additional validation of the analytical method RAM 308/01 for the determination of Glyphosate (N-(phosphonomethyl)glycine (PMG)) and Aminomethyl phosphonic acid (AMPA) in fat, eggs and milk by GC-MSD Report No. S13-04833 ! TK0205528 Date 21.05.2014, not published, ASB2014-9489
Guidelines:	EPA OPPTS 860.1340, SANCO/3029/99 Rev. 4, SANCO/825/00 Rev. 8.1
Deviations:	yes
GLP:	yes
Acceptability:	The study is considered to be not acceptable.

Findings and conclusion:
This study uses the modified Alferness method without stable isotope labelled internal standards. Glyphosate and AMPA in purified extracts are derivatised starting at – 60 °C in a mixture of trifluoroacetic anhydride and heptafluorobutanol. The amount of residues is determined by gas liquid chromatography with mass selective detection.
The efficiency of the derivatisation step was not demonstrated as required in accordance to SANCO/825/00 rev. 8.1. Therefore, the extent of validation of the method is not sufficient.

B.5.2.3 Extractability of Residues

B.5.2.3.1 Extraction efficiency of methods for plant material

The analytical methods for monitoring in plant material are using either water, 0.1 N HCl in water, 6 N HCl in water or 0.1% aqueous formic acid in methanol (96+4, v/v) as extraction solvent. The proposed enforcement method applicable for all parts of residue definition uses 0.1 % aqueous formic acid in methanol (96+4, v/v).
Details on the efficiency of different solvents to extract incurred residues from tolerant crops measured as extracted part of the total radioactive residue (TRR) are outlined in section B.7.1 and summarised in Table B.5.2-43.

Table B.5.2-43: Extractability of the total residue with solvents used in monitoring methods

Reference	Extraction solvent	Matrix	Extractable residue [% TRR (mg/kg)]
Goure, 1994 (RIP9800117)	water	soya bean forage[1] soya bean hay[1] soya bean seeds[1]	104 (24.62) 77 (8.02) 88 (15.38)
Bleeke, 1997 (RIP9700619)	water	cotton forage[2] cotton seeds[1]	97 (14.70) 19 (0.034)
Mehrsheikh, 2000 (RIP2001-906)	water	sugar beet tops[1] sugar beet roots[1]	87 (2.98) 103 (1.44)
George, 1995 (RIP9700618)	water	corn forage[1] corn silage[1] corn fodder[1] corn grain[1]	91 (9.8) 96 (8.20) 93 (17.70) 95 (1.00)
Chapleo, McLachlan, 2010 (ASB2011-13744)	0.1 % aqueous formic acid in methanol (96+4, v/v)	canola foliage[3] canola immature pods[3] canola mature seeds[3]	97 (5.82) 80 (1.01) 97 (2.08)
Green, 2007 (ASB2008-2657)	0.1 % aqueous formic acid in methanol (96+4, v/v)	corn forage[3] corn stover[3] cobs[3] grain[3]	87 (3.20) 85 (10.41) 69 (0.475) 71 (0.195)
MacDonald, 2007 (ASB2008-2658)	0.1 % aqueous formic acid in methanol (96+4, v/v)	soya bean forage[4] soya bean hay[3] soya bean grain[3] soya bean pods[3] soya bean foliage[3]	29 (0.123) 96 (12.89) 88 (2.77) 88 (15.64) 88 (19.48)

[1]) two post-emergence applications
[2]) one post-emergence application
[3]) one pre-emergence soil application and 1 – 3 foliar applications
[4]) single pre-emergence soil application; exposed to soil residues only

In most cases the results show an extraction of more than 80 % of TRR with both solvents. The low efficiency of water to extract residues from cotton seeds seems less important due to the very low residue level.
Glyphosate and N-acetyl-glyphosate are identified as the major residue components in stover, cobs, silage, and forage from glyphosate tolerant corn (George, 1995, RIP9700618 and Green, 2007, ASB2008-2657). Also in soya bean grain, soya bean foliage, soya bean hay, soya bean pods and cotton forage glyphosate and N-acetyl-glyphosate (plus AMPA in soya bean grain) represent the largest part of total residue (Goure, 1994; RIP9800117, Bleeke, 1997, RIP9700619 and MacDonald, 2007, ASB2008-2658). In sugar beet tops glyphosate alone formed the mayor residue (Mehrsheikh, 2000, RIP2001-906) and in foliage, immature pods and mature seeds of tolerant canola 51 – 93 % of TRR were identified as N-acetyl-glyphosate (Chapleo, McLachlan, 2010, ASB2011-13744). Therefore; in these matrices the non-extractable part of the total radioactive residue (≤ 20 %) has not to be considered.
In soya bean forage after one pre-emergence application 71 % of TRR are not extracted with 0.1% aqueous formic acid in methanol (MacDonald, 2007, ASB2008-2658). Consequently, the consideration of additional extraction solvents was necessary. The results presented in Table B.5.2-44 show that the majority (57.1 %) of radioactivity was extracted during this exhaustive extraction. In total, 0.048 mg/kg glyphosate and N-acetyl-glyphosate had been identified in the several extracts. Since 0.036 mg/kg (i.e. 75 %) were extracted with 0.1 % aqueous formic acid in methanol (the solvent used in the proposed monitoring method), the extraction efficiency is considered acceptable also in this case.

Table B.5.2-44: Percentage of total glyphosate / N-acetyl-glyphosate extracted from soya bean forage with different solvents (MacDonald, 2007, ASB2008-2658)

Extraction solvent	% of TRR detected in extract	Glyphosate extracted [% of TRR (mg/kg)]	N-acetylglyphosate extracted [% of TRR (mg/kg)]	Sum of both [% of TRR (mg/kg)]
0.1% aqueous formic acid in methanol (96+4, v/v)	28.7	7.6 (0.033)	0.6 (0.003)	8.2 (0.036)
alpha-amylase	14.8	1.5 (0.006)	-	1.5 (0.006)
amyloglucosidase and cellulase	6.0	-	-	-
0.1 N NaOH	4.4	-	1.3 (0.006)	1.3 (0.006)
0.1 N HCl	3.2	-	-	-
total extracted	57.1	9.1 (0.039)	1.9 (0.009)	11.0 (0.048)

B.5.2.3.2 Extraction efficiency of methods for animal material

Glyphosate and its metabolite N-acetyl-glyphosate are the most important components of the total residue in feeding stuffs. If both compounds are administered to lactating goats, relevant amounts of residues were found in kidney and liver. Both compounds remained largely unchanged in tissues (see sections B.7.2 and B.7.8).
The overall extraction efficiency of pure water or 0.1 N aqueous HCl as extraction solvent for tissues of animals treated with glyphosate was tested in the metabolism studies of Patanella and Feng (1988, RIP9501204) and Powles (1994, RIP9501207). For animals treated with N-acetyl-glyphosate the overall extraction efficiency was tested by Lowrie (2007, ASB2008-2660). More details of all three studies are presented in section B.7.2. The high percentage of the total radioactive residue (TRR) extracted with these solvents is summarised in Table B.5.2-45.

Table B.5.2-45: Extractability of the total residue with solvents used in monitoring methods

Reference	Animals treated with	Extraction solvent	Matrix	Extractable residue (ER) [% TRR)]
Pantanella and Feng, 1988 (RIP9501204)	glyphosate	water	kidney liver	99 98
Powles, 1994 (RIP9501207)	glyphosate	0.1 N aqueous HCl	kidney liver	96 97
Lowrie, 2007 (ASB2008-2660)	N-acetylglyphosate	0.2 N aqueous HCl	kidney liver	83 97

Significant amounts of the total radioactive were neither found in other extracts, nor remained unextracted. Therefore, data on the individual extraction efficiency of glyphosate and N-acetyl-glyphosate are not needed.
Similar complete extraction of radioactivity with 0.1 – 0.2 N aqueous HCl or water as extraction solvents was noticed in metabolism study with laying hens after administration of glyphosate and N-acetyl-glyphosate (see section B.7.2).
The extraction solvent of the method proposed by Pentz and Bramble (2007, ASB2008-2636) was 0.1 % aqueous formic acid in methanol (96+4, v/v). In method 2, diluted acetic acid was used. Both solvents were never tested in animal metabolism studies. However, based on the nearly complete extraction of glyphosate residues with either water or diluted HCl a good extraction efficiency of these solvents can be assumed, too. Information on the extraction efficiency of 0.2 N NaOH used in method 3 does not exist.

B.5.3 Analytical methods (residue) soil, water, air (Annex IIA 4.4 to 4.7; Annex IIIA 5.4, 5.6 to 5.7)

B.5.3.1 Analytical methods (residue) for soil

B.5.3.1.1 Acceptable methods/reports

Method 1

Reference: IIA 4.4/02
Report: Schneider, V. (2001)
Validation of an analytical method for the determination of glyphosate soil
UCL GmbH, Cologne, Germany
Report no.: PR01/006
Date 29.08.2001, not published, MET2005-371
Guidelines: SANCO/825/00 Rev. 6
Deviations: No
GLP: yes
Acceptability: The study is considered to be acceptable.

Materials and Methods:
Test material: soil, Standard soil 2.2, Batch F221201 (loamy sand)
For details see http://www.lufa-speyer.de/images/stories/bodanalyse.pdf

Fortified analyte(s): glyphosate (N-(phosphonomethyl) glycine) and AMPA (amino-methylphosphonic acid)

Analyte(s) determined as:
Glyphosate as N-([Bis-(2,2,2-trifluoroethoxy)-phosphinyl] methyl)-N-(trifluor¬acetyl)-glycine,2,2,2-trifluoroethotyl; $C_{11}H_{10}F_{12}O_6NP$, molar mass = 511 g/mol
AMPA as trifluoroacetylamino-methylphosphonic acid, bis(2,2,2-trifluoroethyl ester; $C_6H_7F_9NO_4P$, molar mass = 371 g/mol

Principle of the method: After addition of internal standards, the soil is sonicated in about 1 % aqueous NaOH solution (ca. 0.2 N). After centrifugation the supernatant is loaded on an anion exchange column (Dowex AG1X8-100), which is conditioned with 1 % ammonium hydroxide. The column is washed first with 1 % ammonium hydroxide and than with water. The washing solution is discarded. Residues of glyphosate and AMPA are eluted with 1 N sodium hydroxide solution. After addition of charcoal, the eluate is heated to 100 °C. After cooling the solution is centrifuged and the supernatant is evaporated to dryness.
To the dry residue phosphoric acid and trifluoroacetic acid are added. Re-dissolved residues of glyphosate and AMPA are derivatised with a mixture of trifluoroacetic anhydride and trifluoroethanol (2+1, V/V). The derivatisation mixture is heated to about 70 °C and derivatisation is finalised within one hour. After derivatization, excess reagents and volatile by-products are removed by rotary evaporation. The concentrated remainder is partitioned between dichloromethane and 5 % aqueous ammonium hydroxide solution. The dichloromethane phase containing the derivatives is dried, filtered and evaporated. The remainder is reconstituted in toluene.
The residues are determined by gas liquid chromatography with mass selective detection (GC-MSD) in the select ion monitoring (SIM) mode using a CP-Sil 19 column (14 % cyanopropylphenyl/ 86 % polydimethylsiloxane). The reported results are based on m/z 411 for glyphosate and m/z 302 for AMPA. For internal standards m/z 412 and m/z 303 are used for ^{13}C-glyphosate and ^{15}N-AMPA, respectively.

Findings:
The validated limit of quantification is 0.05 mg/kg. For glyphosate all blank values are below 30 % of LOQ, but not for AMPA (one control sample has shown an artifact peak corresponding to 0.025 mg/kg).
Quadratic calibration is performed for both analytes (glyphosate and AMPA) in all matrices in the range 0.043 – 0.65 mg/kg based on stable isotope labelled standards, i.e. it does not start from 30 % of LOQ. Confirmatory transitions are not validated. Information on calibration is reported in Table B.5.3-1. For validation data see Tables B.5.3-2 and B.5.3-3.

Table B.5.3-1: Calibration data of the GC-MS method for residues of glyphosate and AMPA in soil (Schneider, 2001, MET2005-371)

Calibration parameter	Results of evaluation
Accepted calibration range in concentration units	2 – 30 µg/mL (25 µg/mL ISTD)
Accepted calibration range in mass fraction units	0.043 – 0.65 mg/kg
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)	yes
Where matrix effects investigated?	not required (use of stable-isotope labelled standards)
The absence of interference >30 % of LOQ in blank samples was demonstrated?	yes (except for one AMPA control sample)

Calibration parameter	Results of evaluation
Chromatogram of sample provided spiked at LOQ demonstrating sufficient sensitivity and signal-to-noise ratio?	yes

Conclusion:
The study of Schneider (2001, MET2005-371) describes a successfully validated method for soil. Because of several cleanup steps (anion exchange chromatography, adsorption on charcoal, partition) two evaporations to dryness and a derivatisation, the practical relevance of this monitoring method will be limited. However from formal reasons (requirements of guidance document SANCO/825/00 rev 8.1) the method has to be accepted.

Table B.5.3-2: Validation of the GC-MS method for residues of glyphosate in soil (Schneider, 2001, MET2005-371)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Schneider, 2001b (MET2005-371)	GC-MS, m/z 411	Loamy sand	0.05	105	3.2	5
		Loamy sand	0.5	109	0.4	5

Table B.5.3-3: Validation of the GC-MS method for residues of AMPA in soil (Schneider, 2001, MET2005-371)

Reference	Detection method	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Schneider, 2001b (MET2005-371)	GC-MS, m/z 302	Loamy sand	0.05	104	3.2	5
		Loamy sand	0.5	108	5.1	5

~~**Method 2 (Confirmatory method)**~~

~~**Reference:**~~ ~~no (study was not provided with the dossier)~~
~~**Report:**~~ ~~Szuter, S.L. (1996)~~
~~Glyphosate Acid: Independent laboratory lalidation of the method for determining residues of N-(Phosphonomethyl)glycine and (Aminomethyl)phosphonic acid in soil (WRC-96-082) (WINO 23013)~~
~~Study number ZPMG-96-MT-01, Report number RR 96-059B~~
~~Date 24.06.1996, not published, MET2000-699~~
~~**Guidelines:**~~ ~~no~~
~~**Deviations:**~~
~~**GLP:**~~ ~~yes~~
~~**Acceptability:**~~ ~~The study is considered to be acceptable.~~

~~**Materials and Methods:**~~
~~Test material: The soil was representing 0 – 6 inch depth. Further specification not given. Soil samples are reminders of Zeneca's storage stability study ZPMG-95-SS-01.~~

~~Fortified analyte(s): glyphosate (N-(phosphonomethyl) glycine) and AMPA (aminomethylphosphonic acid)~~

~~Analyte(s) determined as:~~
~~Glyphosate as N-([Bis-(2,2,3,3,4,4,4-heptafluorobutoxy)-phosphinyl]methyl)-N-(trifluoracetyl)-glycine,2,2,3,3,4,4,4-heptafluorobutotyl; $C_{17}H_{10}F_{24}O_6NP$, molar mass = 811 g/mol~~
~~AMPA as trifluoroacetylamino-methylphosphonic acid, bis(2,2,3,3,4,4,4-heptafluorobutyl ester; $C_{11}H_7F_{14}NO_4P$ $C_{11}H_7F_{17}NO_4P$, molar mass = 571 g/mol~~

~~Principle of the method: The soil is extracted with a solution of 0.25 mol/L ammonium hydroxide and 0.1 mol/L KH_2PO_4 by shaking. After centrifugation, the supernatant is cleaned by addition of activated carbon. Glyphosate and AMPA in a filtrated aliquot of this solution are derivatised starting at 50 – 70 °C in a mixture of trifluoroacetic anhydride and heptafluorobutanol. The derivatisation mixture is slowly heated to about 95 °C and derivatisation is finalised within one hour. After derivatisation, excess reagents and volatile by-products are evaporated and the residuum is dissolved in a solution of 0.2 % citral in ethyl acetate. (Citral is used as analyte protectant.) Sample concentration in final extracts is 0.02 g/mL at LOQ and 0.01 g/mL at 10 x LOQ.~~
~~The residues are determined by gas liquid chromatography with mass selective detection (GC-MSD) in the select ion monitoring (SIM) mode using a XTI-5 column (5 % diphenyl polydimethylsiloxane). The reported results are based on m/z 611 for glyphosate and m/z 446 for AMPA and quantified against external standards.~~

~~**Findings:**~~
~~The lowest spiked level was 0.025 mg/kg. For glyphosate and AMPA all blank values were below 30 % of LOQ. The linearity of the calibration graph was shown for both analytes (glyphosate and AMPA) in the range 0.7 – 8 ng/mL. This corresponds to 0.035 – 0.8 mg/kg in soil. Information on calibration is reported in Table B.5.3-4. For validation data see Tables B.5.3-5 and B.5.3-6.~~

~~**Conclusion:**~~
~~Do to missing calibration data below 0.035 mg/kg the lowest validated level is not accepted as LOQ and the next validated level of 0.05 mg/kg is considered as limit of quantification.~~
~~The study of Szuter (1996, MET2000-699) describes a successfully validated method for soil. Because of the derivatization used, the practical relevance of this monitoring method will be limited. However from formal reasons (requirements of guidance document SANCO/825/00 rev 8.1) the method has to be accepted.~~

~~**Table B.5.3-4: Calibration data of the GC-MS method for residues of glyphosate and AMPA in soil (Szuter, 1996, MET2000-699)**~~

~~**Calibration parameter**~~	~~**Results of evaluation**~~
~~Accepted calibration range in concentration units~~	~~0.7 – 8 ng/mL~~
~~Accepted calibration range in mass fraction units~~	~~0.035 – 0.4 mg/kg (at LOQ)~~ ~~0.070 – 0.8 mg/kg (at 10 x LOQ)~~
~~Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)~~	~~yes~~
~~Where matrix effects investigated?~~	~~no~~
~~The absence of interference >30 % of LOQ in blank samples was demonstrated?~~	~~yes~~
~~Chromatogram of sample provided spiked at LOQ demonstrating sufficient sensitivity and signal-to-noise ratio?~~	~~yes~~

~~Table B.5.3-5: Validation of the GC-MS method for residues of glyphosate in soil (Szuter, 1996, MET2000-699)~~

~~Reference~~	~~Detection method~~	~~Matrix~~	~~Fortification level [mg/kg]~~	~~Average recovery [%]~~	~~RSD [%]~~	~~No. of analyses~~
~~Szuter, 1996 (MET2000-699)~~	~~GC-MS, m/z 611~~	~~soil~~	~~0.025~~	~~80~~	~~9~~	~~5~~
		~~soil~~	~~0.05~~	~~84~~	~~14~~	~~5~~
		~~soil~~	~~0.5~~	~~80~~	~~10~~	~~5~~

~~Table B.5.3-6: Validation of the GC-MS method for residues of AMPA in soil (Szuter, 1996, MET2000-699)~~

~~Reference~~	~~Detection method~~	~~Matrix~~	~~Fortification level [mg/kg]~~	~~Average recovery [%]~~	~~RSD [%]~~	~~No. of analyses~~
~~Szuter, 1996 (MET2000-699)~~	~~GC-MS, m/z 411~~	~~soil~~	~~0.025~~	~~110~~	~~9~~	~~5~~
		~~soil~~	~~0.05~~	~~89~~	~~4~~	~~5~~
		~~soil~~	~~0.5~~	~~78~~	~~7~~	~~5~~

B.5.3.1.2 Studies which do not fulfill the requirements

Not accepted study 1:

Reference: IIA, 4.4/01
Report: Alferness, P.L. (1994)
TOUCHDOWN®: Determination of glyphosate and aminomethylphosphonic acid in soil by gas chromatography and mass-selective detection.
Zeneca Ag Products, Western Research Center, Environmental Chemistry Section, Richmond, CA, USA
Report No. RR 92-010B
Date 21.01.1994, not published, MET9500060
Guidelines: Guideline § 171-4 (C)
Deviations: No
GLP: yes
Acceptability: The study is considered to be not acceptable~~because of an insufficient number of validation experiments~~.

Findings and conclusion:
This study uses the Alferness method without stable isotope labelled internal standards. Glyphosate and AMPA in purified extracts are derivatised starting at – 60 °C in a mixture of trifluoroacetic anhydride and heptafluorobutanol. The amount of residues is determined by gas liquid chromatography with mass selective detection.
The number of recovery experiments at each level is not sufficient to accept this study. The efficiency of the derivatisation step was not demonstrated as required in accordance to SANCO/825/00 rev. 8.1. Therefore, the extent of validation of the method is not sufficient.

~~Materials and Methods:~~
~~Test material: soil (sandy loam, characterised)~~

~~Fortified analyte(s): glyphosate (N-(phosphonomethyl) glycine) and AMPA (amino-methylphosphonic acid)~~

~~Analyte(s) determined as:~~
~~Glyphosate as N-([Bis-(2,2,3,3,4,4,4-heptafluorobutoxy)-phosphinyl]methyl)-N-(trifluor-acetyl)-glycine,2,2,3,3,4,4,4-heptafluorobutotyl; $C_{17}H_{10}F_{24}O_6NP$, molar mass = 811 g/mol~~
~~AMPA as trifluoroacetylamino-methylphosphonic acid, bis(2,2,3,3,4,4,4-heptafluorobutyl ester; $C_{11}H_7F_{14}NO_4P$ $C_{11}H_7F_{17}NO_4P$, molar mass = 571 g/mol~~

~~Principle of the method: Samples are shaken with an aqueous solution containing 0.25 M NH_4OH/0.1 M KH_2PO_4. After centrifugation the supernatant is filtered. Glyphosate and AMPA in the filtered extracts are derivatised starting at – 60 °C in a mixture of trifluoroacetic anhydride and heptafluorobutanol. The derivatisation mixture is slowly heated to about 95 °C and derivatisation is finalised within one hour. After derivatisation, excess reagents and volatile by-products are evaporated and the residuum is dissolved in a solution of 0.8 % citral (V/V) in ethyl acetate. Citral is used as analyte protectant. Sample concentration in final extracts is 0.02 g/mL.~~
~~The residues are determined by gas liquid chromatography with mass selective detection (GC-MSD) in the select ion monitoring (SIM) mode using a J&W DB-5 column (5 % phenyl polydimethylsiloxane). The reported results are based on m/z 611 for glyphosate and m/z 446 for AMPA. Fragment ions m/z 611 and 584 are used as qualifier ions for glyphosate and m/z 372 and 502 are proposed as qualifiers for AMPA.~~

~~**Findings:**~~
~~The method is validated with three replicates each at two levels, only.~~
~~Blank values are not reported but chromatograms of control samples demonstrate that the blanks are distinctly below 30 % of LOQ. Linear calibration is performed for both analytes (glyphosate and AMPA) in the range 0.5 – 20 ng/mL using standards in solvent at 5 levels. The calibrated range corresponds to 0.025 – 1.00 mg/kg. For validation data see Tables B.5.3-7 and B.5.3-8.~~

~~**Conclusion:**~~
~~The number of recovery experiments at each level is not sufficient to accept this study. In addition, it is mentioned that the liner and the first part of GC capillary column have to be replaced after 20 – 200 injections, which is questionable for a robust monitoring method.~~
~~**The efficiency of the derivatisation step was not demonstrated as required in accordance to SANCO/825/00 rev. 8.1. Therefore, the extent of validation of the method is not sufficient.**~~

~~**Table B.5.3-7: Validation of the GC-MS method for residues of glyphosate in soil (Alferness, 1994, MET9500060)**~~

~~**Reference**~~	~~**Detection method**~~	~~**Matrix**~~	~~**Fortification level [mg/kg]**~~	~~**Average recovery [%]**~~	~~**RSD [%]**~~	~~**No. of analyses**~~
~~Alferness, 1994 (MET9500060)~~	~~GC-MS, m/z 611~~	~~Sandy loam~~	~~0.05~~	~~95~~	~~4.8~~	~~3~~
		~~Sandy loam~~	~~0.5~~	~~95~~	~~8.0~~	~~3~~

~~**Table B.5.3-8: Validation of the GC-MS method for residues of AMPA in soil (Alferness, 1994, MET9500060)**~~

~~Reference~~	~~Detection method~~	~~Matrix~~	~~Fortification level [mg/kg]~~	~~Average recovery [%]~~	~~RSD [%]~~	~~No. of analyses~~
~~Alferness, 1994 (MET9500060)~~	~~GC-MS, m/z 446~~	~~Sandy loam~~	~~0.05~~	~~94~~	~~2.7~~	~~3~~
		~~Sandy loam~~	~~0.5~~	~~102~~	~~6.3~~	~~3~~

Not accepted study 2:

Reference: IIA 4.4
Report: Schulz, H. (1995)
Summary report: compilation of glyphosate and AMPA analytical methods used for analysis of soil samples from field soil dissipation experiments in Germany and Switzerland conducted in 1990 and 1991.
Institut Fresenius, D-65232 Taunusstein-Neuhof
Report No.: IF -95/11387-00
Date 23.08.1995, not published, MET9600121
Guidelines: Except from GLP, other guidelines applied not mentioned
Deviations:
GLP: yes
Acceptability: The study is considered to be not acceptable for several reasons.

Findings and conclusion:
The chromatogram of an untreated sandy clay soil sample shows a significant peak corresponding to 0.02 mg/kg glyphosate. In a treated sandy loam soil sample a peak corresponding to > 0.4 mg/kg occurs very near to the glyphosate peak, which is not observed in the blank (untreated) sample.
The chromatogram reported for a clay loam sample fortified with 0.02 mg/kg AMPA does not show any acceptable peak, nevertheless a good recovery is reported. A chromatogram obtained at the lowest fortification level of glyphosate (0.02 mg/kg) is not reported. At the lowest level, the RSD is > 25 % for glyphosate.
Based on the reported chromatograms there are doubts about the sensitivity and selectivity of the method. The repeatability is not sufficient for glyphosate at the required LOQ of 0.05 mg/kg.

~~**Materials and Methods:**~~
~~Test material: four different soils (sandy clay, clay loam, sandy loam, sandy soil)~~

~~Fortified analyte(s): glyphosate (N-(phosphonomethyl) glycine) and AMPA (aminomethylphosphonic acid)~~

~~Analyte(s) determined as:~~
~~Glyphosate is determined after oxidation to glycine as fluorescent isoindole derivative prepared postcolumn after reaction with ortho-phthalaldehyde and 2-mercaptoethanol.~~
~~AMPA is determined as fluorescent isoindole derivative prepared postcolumn after reaction with ortho-phthalaldehyde and 2-mercaptoethanol.~~

~~Principle of the method: The method used is based on DFG method No. 405. Extraction is conducted by shaking of soil samples with 1 mol/L ammonium hydroxide solution. After centrifugation the entire extract is adjusted to pH 2 with hydrochloric acid and diluted with water. This diluted extract is loaded on a column containing a ligand exchange resin (Chelex 100, pre-conditioned with Fe (III) ions). After washing steps with water and 0.2 N HCl, residues of glyphosate and AMPA are eluted from the column with 6 N HCl. Co-eluted Fe (III) ions are separated by filtration through a column containing an anion exchange resin (AG 1-X8). The filtrate is evaporated to dryness to remove hydrochloric acid and reconstituted with 0.001 mol/L EDTA solution. Sample concentration in final extracts is 2.5 g/mL. Samples with residue levels above the calibrated range are diluted with 0.001 mol/L EDTA solution.~~
~~Glyphosate and AMPA were quantified by HPLC equipped with a cation exchange column (Bio-Rad Aminex A-9, loaded with potassium), post derivatisation unit and a fluorescence detector (excitation 330 nm, emission 445 nm). For determination an isocratic elution with 0.05 mol/L KH_2PO_4 in 0.01 mol/L H_3PO_4 and a post-column conversion of glyphosate to glycine are used. Glycine and AMPA are derivatised with ortho-phthaldialdehyde and mercaptoethanol to produce fluorescent derivatives.~~

~~**Findings:**~~
~~The chromatogram of an untreated sandy clay soil sample shows a significant peak corresponding to 0.02 mg/kg glyphosate. (This is based on the assumption of a dilution factor of 10 of the treated sample containing 2.065 mg/kg. Unfortunately, dilution factors are not reported.) In a treated sandy loam soil sample a peak corresponding to > 0.4 mg/kg occurs very near to the glyphosate peak, which is not observed in the blank (untreated) sample.~~
~~The chromatogram reported for a clay loam sample fortified with 0.02 mg/kg AMPA does not show any acceptable peak, nevertheless a good recovery is reported. A chromatogram obtained at the lowest fortification level of glyphosate (0.02 mg/kg) is not reported. At the lowest level, the RSD is > 25 % for glyphosate.~~

~~**Conclusion:**~~
~~Based on the reported chromatograms there are some doubts about the sensitivity and selectivity of the method. The repeatability is not sufficient for glyphosate at the required LOQ of 0.05 mg/kg.~~

Not accepted study 3:

Reference:	no (study was not provided with the dossier)
Report:	Szuter, S.L. (1996) Glyphosate Acid: Independent laboratory lalidation of the method for determining residues of N-(Phosphonomethyl)glycine and (Aminomethyl)phosphonic acid in soil (WRC-96-082) (WINO 23013) Study number ZPMG-96-MT-01, Report number RR 96-059B Date 24.06.1996, not published, MET2000-699
Guidelines:	no
Deviations:	
GLP:	yes
Acceptability:	The study is considered to be not acceptable.

Findings and conclusion:
This study uses the Alferness method without stable isotope labelled internal standards. Glyphosate and AMPA in purified extracts are derivatised starting at – 60 °C in a mixture of trifluoroacetic anhydride and heptafluorobutanol. The amount of residues is determined by gas liquid chromatography with mass selective detection.
The efficiency of the derivatisation step was not demonstrated as required in accordance to SANCO/825/00 rev. 8.1. Therefore, the extent of validation of the method is not sufficient.

B.5.3.2 Analytical methods (residue) for water

Reference:	IIA, 4.5/01
Report:	Knoch, E. (2010) Validation of an analytical method: Determination of glyphosate and AMPA in water matrices using FMOC derivatisation, manual SPE cleanup and LC-MS/MS quantitation. SGS INSTITUT FRESENIUS GmbH, Taunusstein, Germany Report No. IF-10/01618859 Date 14.10.2010, not published, ASB2012-12445
Guidelines:	SANCO/825/00 Rev. 7
Deviations:	No
GLP:	yes
Acceptability:	The study is considered to be acceptable.

Materials and Methods:
Test material: drinking water: tap water from D-65232 Taunusstein; TOC 1.33 g/mL, pH 7.43, conductivity 579 µs/cm
Surface water: brook „Burbach“ near D-35716 Dietzhoelztal; TOC 2.18 g/mL, pH 7.66, conductivity 94 µs/cm
Ground water: TOC 0.30 g/mL, pH 6.58, conductivity 28 µs/cm

Fortified analyte(s): glyphosate (N-(phosphonomethyl) glycine) and AMPA (aminomethylphosphonic acid)

Analyte(s) determined as: FMOC derivatives of glyphosate ($C_{18}H_{18}NO_7P$, molar mass 391 g/mol) and AMPA ($C_{16}H_{16}NO_5P$, molar mass 333 g/mol)

Principle of the method: The method does not require extraction of residues. 0.1 mg sodium thiosulfate is added to each mL of drinking water sample. Samples of surface or ground water are used without addition of sodium thiosulfate. To these samples internal standards ($^{13}C_2$,^{15}N-glyphosate ^{13}C,^{15}N-AMPA) and an aqueous borate/EDTA buffer (pH 8.5) are added. After mixing and addition 10 mmol/L 9-Fluorenylmethylchloroformate (FMOC-Cl) in acetonitrile samples are heated to 40 °C for 1 hour. The solution is allowed to cool to ambient temperature and acidified with 2 % phosphoric acid. The acidified solution is diluted with water and the FMOC derivatives of glyphosate and AMPA are extracted by SPE using a Strata X (polymeric reverse phase) cartridge. The cartridge is dried by centrifugation and rinsed with dichloromethane. After drying, the derivatised analytes are eluted with methanol. The eluate is evaporated to a small remainder and diluted with ammonium acetate solution. The sample concentration in final extracts is 20 mL /mL
Derivatised residues of glyphosate and AMPA are determined by liquid chromatography with tandem mass spectrometer (LC-MS/MS) in negative multiple reaction monitoring (MRM)

mode using a Synergy Fusion RP column with gradient elution using ammonium acetate buffer (pH7.4) and methanol. Quantification is based on the added internal standards. For each analyte two transitions are monitored (FMOC-glyphosate: m/z 390→168, m/z 390→150, FMOC-$^{13}C_2$,^{15}N-glyphosate: m/z 393→171, m/z 393→153, FMOC-AMPA: m/z 332→110, m/z 332→136, FMOC-^{13}C,^{15}N-AMPA: m/z 334→112, m/z 334→138).

Findings:
The validated limit of quantification for glyphosate and AMPA is 0.03 µg/L. In all control samples the intensity of signals at the retention time of glyphosate and AMPA was below 30 % LOQ. Acceptable chromatograms from derivatised standard solutions, samples and blank materials, as well as adequate recovery data and information on the precision of the method are presented.
Linear calibration is performed for both analytes (glyphosate and AMPA) in a range corresponding to 0.01 – 0.5 µg/L using standards in solvent at eight levels. Correlation coefficients of calibration graphs are > 0.9998. Information on calibration is reported in Table B.5.3-12. For validation data see Tables B.5.2-13 to B.5.2-16.

Conclusion:
The study of Knoch (2010, ASB2012-12445) describes a simple, fully validated and highly specific method for the determination of residues of glyphosate and AMPA in water samples. The proposed method includes a fully validated method for confirmation of results based on a second MS/MS transition for each analyte.

Table B.5.3-9: Calibration data of the proposed LC-MS/MS method for derivatised residues of glyphosate and AMPA in water (Knoch, 2010, ASB2012-12445)

Calibration parameter	Results of evaluation
Accepted calibration range in concentration units	0.2 – 10 ng/mL
Accepted calibration range in mass fraction units	0.01 – 0.50 µg/L
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)	yes
Where matrix effects investigated?	no
The absence of interference >30 % of LOQ in blank samples was demonstrated?	yes
Chromatogram of sample provided spiked at LOQ demonstrating sufficient sensitivity and signal-to-noise ratio?	yes

Table B.5.3-10: Validation of the primary LC-MS/MS method for derivatised residues of glyphosate in water (Knoch, 2010, ASB2012-12445)

Reference	Detection method	Matrix	Fortification level [µg/L]	Average recovery [%]	RSD [%]	No. of analyses
Knoch, 2010 (ASB2012-12445)	LC-MS/MS, Synergy Fusion RP column, m/z 390→168	surface water	0.03	100	4.1	5
			0.30	99	2.1	5
		drinking water	0.03	98	2.3	5
			0.30	97	0.8	5
		ground water	0.03	98	2.2	5
			0.30	98	2.3	5

Table B.5.3-11: Validation of the primary LC-MS/MS method for derivatised residues of AMPA in water (Knoch, 2010, ASB2012-12445)

Reference	Detection method	Matrix	Fortification level [µg/L]	Average recovery [%]	RSD [%]	No. of analyses
Knoch, 2010 (ASB2012-12445)	LC-MS/MS, Synergy Fusion RP column, m/z 332→110	surface water	0.03	102	8.4	5
			0.30	100	8.3	5
		drinking water	0.03	104	3.6	5
			0.30	97	2.5	5
		ground water	0.03	101	5.0	5
			0.30	96	5.4	5

Table B.5.3-12: Validation of the confirmatory LC-MS/MS method for derivatised residues of glyphosate in water (Knoch, 2010, ASB2012-12445)

Reference	Detection method	Matrix	Fortification level [µg/L]	Average recovery [%]	RSD [%]	No. of analyses
Knoch, 2010 (ASB2012-12445)	LC-MS/MS, Synergy Fusion RP column, m/z 390→150	surface water	0.03	103	5.0	5
			0.30	96	2.5	5
		drinking water	0.03	98	2.8	5
			0.30	97	2.9	5
		ground water	0.03	95	3.8	5
			0.30	97	3.2	5

Table B.5.3-13: Validation of the confirmatory LC-MS/MS method for derivatised residues of AMPA in water (Knoch, 2010, ASB2012-12445)

Reference	Detection method	Matrix	Fortification level [µg/L]	Average recovery [%]	RSD [%]	No. of analyses
Knoch, 2010 (ASB2012-12445)	LC-MS/MS, Synergy Fusion RP column, m/z 332→136	surface water	0.03	96	5.3	5
			0.30	99	3.9	5
		drinking water	0.03	101	2.2	5
			0.30	98	3.2	5
		ground water	0.03	98	3.0	5
			0.30	99	3.4	5

Independent laboratory validation of the LC-MS/MS method of Knoch (2010, ASB2012-12445)

Reference: IIA, 4.5/02
Report: Geschke, S. (2011)
Independent laboratory validation of an analytical method for the determination of residues of glyphosate and AMPA in drinking water.
Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany
Report No. S10-02882, Date 11.05.2011 , not published, ASB2012-12426
Guidelines: SANCO/825/00 Rev. 7
Deviations: No

GLP: yes
Acceptability: The study is considered to be acceptable.
Materials and Methods:
Test material: tap water from D-75223 Niefern-Oeschelbrunn; DOC 0.44 g/mL, pH 7.62, conductivity 754 µS/cm

Fortified analyte(s):
Glyphosate (N-(phosphonomethyl) glycine) and AMPA (aminomethylphosphonic acid)

Analyte(s) determined as:
FMOC derivatives of glyphosate ($C_{18}H_{18}NO_7P$, molar mass 391 g/mol) and AMPA ($C_{16}H_{16}NO_5P$, molar mass 333 g/mol)

Principle of the method: The procedure of Knoch, 2010 developed for drinking water was used without deviations. Also identical LC-MS/MS conditions were used.

Findings:
The validated limit of quantification for glyphosate and AMPA is 0.03 µg/L. In all control samples the intensity of signals at the retention time of glyphosate and AMPS did not exceed 30 % LOQ. Acceptable chromatograms from derivatised standard solutions, samples and blank materials, as well as adequate recovery data and information on the precision of the method are presented.
Linear calibration is performed for both analytes (glyphosate and AMPA) in a range corresponding to 0.01 – 0.5 µg/L using standards in solvent at eight levels. Correlation coefficients of calibration graphs are > 0.996. Information on calibration is reported in Table B.5.3-17. For validation data see Tables B.5.2-18 to B.5.2-21.

Conclusion:
The study of Geschke (2011, ASB2012-12426) describes a successful independent laboratory validation of the highly specific method of Knoch (2010, ASB2012-12445) with the matrix drinking water.

Table B.5.3-14: Calibration data of the proposed LC-MS/MS method for derivatised residues of glyphosate and AMPA in water (Geschke, 2011, ASB2012-12426)

Calibration parameter	Results of evaluation
Accepted calibration range in concentration units	0.2 – 10 ng/mL
Accepted calibration range in mass fraction units	0.01 – 0.50 µg/L
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)	yes
Where matrix effects investigated?	no
The absence of interference >30 % of LOQ in blank samples was demonstrated?	yes
Chromatogram of sample provided spiked at LOQ demonstrating sufficient sensitivity and signal-to-noise ratio?	yes

Table B.5.3-15: Validation of the primary LC-MS/MS method for derivatised residues of glyphosate in water (Geschke, 2011, ASB2012-12426)

Reference	Detection method	Matrix	Fortification level [µg/L]	Average recovery [%]	RSD [%]	No. of analyses
Geschke, 2011 (ASB2012-12426)	LC-MS/MS, Synergy Fusion RP, m/z 390→168	drinking water	0.03	108	7	5
			0.30	99	3	5

Table B.5.3-16: Validation of the primary LC-MS/MS method for derivatised residues of AMPA in water (Geschke, 2011, ASB2012-12426)

Reference	Detection method	Matrix	Fortification level [µg/L]	Average recovery [%]	RSD [%]	No. of analyses
Geschke, 2011 (ASB2012-12426)	LC-MS/MS, Synergy Fusion RP, m/z 332→110	drinking water	0.03	102	7	5
			0.30	105	5	5

Table B.5.3-17: Validation of the confirmatory LC-MS/MS method for derivatised residues of glyphosate in water (Geschke, 2011, ASB2012-12426)

Reference	Detection method	Matrix	Fortification level [µg/L]	Average recovery [%]	RSD [%]	No. of analyses
Geschke, 2011 (ASB2012-12426)	LC-MS/MS, Synergy Fusion RP, m/z 390→150	drinking water	0.03	87	14	5
			0.30	97	3	5

Table B.5.3-18: Validation of the confirmatory LC-MS/MS method for derivatised residues of AMPA in water (Geschke, 2011, ASB2012-12426)

Reference	Detection method	Matrix	Fortification level [µg/L]	Average recovery [%]	RSD [%]	No. of analyses
Geschke, 2011 (ASB2012-12426)	LC-MS/MS, Synergy Fusion RP, m/z 332→136	drinking water	0.03	102	9	5
			0.30	105	8	5

Further statements provided during Peer Review

Reference: IIA, 4.5
Report: Reding, M.-A. (2014)
Letter to the Editor regarding "Determination of Glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid phase extraction followed by liquid chromatography coupled to tandem mass spectrometry"
ASB2014-9627

Reference: IIA, 4.5
Report: Sanchis, J.; Kantiani, L.; Llorca, M. et al. (2012)

Letter to the Editor regarding "Determination of Glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid phase extraction followed by liquid chromatography coupled to tandem mass spectrometry"
ASB2014-9630

Both statements refer to the paper "Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry" of Sanchís, J.; Kantiani, L.; Llorca, M.; Rubio, F.; Ginebreda, A.; Fraile, J.; Garrido, T.and Farré, M . published in Anal Bioanal Chem (2012) 402:2335–2345. The extent of reported validation data in this paper does not meet the requirements of SANCO/825/00 rev 8.1.
The statements mainly refer to the classification of water samples as groundwater or not.

B.5.3.3 Analytical methods for air

Reference:	IIA, 4.7/01
Report:	Schneider V (2001) Validation of an analytical method for the determination of glyphosate in air. UCL GmbH, Cologne, Germany Report No. PR01/007, Date 04.09.2001, not published MET2005-368
Guidelines:	SANCO/825/00 Rev. 6
Deviations:	No
GLP:	yes
Acceptability:	The study is considered to be acceptable.

Materials and Methods:
Test material: air (35 °C, 80 % rel. humidity)

Fortified analyte(s): glyphosate (N-(phosphonomethyl) glycine)

Analyte(s) determined as: N-([Bis-(2,2,2-trifluoroethoxy)-phosphinyl] methyl)-N-(trifluor¬acetyl)-glycine,2,2,2-trifluoroethotyl; $C_{11}H_{10}F_{12}O_6NP$, molar mass = 511 g/mol

Principle of the method: Residues in air are enriched on a SAX cartridge (BondElute # 1812801). During method validation glyphosate is fortified to the cartridge and afterwards for 6 hours in total 73 – 202 L air is drawn through the fortified cartridge.
^{13}C-glyphosate is added to the cartridge as internal standard. Glyphosate and its internal standard are eleuted from SAX cartridge using 1 N HCl. The eluate is evaporated to dryness. To the dry residue phosphoric acid and trifluoroacetic acid are added. Glyphosate is derivatised with a mixture of trifluoroacetic anhydride and trifluoroethanol (2+1, V/V). The derivatisation mixture is heated to about 70 °C and derivatisation is finalised within one hour. After derivatisation, excess reagents and volatile by-products are removed by rotary evaporation. The concentrated remainder is partitioned between dichloromethane and 5 % aqueous ammonium hydroxide solution. The dichloromethane phase containing the derivatives is dried, filtered and evaporated. The remainder is reconstituted in toluene. Sample concentration in final extracts is ≥ 146 L/mL.

The residues are determined by gas liquid chromatography with mass selective detection (GC-MSD) in the select ion monitoring (SIM) mode using a CP-Sil 19 column (14 % cyanopropylphenyl/ 86 % polydimethylsiloxane). The reported results are based on m/z 411 for glyphosate and m/z 412 for ^{13}C-glyphosate.

Findings:
The validated limit of quantification is 5 µg/m^3, assuming the addition of 5 µg internal standard on each cartridge. (Note: The description of the method contains ambiguous information with regard to the concentration of the internal standard in the fortification solution, resulting in the addition of 5 µg and 10 µg of ISTD on each cartridge, respectively. Also the concentration of ISTD in calibration standards is unclear, i.e. either 5.08 µg/mL or 25.4 µg/mL).
Breakthrough was tested with a second cartridge downstream to the fortified cartridge, but no breakthrough was found. The storage stability was demonstrated for a period of 8 days. Blank values in control samples are not detected. The sorbent material is fixed by porous polypropylene frits of 20 µm pore size to the cartridge. Thus the cartridges are suitable to retain particle associated residues as well.
Quadratic calibration is performed in the range 4 – 60 µg/m^3 based on stable isotope labelled standards. Confirmatory transitions are not validated. Information on calibration is reported in Table B.5.2-22. For validation data see Table B.5.2-23.

Conclusion:
The study of Schneider (2001, MET2005-368) describes a successfully validated method for the determination of glyphosate in air.

Table B.5.3-19: Calibration data of the proposed GC-MS method for derivatised residues of glyphosate in air (Schneider, 2001, MET2005-368)

Calibration parameter	Results of evaluation
Accepted calibration range in concentration units	0.4 – 6.0 µg/mL (each containing 5.0 µg/mL ISTD)
Accepted calibration range in mass fraction units	4 – 60 µg/m^3 (based on 100 L sampling volume)
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)	yes
Where matrix effects investigated?	no
The absence of interference >30 % of LOQ in blank samples was demonstrated?	yes
Chromatogram of sample provided spiked at LOQ demonstrating sufficient sensitivity and signal-to-noise ratio?	yes

Table B.5.3-20: Validation of the GC-MS method for derivatised residues of glyphosate in air (Schneider, 2001, MET2005-368)

Reference	Detection method	Matrix	Fortification level [µg/m^3]	Average recovery [%]	RSD [%]	No. of analyses
Schneider, 2001c (MET2005-368)	GC-MS, CP-Sil 19 column, m/z 411	Air	5	76	2.4	5
			50	81	1.5	5

B.5.4 Analytical methods (residue) for body fluids and tissues (Annex IIA 4.8; Annex IIIA 5.8)

Methods for the determination of glyphosate in body fluids and tissues are not needed, since glyphosate is not classified as acute toxic.

B.5.5 Evaluation and assessment

B.5.5.1 Formulation analysis

Sufficient analyticla methods are available to ensure the determiantion of the glyphosate and the impurities in technical material as well as the determiantion of glyphosate and the relevant impurities in the representative formulation.

B.5.5.2 Residue analysis

For the assessment of the analytical methods proposed for the enforcement of residues of glyphosate the following criteria were used:

The submitted methods enable the enforcement of the following relevant residue definitions and limits (at the time of evaluation) are listed below:

Matrix	Limit		Comment
Plants and plant products			
Commodities with high water content	0.05	mg/kg	residue definition: For ~~lentils~~ sweet corn, oilseed rape, soya beans and maize: sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate
Commodities with high fat content	0.05	mg/kg	
Acidic commodities	0.05	mg/kg	
Dry commodities	0.05	mg/kg	other commodities: glyphosate
Animal products			
Milk	0.05	mg/kg	residue definition: sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate
Meat, egg, fat, liver/ kidney	0.05	mg/kg	
Soil	0.05	mg/kg	typical limit of quantification, if pesticde is not highly phytotoxic (SANCO 825/00); residue definition: glyphosate and AMPA
Drinking water	0.1	µg/L	EU drinking water limit; residue definition: glyphosate
Surface water	38000	µg/L	glyphosate based on the LC_{50} of *Oncorhynchus mykiss*;
	12000	µg/L	AMPA based on the NOEC of *Pimephales promeales*; residue definition: glyphosate and AMPA
Air	30	µg/m³	based on a proposed AOEL-S of 0.1 mg/kg bw/d; residue definition: glyphosate

Tissues	not required, not classified as toxic or very toxic
Body fluids	not required, not classified as toxic or very toxic

An overview of the accepted enforcement methods (incl. confirmatory methods and independent lab validation) is given in the following table.

Table B.5.5-1: Studies, which describe appropriate analytical procedures (Completeness check of analytical methods for monitoring purposes and post-registration control in accordance to guidance document SANCO/825/00 rev. 8.1)

Matrix type/ crop group	Primary Method	Confirmatory method	Independent Lab Validation
Cereals and other dry crops	Pentz and Bramble, 2007, ASB2008-2635	Klimmek, 2007, ASB2008-5606 **(for N-acetylglyphosate missing)**	Seal and Dillon, 2007, ASB2008-2637
Commodities with high water content	Pentz and Bramble, 2007, ASB2008-2635	Weber, 2012, ASB2012-12489 **(for N-acetylglyphosate missing)**	Seal and Dillon, 2007, ASB2008-2637
Commodities with high fat content	Pentz and Bramble, 2007, ASB2008-2635	Klimmek and Weber, 2008 ASB2008-5607 **(for N-acetylglyphosate missing)**	Seal and Dillon, 2007, ASB2008-2637
Fruits with high acid content	Pentz and Bramble, 2007, ASB2008-2635	Klimmek and Weber, 2008 ASB2008-5607 (not required for N-acetylglyphosate)	Seal and Dillon, 2007, ASB2008-2637
Commodities which are difficult to analyse	~~Anderson and Ely, 2001, ASB2012-12364 (coffee, glyphosate only)~~ not required (no intended use in difficult matrices)	not required (no intended use in difficult matrices)	not required (no intended use in difficult matrices)
Milk	Pentz and Bramble, 2007, ASB2008-2636	Schneider, 2001, MET2005-367 **(missing for N-acetylglyphosate)**	Karnik and Dillon, 2007, ASB2008-2634
Egg	Pentz and Bramble, 2007, ASB2008-2636	Schneider, 2001, MET2005-367 **(missing for N-acetylglyphosate)**	Karnik and Dillon, 2007, ASB2008-2634
Meat	Pentz and Bramble, 2007, ASB2008-2636	Schneider, 2001, MET2005-367 **(missing for N-acetylglyphosate)**	Karnik and Dillon, 2007, ASB2008-2634
Fat	Pentz and Bramble, 2007, ASB2008-2636	**missing for glyphosate and N-acetylglyphosate**	not required
Kidney/liver	Pentz and Bramble, 2007, ASB2008-2636	**missing for glyphosate and N-acetylglyphosate**	Karnik and Dillon, 2007, ASB2008-2634
Soil	Schneider, 2001, MET2005-371	~~Szuter, 1996, MET2000-699~~ **missing**	Generally not required
Drinking water	Knoch, 2010, ASB2012-12445	Knoch, 2010, ASB2012-12445	Geschke, 2011, ASB2012-12426

Matrix type/ crop group	Primary Method	Confirmatory method	Independent Lab Validation
Surface water	Knoch, 2010, ASB2012-12445	Knoch, 2010, ASB2012-12445	Generally not required
Air	Schneider, 2001, MET2005-368	Not required	Generally not required
Body tissues	Not required	Not required	Generally not required
Body fluids	Not required	Not required	Generally not required

This overview shows that the following methods for monitoring are missing:

- A confirmatory method for glyphosate in fat and kidney/liver.
- A confirmatory method for N-acetyl-glyphosate in dry plant materials and those with high water and high fat content.
- A confirmatory method for N-acetyl-glyphosate in all kinds of animal matrices.
- A confirmatory method for residues in soil.

B.5.6 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIA 4.2 (OECD)	Probst, D.A.	2012	NNG and formaldehyde method validations in MON 52276 and MON 77973 MSL0024115 GLP: Y, published: N 2309030	Y	EGT
KIIA 4.2.1 (OECD)	Norris, D.	2009	Validation of the Methods of Analysis used for Glyphosate and Specified Impurities DNA0686 GLP: Y, published: N 2315362	Y	PUJ
KIIA 4.2.1 (OECD)	Norris, D.	2009	Validation of the Methods of Analysis used for Glyphosate and Specified Impurities (1st addendum) DNA0686 GLP: N, published: N 2315364	Y	PUJ
KIIA 4.2.1 (OECD)	Diehl, B.	2008	Glyphosate TC: Method Validation for the Analysis of Manufacturing Process Related Impurities SSL 08807 GLP: Y, published: N 2315403	Y	ALB

[1] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIA 4.2.1 (OECD)	Norris, D.	2009	Validation of the method of analysis used for Glyphosate and specified impurities DNA 0775 GLP: Y, published: N 2315439	Y	JGH
KIIA 4.2.1 (OECD)	Fox, O.	2005	Validation of a HPLC method for the Quantitative analysis of impurities in Glyphosate technical acid and 5-batch analysis of glyphosate technical acid for active ingredient, material insoluble in NaOH and moisture content BAR-001 GLP: Y, published: N 2315440	Y	BCL
KIIA 4.2.1 (OECD)	Norris, D.	2006	Analysis of a sample of glyphosate technical material, to show the method specificity DNA0055 GLP: Y, published: N 2315446	Y	BCL
KIIA 4.2.1 (OECD)	Pedersen, S.N.	2004	Analytical Method VAM 002-04: Determination of Glyphosate (CAS No. 1071-83-6) in Glyphosate Technical, Glyphosate Concentrate and Glyphosate SL and SG Formulations VAM 002-04 version 4 GLP: Y, published: N 2315498	Y	CHE
KIIA 4.2.1 (OECD)	Pedersen, S.N.	2002	Validation of Analytical Method VAM 002-04 for determination of Glyphosate (CAS No. 1071-83-6) in Glyphosate Technical, Glyphosate Concentrate and Glyphosate Formulations VAL 002-04 GLP: Y, published: N 2315500	Y	CHE
KIIA 4.2.1 (OECD)	Hinz, B.	2009	Analytical Method VAM 002-04 : Determination of Glyphosate (CAS No. 1071-83-6) in Glyphosate Technical, Glyphosate Concentrate and Glyphosate SL and SG Formulations VAM 002-04 version 5 GLP: N, published: N 2315506	Y	CHE

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIA 4.2.1 (OECD)	Comb, A.L.	2005	Glyphosate technical - Five-batch analysis - Method validation NUF/083 GLP: Y, published: N 2315594	Y	NUF
KIIA 4.2.1 (OECD)	Norris, D.	2009	Validation of the methods of analysis used for glyphosate and specified impurities, in compliance with Good Laboratory Practice DNA0775 GLP: Y, published: N 2315649	Y	HEL
KIIA 4.2.1 (OECD)	Diehl, B.	2010	Glyphosate TC - Method Validation for the Analysis of Manufacturing Process Related Impurities SSL04309 GLP: Y, published: N 2315697	Y	HEL
KIIA 4.2.1 (OECD)	Diehl, B.	2010	Glyphosate TC - Method Validation for the Analysis of Manufacturing Process Related Impurities SSL04309 GLP: Y, published: N 2315730	Y	NUF
KIIA 4.2.1 (OECD)	Huazhen Wen	2009	Spectroscopy complementary data regarding the methods of analysis used in the study NC-2007-012 NC-2007-012 GLP: N, published: N 2315820	Y	SFP
KIIA 4.2.1 (OECD)	Clarke, P.M.; Massey, P.R.	2002	AMP10089-03B - The Determination of Glyphosate and associated Impurities in technical Material by Ion Chromatography R61837/0272 GLP: N, published: N 2315873	Y	SYD
KIIA 4.2.1 (OECD)	Diehl, B.	2010	Glyphosate TC-Method Validation for the Analysis of Manufacturing Process Related Impurities SSL04309 GLP: Y, published: N 2315935	Y	ZXC

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIA 4.2.1 (OECD)	Yusuf Vohra	2009	Preliminary analyses of five representative production batches of glyphosate acid technical grade active ingredient (TGAI) to determine % glyphosate and to quantify its associated impurities (Volume I and II) 8880 GLP: Y, published: N 2317346	Y	AGT
KIIA 4.2.1 (OECD)	Norris, D.	2012	Validation of the method of determination of active ingredient and specified impurities in glyphosate technical material DNA1301 GLP: Y, published: N 2317380	Y	INA
KIIA 4.2.1 (OECD)	Ravi, P.E.	2007	Studies on the chemical composition of five batches of glyphosate technical 05197 GLP: Y, published: N 2317477	Y	IMA
KIIA 4.2.1 (OECD)	Hubbard, S.	2009	Analysis of 5 batches of Technical glyphosate for the active ingredient and relevant impurities with associated validation OA01682 ALS GLP: Y, published: N 2317612	Y	AAP
KIIA 4.2.1 (OECD)	Wasser, C.	2008	Purity profile for 5 batches of glyphosate technical A7190 GLP: Y, published: N 2317738	Y	BRK
KIIA 4.2.1 (OECD)	Hongxia Li	2010	Preliminary Analysis and Enforcement Analytical Method of Glyphosate TGAI - Validation of Analytical Methodology for the Assay of Active Ingredients and related Significant Impurities and Subsequent 5 batch analysis of Glyphosate TGAI NC-2009-019 GLP: Y, published: N 2317822	Y	[redacted]
KIIA 4.2.1 (OECD)	Denny, O.	2010	Purity Profile of 5 Batches of Glyphosate Technical R A9033 GLP: Y, published: N 2320229	Y	MAH

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIA 4.2.1 (OECD)	Clipston, A.S.	2010	5-Batch analysis of Glyphosate acid TGAI in accordance with EC Commission Directive 94/37/EC Article 1.9 to 1.11 (amending EEC Council Directive 91/414/EEC) EPP00009 GLP: Y, published: N 2320514	Y	HEL
KIIA 4.2.1 (OECD)	Sardinha, R.G.	2008	Qualitative and Quantitative Profile of the test substance GLYPHOSATE TECHNICAL (Five Batch Analysis) 3996.030.288.07 GLP: Y, published: N 2320537	Y	HEL
KIIA 4.2.1 (OECD)	Zepperitz, C	2011	Glyphosate 95% TC: Quantification and Identification of the Active Ingredient and Impurities in Five Batches by Validated Methods C86955 GLP: Y, published: N 2320584	Y	HEL
KIIA 4.2.1 (OECD)	Skopec, J.	2009	Determination of Active Content and Impurity Profile of Glyphosate RS-001 GLP: Y, published: N 2320625	Y	HEL
KIIA 4.2.1 (OECD)	Skopec, J.	2009	Determination of active content and impurity profile of Glyphosate OS-012 GLP: Y, published: N 2320658	Y	HEL
KIIA 4.2.1 (OECD)	Mcdonald, E.; Craig, W.B.	2002	Glyphosate TGAI/MP: Analysis and certification of Limits for Glyphosate TGAI/MP: Selected studies to fulfill OPPTS Guidelines 830.1700, 830.1750 and 830.1600 in Accordance with 40 CFR part 158 and EEC Directive 94/37/EEC 20733 GLP: Y, published: N 2321334	Y	SAO
KIIA 4.2.1 (OECD)	Pinto, V	2008	Glyphosate Analytical Profile AI/21/08 Final report GLP: Y, published: N 2321404	Y	SAP

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIA 4.2.1 (OECD)	Shen Lu	2007	Preliminary analysis and enforcement analytical method of Glyphosate TGAI - Validation of analytical methodology for the assay of active ingredient and related significant impurities and subsequent 5-Batch analysis of Glyphosate TGAI NC-2007-012 GLP: Y, published: N 2322253	Y	SFP
~~KIIA 4.2.1 (OECD)~~	~~Wang, S. M.~~	~~2004~~	~~Five Batches Analysis of Technical Grade Active Ingredient (TGAI) Glyphosate Acid~~ ~~CB01-01~~ ~~GLP: Y, published: N~~ ~~2322339~~	~~Y~~	~~SNN~~
KIIA 4.2.1	Jwo, S.-S.	2014	The five batches analysis of technical grade active ingredient (TGAI) glyphosate acid M302 GLP: Y, published: N 2717921	Y	SNN
KIIA 4.2.1 (OECD)	Doyle, T.	2009	5 Batch Analysis of Glyphosate Technical Material for significant impurities, known impurities and materials insoluble in NaOH, including amendment UPL-007 GLP: Y, published: N 2325565	Y	UPL
KIIA 4.2.1 (OECD)	Silva, S.	2011	Analytical profile of glyphosate (Annexes) AI/61/10 Final Rep. GLP: Y, published: N 2326001	Y	SAP
KIIA 4.2.1 (OECD)	Silva, S.	2011	Analytical profile of glyphosate AI/61/10 Final Rep. GLP: Y, published: N 2326020	Y	SAP
KIIA 4.2.1 (OECD)	Bockholt, K.	2010	Glyphosate TC-Analytical Profile of 5 Batches SSL04409 GLP: Y, published: N 2404947	Y	ZXC
KIIA 4.2.1 (OECD)	Clarke, P.M.	2002	Method Validation: AMP10089-03B 'The Determination of Glyphosate and Associated Impurities in Technical Material by Ion Chromatography' R61837/0273! RJ3310B GLP: Y, published: N 2415613	Y	SYD

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIA 4.2.1 (OECD)	Nelson, R. Godbey, J. Jones, J.	2008	Analysis of Product Samples for Active Ingredient and Impurities in Technical Grade of Glyphosate FOR-07-044 GLP: Y, published: N 2431693	Y	DOW
KIIA 4.2.1 (OECD)	Nelson, R. Jones, J. Linder, S. Stock, M.	2006	Analysis of Product Samples for Active Ingredient and Impurities in Technical Grade of Glyphosate (GF-1548) FOR04019 GLP: Y, published: N 2431695	Y	DOW
KIIA 4.2.1 (OECD)	Nelson, R. Jennings, C. Jones, J. Rodriguez-Rosas, M.	2008	Analysis of Product Samples for Active Ingredient and Impurities in Technical Grade of Glyphosate FOR-07-062 GLP: Y, published: N 2431697	Y	DOW
KIIA 4.2.1 (OECD)	Bockholt, K.	2010	Glyphosate TC - Analytical Profile of 5 Batches SSL04409 GLP: Y, published: N 2434971	Y	HEL
KIIA 4.2.1 (OECD)	Desai, H	2008	Preliminary Analysis of Five Representative Production Batches of Glyphosate Technical Grade Active Ingredient (TGAI) to Determine % Glyphosate and to Quantify Its Associated Impurities 8247 GLP: Y, published: N 2435558	Y	RTA
KIIA 4.2.2 (OECD)	Russchen, A.	2007	Validation of the HPLC-method to determine the content of Glyphosate in Glyphosate technical 0702019.00 GLP: Y, published: N 2315366	Y	AGC
KIIA 4.3 (OECD)	Anderson, L.; Ely, S. V.	2001	N-(phosphonomethyl)glycine (PMG) and Aminomethyl Phosphonic acid (AMPA) validation of a residue analytical method for the determination of the residues in various crops RJ3119B BVL-2309035, ASB2012-12364	N	EGT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIA 4.3 (OECD)	Clarke D. M.; Robinson, N. J.	1998	Validation of an analytical method for the determination of [N-(Phosphonomethyl)glycine] (PMG) and Aminomethylphosphonic acid (AMPA) in kidney, milk and eggs by gas chromatography with mass selective detection RJ2585B BVL-2309049, ASB2012-12398	N	EGT
KIIA 4.3 (OECD)	Crook, S. J.	2007	Glyphosate: Provision of independent laboratory validation data to support analytical methods RAM 328/01 and RAM 308/01 J6696/01 BVL-2309039, ASB2012-12387	Y	EGT
KIIA 4.3 (OECD)	Klimmek, S.	2007	Validation of the analytical method DFG Method 405 for determination of residues of Glyphosate and its Metabolite AMPA in various plant materials 0FC00014427 ! FCS-0703V BVL-2309041, ASB2008-5606	Y	EGT
KIIA 4.3 (OECD)	Klimmek, S.; Weber, H.	2008	First Amendment to final report - Validation of the analytical method DFG Method 405 for the determination of residues of Glyphosate and its metabolite AMPA in various plant materials FCS-0703V BVL-2309043, ASB2008-5607	Y	EGT
KIIA 4.3 (OECD)	Robinson, N. J.	1998	GLYPHOSATE TRIMESIUM: Replacement of Chloroform in method RR93-104B RES for analysis of N-(Phosphonomethyl)Glycine and Aminomethyl-Phosphonic acid in animal matrices. A method development summary 36546/01 BVL-2309051, ASB2012-12465	N	EGT
KIIA 4.3 (OECD)	Runnels, J. K.	1993	Applicability of multi-method S19 to the analysis of glyphosate-trimesium BVL-2309059, ASB2009-5598	N	EGT
KIIA 4.3 (OECD)	Schneider, E.	2001 a	Validation of an analytical method for the determination of Glyphosate in foodstuff of animal origin (meet, eggs, milk) (Monitoring method) PR01/005 BVL-2309057, MET2005-367	N	EGT
KIIA 4.3 (OECD)	Weber, H.	2012	Validation of an analytical method for the determination of Glyphosate and AMPA in Raw Agricultural Commodities using LC/MS/MS S11-03331 BVL-2309045, ASB2012-12489	Y	EGT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIA 4.3 (OECD)	Alferness P. L. and Wiebe L. A.	2001	Determination of Glyphosate and Aminomethylphosphonic Acid in Crops by Capillary Gas Chromatography with Mass-Selective Detection: Collaborative Study, J AOAC International 84 (2001) 823 – 846, ASB2012-12387	N	published
KIIA 4.3 (OECD)	Karnik, S., Dillon, R.	2007	Independent laboratory validation of DuPont-20009, "Analytical method for the determination of N-acetalglyphosate and other analytes in various animal matrices using LC/MS/MS" DuPont-21372, Pyxant Labs Inc. ID: 1806 BVL-1748764, ASB2008-2634	Y	DPB
KIIA 4.3 (OECD)	Pentz, A.M., Bramble, F.Q.	2007 a	Analytical method for the determination of glyphosate and degradate residues in various crop matrices using LC/MS/MS DuPont-15444 Revision-1 BVL-1748765, ASB2008-2635	Y	DPB
KIIA 4.3 (OECD)	Pentz, A. M., Bramble, F. Q.	2007 b	Analytical method for the determination of N-acetylglyphosate and other analytes in various animal matrices using LC/MS/MS DuPont-20009 BVL-1748766, ASB2008-2636	Y	DPB
KIIA 4.3 (OECD)	Seal, S., Dillon, R.	2007	Independent laboratory validation of DuPont-15444, "Analytical method for the determination of glyphosate and relevant metabolite residues in various crop matrices using LC/MS/MS" DuPont-21313, Pyxant Labs Project no. 1763 BVL-1748767, ASB2008-2637	Y	DPB
KIIA 4.4 (OECD)	Schneider, E.	2001 b	Validation of an analytical method for the determination of Glyphosate in soil PR01/006 BVL-2309063, MET2005-371	N	EGT
KIIA 4.4 (OECD)	Szuter, S. L.	1996	Glyphosate Acid: Independent laboratory lalidation of the method for determining residues of N-(Phosphonomethyl)glycine and (Aminomethyl)phosphonic acid in soil (WRC-96-082) (WINO 23013) Study number ZPMG-96-MT-01, Report number RR 96-059B not published, MET2000-699	N	MOD
KIIA 4.5 (OECD)	Geschke, S.	2011	Independent laboratory validation of an analytical method for the determination of residues of Glyphosate and AMPA in drinking water S10-02882 BVL-2309067, ASB2012-12426	Y	EGT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIA 4.5 (OECD)	Knoch, E.	2010	Validation of an analytical method: Determination of Glyphosate and AMPA in water matrices using FMOC derivatization, manual SPE cleanup and LC-MS/MS quantitation IF-10/01618859 BVL-2309065, ASB2012-12445	Y	EGT
KIIA 4.7 (OECD)	Schneider, E.	2001 c	Validation of an analytical method for the determination of Glyphosate in air PR01/007 BVL-2309069, MET2005-368	N	EGT
Extraction efficiency	Bleeke, M. S.	1997	Nature of Glyphosate residues in cotton plants tolerant to Roundup herbicide. Report No. MSL-14113 GLP: yes not published RIP9700619	Y	MOD
Extraction efficiency	Chapleo, S.; McLachlan, T.	2010	The metabolism of [14C]Glyphosate in 0827 canola Sponsor Study No: DuPont-26109 GLP: yes not published BVL-2200198, ASB2011-13744	Y	DPB
Extraction efficiency	George, Ch.	1995	Nature of Glyphosate residues in corn plants which are tolerant to Roundup herbicide Report No. MSL-14018 GLP: yes not published RIP9700618	Y	MOD
Extraction efficiency	Goure, W. F.	1994	Nature of Glyphosate residues in soybeans tolerant to Roundup herbicide Report No. MSL-13520 GLP: yes not published RIP9800117	Y	MOD
Extraction efficiency	Green, M.	2007	The metabolism of [14C]Glyphosate in Optimum GAT (Event DP-Ø9814Ø-6) field corn DuPont-19529 BVL-1748787, ASB2008-2657	Y	DPB
Extraction efficiency	Lowrie, Ch.	2007	Metabolism of [^{14}C]-N-Acetylglyphosate (INMCX20) in the lactating goat Report No.: DuPont-19796 GLP: yes not published BVL-1748790, ASB2008-2660	Y	DPB

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
Extraction efficiency	MacDonald, A. M. G.	2007	The metabolism of [14C]Glyphosate in GAT/GM-HRA (DP-356Ø43-5, PHP20163a) soybeans DuPont-19530 BVL-1748788, ASB2008-2658	Y	DPB
Extraction efficiency	Mehrsheikh, A.	2000	Metabolism of Glyphosate in Roundup Ready Sugarbeet Report No. MSL-16247 GLP: yes not published RIP2001-906	Y	MOD
Extraction efficiency	Patanella, J. E.; Feng, P.	1988	Metabolism study of synthetic $^{13}C/^{14}C$-labeled Glyphosate and Aminomethylphosphonic acid in lactating goats. Part II Report: MSL-7458 GLP: yes not published RIP9501204	Y	MOD
Extraction efficiency	Powles, P.	1994	(^{14}C-Glyphosate): Absorption, distribution, metabolism and excretion following repeated oral administration to the dairy goat Report No. 676/9-1011 GLP: yes not published RIP9501207	Y	MOD
KIIIA1 5.2.1 (OECD)	Bates, C.	2001	Determination of glyphosate content in formulations MON 78043, MON 78044 and MON 2139 (glyphosate 360g/l) SL by HPLC : validation of the analytical method MSL-17401 GLP: Y, published: N 2315973	N	EGT
KIIIA1 5.2.4 (OECD)	Probst, D. A.	2012	NNG and formaldehyde method validations in MON 52276 and MON 77973 MSL0024115 GLP: Y, published: N 2315974	Y	EGT

Codes of owner

AAP Arysta LifeScience S.A.S
AGC AgriChem B.V.
AGT Agro Trade GmbH
ALB Albaugh UK Limited
BCL Barclay Chemicals Manufacturing Ltd.
BRK BROKDEN, SL
CHE Cheminova A/S
DOW Dow AgroSciences GmbH

DPB	DuPont de Nemours
EGT	European Glyphosate Task Force AIR 2
HEL	HELM AG
IMA	Imaspro Resources Sdn. Bhd.
INA	Industrias Afrasa
[redacted]	[redacted]
JGH	Jiangsu Good Harvest
MAH	Makhteshim-AGAN Group
MOD	Monsanto Europe S.A./N.V.
NUF	Nufarm GmbH & Co KG
PUJ	Punjab Chemicals and Crop protection Ltd
RTA	Rotam Agrochemicals Europe Ltd.
SAO	Sabero Organics Gujarat Ltd
SAP	Sapec Agro S.A.
SFP	Société Financière de Pontarlier
SNN	Sinon Corporation
SYD	Syngenta Agro GmbH
UPL	United Phosphorus Ltd.
ZXC	Zhejiang Xinan Chemical Industrial Group Co., Ltd.

Studies marked in yellow are not part of the dossier for renewal.

Renewal Assessment Report

18 December 2013

Revised
29 January 2015
31 March 2015

Glyphosate

Volume 3

Annex B.6.~~1~~

Toxicology and metabolism

RMS: Germany **Co-RMS: Slovakia**

Version history (Volume 3, chapter B.6)

Date	**Reason for revision**
19 December 2013	Initial Renewal Assessment Report
29 January 2015	Updated following RAR peer review, taking into account the reporting table, the evaluation table and additional data
31 March 2015	Updated following Pestidices Peer Review 125 expert meeting (25-27 February 2015)

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B.6 Toxicology and metabolism

General introduction and explanation of the approach taken by RMS

This health evaluation of glyphosate is based on the following sources:

- *Toxicological and ADME studies that were submitted by the GTF for this re-evaluation.*
- *Toxicological studies and ADME studies that had been reported in the previous DAR (1998, ASB2010-10302) already and, thus, were part of previous EU evaluation. However, they were subject to re-assessment by the RMS according to current quality standards and were used only when regarded as acceptable or at least supplementary. In very few cases, NOAELs/LOAELs were revised. Unacceptable (old or new) studies were usually deleted with justifications given in the respective sections of Volume 3. In exceptional cases, such studies are still mentioned, i.e., if they were formerly taken into consideration for, e.g., ADI setting.*
- *Scientific publications and other relevant information that were submitted either by the GTF or by third parties or of which the RMS was aware before. It must be emphasised that a large part of the publications was on formulations different from the representative one and, thus, is of limited value for the toxicological evaluation of the active ingredient. With rather few exceptions in the areas of genotoxicity and human data, mainly scientific literature published since 2000 was assessed.*

Due to the large numer of submitted toxicological studies, the RMS was not able to report the original studies in detail and an alternative approach was taken instead. The study descriptions and assessments as provided by GTF were amended by deletion of redundant parts (such as the so-called "executive summaries") and new enumeration of tables. Obvious errors were corrected. Each new study was commented by the RMS. These remarks are clearly distinguished from the original submission by a caption, are always written in italics and may be found on the bottom of the individual study summaries.

Furthermore, in Volume 3, assessment was performed on the individual study level. Overall evaluation of the diverse toxicological endpoints was transferred into Volume 1 (section 2.6).

The technical databases that have been used for the literature search include: Web of Science^{SM}, BIOSIS Previews®, CAB Abstracts® (CABI), MEDLINE®, and CA Plus (Chemical Abstracts Plus). The searches were made on glyphosate acid, glyphosate salts (including isopropyl amine, potassium, ammonium, and methylamine), and AMPA, and their related chemical names and CAS numbers. Searches based on these search terms were also found suitable to identify publications that consider glyphosate and surfactants (such as polyoxyethylenealkylamines, or POEA) in the context of glyphosate formulations.

Additional publications cited in a recent document prepared by the NGO "Earth Open Source" (Antoniou M, et al., 2011, ASB2011-7202) *have also been included in the literature review.*

The peer-reviewed publications identified for inclusion during the literature search were reviewed and classified into one of the categories listed below.

- ***Category 0 publications**: These are publications in which glyphosate is only mentioned as an example substance or is discussed/studied in a context that is not*

relevant or related to any of the regulatory sections or the exposure/hazard assessments within this submission; the publication is therefore outside of the scope of this submission.

- ***Category 1 publications****: These are publications which discuss glyphosate in a context relevant or related to the regulatory dossier sections and the conclusions fall within the conclusions of the exposure/hazard assessment. The publication is submitted with minimal or no comment or discussion.*
- ***Category 2 publications****: These are publications which discuss glyphosate in a context relevant or related to the regulatory dossier sections and have conclusions that call into question the endpoints/conclusions in the exposure/hazard assessment. Additionally, Category 2 also includes publications with conclusions that support the risk/hazard assessment, and may be included in discussion of other relevant publications. For selected Category 2 publications, an OECD Tier-II type summary is provided in addition to a reliability assessment (Klimisch rating, see Klimisch et al. 1997, ASB2010-14388); limited comments and critical remarks are provided, as appropriate.*
- ***Category 3 publications****: These are publications that discuss glyphosate in a context relevant or related to (1) non-regulatory endpoints that need to be addressed as per new Regulation (EC) 1107/2009; or (2) in a context relevant to sensitive allegations that have emerged or could emerge in the media; or (3) in a context relevant to the regulatory dossier sections and have conclusions that are in disagreement with endpoints/conclusions in the exposure/hazard assessment (although the experimental design seems relevant at first glance). An OECD Tier-II type summary is provided and a Klimisch rating assigned, and supplemented with critical review and discussion.*
- ***Category 'E' publications****: These are peer-reviewed publications that were cited in the Earth Open Source document. This category includes publications that were already captured by the literature search and are addressed within the appropriate discipline, as well as publications that were out of scope of the search (primarily as a result of being published prior to 2001). Publications already captured in the literature search were assigned a Category 1, 2 or 3 rating (as appropriate) in addition to a Category 'E' rating. An OECD Tier-II type summary has been prepared and a Klimisch rating assigned for each of the Category E publications. All Category 'E' publications are reviewed within the appropriate discipline, with most of the reviews provided within the toxicology dossier under Section IIA 5.10.*

A full description of the literature search methodology was provided by the GTF in a separate document (Carr and Bleeke, 2012, ASB2012-11583).
Five separate publication subject areas are addressed in the literature review.

1. *Developmental and Reproductive Toxicity (DART) and Endocrine Disruption (ED)*
2. *Neurotoxicity*
3. *Carcinogenicity*
4. *Genotoxicity*
5. *Category E and other publications*

The publications on subject areas 1-4 are presented in the chapters on Genotoxicity, Long term toxicicity and carcinogenicity, Reproductive Toxicity and Neurotoxicity of the report.
Furthermore, publications are presented in the chapters "Further toxicological studies" and "Medical data".
Important publications are presented in summaries as quoted from the articles followed by Klimisch ratings and by RMS comments on the paper.

In the process of public consultation after the submission of the first draft of this RAR PAN-Europe, PAN-Germany and PAN-UK conducted a PubMed literature search on the keywords 'glyphosate' and 'toxicity' and stated they got significant differences in comparison conducted by the notifier. The GTF repeated the PubMed search on June 11, 2014, using the same keywords (Glyphosate Task Force 2014, ASB2014-9624).

Overall, a total of 504 articles were identified in the search. Of those, 349 were from the time period of 2001 to 2012, and thus were considered relevant to the glyphosate submission, and were further evaluated as to whether or not they were included in either the original literature search, included in the May 2012 submission, or as part of the ongoing update of the search, as of the time of June 11 PubMed search. There were 266 reviewed for the submission (222 were included), with an additional 34 reviewed after the submission (29 selected for submission). Of the 49 remaining articles, 43 were considered to be not relevant based on the subject of the article (the majority were either on GM crops, efficacy or weed resistance). The remaining 6 were added to the literature review, and of those 4 were considered to be relevant and were selected for submission in the update.

Thus, of the 349 articles identified in the search, only 4 were determined to be relevant and were not already identified in the GTF literature search process.

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1)

Introduction into this chapter by the RMS
In this section, only studies on toxicokinetics and metabolism of glyphosate are reported in detail and commented by the RMS that were not available when the previous evaluation by the EU in the 1990ies was performed. In addition, re-evaluation of those studies which were mentioned in the original monograph (DAR, 1998, ASB2010-10302) has been performed by RMS, mainly with regard to their quality and reliability. For more detailed description of the individual studies from this group, we refer to the old DAR. The outcome of this re-evaluation is briefly given at the end of this section, as well as a short summary of published information. Overall evaluation is presented in Volume 1, including two tables in which the (new and previously known) critical studies and the distribution of glyphosate in excreta and tissues are summarised.

B.6.1.1 Toxicokinetic and metabolism studies in rats that were not previously evaluated in the EU

1st study ([redacted] 1995)

Reference:	IIA, 5.1.1/01
Report:	[redacted] (1995) HR-001: Metabolism in the rat [redacted] Data owner: Arysta Lifescience SAS Report No.: SNY 332/951256 Date:1995-08-16, not published, ASB2012-11379
Guidelines:	Japanese MAFF, 59 NohSan, Notification No. 4200 (1985) OECD 417 US-EPA FIFRA 85-1
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Materials and methods

Non-labelled test material:	HR-001
Identification:	N-(phosphonomethyl)glycine (CAS No. 1071-83-6)
Description:	Solid
Lot/Batch #:	061221
Purity:	98.9 %
Stability of test compound:	Expiry date 1996-12-20
Radiolabelled test material:	[^{14}C] - HR001
Identification:	[^{14}C] - N-(phosphonomethyl)glycine (CAS No. 1071-83-6)
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Not reported
Purity:	> 98 % (TLC, followed by radioscanning)
Specific activity:	327.7 µCi/mg, 56 mCi/mmol

Stability of test compound:	Not reported
Reference substances:	
Identification:	Aminomethylphosphonic acid (AMPA) (CAS No. 1066-51-9)
Description:	Solid
Lot/Batch #:	09203L2
Purity:	99 %
Stability of test compound:	Not reported
Radiolabelled reference substance:	
Identification:	[^{14}C] -Aminomethylphosphonic acid (^{14}C-AMPA)
Position of radiolabel:	Amino[^{14}C]methyl
Lot/Batch #:	No
Purity:	97.4 %
Specific activity:	2.0 GBq/mmol, 54 mCi/mmol
Stability of test compound:	Not reported
Vehicle and/or positive control:	Water, solubility was increased by addition of sodium hydrogen carbonate
Test animals:	
Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	6-8 weeks (males), 7-9 weeks (females)
Sex:	Males and females
Weight at dosing:	approximately 200 g
Acclimation period:	At least 5 days
Diet/Food:	Standard Laboratory Diet LAD 1 (Special Diet Services, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Individual housing in suspended, wire bottom, stainless steel cages. After dosing: Excretion-balance experiments - individually in glass metabolism cages Blood/plasma kinetics - in stainless steel battery cages Tissue distribution – in stainless steel battery cages
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 40 - 60 % Air changes: not reported 12-hour light/dark cycle

Study design and methods
In life dates: not reported

Animal assignment and treatment: Preliminary excretion studies
In two independent experiments two rats (1 male, 1 female) received single oral doses of either 10 or 600 mg/kg bw/day by gavage and were placed in glass metabolism cages immediately thereafter. Urine was collected at 0-6, 6-24, and every 24 h for 7 days in receivers cooled with solid CO_2. Faeces were collected every 24 h for 7 days. Expired air was passed through traps containing an ethanolamine/2-ethoxyethanol mixture (1:3, v/v). These traps were changed every 24 h for 7 days after dosing. The interior of the cages were washed with water at sacrifice after 7 days. Samples were analysed immediately or were stored at -20 °C until taken for analysis.

Animal assignment and treatment: Excretion studies
In two independent experiments 10 rats (5 male, 5 female) received single oral doses of either 10 or 600 mg/kg bw/day by gavage and were placed in glass metabolism cages immediately thereafter. Urine and faeces were collected as described in the preliminary study. Blood was drawn by cardiac puncture (following light halothane anaesthesia) prior to sacrifice by cervical dislocation and plasma was obtained by centrifugation. The following tissues/organs were taken or sampled for radioactivity measurement:
Adrenals, bone, bone marrow (femur), brain, eyes, fat (abdominal), gastrointestinal tract, heart, kidneys, liver, lungs, lymph nodes (mesenteric), muscle (skeletal), ovaries, pancreas, pituitary gland, plasma, skin, spleen, stomach, testes, thymus, thyroid with parathyroid, urinary bladder, uterus and residual carcass. The contents of the gastrointestinal tract and stomach were analysed separately.

Animal assignment and treatment: Plasma concentrations
In two independent experiments 18 rats (9 male, 9 female) received single oral doses of either 10 or 600 mg/kg bw/day by gavage. The animals were divided into three groups of six (3 per sex) and blood samples (0.5 mL) were taken from the tail vein into heparinised tubes at the following times from each group.
Group 1: prior to administration, 1, 4, 24 and 96 h
Group 2: 0.25, 2, 6, 48 and 120 h
Group 3: 0.5, 3, 12, 72 and 168 h
Each group was sacrificed upon completion of the specified sampling schedule.

Animal assignment and treatment: Quantitative tissue distribution
In two independent experiments 12 rats (6 male, 6 female) received single oral doses of either 10 or 600 mg/kg bw/day by gavage. The animals were divided into two groups of six (3 per sex) and sacrificed by cervical dislocation 6 and 18 h (low dose) or 3 and 9 h (high dose) after dosing, depending on the peak plasma concentrations and half the plasma concentration derived in the blood/plasma kinetics experiments. Data for 168 h (7 days) was provided by the excretion studies. Blood samples were taken by cardiac puncture (following light halothane anaesthesia) prior to sacrifice by cervical dislocation and plasma was obtained by centrifugation. The following tissues/organs were taken or sampled for radioactivity measurement: adrenals, bone, bone marrow (femur), brain, eyes, fat (abdominal), gastrointestinal tract, heart, kidneys, liver, lungs, lymph nodes (mesenteric), muscle (skeletal), ovaries, pancreas, pituitary gland, plasma, skin, spleen, stomach, testes, thymus, thyroid with parathyroid, urinary bladder, uterus and residual carcass. The contents of the gastrointestinal tract and stomach were analysed separately.

Measurement of radioactivity
Faeces were initially extracted by homogenisation with chloroform : 1N HCl (1:1, v/v) followed by further extracts with 1N HCl. After centrifugation radioactivity was measured in both extracts and residues. Samples of urine, plasma, solvent extracts contents of expired air traps, cage washings and other liquid samples were mixed with Special Scintillator MI-31 (Packard Instrument Co. Ltd, Reading, UK) for measurement of radioactivity by liquid scintillation counting (LSC). Samples of faecal residues, gastro-intestinal tract, liver, spleen and whole blood were combusted, absorbed, mixed with scintillation cocktail and analyzed thereafter. Carcasses were digested for 48 h at 55 °C in a solution of 2M NaOH in 30 % Methanol containing Triton X-405 (10 % v/v). Tissue samples suitable for solubilisation were incubated at around 50 °C for 18-24 h with solubiliser and mixed with scintillation cocktail and analyzed thereafter. Radioactivity with less than twice background counts was considered to be below the limit of accurate quantification when performing LSC.

Isolation of the major urinary and faecal metabolites
Samples of urine and faecal extracts from male and female rats were pooled and analysed directly by TLC or HPLC. Radiolabelled metabolite products formed in the rat were identified by co-chromatographic comparison using different systems with the reference compound aminomethyl phosphonic acid (AMPA) or ^{14}C-AMPA.

Thin layer chromatography (TLC)
TLC was carried out on pre-layered Merck cellulose F plates (0.2 mm, BDH Chemicals Ltd., Poole, UK) using the following development systems:
System 1: Ethanol : water : ammonium hydroxide : trichloroacetic acid : acetic acid (55 : 35: 5: 3.5 : 2, v/v/v/w/v)
System 2: Ethanol : water : ammonium hydroxide : trichloroacetic acid : acetic acid (65 : 35: 2.5: 3.5 : 2, v/v/v/w/v)
System 5: Methanol : water : acetic acid (67 : 33 : 1, v/v/v)

Radioactivity was detected with a Berthold Linear Analyser controlled by a computer system (Berthold Instruments (UK)) and proportions of radioactive components were measured by integrating the areas under the peaks on the radio chromatogram following subtraction of background levels. Alternatively, components were detected and quantified using a Fuji BAS 2000 Bioimage Analyser. The produced images of radioactive TLC plates were processed to generate quantitative data.

High performance liquid chromatography (HPLC)
Two HPLC methods were used. HPLC system 1 (gradient elution method; column: Sperisorp SAX HPLC column (Hichrom, UK) and guard column, eluent A: water, eluent B: 0.75 M KH_2PO_4, pH 3.35) and HPLC system 2 (isocratic method; column: glyphosate analytical column (BioRad, USA), eluent: 0.005 M KH_2PO_4 + 4 % methanol v/v, pH 2.1) were both linked to an UV- and a radio-detector. A Compaq Prolinea computer with Labchrom software was used to collect and process data from the UV and radio detectors. Samples were co-injected with a mixture of the reference standards. Fractions were collected and radio assayed by LSC.

Results and discussion
Excretion and retention of radioactivity
A preliminary study on two rats per dose (male/female) indicated that more than 90 % of the administered radioactivity was excreted within 7 days by both the low and the high dose

group after a single application of the test substance. Almost no radioactivity could be detected in expired air (about 0.15 %).
The main study with 10 rats per dose confirmed the initial observation (see Table B.6.1-1). During the 7 days observation period 23 % and 19 % (male/female) were excreted in the urine of the low dose group. Slightly higher percentages, 30 % and 29 % (male/female), of total administered radioactivity were detected in urine of the high dose group. The main portion of the radioactivity was detected at both dose levels within the first 48 h in males and females (21 % and 18 %, 10 mg/kg bw; 28 % and 27 %, 600 mg/kg bw). In both dose groups about 75 % of the administered radioactivity could be detected in the faeces of males and females within 7 days (75 % and 84 %, 10 mg/kg bw; 75 % and 74 %, 600 mg/kg bw). Again most of the radioactivity was detected within 48 h after dosing (72 % and 82 %, 10 mg/kg bw; 72 % and 69 %, 600 mg/kg bw). About 0.3 % of the radioactivity remained in the carcasses of the sacrificed animals after 7 days. Thus, in male and female rats almost all the administered radioactivity was excreted via in the urine and faeces within 7 days (97 % and 104 %, 10 mg/kg bw; 105 % and 104 %, 600 mg/kg bw).

Table B.6.1-1: Excretion balance (in mean % of applied dose) up to 168 h post dosing

Balance/Excretion	**10 mg/kg bw**		**600 mg/kg bw**	
	Males	Females	Males	Females
Urine 0-6	2.63	3.25	11.55	9.08
Urine 6-24	15.85	12.69	13.85	13.36
Urine 24-48	2.82	2.41	2.33	4.40
Urine 48-72	0.54	0.44	0.59	1.07
Urine 72-96	0.24	0.19	0.30	0.40
Urine 96-120	0.15	0.13	0.21	0.24
Urine 120-144	0.09	0.07	0.17	0.17
Urine 144-168	0.07	0.05	0.13	0.18
Cage wash	0.12	0.14	1.13	0.60
Subtotal urine + cage wash	22.51	19.37	30.26	29.50
Faeces 0-24	60.28	74.59	58.94	46.28
Faeces 24-48	11.72	7.56	13.41	22.87
Faeces 48-72	1.18	1.34	1.36	3.83
Faeces 72-96	0.29	0.36	0.35	0.47
Faeces 96-120	0.17	0.27	0.36	0.23
Faeces 120-144	0.35	0.08	0.08	0.12
Faeces 144-168	0.64	0.10	0.15	0.35
Subtotal faeces	74.63	84.30	74.65	74.15
Carcass	0.33	0.27	0.31	0.39
Total	97.47	103.94	105.22	104.04

Concentration of radioactivity in the plasma
After a single oral dose of 10 mg/kg bw ^{14}C HR-001 to rats peak mean concentrations of radioactivity in plasma occurred at 6 and 2 h in males (0.22 µg equiv./mL) and females (0.28 µg equiv./mL), respectively. The absorption rate constants were 0.2963 h^{-1} in males and 0.4239 h^{-1} in females. Concentrations declined with an approximate half-life of 8.3 h in males and 7.8 h in females. The area under the concentration *versus* time curve (AUC_t) was 3.2 and 3.7 µg equiv./mL *h in males and females, respectively (see Table B.6.1-2).

After a single oral dose of 600 mg/kg bw ^{14}C HR-001 to rats peak mean concentrations of radioactivity in plasma occurred at 3 h in males (26 µg equiv./mL) and females (29 µg

equiv./mL), respectively (see Table B.6.1-3). The absorption rate constants were with 0.2845 h^{-1} in males and 0.4477 h^{-1} in females comparable with the low dose group, thus absorption did not increase with dose. Concentrations declined with an approximate half-life of 5.9 h in males. The terminal half life could not be calculated for females of the high dose group due to rapid clearance from plasma. The area under the concentration *versus* time curve (AUC_t) was calculated at 400 and 355 µg equiv./mL*h in males and females, respectively. These values were around 120 fold higher than the AUC_t obtained in the low dose group.

Table B.6.1-2: Kinetic parameters in plasma after single oral doses of 10 or 600 mg/kg bw

	10 mg/kg bw		**600 mg/kg bw**	
	Males	Females	Males	Females
C_{max} (µg equiv./mL)	0.2219	0.2789	25.97	28.84
T_{max} (h)	6.00	2.00	3.00	3.00
AUC_t (µg equiv./mL*h)	3.20	3.70	399.90	355.30
AUC (µg equiv./mL*h)	3.80	4.20	419.00	*
Terminal rate constant (h^{-1})	0.0840	0.0887	0.1174	*
Terminal half life (h)	8.30	7.80	5.90	*
Absorption rate constant (h^{-1})	0.2963	0.4239	0.2845	0.4477

* could not be calculated

Measurements in whole blood in general lead to the same result.

Distribution of radioactivity in tissue
Radioactivity concentrations in tissues were very low at all times (see Table B.6.1-3 and Table B.6.1-4). There was no indication of accumulation of radioactivity in any tissue. Only the gastrointestinal tract (GIT) the stomach, muscles and the kidneys, the organs of excretion contained higher concentrations of radioactivity than the plasma. High levels of radioactivity were detected in the content of stomach and GIT. At 7 days p.a. the radioactivity in most tissues had decreased to around the limit of detection. Highest remaining concentrations were detected in carcass (<0.4 %).

Table B.6.1-3: Radioactivity in tissues after single oral dose of 10 mg/kg bw (in mean % of applied dose, except bone and skin expressed as % of applied dose/g)

Tissue	**Males**			**Females**		
	6 h (n=3)	18 h (n=3)	168 h (n=5)	6 h (n=3)	18 h (n=3)	168 h (n=5)
Adrenal glands	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Bone	0.12	0.10	0.02	0.10	0.09	0.03
Bone marrow	0.01	0.01	<0.01	0.01	0.01	0.01
Brain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carcass	2.00	2.69	0.33	1.69	3.03	0.27
Eyes	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fat (abdominal)	0.06	0.04	0.01	0.04	0.03	0.01
Gastrointestinal tract	19.05	10.04	0.01	16.47	5.41	0.01
GIT contents	31.56	4.89	0.01	34.54	14.30	0.01
Heart	0.01	<0.01	<0.01	0.01	<0.01	<0.01
Kidneys	0.79	0.36	<0.01	0.67	0.26	<0.01
Liver	0.07	0.09	0.01	0.06	0.07	0.01
Lungs	0.01	0.01	<0.01	0.01	0.01	<0.01
Lymph nodes	0.09	0.05	<0.01	0.04	0.04	<0.01
Muscle (skeletal)	0.23	0.13	0.04	0.24	0.11	<0.03

Tissue	Males			Females		
	6 h (n=3)	18 h (n=3)	168 h (n=5)	6 h (n=3)	18 h (n=3)	168 h (n=5)
Ovaries	-	-	-	<0.01	<0.01	<0.01
Pancreas	0.02	<0.01	<0.01	0.01	<0.01	<0.01
Pituitary gland	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Plasma	0.12	0.03	<0.01	0.13	0.03	<0.01
Skin	0.01	0.01	<0.01	0.01	0.01	<0.01
Spleen	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Stomach	3.47	0.60	0.60	2.56	0.62	<0.01
Stomach contents	25.16	5.05	0.01	22.90	6.96	0.01
Testes	0.01	0.01	<0.01	-	-	-
Thymus	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thyroid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Urinary bladder	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Uterus	-	-	-	0.01	<0.01	<0.01
Whole blood	0.20	0.04	<0.03	0.15	0.05	<0.03

Table B.6.1-4: Radioactivity in tissues after single oral dose of 600 mg/kg bw (in mean % of applied dose, except bone and skin expressed as mean % of applied dose/g)

Tissue	Males			Females		
	3 h (n=3)	9 h (n=3)	168 h (n=5)	3 h (n=3)	9 h (n=3)	168 h (n=5)
Adrenal glands	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Bone	0.09	0.10	0.02	0.09	0.05	0.02
Bone marrow	0.01	0.01	<0.01	0.01	0.01	<0.01
Brain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carcass	1.87	1.70	0.31	2.85	2.41	0.39
Eyes	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fat (abdominal)	0.09	0.05	<0.01	0.09	0.02	<0.01
Gastrointestinal tract	19.71	9.99	0.01	20.90	9.33	0.01
GIT contents	30.48	13.19	0.02	22.65	12.86	0.03
Heart	0.01	<0.01	<0.01	0.01	<0.01	<0.01
Kidneys	1.00	0.55	<0.01	0.82	0.21	<0.01
Liver	0.06	0.14	0.01	0.07	0.06	0.02
Lungs	0.02	0.01	<0.01	0.02	0.01	<0.01
Lymph nodes	0.07	0.02	<0.01	0.04	0.01	<0.01
Muscle (skeletal)	0.38	0.19	<0.05	0.48	0.18	<0.05
Ovaries	-	-	-	<0.01	<0.01	<0.01
Pancreas	0.01	0.01	<0.01	0.01	0.01	<0.01
Pituitary gland	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Plasma	0.26	0.07	<0.01	0.30	<0.01	<0.01
Skin	0.01	<0.01	<0.01	0.01	<0.01	<0.01
Spleen	0.01	0.01	<0.01	<0.01	<0.01	<0.01
Stomach	3.53	3.36	<0.01	4.33	3.14	<0.01
Stomach contents	28.73	32.70	0.02	34.20	45.01	0.02
Testes	0.01	0.01	<0.01	-	-	-
Thymus	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thyroid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Urinary bladder	0.01	0.01	<0.01	<0.01	<0.01	<0.01
Uterus	-	-	-	0.01	<0.01	<0.01
Whole blood	0.28	0.08	0.01	0.30	0.05	<0.01

Proportion of radioactive components in urine

Major urinary (18 - 27 %) and faecal (65 - 78 %) component was the parent compound. One further minor component was also observed in urine (0.1 – 0.3 %) and identified as aminomethyl phosphonic acid by TLC and HPLC co-chromatography. In faeces two further minor components were detected (1-2 %, low dose; 0.3-0.6 %, high dose). One of them could be identified as aminomethyl phosphonic acid by TLC and HPLC co-chromatography.

Conclusion by the Notifiers
After oral administration of glyphosate (HR-001) at least about 25 % are absorbed. Absorption was similar in both sexes. About 75 % and 25 % of the parent compound are excreted via faeces and urine, respectively. There was no indication for accumulation of glyphosate.

RMS comments
The study is considered acceptable. As in some other studies of this section, it is not clear if the animals were fasted before sacrifice but this will not have affected the outcome of the study. The results confirmed previous knowledge, in particular with regard to rather poor oral absorption from the gut (based on renal excretion), distribution of radioactivity to the various body compartments with certain affinity to the bones, virtually complete excretion and very minor metabolism. The identity of the second minor component in faeces (beside AMPA) should have been elucidated but it may be assumed to be a transformation product of bacterial activity in the gut. Unfortunately, no experimental group receiving multiple treatment was included in this study. Thus, final proof for the absence of an accumulating potential cannot be taken from the results of this study alone. However, such investigations have been performed by other researchers ([redacted] 1996 (TOX2000-1979); [redacted] 1992 (TOX9300343); [redacted], 1988 (TOX9552356)) giving sufficient information on this endpoint.

2nd study [redacted], 1996)

Reference:	IIA, 5.1.1/02
Report:	[redacted]. (1996) [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat [redacted] Data owner: Nufarm Report No.: 1413/2-1011 Date:1996-10-23, not published, ASB2012-11380
Guidelines:	Japanese MAFF, 59 NohSan, Notification No. 4200 (1995)
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Materials and methods

Non-labelled test material:	
Identification:	Glyphosate
Description:	Not reported
Lot/Batch #:	08808TG and H95D161A
Purity:	96 % and 95.3 %, respectively
Stability of test compound:	Not reported
Radiolabelled test material:	
Identification:	[^{14}C] – glyphosate
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Description:	Not reported
Lot/Batch #:	24, lot 3 and 25, lot 4-7
Purity:	> 99 % (HPLC and TLC)
Specific activity:	310 µCi/mg, 53 mCi/mmol
Stability of test compound:	Stable over 24 h under the conditions of the study
Reference substance:	
Identification:	Aminomethylphosphonic acid (AMPA) (CAS No. 1066-51-9)
Description:	Not reported
Lot/Batch #:	50526010
Purity:	Not reported
Stability of test compound:	Not reported
Vehicle:	Deionised water
Test animals:	
Species:	Rat
Strain:	Sprague-Dawley (Crl:CD BR)
Source:	[redacted]
Age:	6-10 weeks
Sex:	Males and females
Weight at dosing:	179 - 280 g (males) and 167 - 205 g (females)
Acclimation period:	Approximately 1 week
Diet/Food:	SQC Rat and Mouse Maintenance Diet No. 1, Expanded (Special Diet Services, Stepfield, Witham, Essex, UK), *ad libitum* Diet was removed the evening before and returned 4 h after administration.
Water:	Tap water, *ad libitum*

Housing:	During acclimatisation: Groups of 5 per cage, in wire floor polypropylene cages suspended over polypropylene dirt trays containing wood saw dust After dosing: Excretion-balance experiments - individually in glass metabolism cages Blood/plasma kinetics - in wire floor cages Tissue distribution – in wire floor cages
Environmental conditions:	Temperature: 21 ± 2 °C (24 and 26 °C on two consecutive days) This deviation did not affect the study outcome Humidity: 40 - 70 % Air changes: not reported 12-hour light/dark cycle

Study design and methods
In life dates: not reported

Animal assignment and treatment: Preliminary excretion study
Four fasted rats (2 males, 2 females) received single oral doses of 100 mg/kg bw/day by gavage and were placed in glass metabolism cages immediately thereafter. Urine was collected at 0-12, 12-24, and every 24 h for 7 days in receivers cooled with solid CO_2. Faeces were collected every 24 h for 7 days. Expired air was passed through duplicate traps containing an ethanolamine/2-ethoxyethanol mixture (1:3, v/v). These traps were changed after 12, 24, 48 and 72 h after dosing. The interiors of the cages were rinsed with water after each collection time. At the end of the collection period cages were rinsed with water and methanol. Samples were analysed accordingly.

Animal assignment and treatment: Excretion studies
In two independent experiments 10 fasted rats (5 males, 5 females) received single oral doses of either 1 or 100 mg/kg bw/day by gavage and were placed in glass metabolism cages immediately thereafter. Urine and faeces were collected as described in the preliminary study.

Animal assignment and treatment: Plasma concentrations
In two independent experiments 10 fasted rats (5 males, 5 females) received single oral doses of either 1 or 100 mg/kg bw/day by gavage. Blood samples (0.1 mL) were taken from the tail vein into heparinised tubes at the following times from each animal:
Prior to administration, and 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 36, 48 and 72 h after administration.
Blood was centrifuges to separate plasma and radioactivity was determined in plasma.

Animal assignment and treatment: Quantitative tissue distribution
In two independent experiments 24 fasted rats (12 male, 12 female) received single oral doses of either 1 or 100 mg/kg bw/day by gavage. The animals were divided into four groups of six (3 per sex) and sacrificed 4, 12, 24 and 72 (low dose) or 4, 6, 24 and 72 h (high dose) after dosing. Animals were exsanguinated under halothane anaesthesia. Following tissues/organs were taken or sampled for radioactivity measurements:

Adrenals, bone, brain, fat (abdominal), gastrointestinal tract, heart, kidneys, liver, lungs, muscle (quadriceps), gonads, plasma, skin, spleen, and residual carcass

The contents of the gastrointestinal tract was analysed separately.

Animal assignment and treatment: Biliary excretion study
16 rats were cannulated (8 males, 8 females), of which 14 received single oral doses of 1 mg/kg bw/day by gavage. Following incision animals were placed in metabolism cages and allowed to recover for 24 h. Fully recovered animals were dosed after they were fasted overnight, and bile, urine and faeces were taken from the animals at the following times:
Bile: prior to administration, 0 - 1, 1 - 4, 4 - 6, 6 - 12, 12 - 24 and 24 - 48 h p.a.
Urine, faeces: 0 - 24 and 24 - 48 h p.a. in vessels cooled with solid CO_2.

The interiors of the cages were rinsed with water after each collection time. At the end of the collection period cages were rinsed with water and methanol. Samples were analysed accordingly.

Measurement of radioactivity
Pooled faecal samples were extracted with water prior to solid phase extraction. Urine samples were diluted with water prior to solid phase extraction. Solid phase extraction was performed using columns, conditioned with methanol and de-ionised water. The samples were loaded onto the cartridge washed with de-ionised water, methanol and again water. Radioactivity was eluted using formic acid (5 % v/v). The eluate was freeze dried and reconstituted in water prior to HPLC analysis and where appropriate LC - MS (samples of 100 mg/kg dose group).

A suitable volume of solubilising agent was added to tissue samples. After an appropriate incubation time, liquid scintillant was added and samples were subjected to liquid scintillation counting (LSC). Samples of faecal residues, cage debris homogenates, blood and bone were combusted, absorbed, mixed with scintillation cocktail and analysed by LSC thereafter. Combustion and trapping efficiencies were found to be in excess of 96 % and all reported data are, therefore, uncorrected. Radioassays were performed in duplicats.

Isolation of the major urinary and faecal metabolites
Samples of urine and faecal extracts from male and female rats of the excretion studies were pooled and analysed directly by HPLC. Representative samples were then submitted for analysis by mass spectrometry. The samples were analysed for the presence of glyphosate and the potential metabolite aminomethyl phosphonic acid (AMPA). Following samples were pooled and analysed for each dose group and sex:
Excretion study: Urine 12 - 24 h, Faeces 24 - 48 h
Biliary excretion study: Urine 24 - 48 h, Faeces 24 - 48 h

High performance liquid chromatography (HPLC)
The gradient elution method was used for sample analysis (column: Sperisorp SAX 250 x 4.6 mm id, eluent A: water, eluent B: 0.75 M KH_2PO_4, pH 3.35). The system was linked to a radio-detector. Following HPLC analysis, representative samples were submitted for analysis by mass spectroscopy (samples of 100 mg/kg bw dose group).

Liquid chromatography - Mass Spectrometry (LC - MS)
A VG Quattro triple quadrupole mass spectrometer with electrospray LCMS interface connected to a Jasco ternary gradient HPLC system and a Lablogic β-Ram radio detector were used.

Mode: positive ion electrospray
Scan range: m/z 50 – 250
Mobile phases: water or 1 M formic acid

Glyphosate was detected using Multiple Reaction Monitoring (MRM) of m/z 170 → 88. AMPA was detected using Selected Ion Recording (SIR) of m/z 112.

Results and discussion

Excretion and retention of radioactivity

In a preliminary study with a single dose of 100 mg/kg bw (two rats/sex) the mean total recovery of radioactivity within 7 days was 100.3 % (male) and 95.15 % (female). No relevant radioactivity could be detected in expired air or carcass.

The initial observation was confirmed in the main study with 10 rats per dose (see Table B.6.1-5).

Mean total recovery of radioactivity in rats receiving a single dose of 1 mg/kg bw was 98.31 % in males and 98.81 % in females. Elimination of radioactivity was almost complete within the first 48 h after dosing. The major route of elimination after oral dosing was faeces with 72.62 % and 62.39 % recovered in males and females, respectively, with most of the radioactivity being excreted within the first 24 h after dosing, suggesting this proportion of the dose was not systemically absorbed. During the 7 days observation period 24.92 % (male) and 34.86% (female) of radioactivity were recovered in the urine, representing the systemically absorbed dose.

After administration of 100 mg/kg bw to rats mean total recovery of radioactivity was 96.31 % in males and 98.50 % in females. Elimination of radioactivity in the urine (including cage wash 53.27 % in males and 55.04 % in females) was quantitatively more significant compared to the low dose group. Faecal elimination accounted for 41.23 % in males and 42.37 % in females. Again most of the radioactivity was recovered within the first 48 h after dosing.

Table B.6.1-5: Excretion balance (in mean % of applied dose) up to 168 h post dosing

Balance/Excretion	**1 mg/kg bw**		**100 mg/kg bw**	
	Males	Females	Males	Females
Urine 0-12	9.52	15.47	31.30	34.93
Urine 12-24	6.14	7.59	4.68	4.46
Urine 24-48	2.10	3.03	2.40	2.32
Urine 48-72	0.35	0.56	0.46	0.71
Urine 72-96	0.15	0.20	0.27	0.33
Urine 96-120	0.09	0.14	0.14	0.15
Urine 120-144	0.06	0.10	0.10	0.10
Urine 144-168	0.04	0.06	0.08	0.07
Cage wash	6.48	7.71	13.85	11.96
Subtotal urine + cage wash	24.92	34.86	53.27	55.04
Faeces 0-24	63.93	49.69	30.46	32.28
Faeces 24-48	7.21	10.93	9.96	4.46
Faeces 48-72	0.65	1.46	0.55	1.10
Faeces 72-96	0.09	0.16	0.12	4.42
Faeces 96-120	0.03	0.06	0.06	0.06
Faeces 120-144	ND	0.07	0.06	0.04
Faeces 144-168	0.71	0.02	0.03	0.01
Subtotal faeces	72.62	62.39	41.23	42.37
Cage debris	0.03	0.58	0.98	0.10

Balance/Excretion	**1 mg/kg bw**		**100 mg/kg bw**	
	Males	Females	Males	Females
Carcass	0.75	0.98	0.84	0.98
Total	98.31	98.81	96.31	98.50

Biliary excretion of radioactivity

Biliary excretion was determined in biliary cannulated rats receiving 1 mg/ kg bw. Within 48 h 94.63 % and 95.99 % of radioactivity were recovered in males and females, respectively. Major route of elimination was faeces. Negligible amounts of radioactivity were detected in the bile (see Table B.6.1-6) providing strong evidence that low doses of systemic glyphosate are eliminated almost exclusively in the urine.

Table B.6.1-6: Excretion balance (in mean % of applied dose) at 48 h post dosing in biliary excretion study

	1 mg/kg bw	
	Males	Females
Urine	27.45	24.21
Faeces	55.33	60.97
Bile	0.031	0.076
Cage wash	6.571	6.769
Cage debris	0.262	0.146
Carcass	4.989	3.817
Total	94.63	95.99

Concentration of radioactivity in the plasma

Following a single oral dose of 1 mg/kg bw of the test substance, low levels of radioactivity were detected in plasma (see Table B.6.1-7). Concentrations of radioactivity declined rapidly such that the levels of radioactivity were below the detection limit in most animals by 24 h. The mean terminal elimination half-lives were 10.86 h and 8.07 h with corresponding AUC of 0.319 and 0.340 µg equiv./mL*h in males and females respectively. As the elimination half-lives could not be calculated for several animals of the high dose group mean AUC_{0-24} (0.257 and 0.338 µg equiv./mL*h in males and females) were calculated to compare the results of both groups.

Following a single oral dose of 100 mg/kg bw of the test substance mean maximal plasma concentration of 8.91 (male) and 7.63 µg equiv./mL (female) were observed 2-4 h post-dose in males and 4 h post dose in females (see Table B.6.1-7). Mean AUC_{0-24} were 58.2 and 50.7 µg equiv./mL*h in males and females, respectively. Levels of radioactivity were below the detection limit in males by 48 and in females by 72 h.

Table B.6.1-7: Kinetic parameters in plasma after single oral dose of 1 or 100 mg/kg bw (n = 5)

	1 mg/kg bw		**100 mg/kg bw**	
	Males	Females	Males	Females
C_{max} (µg equiv./mL)	0.016	0.037	8.909	7.634
T_{max} (h)	3.900	8.000	3.600	4.000
AUC_{0-24} (µg equiv./mL*h)	0.257	0.338	58.200	50.700
AUC (µg equiv./mL*h)	0.319	0.340	*	*
Terminal half life (h)	10.860	8.065	*	*

* could not be calculated

Distribution of radioactivity in tissue

After administration of 1 mg/kg bw radioactivity concentrations were detected in all tissues by 4 h post-dose (see Table B.6.1-8 and Table B.6.1-9). Apart from the gastrointestinal tract (and content) and carcass, the kidney was the only tissue with a notable content of radioactivity throughout the observation period. By 72 h, post-dose concentrations had decreased or plateaued to less than 2 % of the administered dose in all tissues of either sex, with carcass containing most of the remaining radioactivity. After administration of 100 mg/kg bw all tissues were exposed to radiolabelled material by 4 h post-dose (see Table B.6.1-10 and Table B.6.1-11). Again, only gastrointestinal tract, carcass and kidney contained significant amounts of radioactivity. By 72 h post-dose concentrations had decreased or plateaued to less than 2 % of the administered dose in all tissues of either sex, with carcass containing most of the remaining radioactivity.

Table B.6.1-8: Radioactivity in tissues after single oral dose of 1 mg/kg bw (in mean µg equiv./g)

Tissue	Males				Females			
	4 h	12 h	24 h	72 h	4 h	12 h	24 h	72 h
Adrenals	0.014	0.024	0.020	0.009	0.023	0.031	0.022	0.009
Blood	0.010	0.015	0.001	0.002	0.020	0.009	0.002	<0.001
Bone	0.062	0.105	0.201	0.123	0.091	0.140	0.134	0.112
Brain	<0.001	0.003	0.003	0.002	0.002	0.001	0.002	0.002
Carcass	0.021	0.028	0.049	0.016	0.035	0.076	0.045	0.024
Fat	0.022	0.005	0.003	0.002	0.013	0.010	0.006	0.002
GIT + contents	13.040	1.333	1.272	0.026	11.630	3.531	1.314	0.075
Heart	0.006	0.004	0.003	0.002	0.010	0.006	0.004	0.001
Kidney	0.463	0.380	0.307	0.020	0.424	0.387	0.129	0.012
Liver	0.012	0.013	0.022	0.012	0.016	0.018	0.015	0.012
Lung	0.009	0.009	0.013	0.006	0.019	0.013	0.009	0.006
Muscle	0.003	0.001	0.002	<0.001	0.006	0.003	0.002	0.001
Ovaries	-	-	-	-	0.031	0.018	0.021	0.007
Plasma	0.017	0.011	0.006	<0.001	0.027	0.015	0.004	<0.001
Skin	0.010	0.026	0.016	0.006	0.029	0.016	0.106	0.014
Spleen	0.004	0.009	0.010	0.005	0.010	0.009	0.010	0.005
Testes	0.004	0.002	0.001	0.001	-	-	-	-

Table B.6.1-9: Radioactivity in tissues after single oral dose of 1 mg/kg bw (in mean % of applied dose)

Tissue	Males				Females			
	4 h	12 h	24 h	72 h	4 h	12 h	24 h	72 h
Adrenals	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Brain	<0.001	0.003	0.002	0.002	0.002	0.001	0.002	0.002
Carcass	1.236	1.668	3.048	1.045	1.887	4.115	2.542	1.405
GIT + contents	94.310	17.670	12.990	0.342	89.940	41.740	13.760	0.910
Heart	0.002	0.001	0.001	0.001	0.005	0.003	0.002	0.001
Kidney	0.392	0.304	0.255	0.016	0.348	0.341	0.110	0.011
Liver	0.040	0.050	0.113	0.059	0.055	0.070	0.073	0.057
Lung	0.005	0.005	0.007	0.003	0.012	0.008	0.005	0.004
Ovaries	-	-	-	-	0.002	0.001	0.001	<0.001
Spleen	0.001	0.002	0.002	0.001	0.002	0.002	0.002	0.001
Testes	0.004	0.002	0.002	0.001	-	-	-	-

Table B.6.1-10: Radioactivity in tissues after single oral dose of 100 mg/kg bw (in mean µg equiv./g)

Tissue	Males				Females			
	4 h	6 h	24 h	72 h	4 h	6 h	24 h	72 h
Adrenals	2.936	5.610	1.856	0.338	8.161	7.244	1.522	0.504
Blood	4.545	4.900	0.016	ND	5.719	1.923	0.218	ND
Bone	24.660	31.360	18.600	11.140	35.450	24.420	17.010	10.420
Brain	0.344	0.699	0.269	0.221	0.619	0.630	0.293	0.215
Carcass	6.097	26.530	4.978	1.843	10.910	38.410	7.206	3.057
Fat	1.366	1.547	0.290	0.120	3.826	2.042	0.393	0.115
GIT+contents	1155.000	544.600	47.750	1.279	1057.000	401.800	59.580	4.320
Heart	2.063	3.424	0.363	0.140	3.704	2.282	0.314	0.092
Kidneys	105.500	127.700	17.440	1.433	132.200	55.770	10.800	1.191
Liver	2.942	4.970	1.831	1.165	5.105	5.564	1.552	0.981
Lung	3.495	4.206	1.069	0.423	6.476	4.623	0.999	0.443
Muscle	0.827	0.887	0.168	0.026	1.698	1.141	0.213	0.051
Ovaries	-	-	-	-	7.532	5.407	1.260	0.438
Plasma	6.479	5.406	0.359	ND	10.830	3.033	0.403	ND
Skin	2.884	3.520	1.293	0.313	6.106	22.480	1.543	0.435
Spleen	1.277	2.678	0.974	0.479	2.337	1.237	0.937	0.395
Testes	0.949	0.942	0.203	0.104	-	-	-	-

ND not detected

Table B.6.1-11: Radioactivity in tissues after single oral dose of 100 mg/kg bw (in mean % of applied dose)

Tissue	Males				Females			
	4 h	6 h	24 h	72 h	4 h	6 h	24 h	72 h
Adrenals	0.001	0.001	<0.001	<0.001	0.002	0.001	<0.001	<0.001
Brain	0.003	0.005	0.002	0.002	0.006	0.006	0.003	0.002
Carcass	4.620	8.549	2.402	1.014	8.288	5.879	2.752	1.254
GIT + contents	85.430	64.870	5.456	0.199	75.050	48.910	7.509	0.676
Heart	0.009	0.015	0.001	0.001	0.016	0.009	0.001	<0.001
Kidney	0.870	1.109	0.151	0.012	1.165	0.535	0.096	0.011
Liver	0.104	0.180	0.110	0.060	0.183	0.214	0.088	0.050
Lung	0.027	0.024	0.006	0.003	0.040	0.027	0.007	0.003
Ovaries	-	-	-	-	0.004	0.002	0.001	<0.001
Spleen	0.003	0.007	0.002	0.001	0.006	0.003	0.003	0.001
Testes	0.011	0.010	0.003	0.001	-	-	-	-

Metabolite profiling

After analysis of the pooled samples by HPLC a comparison of chromatograms indicated that the metabolism of the compound was not influenced by the sex or dose level. The peak with the majority of radioactivity could be allocated to [^{14}C]-glyphosate standard. A peak with <1 % of the total radioactivity was thought to correspond to AMPA. The presence of glyphosate could be confirmed by mass spectroscopy, whereas the presence of AMPA could not be verified due to technical problems.

Conclusion by the Notifiers

After oral administration of glyphosate absorption, distribution, metabolism and excretion were independent of dose level and sex. Absorption was limited and distribution was rapid and extensive. Metabolism was negligible. Elimination was essentially complete within 48 h, with the majority of radioactivity recovered in faeces, likely being the unabsorbed dose. The

remaining radioactivity was excreted with the urine. There was no indication for accumulation of glyphosate.

RMS comments

The study is considered acceptable. Again, poor absortion at least of the low dose, wide distribution and fast excretion of the compound as well as the virtual absence of metabolism were confirmed. Limited oral absorption rate was further supported by the very low amount of biliary excretion that was seldom investigated in ADME studies with glyphosate. In contrast, as compared to other studies, urinary elimination following the high dose was strikingly high. A much higher percentage of radioactivity than in other studies was found in cagewash and may be summed up with the material excreted in urine. The rather high organ/tissue residues in bile-cannulated rats at termination are due to the shorter duration of this experiment and are equal to what was measured in non-cannulated rats after 24 or 72 hours. It should be emphasised that the by far highest residues were found in bone since this was not mentioned in the GTF dossier. Elimination of bone-bound residues was apparently slower than of the radioactivity that had been retained in other tissues.
Unfortunately, no experimental group receiving multiple treatment was included in this study. Thus, final proof for the absence of an accumulating potential cannot be taken from the results of this study alone. However, such investigations have been performed by other researchers ([redacted] 1996 (TOX2000-1979); [redacted] 1992 (TOX9300343); [redacted], 1988 (TOX9552356)) giving sufficient information on this endpoint.

The following studies ([redacted] 1996 (TOX2000-1977, TOX2000-1978, TOX2000-1979, TOX2000-1980), [redacted] 1996 (TOX2000-1981)) have been performed to adress certain aspects of toxicokinetics and metabolism of glyphosate. Taken together, they allow comprehensive evaluation.

3d study ([redacted] 1996)

Reference:	IIA, 5.1.1/03
Report:	[redacted] (1996) Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat [redacted] Data owner: Syngenta Report No.: [redacted]/4940 Date:1996-04-26, not published, TOX2000-1977
Guidelines:	MAFF (Japan) Metabolism Study (1985), OECD 417 (1984), US-EPA FIFRA 85-1, EEC B.36 (1987)
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*.

Materials and methods

Non-labelled test material:	Glyphosate acid
Identification:	N-phosphonomethyl glycine
Description:	White solid
Lot/Batch #:	Y04707/045
Purity:	99.2 %
Stability of test compound:	Stable throughout the experiment
Radiolabelled test material:	
Identification:	[^{14}C]–glyphosate acid
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Y04707/047
Purity:	> 98 %
Specific activity:	1.580 GBq/mMol
Stability of test compound:	Stable throughout the experiment
Vehicle and/or positive control:	Deionised water
Test animals:	
Species:	Rat
Strain:	Alpk:AP$_f$SD
Source:	[redacted]
Age:	Not reported
Sex:	Male/female
Weight at dosing:	195 - 235 g
Acclimation period:	At least 5 days
Diet/Food:	PCD rat diet (SDS Ltd. Stepfield, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Groups of the same sex, in stock rat cages, 24 h prior to dosing transfer individually into metabolism cages After dosing: Excretion-balance experiments - individually in metabolism cages
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 55 ± 15 % Air changes: 12/hour 12-hour light/dark cycle

Study design and methods
In life dates: not reported

Animal assignment and treatment:(Excretion study and quantitative tissue distribution)

Ten rats (5 male, 5 female) received a single oral dose of 10 mg [^{14}C]-glyphosate acid/kg bw/day (10 mL/kg, 0.6 MBq/kg of dosing solution) by gavage and were placed back in glass metabolism cages immediately thereafter. Urine was collected at 6, 12, 24, 36, 48 and 72 h after dosing in receivers cooled with solid CO_2. Faeces were collected at 12, 24, 36, 48 and 72 h. The interior of the cages were washed with water after each collection time. At the end of the study cages were washed with ethanol/water 1:1 (v/v). Samples were stored at -20 °C until taken for analysis.
Animals were exsanguinated by cardiac puncture under halothane anaesthesia. Two blood samples were collected in heparinised vials. One was centrifuged to separate plasma. Following tissues/organs were taken or sampled for radioactivity measurements: bone (femur), brain, fat (abdominal), gastrointestinal tract and its contents, heart, kidneys, liver, lungs, muscle (femoral), gonads, spleen, salivary glands, residual carcass.

Measurement of radioactivity
Samples of urine, cage wash and plasma were taken, without further processing, for liquid scintillation counting (LSC). Faecal samples were ground with an approximately equal weight of anhydrous magnesium sulphate until homogeneous. Samples were analysed by sample oxidation followed by LSC. Bone (cut into pieces) and whole blood were analysed by sample oxidation followed by LSC. Liver, fat, residual carcass and gastrointestinal tract (GIT) plus contents were homogenised. Liver, fat and residual carcass were than solubilised in Soluene-350® whereas GIT plus contents were oxidised. All other tissues were solubilised without prior homogenisation.

Sample oxidation
Samples were oxidised in a Packard Tricarb sample oxidiser. The [^{14}C]-carbon dioxide generated was absorbed into Carbo-sorb E® and mixed with Permafluor E+® scintillant prior to analysis by LSC.

Liquid scintillation counting (LSC)
Samples and dilutions of the dosing preparation were mixed with Optiphase Hi-Safe 3® and counted for [^{14}C]-radioactivity to a 1% standard deviation of the count or for a maximum of 10 min, whichever occurred first. The results obtained were corrected for background activity and counting efficiency using [^{135}Ba] as the external source.

Results and discussion
Excretion and retention of radioactivity
After a single oral dose to rats, excretion was rapid for both sexes with most of the radioactivity being eliminated in the faeces during the first 24 h after dosing (means of 77.8 % in males, and 80.7 % in females). In the urine, means of 11.5 % and 9.4 % of the radioactivity were eliminated in the first 24 h in male and female rats, respectively. Within the observation period of 72 h, means of 101.8 % (male) and 99.6 % (female) of the administered radioactivity were excreted (see Table B.6.1-12). There were no differences in the cumulative excretion patterns between the sexes.

Table B.6.1-12: Excretion balance (in mean % of applied dose) at 72 h post dosing

Balance/Excretion	**10 mg/kg bw (oral gavage)**	
	Males	Females
Urine 0-6	3.7	3.5
Urine 6-12	4.5	3.3
Urine 12-24	3.3	2.6
Urine 24-36	0.8	0.7
Urine 36-48	0.4	0.4
Urine 48-72	0.3	0.2
Cage wash	0.3	0.4
Subtotal urine + cage wash	13.3	11.1
Faeces 0-12	42.3	48.1
Faeces 12-24	35.5	32.6
Faeces 24-36	6.6	3.9
Faeces 36-48	2.8	2.9
Faeces 48-72	1.3	1.2
Subtotal faeces	88.5	88.7
Total	101.8	99.6

Distribution of radioactivity in tissues

The highest tissue concentration of radioactivity was found in the bone with a mean concentration of 0.51 µg equiv./g (male) and 0.40 µg equiv./g (female), followed by the the intestinal tract plus contents with 0.15 µg equiv./g (males and females). Lower mean concentrations between 0.07 and 0.01 µg equiv./g were found in kidneys, liver, lungs, spleen, salivary glands and ovaries. Mean concentrations of 0.06 µg equiv./g were found in the residual carcass (which also included the skeletal bone) of either sex. All other concentrations were either similar to or lower than the corresponding blood concentrations (see Table B.6.1-13). The mean total percentage of administered radioactivity present in all of the tissues examined and the residual carcass was 0.6 % for males and 0.5 % for females. The amounts in the intestinal tract plus contents were about 0.2 % for both sexes.

Table B.6.1-13: Radioactivity in tissues after a single oral dose of 10 mg/kg bw at 72 h

Tissue	**Males**		**Females**	
	% of dose	µg equiv./g	% of dose	µg equiv./g
Blood	N/A	0.011	N/A	0.009
Bone (femur)	N/A	0.511	N/A	0.395
Brain	0.001	0.011	0.001	0.009
Fat (abdominal)	N/A	0.007	N/A	<0.004
Heart	<0.001	0.012	<0.001	0.011
Kidneys	0.007	0.068	0.004	0.049
Liver	0.036	0.059	0.022	0.044
Lungs	0.002	0.031	0.001	0.026
Muscle (femoral)	N/A	0.007	N/A	0.006
Ovary	-	-	<0.001	0.024
Plasma	N/A	N/A	N/A	<0.004
Residual Carcass	0.542	0.062	0.458	0.056
Salivary glands	<0.001	0.017	<0.001	0.018
Spleen	0.001	0.026	0.001	0.024
Testes	0.001	0.007	-	-
Total	0.590	N/A	0.488	N/A
GIT plus contents	0.186	0.152	0.172	0.152

N/A not applicable

Conclusion by the Notifiers

After a single oral dose glyphosate acid was excreted rapidly and predominantly in faeces. Elimination was essentially complete within 72 h. There was no indication for accumulation of glyphosate acid.

RMS comments

The study is considered acceptable. Sufficient data was provided to describe toxicokinetics and tissue residues of glyphosate following administration of a low dose of 10 mg/kg bw. Plasma kinetics was not followed and metabolism was not investigated but enough information on these parameters is available from other sources.

*Taking into account the negligible biliary excretion ([redacted], 1996 (*ASB2012-11380*); [redacted] 1996 (TOX2000-1981)), oral absorption rate is estimated on the basis of renal elimination. As compared to other studies in which the same dose was applied ([redacted] 1995 (*ASB2012-11379*); [redacted], 1988 (TOX9552356)), urinary excretion in this study was remarkably low, ~~accounted~~ accounting for only 50 to 60% of the previously measured excretion rates in both sexes. The reason for this difference is not known but the lower figure established by [redacted] (1996, TOX2000-1977) must not be ignored when correction of the AOEL for an internal dose is considered.*

Affinity of the compound to bone tissue was confirmed once more.

4th study ([redacted] 1996)

Reference:	IIA, 5.1.1/04
Report:	[redacted] (1996) Glyphosate acid: Excretion and tissue retention of a single oral dose (1000 mg/kg) in the rat [redacted] Data owner: Syngenta Report No.: [redacted] Date:1996-06-19, not published, TOX2000-1978
Guidelines:	MAFF (Japan) Metabolism Study (1985), OECD 417 (1984), US-EPA FIFRA 85-1, EEC B.36 (1987)
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Materials and methods

Non-labelled test material:	Glyphosate acid
Identification:	N-phosphonomethyl glycine
Description:	White solid
Lot/Batch #:	Y04707/048
Purity:	99.5 %
Stability of test compound:	Stable throughout the experiment
Radiolabelled test material:	
Identification:	[^{14}C]–glyphosate acid
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Y04707/047
Purity:	> 98 %

Specific activity:	1.580GBq/mMol
Stability of test compound:	Stable throughout the experiment
Vehicle and/or positive control:	Deionised water
Test animals:	
Species:	Rat
Strain:	Alpk:AP_fSD
Source:	[redacted]
Age:	Not reported
Sex:	Male/female
Weight at dosing:	182 - 235 g
Acclimation period:	At least 4 days
Diet/Food:	PCD rat diet (SDS Ltd. Stepfield, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Groups of 6 per cage and sex, in stock rat cages After dosing: Excretion-balance experiments - individually in metabolism cages
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 40 - 70 % Air changes: 12/hour 12-hour light/dark cycle

Study design and methods
In life dates: not reported

Animal assignment and treatment (Excretion study and quantitative tissue distribution)
Ten non-fasted rats (5 male, 5 female) received single oral doses of 1000 mg/kg bw/day (10 mL/kg, 6MBq/kg) by gavage and were placed in glass metabolism cages immediately thereafter. Urine was collected at 6, 12, 24, 36, 48 and 72 h after dosing in receivers cooled with solid CO_2. Faeces were collected at 12, 24, 36, 48 and 72 h. The interior of the cages were washed with water after each collection time. At the end of the study cages were washed with ethanol/water 1:1 (v/v). Samples were stored at -20 °C until taken for analysis.
Animals were exsanguinated by cardiac puncture under halothane anaesthesia. Two blood samples were collected in heparinised vials. One was centrifuged to separate plasma. Following tissues/organs were taken or sampled for radioactivity measurements:
Bone (femur), brain, fat (abdominal), gastrointestinal tract and its contents, heart, kidneys, liver, lungs, muscle (femoral), gonads, spleen, salivary glands, residual carcass.

Measurement of radioactivity
Samples of urine cage wash and plasma were taken, without further processing, for liquid scintillation counting (LSC). Faecal samples were ground with an approximately equal weight of anhydrous magnesium sulphate until homogeneous. Samples were analysed by sample oxidation followed by LSC. Bone (cut into pieces) and whole blood were analysed by sample oxidation followed by LSC. Liver, fat, residual carcass and gastrointestinal tract (GIT) plus

contents were homogenised. Liver and fat were than solubilised in Soluene-350® whereas GIT and residual carcass were oxidised. All other tissues were solubilised without prior homogenisation.

Sample oxidation
Samples were oxidised in a Packard Tricarb sample oxidiser. The [^{14}C]-carbon dioxide generated was absorbed into Carbo-sorb E® and mixed with Permafluor E+® scintillant prior to analysis by LSC.

Liquid scintillation counting (LSC)
Samples and dilutions of the dosing preparation were mixed with Optiphase Hi-Safe 3® and counted for a maximum of 10 min in Packard Tricarb instruments. The results obtained were corrected for background activity and counting efficiency using [^{135}Ba] as the external source.

Results and discussion
Excretion and retention of radioactivity
Excretion was rapid for both sexes with most of the radioactivity being eliminated in the faeces during the first 24 h after dosing (78.7 % in males, and 71.3 % in females). In the urine, means of 15.3 % and 16.0 % of the radioactivity were eliminated in the first 24 h in males and females, respectively. Within the observation period of 72 h, means of 106.4 % (male) and 102.3 % (female) of the administered radioactivity were excreted in total (see Table B.6.1-14).

Table B.6.1-14: Excretion balance (in mean % of applied dose) at 72 h post dosing

Balance/Excretion	**1000 mg/kg bw**	
	Males	Females
Urine 0-6	7.9	9.7
Urine 6-12	5.0	3.9
Urine 12-24	2.5	2.4
Urine 24-36	0.7	0.8
Urine 36-48	0.4	0.5
Urine 48-72	0.3	0.3
Cage wash	0.1	0.2
Subtotal urine + cage wash	16.9	17.8
Faeces 0-12	36.4	19.7
Faeces 12-24	42.2	51.6
Faeces 24-36	6.6	8.5
Faeces 36-48	2.9	3.5
Faeces 48-72	1.4	1.3
Subtotal faeces	89.5	84.6
Total	106.4	102.4

Distribution of radioactivity in tissues
The highest tissue concentration of radioactivity was found in the bone with a mean concentration of 49.8 µg equiv./g (male) and 44.9 µg equiv./g (female), followed by the gastrointestinal tract (and contents) with 13.3 µg equiv./g (male and 16.3 µg equiv./g (female). Lower mean concentrations between 6.6 and 1.1 µg equiv./g were found in kidneys, liver, heart, lungs, spleen, brain, gonads and salivary glands of both sexes. Mean concentrations of 4.8 and 5.9 µg equiv./g were found in the residual carcass (which also included the skeletal bone) of males and females respectively. All other concentrations were either similar to or lower than the corresponding blood concentrations (see Table B.6.1-15).

Table B.6.1-15: Radioactivity in tissues after single oral dose of 1000 mg/kg bw at 72 h

	Males		**Females**	
Tissue	% of dose	µg equiv./g	% of dose	µg equiv./g
Blood	N/A	0.894	N/A	0.803
Bone	N/A	49.792	N/A	44.925
Brain	0.001	1.233	0.001	1.164
Fat	N/A	0.536	N/A	0.496
GIT plus contents	0.2	13.276	0.219	16.329
Heart	0.001	1.111	0.001	1.254
Kidneys	0.007	6.511	0.005	6.046
Liver	0.039	5.480	0.029	5.226
Lungs	0.002	2.870	0.002	3.535
Muscle	N/A	0.816	N/A	0.825
Ovary	-	-	<0.001	2.940
Plasma	N/A	<0.392	N/A	<0.396
Residual carcass	0.466	4.772	0.537	5.858
Salivary glands	<0.001	1.811	<0.001	2.089
Spleen	0.001	2.441	0.001	3.106
Testes	0.001	0.905	-	-

N/A not applicable

Conclusion by the Notifiers

Oral doses of glyphosate acid were excreted rapidly and predominantly in the faeces. Elimination was essentially complete within 48 h. The remaining radioactivity was excreted with the urine. Negligible traces of radioactivity (<0.6 %) were still present in the tissues and residual carcass at 72 h, with bone representing the highest tissue residue. Thus, there was no indication for accumulation of glyphosate acid.

RMS comments

The study is considered acceptable. The conclusion is agreed with. Again, oral absorption rate (expressed as percentage of urinary excretion) was rather low in this study but there is no direct comparison with other studies from other laboratories possible since 1000 mg/kg bw was by far the highest dose that was administered in ADME studies. However, the urinary excretion rates were in line with data obtained by [redacted] (1996, TOX2000-1981).

Plasma kinetics was not followed and metabolism was not investigated but enough information on these parameters is available from other sources.

The tissue distribution pattern observed by other researchers was confirmed.

5th study ([redacted] 1996)

Reference: IIA, 5.1.3/03

Report: [redacted] (1996) Glyphosate acid: Biotransformation in the rat [redacted]
Data owner: Syngenta
Report No.: [redacted]/P/5058
Date: 1996-06-28, not published, TOX2000-1981

Guidelines: OECD 417 (1984)

Deviations: None

GLP: Yes

Acceptability: *See RMS comment*

Materials and methods

Non-labelled test material:	Glyphosate acid
Identification:	N-(phosphonomethyl) glycine
Description:	White solid
Lot/Batch #:	Y04707/048
Purity:	99.5 % w/w
Stability of test compound:	Not reported
Radiolabelled test material:	[^{14}C]-phosphonomethyl-labelled glyphosate acid
Identification:	[^{14}C]-phosphonomethyl-labelled glyphosate acid
Description:	Not reported
Lot/Batch #:	Y04707/047
Purity:	97.8 %
Specific activity :	1.580 GBq/mMol
Stability of test compound:	The test substance was shown to be stable in vehicle for longer than the period of use during this study.
Reference substance:	
Identification:	Aminomethylphosphonic acid (AMPA)
Description:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported
Stability of test compound:	Not reported
Test animals:	
Species:	Rat
Strain:	Alpk:AP$_f$SD
Source:	[redacted]
Age:	Not reported
Sex:	Males and females
Weight at dosing:	260- 305 g
Acclimation period:	At least 4 days in stock rat cages and 24 hours prior to surgery in metabolism cages
Diet/Food:	Pelleted PCD rat diet (Special Diets Services Ltd., Stepfield, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Housed individually in glass metabolism cages
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 55 ± 15 % Air changes: At least 12 change/hour 12-hour light/dark cycle

Study design and methods

In life dates: 1995-11-26 to 1996 May

Animal assignment and treatment
Two male and two female non-fasted rats were administered with a single oral dose of 1000 mg [^{14}C]-glyphosate acid/kg by gavage after bile duct cannulation. 48 hours after dosing all animals were sacrificed.

Bile duct cannulation
The abdominal cavity was opened after anaesthesia and the bile duct exposed. A fine plastic cannula was inserted into the bile duct and externalised by passing through the abdominal wall and under the skin to an exit point at the back of the neck. The incisions in the abdominal and body walls were saturated and the exposed cannula was protected within a flexible metal sheath anchored to the skin at the back of the neck. Following surgery each animal was returned to its cage and allowed to recover overnight prior dosing.

Dosing Formulation Analysis
The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The radiochemical purity of the [^{14}C]-labelled test substance was determined by the high performance liquid chromatography (HPLC) following formulation in the dosing.

Collection of excreta and bile
Urine only was collected 6 hours after dosing, and separately together with faeces 12, 24, 36 and 48 hours after dosing from all surviving animals.
Bile was collected at 2, 4, 6, 8, 12, 24, 36 and 48 hours after dosing.
Faecal samples were analysed by sample oxidation followed by liquid scintillation counting whereas samples of urine and bile were analysed without intermediate processing.

Quantification of metabolites
Urine and faecal samples obtained from previous excretion and tissue distribution studies ([redacted] 1996 TOX2000-1977, TOX2000-1978, TOX2000-1979) over 72 hours were used for the quantification of metabolites.
Urine samples were combined by taking a fixed percentage by weight to give separate male and female pools for each of the sample collections intervals. Subsamples of these pools were further combined to give pools representing the entire sample collection period. Each pool was analysed by TLC and HPLC. A representative urine sample was analysed by ^{1}H-NMR.
Faecal samples were combined in the same way as described above for urine samples. Subsamples of pooled faecal samples were mixed with distilled water and sonicated for several hours, the samples were filtered through filter paper and the solid material was re-extracted a second time with distilled water and a third time with 10% aqueous HCl. Extract volumes were measured and aliquots taken for scintillation counting to allow the calculation of extraction efficiencies.

Measurement of radioactivity
Liquid scintillation counting
Radioactivity was measured by liquid scintillation analysis by means of Packard Tricarb instruments. The results obtained were corrected for background activity and counting efficacy using [^{133}Ba] as the external source. Disintegrations per minute (dpm) values were calculated using the appropriate quench curve data entered into instrument's computer.
Where sample oxidation had to be performed, samples were oxidised in a Packard Tricarb sample oxidiser.

Thin layer chromatography (TLC)
TLC was conducted using a normal phase silica-gel ($60F_{254}$) with the following solvent system: methanol: water: 28 % ammonium hydroxide: 10 % trichloroacetic acid (60: 30: 15: 15 v/v/v/v).
Radioactivity on the TLC plate was measured using a Berthold Tracemaster linear analyser or a Bioscan System 200 imaging scanner. Glyphosate acid and AMPA standards were located by spraying the plates with a solution of 300 mg ninhydrin in 100 mL of butanol and 3 mL of glacial acetic acid.

High performance liquid chromatography (HPLC)
Two different HPLC methods were employed:
HPLC method 1 was used for the analysis of dosing solutions. Prior to analysis samples were derivatised. A Hichrom S5NH column (250 x 4.6 mm) was eluted with acetonitrile buffered with 25 mMol aqueous potassium dihydrogen phosphate (60:40 v/v) at a flow rate of 1.5 mL/min.
HPLC method 2 was used for the analysis of urine and faecal extracts and for the quantification of glyphosate acid and AMPA. Prior to analysis samples were filtered. A Biorad's HLRC acid analysis column (250 x 4.6 mm) was eluted with 5 mM aqueous potassium dihydrogen phosphate with 4 % methanol at a flow rat of 0.5 mL/min.
Radioactivity was detected in both methods by liquid cell.

Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR)
Proton and phosphorus NMR spectra were acquired using a Brucker 400MHz instrument. Samples of glyphosate acid and AMPA were dissolved in D_2O and analysed by both phosphorus and proton NMR. Control urine and urine from a bile duct cannulated rat administered an oral dose of glyphosate acid were analysed by phosphorus NMR. The urine sample from the rat that had been administered glyphosate was subsequently fortified with AMPA then glyphosate acid and reanalysed by phosphorus NMR.

Data evaluation
Dosing and excretion of radioactivity data were processed using the Debra computerised acquisition and processing system. Metabolites were quantified using the Flo_One integration software for HPLC.

Results and discussion
Excretion of radioactivity
The results showed that 48 hours after dosing, excreted means of the administered dose in the urine and faeces amounted to 20.8 % and 39.1 % in males and 16.3 % and 30.5 % in females, respectively, whereas biliary excretion of radioactivity was negligible (see Table B.6.1-16).
The total excreted radioactivity after 48 hours accounted for 62.5 % and 52.0 % in males and females, respectively.

Table B.6.1-16: Excretion of radioactivity in urine, faeces and bile by male and female bile duct cannuled rats given a single oral dose of 1000 mg [^{14}C]-glyphosate acid/kg bw (mean of two rats expressed as % of applied dose)

	Males rats			**Female rats**		
	% of applied dose			% of applied dose		
Time after dosing (hours)	Urine	Faeces	Bile	Urine	Faeces	Bile
0-2	N/A	N/A	0.004	N/A	N/A	0.002
2-4	N/A	N/A	0.004	N/A	N/A	0.011
4-6	N/A	N/A	0.002	N/A	N/A	0.011
0-6	2.137	N/A	N/A	8.718	N/A	N/A
6-8	N/A	N/A	0.005	N/A	N/A	0.005
0-12	N/A	3.776	N/A	N/A	1.392	N/A
6-12	6.765	N/A	N/A	2.495	N/A	N/A
8-12	N/A	N/A	0.008	N/A	N/A	0.007
12-24	5.432	12.333	0.016	3.631	12.115	0.010
24-36	3.468	18.079	0.009	1.004	8.712	0.008
36-48	3.013	4.946	0.007	0.427	8.325	0.007
0-48	20.815	39.134	0.055	16.275	30.544	0.062
Cage wash at 48 hours	2.534 (mean)			5.097 (mean)		
Total excreted	62.538			51.978		

Values are expressed as percentages of administered dose and are then mean of two rats

Characterisation of radioactivity

The negligible levels of radioactivity in bile samples made chromatographic analysis unnecessary. Corresponding to this result, faecal extracts analysed by HPLC and NMR confirmed the radioactivity as glyphosate acid.

Analyses by chromatography and phosphorus NMR of urine pools from former studies ([redacted] 1996, TOX2000-1977, TOX2000-1978, TOX2000-1979) covering the 0-72 hour period demonstrated a single peak identified as glyphosate acid. Earlier timepoints demonstrated a second peak identified as aminomethyl phosphonic acid (AMPA) that occurred in measurable quantities.

The percentages of dose accounted for glyphosate acid and AMPA following a low, high or repeated dose of glyphosate acid are given in Table B.6.1-17. For glyphosate acid and AMPA, the values range from 63.3-95.3 % and 0.07-0.66 %, respectively.

Table B.6.1-17: Percentage of administered radioactivity identified as glyphosate acid and AMPA

		Low dose study 10 mg/kg bw		**High dose study 1000 mg/kg bw**		**Repeated dose study 10 mg/kg bw**	
		Male	Female	Male	Female	Male	Female
		(% of applied radioactivity)					
Urine	Glyphosate acid	12.71	10.51	16.00	16.73	10.46	10.47
	AMPA	0.19	0.11	0.63	0.66	0.07	0.08
Faeces	Glyphosate acid	74.80	55.22	79.25	63.88	52.86	72.09
Total	Glyphosate acid	87.52	65.73	95.25	80.61	63.33	82.57
	AMPA	0.19	0.11	0.63	0.66	0.07	0.08

Conclusion by the Notifiers
Following an oral dose of glyphosate acid to rats approximately 10-20 % of the dose was absorbed. The unabsorbed glyphosate acid was excreted unchanged in faeces. The absorbed dose was excreted in urine as glyphosate and trace amounts of aminomethyl phosphonic acid (AMPA).

RMS comments
The study is considered acceptable. Data obtained in bile-canulated rats and on metabolism amend the information provided by [redacted] (1996, TOX2000-1977, TOX2000-1978) and confirm low urinary excretion (although some underestimation is possible since 2-5 – 5 % radioactivity in cage was was not included), negligible biliary excretion also following administration of a high dose and virtually absent metabolism.

6th study ([redacted] 1996)

Reference: IIA, 5.1.3/01
Report: [redacted] (1996) Glyphosate acid: Excretion and Tissue Retention of a Single Oral Dose (10 mg/kg) in the Rat Following Repeat Dosing
[redacted]
Data owner: Syngenta
Report No.: [redacted]/P/4944
Date: 1996-05-22, not published, TOX2000-1979
Guidelines: OECD 417 (1984)
Deviations: None
GLP: Yes
Acceptability: *See RMS comment*

Materials and methods

Non-labelled test material:	Glyphosate acid
Identification:	N-phosphonomethyl glycine
Description:	White solid
Lot/Batch #:	Y04707/045
Purity:	99.2 % w/w
Stability of test compound:	Not reported
Radiolabelled test material:	[^{14}C]-phosphonomethyl-labelled glyphosate acid
Identification:	[^{14}C]-phosphonomethyl-labelled glyphosate acid
Description:	Not reported
Lot/Batch #:	Y04707/047
Purity:	> 98 %
Specific activity :	1.580 GBq/mMol
Stability of test compound:	The test substance was shown to be stable in the vehicle for longer than a period of use during the study.
Vehicle and/or positive control:	Deionised water
Test animals:	
Species:	Rat
Strain:	Alpk:AP$_f$SD
Source:	[redacted]
Age:	Not reported
Sex:	Males and females
Weight at dosing:	225 - 328 g
Acclimation period:	At least 4 days prior to the study start and 24 hours prior to dosing with the radiolabelled preparation
Diet/Food:	Pelleted PCD rat diet (Special Diets Services Ltd., Stepfield, Wiltham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	After administration of the 14th unlabelled dose: Individually in stainless steel metabolism cages
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 55 ± 15 % Air changes: At least 12 change/hour 12-hour light/dark cycle

Study design and methods
In life dates: 1995-10-16 to 1996-03-26

Animal assignment and treatment
Eight male and eight female non-fasted rats received a single oral dose of the unlabelled test substance (10 mg/kg) daily for 14 days by gavage. 24 hours after the 14th dose of unlabelled glyphosate acid, five male rats and five female rats were given a single oral dose (10 mg/kg)

of [^{14}C]-phosphonomethyl glyphosate acid. 72 hours after dosing, the rats were killed and the residual radioactivity was measured in blood, selected tissues and in the residual carcasses.

Dosing Formulation Analysis
The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The (radiochemical) purity of the [^{14}C]-labelled test substance prior to and following formulation in the vehicle was determined by thin layer chromatography (TLC) high performance liquid chromatography (HPLC).

Collection of excreta
Urine only was collected at 6 hours after dosing, while urine together with faeces were separately collected at 12, 24, 36, 48 and 72 hours after dosing.
Urine collections comprised rinsing of each cage at each time point together with a thorough washing at the end of the study. Faecal samples were analysed by sample oxidation followed by liquid scintillation counting whereas samples of urine and cage washings were analysed without intermediate processing.

Collection of blood and tissues
72 hours after dosing, the rats were sacrificed and a blood sample was retained and divided into two portions. A portion of each blood sample was centrifuged to obtain plasma, which was analysed for liquid scintillation counting. Whole blood was analysed by sample oxidation.
The following tissues together with representative samples of fat (abdominal), bone (femur), and muscle (femoral) were removed from each rat: brain, liver, testes or ovaries, lungs, heart, spleen, kidneys, salivary glands, intestinal tract plus contents and residual carcasses.

Measurement of radioactivity - Liquid scintillation counting
Radioactivity was measured by liquid scintillation counting by means of Packard Tricarb instruments. The results obtained from the counting were corrected for background activity and counting efficacy using [^{133}Ba] as the external source. Disintegrations per minute (dpm) values were calculated using the appropriate quench curve data entered into instrument's computer.
Where sample oxidation had to be performed, samples were oxidised in a Packard Tricarb sample oxidiser.

Thin layer chromatography (TLC)
TLC was conducted using a normal phase silica-gel ($60F_{254}$) with the following solvent system: methanol: water: 28 % ammonium hydroxide: 10 % trichloroacetic acid (60: 30: 15:5 v/v/v/v).
Radioactivity on the TLC plate was measured using a Berthold Tracemaster linear analyser. Unlabelled glyphosate acid was visualised by spraying the TLC plate with a 0.2 % ethanoic ninhydrin solution.

High performance liquid chromatography (HPLC)
To facilitate analysis a mixture of the unlabelled and radiolabelled test substance was derivatised. Sample analysis was performed by a Hichrom S5NH column (250 x 4.6 mm) which was eluted with acetonitrile buffered with 25 mMol aqueous potassium dihydrogen phosphate (60:40 v/v) at a flow rate of 1.5 mL/min. Radioactivity was detected using an on-line flow detector (liquid cell) and with UV absorption at 230 nm.

Data evaluation
Data were processed using the Debra (Version 4.1) computerised acquisition and processing system. The limit of detection (LOD) of radioactivity measurement during this study was taken as 50 dpm per sample which was twice the liquid scintillation counter's background rate. For the purpose of calculating group mean results, individual values below the LOD are accepted as being equal to the limit of detection. Means which include one or more values which are below the LOD are reported as "<" the mean result and without a standard deviation. The limit of detection obtained for all tissues in this study was 0.004/µg equivalents glyphosate acid/g of tissue (µg equiv/g). This value is based upon a sample size of 200 mg of all determinations. Organs of less than this weight were analysed as a single sample and hence this figure represent a limiting value.

Results and discussion

Excretion of radioactivity
The results showed that the excretion was rapid for both sexes with most of the radioactivity being eliminated in the faeces during the first 24 hours after dosing (average of 80.6 % for males and 85.8 % for females).
Excretion of radioactivity in the urine during this period accounted for means of 9.2 % and 9.1 % of the administered dose in the male and female rats respectively. The total percentage of the administered radioactivity eliminated in excreta 72 hours after dosing were means of 97.5 % for males and 101.7 % for females (Table B.6.1-18)
Comparison of the cumulative excretion data showed that there were no marked differences in the rates of excretion of radioactivity in the urine or faeces for male and female rats.

Table B.6.1-18: Excretion of radioactivity in urine and faeces in male and female rats

	Excretion of radioactivity [%]			
	Males			
Time after dosing (hours)	Urine		Faeces	
	Mean[a]	SD	Mean	SD
0-6	3.1	0.8	N/A	N/A
6-12	2.7	0.7	N/A	N/A
0-12	N/A	N/A	50.2	15.5
12-24	3.4	1.6	30.3	9.0
24-36	0.9	0.3	3.6	1.5
36-48	0.3	<0.1	1.3	0.7
48-72	0.2	<0.1	1.1	0.6
0-72	10.6	3.0	86.6	5.2
	Mean	SD	N/A	N/A
Cage wash at 72 h	0.2	<0.1	N/A	N/A
Total excreted	97.5	2.7	N/A	N/A

	Excretion of radioactivity [%]			
	Females			
Time after dosing (hours)	Urine		Faeces	
	Mean[b]	SD	Mean	SD
0-6	3.3	0.6	N/A	N/A
6-12	2.5	0.3	N/A	N/A
0-12	N/A	N/A	44.7	34.1
12-24	3.2	0.6	41.0	31.1
24-36	0.9	0.3	2.7	1.1
36-48	0.4	0.2	1.2	0.2
48-72	0.3	0.1	1.1	0.7
0-72	10.7	1.1	90.7	4.2
	Mean	SD	N/A	N/A
Cage wash at 72 h	0.2	<0.1	N/A	N/A
Total excreted	101.7	4.0	N/A	N/A

N/A not applicable
[a] Mean of 4 animals
[b] Mean of 5 animals

Tissue distribution of radioactivity
The highest tissue concentration of radioactivity was found in bone with a mean concentration of 0.36 µg equiv/g for males and 0.35 µg equiv/g for females, followed by the intestinal tract plus contents, kidneys, liver, lungs, salivary glands, and ovaries.
Mean concentrations of 0.05 µg equiv/g were found in the residual carcass of either sex which also includes the remaining skeletal bone.
All other concentrations were either similar to or lower than the corresponding blood concentrations.

The mean total percentage of administered radioactivity present in all of the tissues examined and the residual carcass was 0.5 % for males and 0.4 % for females. The amounts present in the intestinal tract plus contents were 0.1 % for males and females (see Table B.6.1-19)

Table B.6.1-19: Tissue and carcass residues of radioactivity in male and female rats

	Residue of radioactivity			
	Males			
Tissue	% radioactivity of dose		µg equivalents/g	
	Mean [a]	SD	Mean	SD
Brain	0.001	<0.001	0.010	0.002
Testes	0.001	<0.001	0.007	0.001
Heart	<0.001	<0.001	0.011	0.002
Kidneys	0.005	0.002	0.061	0.015
Liver	0.031	0.009	0.055	0.014
Lungs	0.001	<0.001	0.026	0.004
Spleen	0.001	<0.001	0.022	0.003
Salivary glands	<0.001	<0.001	0.019	0.004
Bone (femur)	N/A	N/A	0.358	0.177
Fat (abdominal)	N/A	N/A	0.008	0.001
Muscle (femoral)	N/A	N/A	0.008	0.001
Blood	N/A	N/A	0.014	0.006
Plasma	N/A	N/A	<0.004	
Residual carcass	0.423	0.090	0.050	0.011
Total	0.463	0.101	N/A	N/A
Intestinal tract plus contents	0.108	0.040	0.109	0.041

	Residue of radioactivity			
	Females			
Tissue	% radioactivity of dose		µg equivalents/g	
	Mean [b]	SD	Mean	SD
Brain	0.001	<0.001	0.010	0.002
Ovaries	<0.001	<0.001	0.026	0.006
Heart	<0.001	<0.001	0.012	0.004
Kidneys	0.004	0.001	0.049	0.011
Liver	0.021	0.005	0.045	0.010
Lungs	0.001	<0.001	0.029	0.006
Spleen	0.001	<0.001	0.025	0.006
Salivary glands	<0.001	<0.001	0.027	0.006
Bone (femur)	N/A	N/A	0.345	0.081
Fat (abdominal)	N/A	N/A	0.006	0.002
Muscle (femoral)	N/A	N/A	0.007	0.002
Blood	N/A	N/A	0.010	0.002
Plasma	N/A	N/A	<0.005	
Residual carcass	0.382	0.067	0.046	0.008
Total	0.411	0.073	N/A	N/A
Intestinal tract plus contents	0.115	0.014	0.117	0.015

N/A not applicable
Residual carcass values include partial tissue percentages
[a] Mean of 4 animals
[b] Mean of 5 animals

Recovery of radioactivity
The total mean percentage recoveries, including excreta, tissues and residual carcass was 98.0 % for male rats and 102.2 % for females.

Conclusion by the Notifiers
Comparison of the results with those obtained at the same dose level but without pre-administration of unlabelled test substance ([redacted] 1996, TOX2000-1977) showed no significant differences on either the routes or rates of elimination after oral dosing. In both studies the test substance was excreted rapidly and predominantly in the faeces by rats of both sex and low amounts of radioactivity were detected in all the tissue examined.

RMS comments
The study is considered acceptable. No significant differences were observed when the results were compared to the data obtained following single administration. In particular, there was no evidence of accumulation when, e.g., the tissue residues were considered. Urinary excretion that may be taken as surrogate for oral absorption was even lower than in the single-dose experiments without pre-treatment, and so was radioactivity in cage wash.

7th study ([redacted] 1996)

Reference: IIA, 5.1.3/02
Report: [redacted] 1996) Glyphosate acid: Whole body autoradiography in the rat (10 mg/kg)
[redacted]
Data owner: Syngenta
Report No.: [redacted]/P/4943
Date: 1996-06-10, not published, TOX2000-1980

Guidelines: OECD 417 (1984)
Deviations: None
GLP: Yes
Acceptability: *See RMS comment*

Materials and methods

Non-labelled test material:	Glyphosate acid
Identification:	N-phosphonomethyl glycine
Description:	White solid
Lot/Batch #:	Y04707/045
Purity:	99.2 % w/w
Stability of test compound:	Not reported
Radiolabelled test material:	[^{14}C]-phosphonomethyl-labelled glyphosate acid
Identification:	[^{14}C]-phosphonomethyl-labelled glyphosate acid
Description:	Not reported
Lot/Batch #:	Y04707/047
Purity:	> 96 %
Specific activity :	1.580 GBq/mMol
Stability of test compound:	The test substance was shown to be stable in the vehicle for longer than a period of use during the study
Vehicle and/or positive control:	Deionised water
Test animals:	
Species:	Rat
Strain:	Alpk:AP$_f$SD
Source:	[redacted]
Age:	Not reported
Sex:	Males and females
Weight at dosing:	215- 271 g
Acclimation period:	At least 5 days in stock rat cages and 24 hours prior to dosing in metabolism cages
Diet/Food:	Pelleted PCD rat diet (Special Diets Services Ltd., Stepfield, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Housed individually in glass metabolism cages
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 55 ± 15 % Air changes: At least 12 change/hour 12-hour light/dark cycle

Study design and methods
In life dates: 1995-10-25 to 1996-04-04

Animal assignment and treatment
Two male and two female non-fasted rats were administered with a single oral dose of 10 mg [^{14}C]-glyphosate acid/kg by gavage. 24 and 48 hours after dosing, a heterosexual pair was killed and a qualitative whole body autoradiogram was performed. In addition, radioactivity was measured in urine, faeces and exhaled air.

Dosing Formulation Analysis
The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The (radiochemical) purity of the [^{14}C]-labelled test substance prior to and following formulation in the vehicle was determined by thin layer chromatography (TLC) high performance liquid chromatography (HPLC).

Collection of excreta
Urine only was collected 6 hours after dosing, and separately together with faeces 12, 24, 36 and 48 hours after dosing from all surviving animals.
Urine collections comprised rinsing of each cage at each time point together with a thorough washing at the end of the study.
Faecal samples were analysed by sample oxidation followed by liquid scintillation counting whereas samples of urine and cage washings were analysed without intermediate processing.

Collection of exhaled air
The exhaled air from one heterosexual pair was passed through sodium hydroxide to trap any radioactivity expired as [^{14}C]-carbon dioxide. Subsamples of the contents of each trap were removed for radiochemical analysis at 6, 12 and 24 hours after dosing and were taken, without further processing, for liquid scintillation counting.

Whole body autoradiography
Immediately after scheduled sacrifice, each carcass was frozen rapidly and embedded in blocks of 2 % (w/v) aqueous carboxymethylcellulose. Longitudinal sagittal sections, 30 µm thick, were taken, mounted on adhesive tape and freeze-dried for approximately 48 hours. Autoradiograms were prepared by contact with autoradiographic film and exposed for periods of 2, 4 or 6 weeks.

Measurement of radioactivity - Liquid scintillation counting
Radioactivity was measured by liquid scintillation analysis by means of Packard Tricarb instruments. The results obtained were corrected for background activity and counting efficacy using [^{133}Ba] as the external source. Disintegrations per minute (dpm) values were calculated using the appropriate quench curve data entered into instrument's computer.
Where sample oxidation had to be performed, samples were oxidised in a Packard Tricarb sample oxidiser.

Thin layer chromatography (TLC)
TLC was conducted using a normal phase silica-gel ($60F_{254}$) with the following solvent system: methanol: water: 28 % ammonium hydroxide: 10 % trichloroacetic acid (60: 30: 15:5 v/v/v/v).

Radioactivity on the TLC plate was measured using a Berthold Tracemaster linear analyser. Unlabelled glyphosate acid was visualised by spraying the TLC plate with a 0.2 % ethanoic ninhydrin solution.

High performance liquid chromatography (HPLC)
To facilitate analysis a mixture of the unlabelled and radiolabelled test substance was derivatised. Sample analysis was performed by a Hichrom S5NH column (250 x 4.6 mm) which was eluted with acetonitrile buffered with 25 mMol aqueous potassium dihydrogen phosphate (60:40 v/v) at a flow rate of 1.5 mL/min. Radioactivity was detected using an on-line flow detector (liquid cell) and with UV absorption at 230 nm.

Data evaluation
Data were processed using the Debra (Version 4.1) computerised acquisition and processing system. The limit of detection (LOD) of radioactivity measurement during this study was taken as 50 dpm per sample, which was twice the liquid scintillation counter's background rate. The LOD for each carbon dioxide trap in this study was 0.01 % of the administered dose.

Results and discussion
Excretion of radioactivity
The results showed that 24 hours after dosing, excreted means of the administered dose in the urine and faeces amounted to 22.3 % and 55.5 % in males and 11.9 % and 83.8 % in females, respectively, whereas less than 0.2 % was excreted as carbon dioxide.
48 hours after dosing, excreted means of the administered dose in the urine and faeces increased to 34.0 % and 60.5 % in males and 12.5 % and 91.2 % in females, respectively.

The results of the excreted radioactivity in urine, faeces and exhaled air expressed as percentages of the administered radioactivity, together with the results for cage washings, are listed in Table B.6.1-20.

Table B.6.1-20: Excretion of radioactivity in urine, faeces, cage wash and expired air by male and female rats

	Time After Dosing (hours)	**Males**		**Females**	
		Rat 1	Rat 2	Rat 3	Rat 4
		(% of applied dose)		(% of applied dose)	
Urine	0-6	5.00	8.21	4.06	3.67
	6-12	5.30	8.67	4.32	4.12
	12-24	7.57	9.92	4.39	3.23
	24-36	N/A	4.30	N/A	0.93
	36-48	N/A	2.90	N/A	0.54
	Total	17.86	34.00	12.77	12.48
Faeces	0-12	30.62	0.21	54.27	32.63
	12-24	28.69	51.48	26.03	54.76
	24-36	N/A	1.65	N/A	2.71
	36-48	N/A	7.12	N/A	1.13
	Total	59.32	60.46	80.30	91.23
Exhaled $^{14}CO_2$	0-6	0.07	N/A	0.08	N/A
	6-12	0.02	N/A	0.04	N/A
	12-24	0.02	N/A	0.03	N/A
	Total	0.11	N/A	0.14	N/A
Terminal Cage wash		1.12	0.98	1.43	0.41
Total		78.40	95.44	94.64	104.12

N/A not applicable

Whole body autoradiography
The whole body autoradiograms showed no marked differences in the distribution of radioactivity between male and female rats. The greatest intensity of labelling was present in the bone for both sexes, followed by the intestinal tract and the kidneys 24 hours after dosing with lesser to negligible amounts being present after 48 hours.

Conclusion by the Notifiers
Orally dosed glyphosate acid was excreted rapidly and predominantly in the faeces. 48 hours after dosing the greatest intensity of radiolabelling was in the bone and intestinal tract plus contents.

RMS comments
The study is considered supplementary despite its good quality. However, the number of animals of one per sex and timepoint is too low for definitive conclusions. All the parameters examined (i.e., excretion and distribution) had been adressed in other studies before. This previous knowledge was confirmed. Oral absorption was generally low but showed a remarkable interindividual variability.

B.6.1.2 Re-evaluation of previously known studies (mentioned in the 1998 DAR, ASB2010-10302) by the RMS

For the first EU evaluation of glyphosate, two separate "pairs" of ADME studies have been submitted which consisted of an in-life part, *i.e.*, a complete ADME study according to OECD 417, and a subsequent metabolism part in which the metabolite pattern in urine and faeces was investigated.

- Monsanto studies
  In life part: [redacted] (1988, TOX9552356)
  Metabolism part: [redacted] (1988, TOX9552357)
- ADAMA studies
  In life part: [redacted] (1995, TOX9650071)
  Metabolism (and organ/tissue distribution) part: [redacted] (1995, TOX9552251)

Furthermore, on behalf of Cheminova, [redacted] (1992, TOX9300343) performed a full-range ADME study in rats.

Re-evaluation by the RMS revealed that, although partly carried out before GLP became compulsory, all these studies mostly comply to modern standards and provide reliable information on kinetics and metabolism of glyphosate in the rat. In line with the original DAR, the studies by [redacted] (1988, TOX9552356), by [redacted] (1988, TOX9552357) and by [redacted] (1992, TOX9300343) are still regarded as fully acceptable by the RMS allowing comprehensive evaluation.

In contrast, quality rating of the studies by [redacted] (1995, TOX9650071) and [redacted] (1995, TOX9552251) has been declined to "supplementary". This is due to the fact that radioactive residues in the organs, tissues and carcass were only investigated in one group receiving a low intravenous dose and, thus, do not reflect real exposure conditions. Even for this group that was treated by a highly artifical route, radioactivity in bone was not

determined. Furthermore, search for metabolites was confined to urine samples taken from high dose animals and possible abundance of faecal metabolites was not considered. Surprisingly, there was a different concentration given for the low oral dose by [redacted] (0.2 mg/kg bw) and [redacted] (0.3 mg/kg bw).
In addition, it must be emphasised that this study [as well as those by [redacted] (1995, ASB2012-11379) and by [redacted] (1996, ASB2012-11380)] did not include a group of rats receiving multiple administrations. Thus, a possible different kinetic behaviour following repeated exposure would not have been elucidated and information from other sources must be taken into account to cover this endpoint.
It could be argued that [redacted] (1995, TOX9650071) had employed a rather low dose of only 0.2 mg/kg bw(/d) but the use of this concentration might even better reflect real exposure conditions than higher "low" doses would do. The advantage of that many ADME studies with glyphosate is in covering a wide dose range.

A preliminary study by [redacted] (1992, TOX9552358) included determination of pharmacokinetic parameters in the blood and whole-body autoradiography at a low dose of 30 mg/kg bw and is, because of its limited focus, now considered supplementary, too.

Two more (and rather old) studies were considered supplementary during the previous evaluation yet:
A study by [redacted] (1973, TOX9552355) is unique in that way that it was the only toxicokinetic experiment in which glyphosate was administered (for two weeks) to rats via the diet. Indeed, the dietary dose levels of 1 to 100 ppm appear rather low but these concentrations might reflect an actual residue concentration.
A second study by the same authors ([redacted], 1973, TOX9552353) is about toxicokinetics of glyphosate in male rabbits following single oral (gavage) administration of low doses in the range of 5.7 – 8.8 mg/kg bw. The results indicate similarities and differences between species such as a lower urinary clearance rate and higher tissue retention in rabbits as compared to the rat.

Although no batch number and no purity of the applied glyphosate was given and, therefore, in principle, assessment of these studies should have been turned into "unacceptable" now, the RMS suggests to keep them available as sources of useful additional information.

In contrast, there was another study also considered "supplementary" in the original DAR for which this rating actually cannot be maintained:
The metabolism study by [redacted] (1990, TOX9551961) is considered not acceptable due to serious reporting deficiencies. Thus, no information on the test material (batch, purity) is given and the applied dose not mentioned. The application method is not clear since on one hand "gastric lavage" (gavage?) is stated as the route but on the next page it is said that "rats were fed with the radioactive material". Description of animals and animal handling is very poor. No information on toxicokinetics was obtained but this was apparently beyond the scope of the study that was performed to find out wheter glyphosate was metabolised or not.

Likewise, a brief information ([redacted], 1973, TOX9552354) concerning kinetics and (absent) metabolism of aminomethylphosphonic acid (AMPA) is very poor and not acceptable from a today's regulatory view.

B.6.1.3 Published information

Toxicokinetics and metabolism of glyphosate were seldom subject to investigations of industry-indpendent researchers and, thus, experimental data in open literature is scarce. The following paragraphs were transferred from the original DAR (1998, ASB2010-10302) and slightly amended for purposes of the RAR:

[redacted] *(1991, TOX9551791) reported an absorption rate from the gastrointestina tract of 35 - 40 % of the total dose following the single oral administration of 10 mg of a mixture of ^{12}C- and ^{14}C-glyphosate per kg bw to male Sprague-Dawley rats. Urine and faeces were considered equally important routes of elimination. 7 days after application, total body burden was approximately 1 % of the administered dose and was primarily associated with the bone. Two hours following a single dose of the mixed test material, traces of a minor metabolite (<0.1 % of the dose applied) were detected beside the predominating parent in the colon tissue. This compound was also found in the gastrointestinal tract content of one rat at 28 hours post dosing and was considered likely to be aminomethyl phosphonic acid (AMPA) although the retention time for this metabolite was not identical to that for AMPA. The authors reported AMPA to be a product of metabolic activity of intestinal microbes.*

[redacted] *(1992, TOX9551954) investigated (with contributions of* [redacted] *who are mentioned above) the elimination and tissue distribution of ^{14}C-glyphosate in male F344/N rats following oral and intravenous administration. After single low (5.6 mg/kg bw) or high (56 mg/kg bw) oral doses, more than 90 % of the applied radioactivity was eliminated within 72 hours. During the first 24 hours, approximately 50 % had been excreted in the faeces and nearly 30 % via the urine. It was assumed that the urinary radioactivity represented the amount of glyphosate absorbed. The peak blood levels occurred at 1 (low dose) or 2 (high dose) hours after dosing. Following an i.v. dose of 5.6 mg/kg bw, 90 % of the radioactivity was excreted in urine within the first 6 hours already. Glyphosate did not accumulate in the body. In a further group of rats receiving 5.6 mg/kg bw by oral gavage, only 1 % of the dose remained in the tissues after 24 hour. It is also stated that pretreatment with Roundup® via drinking water did not change the elimination pattern of glyphosate.*

In a more recent paper that was included in the GTF dossier submitted for current evaluation, [redacted] (2009, ASB2012-11542) reported some parts of toxicokinetics of glyphosate (obtained from SIGMA CHEMICALS) in rats after single intravenous (i.v.) administration of 100 mg/kg bw or a single oral dose of 400 mg/kg bw. The focus was on plasma characteristics and distribution to the different compartments: "Serial blood samples were obtained after i.v. and oral administration. Plasma concentrations of glyphosate and its metabolite amiomethyl phosphonic acid (AMPA) were determined by HPLC method. After i.v. and oral administration, plasma concentration-time curves were best described by a two-compartment open model. For glyphosate, the elimination half-lives ($T_{1/2\beta}$) from plasma were 9.99 h after i.v. and 14.38 h after oral administration. The total plasma clearance was not influenced by dose concentration or route and reached a value of 0.995 l $h^{-1}kg^{-1}$. After i.v. administration, the apparent volume of distribution in the second compartment (V_2) and volume of distribution at steady state (V_{ss}) were 2.39 and 2.99 l kg^{-1}, respectively, suggesting a considerable diffusion of the herbicide into tissues. After oral administration, glyphosate was partially and slowly absorbed with a T_{max} of 5.16 h. The oral bioavailability of glyphosate was found to be 23.21 %. Glyphosate was converted to AMPA. The metabolite AMPA represented 6.49 % of the parent drug plasma concentrations. The maximum plasma concentrations of glyphosate and AMPA were 4.62 and 0.416 μml^{-1}, respectively. The

maximum plasma concentration of AMPA was achieved at 2.42 h. For AMPA, the elimination half-life ($T_{1/2\beta}$) was 15.08 h after oral administration of glyphosate parent compound (*quoted from original article*)."

The RMS is not aware of any further scientific publications dealing with toxicokinetics and metabolism of glyphosate in laboratory animals or man. However, ~~interesting additional~~ information on urinary excretion of glyphosate in humans was provided ~~that, however, did not alter the conclusions that were drawn from the many studies described above~~. In the original draft, this data was reported here but, for the revised version, the information was substantially amended (because more data had become available in the meantime) and transferred to section B.6.9.3 where a new sub-section on human biomonitoring was created.

~~Data on urinary excretion in man following systemic exposure (presumed to be mainly dermal and inhalative) were obtained by Acquavella *et al.* (2004, ASB2012-11528) as part of the "Farm Family Exposure Study". In this study, urinary glyphosate concentrations obtained from 48 farmers, their spouses, and their 79 children (4–18 years of age) were measured and evaluated. 24-hr composite urine samples were collected for each family member the day before, the day of, and for 3 days after a proven occupational glyphosate application. Sixty percent of the farmers had detectable levels of glyphosate in their urine on the day of application. The geometric mean (GM) concentration was 3 ppb, the maximum value was 233 ppb, and the highest estimated systemic dose (based on the latter measurement) was 0.004 mg/kg bw. Farmers who did not use rubber gloves had higher GM urinary concentrations than found in the other men (10 ppb vs. 2.0 ppb). Among spouses, only 4 % had detectable levels in their urine on the day of application. The maximum value was 3 ppb. In children, 12 % had detectable glyphosate in their urine on the day of application, with a maximum concentration of 29 ppb. All but one of the children with detectable concentrations had helped with the application or were present during herbicide mixing, loading, or application. This data is considered important because it is the only one that might give an idea about urinary excretion of glyphosate in humans following occupatinal exposure although Mage (2006, ASB2012-11888) had claimed some methodological deficiencies with regard to urine collection and absent correction for prior glyphosate exposure.~~

~~More recently, in 2013, a biomonitoring study was performed on behalf oft he NGO "Friends of the earth" and its German partner organisation BUND by Hoppe (Medical Laboratory Bremen, Haferwende 12, D-28357 Bremen, Germany) and submitted to the RMS (ASB2013-8037). To our knowledge, this data has not been published in a scientific journal so far but is available in the internet. 182 urine samples from 18 European (EU and non-EU) countries (6 – 12 per country but mostly 10) were examined for glyphosate and AMPA by means of a modern analytical method (transformation of both compounds to two different derivatives followed by GC-MS/MS). This method appears very selective but it is not known whether it has been sufficiently validated so far. The LOQ for both, glyphosate and AMPA, was 0.15 µg/L. Creatinine was also measured as an internal proof for validity of the urine measurements.~~

~~For glyphosate, nearly one half (80) and for AMPA, more than one third (65) of the participants had urine concentations above the LOQ. Maximum values of 1.82, 1.64 or 1.55 µg/L for glyphosate were found in samples obtained from Latvia, the UK, and Malta, respectively, but the mean value of 0.21 µg/L was much lower. (For calculation, the study~~

~~author had included the samples with values below the LOQ and assumed a concentration of 0.075 µg/L, i.e., half the LOQ.) For AMPA, the maximum values of 2.63, 1.26, and 0.89 µg/L were measured in samples from Croatia, Belgium, and Malta with a mean urinary concentration of 0.18 µg/L for all involved people. It was surprising that in more than 30 cases the AMPA concentrations were higher than those of glyphosate, sometimes by 10 times or more. In a few samples, AMPA values were rather high with glyphosate concentrations below the LOQ. These findings may arise doubts on the source of AMPA in urine because virtually no metabolism of glyphosate to AMPA may be expected.~~

~~The measured values themselves are considered reliable by the RMS. The results suggest that there is a certain exposure of European population to glyphosate, mainly by dietary intake. This is not surprising since glyphosate is a widely used active substance worldwide. Residues in food and feed may occur and are allowed if below the MRLs. Systemically available glyphosate (*i.e.*, the rather low percentage that is absorbed from the GIT) is excreted via the urine, virtually unchanged. Apparently, there is also some exposure to AMPA although its origin is less clear. However, due to the limited number of involved participants and the absence of any information about them (such as age, gender, body weight, social background, origin from urban or rural environments, nutrition habits) and the way how they were recruited, the study was only explorative and cannot be regarded as representative. The mean dietary exposure level cannot be estimated on this basis, neither for a single country nor for Europe it its whole. Moreover, no conclusion can be drawn to which extent the apparent differences in urinary levels of glyphosate in samples obtained in the different countries might reflect the actual use of glyphosate. (It was reported, *e.g.*, that 8 out of 10 samples from Austria and 10 out of 12 from Switzerland were below the LOQ in contrast to only 3 of 10 from the UK or even 1 of 10 from Malta.)~~

~~In any case, none of the measured concentrations was of health concern since the exposure that may be calculated on this basis is far below the ADI. For an adult with 70 kg body weight, the newly proposed ADI of 0.5 mg/kg bw (see B.6.10 and Volume 1), would mean that the total daily intake of glyphosate might be as high as 35 mg. If 20 % is assumed to be orally absorbable (see above), up to 7 mg might be eliminated via the urine. Since the average urine volume is 1.5 – 2 L, theoretical urine concentrations of glyphosate in the magnitude of 3.5 to 4.7 mg/L would result. Even the maximum values measured by Hoppe (2013, ASB2013-8037) are less than 0.1 % of these expected concentrations proving a very low systemic dose that was received by the participants, presumably via the dietary route. With regard to AMPA, it must be emphasised that the glyphosate ADI also covers this metabolite and that the assessment does not change even when the low AMPA concentrations and those of glyphosate would be summed up.~~

~~It is interesting to note that the mean value of *ca* 0.2 µg/L was by five times lower than the geometric mean of glyphosate concentrations that were measured in a study in U.S. farmers on the third day following application, *i.e.*, with mainly dermal and inhalative exposure to be assumed. However, the maximum value on that post-application day 3 was 68 µg/L and on the day of glyphosate spraying even 233 µg/L (Acquavella *et al.*, 2004, ASB2012-11528). This comparison suggests that exposure of operators will normally exceed that of consumers and that, if operators are not on risk, dietary exposure should not be a matter of concern.~~

B.6.1.4 Data obtained with formulations

Not relevant for this section dealing with toxicokinetic behaviour and metabolism of the active substance. For dermal absorption, see B.6.12.

B.6.2 Acute toxicity including irritancy and skin sensitisation (Annex IIA 5.2)

Introduction to this chapter by RMS:
The acute studies already evaluated in 2001 are only summarised in the tables below. These studies were not re-evaluated for the present renewal procedure. Even if some of these old studies would be considered now as not acceptable according to current standards, the assessment of the acute toxicity potential (incl. irritancy & skin sensitisation) of glyphosate will remain unchanged due to the huge amount of valid (new) studies. For details regarding studies reviewed during the 2001 EU evaluation please refer to the DAR.
The new submitted studies are summarized as well, additionally described in detail and commented by the RMS.
For higher efficiency of the review and for the sake of transparency, the descriptions of methods and study results in the GTF dossier were virtually not amended and even the conclusions were kept as provided. However, each study that is described in detail was commented by RMS. These remarks on bottom of each study description are clearly distinguished from the original submission by a caption and are always written in italics. In addition, the so-called "executive summaries" have been deleted to clearly represented the new studies.

B.6.2.1 Acute oral toxicity

Glyphosate acid and its salts have been extensively tested for acute toxicity, skin and eye irritation, and skin sensitisation. An amount of 145 acute studies was submitted either for the previous EU evaluation or in the present GTF dossier.
For the previous EU evaluation, a large number of oral toxicity studies in rats and mice were submitted that had been conducted with either glyphosate acid or its salts. In the current GTF dossier, a variety of additional studies in rats with administration of glyphosate acid and two more studies in rats and mice with the IPA salt were provided.

Table B.6.2-1: Summary of acute oral toxicity studies with glyphosate acid

Reference (Data owner)		Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
Studies from the 2001 evaluation	Annex B-5.2.1.1.1, Glyphosate Monograph [redacted] 1995 (Sanachem) TOX9650909	Rat, Sprague Dawley	5/sex/2000	97.6	Cotton seed oil	> 2000 (limit test)	Slightly congested lungs, spleno-megaly, Liver: centri-lobular congestion
	Annex B-5.2.1.1.1, Glyphosate Monograph [redacted] 1994 (Herbex) TOX9500245	Rat, Sprague Dawley	1/sex/2000[##] 5/sex/2000	95	Arachis oil	> 2000 (limit test)	No findings

Reference (Data owner)		Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
	Annex B-5.2.1.1.1, Glyphosate Monograph [redacted] 1994 (Alkaloida) TOX9650142	Rat, Wistar	5/sex/0 5/sex/5000	97.2	water	> 5000 (limit test)	♂: heart weights ↓
	Annex B-5.2.1.1.1, Glyphosate Monograph [redacted], 1995 (SIN) TOX9500377	Rat, Sprague Dawley	2/sex/250## 2/sex/500## 2/sex/1000## 2/sex/3000## 2/sex/5000## 5/sex/5000	95	CMC	> 5000 (limit test)	Piloerection, subdued behaviour, hunched appearance
	Annex B-5.2.1.1.1, Glyphosate Monograph [redacted], 1992 (Barclay) TOX9551810	Rat, Sprague Dawley	1/sex/2000## 5/sex/2000	>97	water	> 2000 (limit test)	No findings
	Annex B-5.2.1.1.1, Glyphosate Monograph [redacted] 1991 (ADM) TOX9551088	Rat, Wistar	5/sex/2500 5/sex/5000 5/sex/7500	96.8	Peanut oil	> 7500 (estimated)	7500 mg/kg bw: mortality (2/5 ♂, 2/5 ♀); lethargy, ataxia, dyspnoea, weight loss
Studies from the 2001 evaluation	Annex B-5.2.1.1.1, Glyphosate Monograph [redacted] 1990 (AGC) TOX9500261	Rat, CD	5/sex/0 5/sex/3000 5/sex/5000 5/sex/8000	98.1	1% CMC	> 8000	≥ 5000 mg/kg bw: decreased activity, abnormal gait and/or limb position
	Annex B-5.2.1.1.1, Glyphosate Monograph [redacted] 1989 (CHE) TOX9552319	Rat, Sprague Dawley	5/sex/5000	98.6	0.5 % CMC	> 5000 (limit test)	Piloerection, reduced activity, ataxia (♂ only)
Studies not reviewed in the	IIA 5.2.1/01 [redacted] 2009 (HAG) ASB2012-11381	Rat, Sprague Dawley	5/females/5000	96.4	water	> 5000 (limit test)	decreased activity, diarrhoea, piloerection, polyuria, salivation

Reference (Data owner)		Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
	IIA 5.2.1/02 [redacted], 1995 (ALS) ASB2012-11382	Rat, Sprague Dawley	5/sex/5000	95.68	0.5 % CMC	> 5000 (limit test)	decreased spontaneous motor activity and salivation
	IIA 5.2.1/03 [redacted], 1995 ASB2012-11383	Mice, ICR	5/sex/5000	95.68	0.5 % CMC	> 5000 (limit test)	decreased spontaneous motor activity, sedation and crouching position
	IIA 5.2.1/04 [redacted] 2009 (EXC) ASB2012-11384	Rat, Wistar	3 females/2000 (step 1) 3 females/2000 (step 2)	96.66	water	> 2000	No findings
	IIA 5.2.1/05 [redacted] 2009 (HAG) ASB2012-11385	Rat, CD	3 females/2000 (step 1) 3 females/2000 (step 2)	98.8	0.8 % hydro-xypro-pylme-thylcel-lulose	> 2000 (limit test)	No findings
Studies not reviewed in the 2001 evaluation	IIA 5.2.1/06 [redacted] 2010 (HAG) ASB2012-11386	Rat, CD	3 females/2000 (step 1) 3 females/2000 (step 2)	96.4	0.8 % hydro-xypro-pylme-thylcel-lulose	> 2000 (limit test)	No findings
	IIA 5.2.1/07 [redacted] 2010 (HAG) ASB2012-11387	Rat, CD	3 females/2000 (step 1) 3 females/2000 (step 2)	97.3	0.8 % hydro-xypro-pylme-thylcel-lulose	> 2000 (limit test)	No findings
	IIA 5.2.1/08 [redacted] 2005 (HAG) ASB2012-11388	Rat, Sprague-Dawley	3 females/5000	97.23	water	> 5000 (limit test)	Diarrhea, ano-genital & facial staining, reduced faecal volume
	IIA 5.2.1/09 [redacted] 2008 (HAG) ASB2012-11389	Rat, Wistar	3 females/2000 (step 1) 3 females/2000 (step 2)	98.05	water	> 2000 (limit test)	No findings
	IIA 5.2.1/10 [redacted] 2007 (NUF) ASB2012-11390	Rat, HanRcc: WIST	2 x 3 ♀/2000	95.1	PEG 300	> 2000 (limit test)	Slightly ruffled fur
Studies not reviewed in [redacted]	IIA 5.2.1/11 [redacted] 1988 (MON) Z35389	Rat, Sprague Dawley	5/sex/5000	97.76	water	> 5000	diarrhea, apparent urinary incontinence and hair loss on the abdomen

Reference (Data owner)		Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
	IIA 5.2.1/12 [redacted]., 1979 (MON) Z35541	Rat, Wistar	5/sex/2500 5/sex/3500 5/sex/5000 5/sex/7000 5/sex/9900	99	water	> 5000	Mortalities: 1/10 1/10, 3/10,7/10, 10/10 at 2500, 3500, 5000, 7000 and 9900 mg/kg bw; clinical signs: ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy, and fecal staining of the abdomen
	IIA 5.2.1/13 [redacted] 1996 (SYN) TOX2000-1982	Rat	5/sex/5000	95.6	water	> 5000	No findings
	IIA 5.2.1/14 [redacted] 2007 (SYN) ASB2012-11391	Rat	3 ♀/5000	96.1	Water	> 5000	Ruffled fur, hunched posture
	IIA 5.2.1/15 Tavaszi, 2011 ([redacted] ASB2012-11392	Rat	3 ♀/5000	96.3	0.5 % CMC	>5000	No findings
	IIA 5.21/18 [redacted] 2014 (Albaugh Europe Sàrl) ASB2014-9147	Rat	5 ♀/2000	85.8	DMS	> 2000 (fixed dose method)	Hunched posture
Studies from the 2001 evaluation	Annex B-5.2.1.1.2, Glyphosate Monograph [redacted] 1991 (ADM) TOX9551089	Mice, Swiss albino	5/sex/2500 5/sex/5000 5/sex/7500	96.8	Peanut oil	> 7500	≥ 2500 mg/kg bw: mortality, lethargy, ataxia, dyspnoe, weight loss
	Annex B-5.2.1.1.2, Glyphosate Monograph [redacted] 1994 (I.Pi. Ci) TOX9551624	Mice, Charles River	5/sex/2000	technical	0.5 % CMC	> 2000 (limit test)	Piloerection, hunched posture, hypoactivity

Reference (Data owner)		Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
	Annex B-5.2.1.1.2, Glyphosate Monograph [redacted] 1991 (CHE)TOX9552320	Mice, Bom:NMRI	5/sex/2000	98.6	water	> 2000 (limit test)	Piloerection, sedation

CMC = carboxymethylcellulose

Table B.6.2-2: Summary of acute oral toxicity studies with glyphosate salts

Reference (Data owner)		Species Strain	Number of animals / Dose levels (mg/kg bw)	Salt type Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
Studies from the 2001 evaluation	Annex B-5.2.1.2.1, Glyphosate Monograph [redacted] 1995 (Sanachem) TOX9650910	Rat, Sprague Dawley	5/sex/2000	IPA 61.8	none	> 2000 (limit test)	severely congested lungs, splenomegaly, hepatomegaly with centrilobular congestion, subcapsuloar renal petechiae
	Annex B-5.2.1.2.1, Glyphosate Monograph [redacted] 1994 (MON / CHE) TOX9552322	Rat, Sprague Dawley	5/sex/2000	IPA 62.2	none	> 5000 (limit test)	No findings
	Annex B-5.2.1.2.1, Glyphosate Monograph [redacted] 1989 (I.Pi.Ci) TOX9551623	Rat, Wistar	5/sex/2000	IPA 62% in water	none	> 2000 (limit test)	No findings
Studies from the 2001 evaluation	Annex B-5.2.1.2.1, Glyphosate Monograph [redacted] 1981 (MON / CHE) TOX9552321	Rat, Sprague-Dawley	5/sex/5000	IPA 65	none	> 5000 (limit test)	Pale colored kidneys and hydronephrosis in few ♀

Reference (Data owner)		**Species Strain**	**Number of animals / Dose levels (mg/kg bw)**	**Salt type Purity (%)**	**Vehicle**	**LD_{50} (mg/kg bw)**	**Main effects**
	Annex B-5.2.1.2.2, Glyphosate Monograph [redacted] 1987 (SIN) TOX9500376	Mice, ICR	5/sex/0 (PEG) 5/sex/3125 5/sex/3625 5/sex/4125 5/sex/4625 5/sex/5125	IPA 41	PEG 200	3669 (confidence interval: 3489 – 3858)	Dose-related mortality in all IPA-treated groups; immobility, tremor, hyperemia of the ears
Study from the 2001 evaluation	Annex B-5.2.1.2.2, Glyphosate Monograph [redacted] 1987 (SIN) TOX9500375	Mice, ICR	5/sex/0 (PEG) 5/sex/3125 5/sex/3625 5/sex/4125 5/sex/4625 5/sex/5125	IPA 64	PEG 200	4373 (confidence interval: 4144 – 4644)	Dose-related mortality in all IPA-treated groups; immobility, tremor, hyperemia of the ears
Studies not reviewed in the 2001 evaluation	IIA 5.2.1/16 [redacted] 1995 (MON) ASB2012-11393	Mice CD-1	5/sex/5000	IPA 62.34	none	> 5000	Slight reduction in body weight gain in males at 5000 mg/kg/day versus controls
	IIA 5.2.1/17 [redacted] 1999 (NUF) ASB2012-11394	Rat, Sprague Dawley	5/sex/5000	IPA 62%	none	> 5000 (limit test)	♀: anogenital staining, soft faeces or diarrhoea
Study from the 2001 evaluation	Annex B-5.2.1.2.1, Glyphosate Monograph [redacted] 1987 (MON / CHE) Z85869 & TOX9552323	Rat, Sprague-Dawley	1/sex/2000 5/sex/5000 5/sex/7500	NH_4-salt 90.8	none	4613 (confidence interval: 3511 – 5716)	5000 and 7500 mg/kg bw: mortality; ataxia, decreased activity, diarrhea, labored breathing, color changes of lungs (dark red), GI tract of dead rats with dark/red fluid

Tier II summaries are presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.

Reference:	IIA, 5.2.1/01
Report:	[redacted] (2009) Glyphosate: Acute Oral Toxicity Study (UDP) In Rats. [redacted] Data owner: Helm AG Report No.: 12170-08 Date: 2009-03-11, not published ASB2012-11381
Guidelines:	US EPA OPPTS 870.1100 Equivalent to OECD 425 (2008).
Deviations:	Humidity was in the range of 33-89 % instead of 30-70 %. This deviation did not affect the study outcome
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate Tech Grade Mixed 5-Batch
Description:	White powder
Lot/Batch #:	080704-1 thru 5
Purity:	96.40 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	Deionised water
Test animals:	
Species:	Rat albino
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	7 - 8 weeks
Sex:	Female
Weight at dosing:	160 - 187 g
Acclimation period:	5 days
Diet/Food:	Formulab #5008 (PMI Feeds Inc.), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Individual housing in suspended, wire bottom, stainless steel cages.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 89 % Air changes: 10 - 12/hour 12-hour light/dark cycle

Study design and methods

In life dates: 2008-11-11 to 2008-11-27
Animal assignment and treatment:
A group of three fasted females received the test material at a dose level of 5000 mg/kg bw by oral gavage in a sequential manner according to the up-and-down procedure (limit test). The dosing volume was 12.5 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14.
On Day 14 after dosing, each animal was euthanised by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: Clinical signs in one animal included activity decrease, diarrhoea, piloerection, polyuria and salivation, which were no longer evident at Day 8.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers
The oral LD_{50} of the test material (glyphosate) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate is not to be classified for acute oral toxicity.

Comment by RMS:
The study is considered acceptable and the evaluation is agreed.

Reference:	IIA, 5.2.1/02
Report:	[redacted] (1995) HR-001: Acute Oral Toxicity Study In Rats. [redacted] Data owner: Arysta Life Sciences Report No.: IET 94-0134 Date: 1995-02-20, not published ASB2012-11382
Guidelines:	OECD 401 (1987), JMAFF 59 NohSan 4200 (1995), US EPA (1984)
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:

Identification:	Glyphosate technical, Code: HR-001
Description:	White crystal

Lot/Batch #:	940908-1
Purity:	95.68%
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	0.5% carboxymethyl-cellulose (CMC)
Test animals:	
Species:	Rat
Strain:	Sprague-Dawley (Crj:CD), SPF
Source:	[redacted]
Age:	5 weeks
Sex:	Males and females
Weight at dosing:	♂ 168 - 179 g; ♀ 125 - 142 g
Acclimation period:	7 days
Diet/Food:	Pellet Diet MF (Oriental Yeast Co., Japan), *ad libitum* except for an overnight fast before dosing and about 3 h after dosing
Water:	Tap water, *ad libitum*
Housing:	Wire-mesh stainless steel cages in groups of 5 animals/sex/cage.
Environmental conditions:	Temperature: 23 ± 3°C Humidity: 55 ± 15% Air changes: 12/hour 12-hour light/dark cycle

Study design and methods
In life dates: 1995-01-24 to 1995-02-07

Animal assignment and treatment:
A group of five fasted rats per sex received the test material at a dose level of 5000 mg/kg bw by oral gavage (limit test). The dosing volume was 20 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, each animal was euthanized under ether anesthesia and subjected to gross necropsy.

Results and discussion
Mortality: There were no mortalities during the study.

Clinical observations: Decreased spontaneous motor activity was observed in five males and three females as well as salivation in one male. These signs were observed at 1 and 3 hours after the administration.

Body weight: No body weight losses were recorded on Day 7 and 14 after administration when compared with the body weights on the day of administration.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The oral LD_{50} of the test material (HR-001) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, HR-001 is not to be classified for acute oral toxicity.

Comment by RMS:

The study is considered acceptable and the evaluation is agreed.

Reference:	IIA, 5.2.1/03
Report:	[redacted] (1995) HR-001: Acute Oral Toxicity Study In Mice. [redacted] Data owner: Arysta Life Sciences Report No.: IET 94-0133 Date: 1995-02-20, not published ASB2012-11383
Guidelines:	OECD 401 (1987), JMAFF 59 NohSan 4200 (1995), US EPA (1984)
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate technical, Code: HR-001
Description:	White crystal
Lot/Batch #:	940908-1
Purity:	95.68 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	0.5 % carboxymethyl-cellulose (CMC)
Test animals:	
Species:	Mice
Strain:	ICR (Crj:CD-1), SPF
Source:	[redacted]
Age:	6 weeks
Sex:	Males and females
Weight at dosing:	♂ 29.4 - 32.7 g; ♀ 22.8 - 25.8 g
Acclimation period:	7 days
Diet/Food:	Pellet Diet MF (Oriental Yeast Co., Japan), *ad libitum* except for approx. 2 h before dosing, and 3 h after dosing
Water:	Tap water, *ad libitum*
Housing:	Aluminium cages with wire-mesh floors in groups of 5 animals/sex/cage.

Environmental conditions:	Temperature:	23 ± 3 °C
	Humidity:	55 ± 15 %
	Air changes:	12/hour
	12-hour light/dark cycle	

Study design and methods
In life dates: 1995-01-24 to 1995-02-07

Animal assignment and treatment:
A group of five fasted mice per sex received the test material at a dose level of 5000 mg/kg bw by oral gavage (limit test). The dosing volume was 20 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, each animal was euthanized under ether anesthesia and subjected to gross necropsy.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: Decreased spontaneous motor activity was observed in one male and one female as well as sedation and crouching position in another male. These signs were observed at 1 and 3 hours after the administration.
Body weight: 7 days after administration, a slight body weight loss (0.5 g) was observed in one male when compared with the body weight on the day of administration. No body weight losses were recorded in any animal 14 days after the administration.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers
The oral LD_{50} of the test material (HR-001) in mice was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, HR-001 is not to be classified for acute oral toxicity.

Comment by RMS:
The study is considered acceptable and the oral LD_{50} of > 5000 mg/kg bw in mice is agreed.

Reference:	IIA, 5.2.1/04
Report:	[redacted] (2009) Glyphosate Technical: Acute Oral Toxicity Study in Rats. [redacted] Data owner: Excel Report No.: C22864 Date: 2009-04-02, not published ASB2012-11384
Guidelines:	OECD 423 (2001) Commission Regulation (EC) No 440/2008 (2008), method B.1 tris
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	GI-1045
Purity:	96.66 %
Stability of test compound:	(Stable under storage conditions.) Expiry date: July 2010.
Vehicle and/ or positive control:	Purified water
Test animals:	
Species:	Rat
Strain:	HanRcc: WIST (SPF)
Source:	[redacted]
Age:	11 weeks
Sex:	Female
Weight at dosing:	181.0 – 198.7 g
Acclimation period:	7 days
Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 61/08 (Provimi Kliba AG, 4303 Kaiseaugst / Switzerland) ad libitum (except for the overnight fasting period rior to intubation and approximately 3-4 hours post dose).
Water:	Tap water, *ad libitum*
Housing:	In groups of three in Makrolon type-4 cages with wire mesh tops and standard softwood bedding (‘Lignocel’ Shill AG, 4132 Muttenz / Switzerland).
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: 10 - 15/hour 12-hour light/dark cycle

Study design and methods
In life dates: 2009-02-13 to 2009-02-03 and 2009-02-05

Animal assignment and treatment:
Two groups of three fasted females each received the test material at a dose level of 2000 mg/kg bw by oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical signs of toxicity were made at least five times on the day of dosing (Day 1) and at twice daily thereafter during days 2-15. Individual body weights were recorded just prior to dosing and on Days 8 and 15.
On Day 15 after dosing, each animal was euthanised by CO_2 asphyxiation. All study animals were subjected to gross necropsy and all abnormalities were recorded.

Results and discussion
Mortality: No deaths occurred during the study.
Clinical observations: No clinical signs were observed during the course of the study.
Body weight: The body weight of the animals was within the range commonly recorded for this strain and age.
Necropsy: No macroscopic findings were recorded at necropsy.

Conclusion by the Notifiers
The oral LD_{50} of the test material (glyphosate technical) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate technical is not to be classified for acute oral toxicity.

Comment by RMS:
The study is considered acceptable and the estimated oral LD_{50} > 2000 mg/kg bw is agreed.

Reference:	IIA, 5.2.1/05
Report:	[redacted] (2009) Acute Oral Toxicity Study of Glyphosate TC in Rats. [redacted] Data owner: Helm AG Report No.: 23910 Date: 2009-06-16, not published ASB2012-11385
Guidelines:	EC method B.1 tris (2004/73/EC), OECD 423 (ATC method) and OPPTS 870.1100.
Deviations:	A personnel change in the Head of the Quality Assurance Unit did not affect the validity of the study outcome.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate TC
Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	20080801
Purity:	98.8 %
Stability of test compound:	2010-08-01
Vehicle and/or positive control:	0.8 % aqueous hydroxypropylmethylcellulose gel
Test animals:	
Species:	Rat albino
Strain / Stock:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	50 - 51 days

Sex:	Female
Weight at dosing:	167 - 186 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Groups of 3 animals were kept in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Rel. humidity: 40 - 70 % 12-hour light/dark cycle

Study design and methods:
In life dates: 2009-02-04 to 2009-03-04

Animal assignment and treatment:
A group of three fasted females received the test material at a single dose level of 2000 mg/kg bw by oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6, and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and thereafter in weekly intervals up to the end of the study.
On Day 14 after dosing, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: No clinical signs were observed during the study.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers
The oral LD_{50} of the test material (Glyphosate TC) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate TC is not to be classified for acute oral toxicity.

Comment by RMS:
The study is considered acceptable and evaluation is agreed, the oral LD_{50} is greater than 2000 mg/kg bw.

Reference:	IIA, 5.2.1/06
Report:	[redacted] (2010) Acute Oral Toxicity Study of Glyphosate TC in Rats. [redacted] Data owner: Helm AG Report No.: 24874 Date: 2010-01-06, not published ASB201211386
Guidelines:	EC method B.1 tris (2004/73/EC), OECD 423 (ATC method) and OPPTS 870.1100.
Deviations:	There were no deviations from the study plan.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate TC
Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	2009051501
Purity:	96.4 %
Stability of test compound:	2011-05-15
Vehicle and/ or positive control:	0.8 % aqueous hydroxypropylmethylcellulose
Test animals:	
Species:	Rat albino
Strain / Stock:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Approx. 7 weeks
Sex:	Female
Weight at dosing:	171 - 192 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Groups of 3 animals were kept in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Rel. humidity: 40 - 70 % 12-hour light/dark cycle

Study design and methods:
In life dates: 2010-10-15 to 2010-11-10

Animal assignment and treatment:
A group of three fasted females received the test material at a single dose level of 2000 mg/kg bw by oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and thereafter in weekly intervals up to the end of the study.
On Day 14 after dosing, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: No clinical signs were observed during the study.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers
The oral LD_{50} of the test material (Glyphosate TC) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate TC is not to be classified for acute oral toxicity.

Comment by RMS:
The study is considered acceptable and the oral LD_{50} >2000 mg/kg bw is agreed.

Reference:	IIA, 5.2.1/07
Report:	[redacted] (2010) Acute Oral Toxicity Study of Glyphosate TC in Rats. [redacted] Data owner: Helm AG Report No.: 24602 Date: 2010-02-19, not published ASB2012-11387
Guidelines:	EC method B.1 tris (2004/73/EC), OECD 423 (ATC method) and OPPTS 870.1100.
Deviations:	There were no deviations from the study plan.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate TC
Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	20090506
Purity:	97.3 %

Stability of test compound:	May 2011
Vehicle and/or positive control:	0.8 % aqueous hydroxypropylmethylcellulose
Test animals:	
Species:	Rat albino
Strain / Stock:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Approx. 7 - 8 weeks
Sex:	Female
Weight at dosing:	154 - 196 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Groups of 3 animals were kept in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Rel. humidity: 40 - 70 % 12-hour light/dark cycle

Study design and methods:
In life dates: 2009-10-26 to 2009-11-24

Animal assignment and treatment:
A group of three fasted females received the test material at a single dose level of 2000 mg/kg bw by oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6, and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and thereafter in weekly intervals up to the end of the study.
On Day 14 after dosing, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: No clinical signs were observed during the study.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers
The oral LD_{50} of the test material (Glyphosate TC) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate TC is not to be classified for acute oral toxicity.

Comment by RMS:
The study is considered acceptable and based on the results the oral LD_{50} *>2000 mg/kg bw/d is agreed.*

Reference:	IIA, 5.2.1/08
Report:	[redacted] (2005) Glyphosate Acid Technical – Acute Oral Toxicity Up and Down Procedure in Rats. [redacted] Data owner: Helm AG Report No.: 15274 Date: 2005-04-04, not published ASB2012-11388
Guidelines:	US EPA OPPTS 870.1100 (2002) OECD 425 (2001).
Deviations:	There were no deviations from the study plan.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate Acid Technical
Identification:	Glyphosate Acid Technical
Description:	White crystalline powder
Lot/Batch #:	040205
Purity:	97.23 %
Stability of test compound:	Test substance was expected to be stable for the duration of testing.
Vehicle and/or positive control:	Distilled water
Test animals:	
Species:	Rat albino
Strain:	Sprague-Dawley derived
Source:	[redacted]
Age:	11 weeks
Sex:	Female
Weight at dosing:	222 - 235 g
Acclimation period:	21 or 23 days
Diet/Food:	Purina Rodent Chow #5012, *ad libitum* except for overnight fasting before dosing
Water:	Filtered tap water, *ad libitum*
Housing:	Individual housing in suspended stainless steel cages with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:	Temperature: 19-23 °C 12-hour light/dark cycle

Study design and methods:
In life dates: 2004-05-04 to 2004-05-20

Animal assignment and treatment:
A group of three fasted females received the test material at a dose level of 5000 mg/kg bw by oral gavage in a sequential manner according to the up-and-down procedure (limit test). The test substance was administered as a 50 % w/w suspension in distilled water. Observations for mortality and clinical/behavioural signs of toxicity were made during the first several hours post-dosing and at least once daily thereafter for 14 days after dosing. Individual body weights were recorded just prior to dosing and on Days 7 and 14.
On Day 14 after dosing, each animal was euthanised by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: Clinical signs noted for all animals included diarrhea, ano-genital and facial staining, and/or reduced fecal volume. All animals recovered by Day 4 and appeared active and healthy for the remainder of the 14-day observation period.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers
The oral LD_{50} of the test material (glyphosate acid technical) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate Acid Technical is not to be classified for acute oral toxicity.

Comment by RMS:
The study is considered acceptable and the evaluation is agreed.

Reference:	IIA, 5.2.1/09
Report:	[redacted]2008) Acute Oral Toxicity Study in [redacted] Data owner: Helm AG Report No.: RF-3996.305.475.07 Date: 2008-09-16, not published ASB2012-11389
Guidelines:	OECD guideline 423 (2001).
Deviations:	The experimental phase initiation and conclusion dates were updated. This deviation did not affect the study outcome.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate Technical
Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	20070606
Purity:	98.05 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	Deionised water
Test animals:	
Species:	Rat albino
Strain:	Wistar Hannover
Source:	[redacted]
Age:	8 - 9 weeks
Sex:	Female
Weight at dosing:	172 - 205 g
Acclimation period:	6 days
Diet/Food:	Autoclaved Nuvilab CR-1 pellet diet type for rodents (Nuvital Nutrients Ltda.), *ad libitum* except for fasting overnight before dosing
Water:	Filtered drinking water, *ad libitum*
Housing:	Groups of three rats per cage were held in polypropylene rodents cages with wire mesh tops and bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: min. 10/hour 12-hour light/dark cycle

Study design and methods
In life dates: 2007-09-12 to 2008-06-11

Animal assignment and treatment:
A group of three fasted females received the test material at a dose level of 2000 mg/kg bw by oral gavage in a stepwise manner. Observations for mortality and clinical/behavioural signs of toxicity were made once within the first 30 minutes after dosing, three times more during the first 4 hours after dosing, and daily thereafter for a period of 14 days. Individual body weights were recorded just prior to dosing (Day 0) and on Days 7 and 14. On Day 14 after dosing, each animal was euthanized by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: No clinical signs of toxicity were observed in females treated with 2000 mg/kg bw.
Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers
The acute oral LD_{50} cutoff-value of the test material (glyphosate technical) in female rats was estimated to be 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria Glyphosate Technical is not to be classified for acute oral toxicity.

Comment by RMS:
The study is considered acceptable. The limit dose of 2000 mg/kg bw (not 5000 mg/kg bw/d) did not cause signs of toxicity, the acute oral LD_{50} is >2000 mg/kg bw.

Reference:	IIA, 5.2.1/10
Report:	[redacted] (2007) GLYPHOSATE TECHNICAL (NUP05068) : Acute oral toxicity study in rats [redacted] Data owner: Nufarm Report No.: BO2272 Date: 2007-03-01, unpublished ASB2012-11390
Guidelines:	Japanese guideline Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Preparation of Study Results, Acute oral toxicity studies. Guideline 2"1-1 Notification 12 NohSan No. 8147, as partly revised in 16-Shouan-9260, on 16 March 2005. English translation by ACIS on 17 Oct 2005. Directive 2004173/EC, 8.1 tris "Acute Oral Toxicity-Acute Toxic Class Method", April 29, 2004. OECD Guidelines for the Testing of Chemicals, Number 423 "Acute Oral Toxicity - Acute Toxic Class Method", adopted 17 December 2001.
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate Technical (NUP 05068)
Description:	White powder
Lot/Batch #:	200609062
Purity:	95.1 %
Stability of test compound:	Stable under storage conditions.

Vehicle and/or positive control:	Polyethylene glycol 300 (PEG 300)
Test animals:	
Species:	Rat
Strain:	HanRcc:WIST (SPF)
Source:	[redacted]
Age:	11 weeks
Sex:	Female
Weight at dosing:	160 - 187 gg
Acclimation period:	5 days
Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 67/06 (Provimi Kliba AG, CH-4303 Kaiseraugst/Switzerland) ad libitum.
Water:	Tap water, ad libitum
Housing:	In groups of three in Makrolon type-4 cages with wire mesh tops and standard softwood bedding ('Lignocel' Schill AG, CH-4132 MuttenzlSwitzerland).
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: 10 - 15/hour 12-hour light/dark cycle

Study design and methods:
In life dates: 2006-12-12 to 2007-01-04

Animal assignment and treatment:
The animals received a single dose of the test item by oral gavage administration at 2000 mg/kg body weight after being fasted for approximately 18 to 19 hours (access to water was permitted). Food was provided again approximately 3 hours after dosing. The dosing volume was 10 mL/kg body weight. OBSERVATIONS for Mortality and Viability: Daily during the acclimatization period, during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1 (with the clinical signs) and twice daily during days 2-15.
Body weights: On test days 1 (prior to administration), 8 and 15.
Clinical signs: Daily during the acclimatisation period, during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1. Once daily during days 2-15. All abnormalities were recorded.
NECROPSY: All animals were killed at the end of the observation period by Carbon dioxide asphyxiation.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: Clinical signs in one animal included activity decrease, diarrhoea, piloerection, polyuria and salivation, which were no longer evident at Day 8.
Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers
The median lethal dose of Glyphosate Technical (NUP 05068) after single oral administration to female rats, observed over a period of 14 days is: LD50 (female rat) > than 2000 mg/kg body weight.
Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate Technical (NUP 05068) is not to be classified for this end point.

Comment by RMS:
The study is considered acceptable and evaluation is agreed.

Reference:	IIA, 5.2.1/11
Report:	[redacted]. (1988) Acute Oral Toxicity Study of Glyphosate Batch/Lot/NBR No. XLI-55 in Sprague-Dawley Rats [redacted] Data owner: Monsanto Monsanto Report No.: FD-88-29 Date: 1988-06-08, not published Z35389
Guidelines:	US EPA 81-1
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	XLI-55
Purity:	97.76 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	Distilled water
Test animals:	
Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Not specified
Sex:	Male and female
Weight at dosing:	♂ 300 - 332 g; ♀ 217 - 222 g
Acclimation period:	At least 5 days

Diet/Food:	NIH Open Formula 07 Rat and Mouse Diet, certified feed (Zeigler Brothers, Inc., Gardners, PA, US), *ad libitum* (except when fasted overnight prior to dosing)
Water:	Tap water, *libitum*
Housing:	Wire mesh cages
Environmental conditions:	Temperature: 20 – 23.9 ºC Humidity: 40 – 70 % Air changes: Not specified Light cycle: 12 hour light/dark cycle

Study design and methods:
In life dates: 1988-04-05 to 1988-04-19

Animal assignment and treatment:
Groups of five male and five female rats received the test material at a dose level of 5000 mg/kg body weight by oral gavage as a 50 % w/v aqueous suspension. Observations for mortality and signs of toxicity were made three times on the day of dose administration and twice daily thereafter. Body weights were recorded prior dose administration on study day 1, and on days 8 and 15 (terminal sacrifice). A gross necropsy was performed on all animals at the time terminal sacrifice (day 15) and all abnormalities were recorded.

Results and discussion
Mortality: No mortalities occurred.
Clinical observations: Clinical signs included diarrhoea, apparent urinary incontinence, and hair loss on the abdomen.
Body weight: Body weight gain was noted for all animals.
Necropsy: No internal abnormalities were noted during gross necropsy examination of the animals.

Conclusion by the Notifiers
The oral LD_{50} of the test material (glyphosate) in rats was estimated to be 5000 mg/kg body weight. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate should not be classified for acute oral toxicity.

Comment by RMS:
The study is considered acceptable and evaluation is agreed.

Reference:	IIA, 5.2.1/12
Report:	[redacted] (1979) Acute Oral Toxicity Study In Rats. [redacted] Data owner: Monsanto Monsanto Report No.: BND-77-428 Date: 1979-08-06, not published Z35541
Guidelines:	None (pre-guideline)
Deviations:	Not specified
GLP:	no (pre-GLP)
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate Technical
Description:	Fine white powder
Lot/Batch #:	XHI-180
Purity:	99 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	Distilled water
Test animals:	
Species:	Rat
Strain:	Wistar
Source:	[redacted]
Age:	Not specified
Sex:	Male and female
Weight at dosing:	225 - 294 g
Acclimation period:	Not specified
Diet/Food:	*ad libitum* (except when fasted for approximately 18 hours prior to dosing)
Water:	*ad libitum*
Housing:	Individually
Environmental conditions:	Temperature: Not specified Humidity: Not specified Air changes: Not specified Light cycle: Not specified

Study design and methods:
In life dates: Not specified

Animal assignment and treatment:
Groups of five male and five female rats received the test material at a dose levels of 2.5, 3.5, 5.0, 7.0, and 9.9 g/kg body weight by oral gavage. The test material was administered by oral intubation as a 25 % w/v solution in distilled water. Observations for mortality and overt signs of effect were made at 0-2 and 4-6 hours following dosing and twice daily thereafter (early morning and late afternoon) for fourteen days. Body weights were recorded prior to fasting, on Day 7, and on Day 14 of the study. A gross necropsy was performed on all animals at the time of death or terminal sacrifice (Day 14). All abnormalities were recorded.

Results and discussion
Mortality: Mortalities in the study are as indicated below in Table B.6.2-3.

Table B.6.2-3: Summary of Mortality

Dose Level (g/kg body weight)	Mortality/Total Number of Animals Dosed
2.5	1/10
3.5	1/10
5.0	3/10
7.0	8/10
9.9	10/10

Clinical observations: Clinical signs included ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy, and fecal staining of the abdomen.
Body weight: For the 2.5, 3.5, 5.0, and 7.0 g/kg body weight dose levels, although some animals lost weight between 7 and 14 days, all surviving animals gained weight throughout the study.
Necropsy: A summary of the gross necropsy findings for the decedents and the animals necropsied at the conclusion of the 14-day observation period is presented in Table B.6.2-4.

Table B.6.2-4: Summary of Necropsy Findings

Dose Level (g/kg body weight)	Animals Necropsied at 14 Days	Decedents
2.5	Discoloured lungs, liver, and/or kidneys	Urinary and faecal staining of the abdomen Discoloured lungs Fluid filled stomach Fluid filled and/or distended intestines
3.5	Discoloured lungs, liver, and/or kidneys Air filled intestines	Discoloured lungs
5.0	No observations	Oral and/or nasal discharge Urinary and/or faecal staining of the abdomen Discoloured lungs and/or liver Fluid filled and/or discoloured stomach and/or intestines
7.0	Discoloured lungs, liver, and/or kidneys Air filled intestines	Oral discharge Fluid filled intestines and/or stomach Discoloured liver, and/or kidneys Urinary and/or faecal staining of the abdomen
9.9	Not applicable	Discoloured lungs, liver, and/or kidneys Fluid filled intestines and/or stomach Oral and/or nasal discharge Urinary staining of the abdomen

Conclusion by the Notifiers
The oral LD_{50} of the test material (glyphosate) in rats was estimated to be 5.6 g/kg body weight with 95 % confidence limits of 4.9 to 6.3 g/kg body weight. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate is not to be classified for acute oral toxicity.

Comment by RMS:
This old study was conducted prior to GLP and not according to any Test Guideline, subsequently some reporting deficiencies were apparent. Therefore, this study is considered

to be supplementary. Under the conditions of the present study the oral LD_{50} value of > 5000 mg/kg bw is agreed.

Reference:	IIA, 5.2.1/13
Report:	[redacted] (1996) Glyphosate Acid: Acute Oral Toxicity Study In Rats. [redacted] Data owner: Syngenta Report No.: CTL/P/4660 Date: 1996-08-23, not published TOX2000-1982
Guidelines:	OECD 425 (2001) US EPA OPPTS 870.1100 (2002)
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS.*

Materials and methods

Test material:	
Identification:	Glyphosate Acid
Description:	Technical, white solid
Lot/Batch #:	P24
Purity:	95.6 %
Stability of test compound:	The test substance was used within the expiry date.
Vehicle and/ or positive control:	Deionised water
Test animals:	
Species:	Rat
Strain:	Alpk:AP_fSD (Wistar-derived)
Source:	[redacted]
Age:	Young adult
Sex:	Male and female
Weight at dosing:	233-260 g (males), 197-225 g (females)
Acclimation period:	At least 6 days
Diet/Food:	Diet (PCD), supplied by Special Diet Services Limited, Witham, Essex, UK *ad libitum* (except overnight immediately prior to dosing).
Water:	Mains water *ad libitum*
Housing:	5/cage, sexes separately in multiple rat racks suitable for animals of this strain and the weight range expected during the course of the study.

Environmental conditions:	Temperature:	21 ± 2 °C
	Humidity:	40 – 70 %
	Air changes:	Approximately 25-30/hour
	12-hour light/dark cycle	

Study design and methods:
In life dates: 1995-03-16 to 1995-03-30

Animal assignment and treatment:
In an acute oral toxicity study, a group of five male and five female, fasted, young adult Alpk:AP_fSD (Wistar-derived) rats were given a single oral dose of 5000 mg/kg bw of glyphosate acid by gavage. The test substance was diluted in deionised water. The volume of the dose was calculated for each animal according to its weight at the time of dosing and a standard volume of 10 mL/kg of the dosing preparation was administered.
Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. The animals were observed for signs of systemic toxicity once within 2 hours of dosing and again between 4 and 7 hours after dosing. Subsequent observations were made daily, up to day 15.
The animals were weighed on the day before dosing (day -1), immediately before dosing (day 1) and on days 3, 8, 8 and 15.
All animals were subjected to an examination *post mortem*. This involved an external observation and a careful examination of all thoracic and abdominal viscera.

Results and discussion
Mortality: There were no mortalities.
Clinical observations: There were no signs of systemic toxicity.
Body weight: All animals lost weight initially due to the pre-dose fast, but all had exceeded their initial weight by day 3, and apart from a transient weight loss in one female, continued to gain weight throughout the remainder of the study.
Necropsy: Red or mottled areas in the lung or red areas in the thymus were seen in three males and two females. These are common spontaneous findings in rats of this age and strain and are considered not to be treatment-related.

Conclusion by the Notifiers
The oral LD_{50} of the test material (glyphosate acid) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate acid is not to be classified for acute oral toxicity.

Comment by RMS:
The study is considered acceptable and evaluation is agreed.

Reference:	IIA, 5.2.1/14
Report:	[redacted] (2007) Glyphosate technical material: Acute oral toxicity study in the rat (up and down procedure). [redacted] Data owner: Syngenta Report No.: B02755 Date: 2007-02-08, not published ASB2012-11391

Guidelines:	OECD 425 (2001) US EPA OPPTS 870.1100 (2002) Japanese MAFF 12 NohSan No. 8147
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate technical material
Description:	Technical, white powder
Lot/Batch #:	0507
Purity:	96.1 %
Stability of test compound:	Re-certification date August 2008. Stable under storage conditions (room temperature range 20 ± 5 °C, protected from light and humidity).
Vehicle and/or positive control:	Purified water (deionised water processed and treated by the PURELAB Option-R unit which links four purification technologies: reverse osmosis, adsorption, ion-exchange and photo oxidation).
Test animals:	
Species:	Rat
Strain:	HanRcc:WIST (SPF)
Source:	[redacted]
Age:	11 weeks
Sex:	Female
Weight at dosing:	183.0-188.9 g
Acclimation period:	5-7 days
Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet (Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland) *ad libitum* (except for pre-dose fast).
Water:	Community tap water *ad libitum*
Housing:	Individually in Makrolon type-3 cages with standard softwood bedding
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: 10 - 15/hour 12-hour light/dark cycle

Study design and methods:
In life dates: 2006-12-06 to 2006-12-26

Animal assignment and treatment:
In an acute oral toxicity study, a group of three, fasted, 11 week old, HanRcc:WIST (SPF), female rats was given a single oral dose of glyphosate technical material (96.1 % w/w glyphosate acid) at a concentration of 5000 mg/kg body weight by gavage. The test substance was diluted in vehicle (purified water) and dosed at a volume dosage of 10 mL/kg body weight.
Single animals were dosed sequentially at no less than approximately 48 hour intervals. The time intervals between dosing were determined by the onset, duration and severity of clinical signs. The first animal was treated at a dose level of 5000 mg/kg body weight. As no mortality or significant clinical signs were observed, two additional animals were sequentially dosed at 5000 mg/kg such that a total of 3 animals were tested. No mortalities were observed, therefore the study was terminated.
The animals were examined daily during the acclimatisation period and mortality, viability and clinical signs were recorded. All animals were examined for clinical signs once during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after treatment on day 1 and once daily during test days 2-15. Mortality/viability was recorded once during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1 (with the clinical signs) and twice daily during days 2-15.
Body weights were recorded on day -1 (prior to removal of food), day 1 (prior to administration) and on days 8 and 15.
All animals were killed at the end of the observation period by carbon dioxide asphyxiation, necropsied and examined macroscopically.

Results and discussion
Mortality: There were no mortalities.
Clinical observations: Slight ruffled fur was noted in all animals from the 30-minute reading to the 5-hour reading and persisted in one animal until test day 3. Hunched posture was also noted in the animals from the 1- or 2-hour reading to the 5-hour reading.
Body weight: The body weight of the animals was within the range commonly recorded for this strain and age.
Necropsy: No macroscopic findings were recorded at the scheduled necropsy.

Conclusion by the Notifiers
The oral LD_{50} of the test material (glyphosate technical material) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate technical material is not to be classified for acute oral toxicity.

Comment by RMS:
The study is considered acceptable and the estimated oral LD_{50} >5000 mg/kg bw is agreed.

Reference: IIA, 5.2.1/15
Report: [redacted] 2011) Glyphosate technical: Acute oral toxicity study in the rat (up and down procedure)
[redacted]
Data owner: Syngenta
Report No.: 10/218-001P
Date: 2011-04-15, not published
ASB2012-11392

Guidelines:	OECD 425 (2008) OPPTS 870.1100 (2002)
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate technical
Description:	Technical, dry white powder
Lot/Batch #:	569753(BX20070911)
Purity:	96.3 %
Stability of test compound:	Stable under storage conditions (room temperature range <30 °C), recertification date end August 2011
Vehicle and/or positive control:	0.5% Carboxymethylcellulose (CMC)
Test animals:	
Species:	Rat
Strain:	RjHan:WI
Source:	[redacted]
Age:	10-11 weeks
Sex:	Female
Weight at dosing:	228-231 g
Acclimation period:	At least 21 days
Diet/Food:	ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and rats – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494 Soest Germany *ad libitum* (except for pre-dose fast)
Water:	Tap water *ad libitum*
Housing:	Individually in Type II. polypropylene/polycarbonate cages with Lignocel Bedding for Laboratory Animals
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: 15 - 20/hour 12-hour light/dark cycle

Study design and methods:
In life dates: 2011-01-20 to 2011-02-10

Animal assignment and treatment:
In an acute oral toxicity study, a group of three, fasted, 10-11 week old, RjHan:WI female rats was given a single oral dose of glyphosate technical (96.3 % w/w glyphosate technical) at a concentration of 5000 mg/kg body weight by gavage. The test substance was diluted in vehicle (0.5 % carboxymethylcellulose) and administered at a dosing volume of 10 mL/kg.

Single animals were dosed sequentially at no less than approximately 48 hour intervals. The time intervals between dosing were determined by the onset, duration and severity of clinical signs. Treatment of an animal at the next dose was only performed when no significant clinical signs were noted in the previous animal.
All animals were examined for clinical signs once during the first 30 minutes and at approximately 1, 2, 3, 4 and 6 hours after treatment on day 1 and once daily for 14 days thereafter.
Body weights were recorded on day -1 (prior to removal of food), day 0 (prior to administration) and on days 7 and 14.
All animals were exsanguinated under pentobarbital anaesthesia at the end of the observation period, necropsied and examined macroscopically.

Results and discussion
Mortality: There were no mortalities.
Clinical observations: No clinical signs were observed in the 3 animals treated at 5000 mg/kg bw.
Body weight: The body weight of the animals was within the range commonly recorded for this strain and age.
Necropsy: No macroscopic findings were recorded at the scheduled necropsy.

Conclusion by the Notifiers
The oral LD_{50} of the test material (glyphosate technical) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate technical is not to be classified for acute oral toxicity.

Comment by RMS:
The study is considered acceptable and evaluation is agreed.

Reference:	IIA, 5.2.1/18
Report:	[redacted] (2011) Glyphosate: Acute Oral Toxicity in the Rat – Fixed Dose Method [redacted] Data owner: Albaugh Europe Sàrl Report No.: 4140853 Date: 2014-00-01, not published ASB2014-9147
Guidelines:	OECD 420 (2001) Method B1 bis (EC) No.440/2008
Deviations:	Homogeneity, concentration or stability or test item formulation not determined
GLP:	yes
Acceptability:	Please see comment by RMS

Materials and methods

Test material:

Identification:	Glyphosate technical
Description:	Technical, white crystalline solid

Lot/Batch #:	04062014
Purity:	85.79 %
Vehicle and/or positive control:	Dimethyl sulphoxide
Test animals:	
Species:	Rat
Strain:	RccHan™:Wistar
Source:	[redacted]
Age:	8-12 weeks
Sex:	Female
Weight at dosing:	141 - 171 g
Acclimation period:	At least 5 days
Water:	Tap water ad libitum
Housing:	Animals were housed in groups of up to four
Environmental conditions:	Temperature: 19 to 25 °C Humidity: 30 - 70 % Air changes: at least 15 /hour 12-hour light/dark cycle

Study design and methods:
In life dates: 2014-07-10 to 2014-07-30

Animal assignment and treatment:
Female Wistar (RccHan™:Wist) strain rats were supplied by Harlan Laboratories UK Ltd., Oxon, UK. On receipt the animals were randomly allocated to cages. The females were nulliparous and non-pregnant. The body weight variation did not exceed ±20 % of the body weight of the initially dosed animal.
With the exception of an overnight fast immediately before dosing and for approximately three to four hours after dosing, free access to mains drinking water and food (2014C Teklad Global Rodent diet supplied by Harlan Laboratories UK Ltd., Oxon, UK) was allowed throughout the study.
For the purpose of the study the test item was freshly prepared, as required, as a dispersion/suspension in dimethyl sulphoxide. Dimethyl sulphoxide was used because the test item did not dissolve/suspend in distilled water or arachis oil BP.
The test item was formulated within two hours of being applied to the test system. It is assumed that the formulation was stable for this duration.
No analysis was conducted to determine the homogeneity, concentration or stability of the test item formulation. This is an exception with regard to GLP and has been reflected in the GLP compliance statement.
Using available information on the toxicity of the test item, 2000 mg/kg bwwas chosen as the starting dose (one animal) and administered at a dosing volume of 10 mL/kg. In the absence of toxicity at a dose level of 2000 mg/kg bw, an additional group of four animals was treated.
All animals were dosed once only by gavage, using a metal cannula attached to a graduated syringe.
Clinical observation were made ½, 1, 2, and 4 hours after dosing and then daily for fourteen days. Morbidity and mortality checks were made twice daily.
Individual body weights were recorded on day 0 (the day of dosing) and on days 7 and 14.

At the end of the observation period the animals were killed by cervical dislocation. All animals were subjected to gross necropsy. This consisted of an external examination and opening of the abdominal and thoracic cavities. The appearance of any macroscopic abnormalities was recorded. No tissues were retained.

Results and discussion
Mortality: There were no deaths.
Clinical observations: No signs of sytemic toxicity were noted in the initial treated animal during the observation period. Hunched posture was noted during the day of dosing in four additional treated animals.
Body weight: All animals showed expected gains in body weight over the observation period.
Necropsy: No macroscopic findings were recorded at the scheduled necropsy. No tissues were retained.

Conclusion by the RMS
The study is considered to be acceptable. The oral LD_{50} of the test material (glyphosate technical) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate technical is not to be classified for acute oral toxicity.

Reference:	IIA, 5.2.1/16
Report:	[redacted] 1995) Acute Toxicity Study of MON 0139 by Oral Administration in Mice. [redacted] Data owner: Monsanto Monsanto Report No.: XX-95-205 Date: 1995-10-05, not published ASB2012-11393
Guidelines:	JMAFF 59 NohSan No. 4200 (January 28, 1985)
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	MON 0139
Description:	Light yellow viscous solution
Lot/Batch #:	LBRV-11092
Purity:	62.34% (isopropyl amine salt of glyphosate)
Stability of test compound:	Stable under room temperature, expiry July, 1996
Vehicle and/or positive control:	Water for injection
Test animals:	
Species:	Mouse
Strain:	Crj:CD-1(ICR)
Source:	[redacted]

Age:	6 weeks
Sex:	Male and female
Weight at dosing:	♂ 31.1 – 34.5 g; ♀ 22.2 – 26.2 g
Acclimation period:	Approximately 1 week
Diet/Food:	CRF-1 pelleted diet, sterilised by radiation (Oriental Yeast Co., Ltd.), *ad libitum* except during fasting prior to dosing
Water:	Tap water; *ad libitum*
Housing:	Plastic cages with wood chip bedding in groups of 5 (groups of 5 or 6 during quarantine/acclimation)
Environmental conditions:	Temperature: 23 ± 3 ºC Humidity: 50 ± 20 % Air changes: 11 – 13 per hour Light cycle: 12 hour illumination per day

Study design and methods:
In life dates: 1995-08-16 – 1995-09-06

Animal assignment and treatment:
Preliminary study: During the quarantine/acclimatization period, a preliminary study was conducted using 9 males and 9 females. The animals were fasted for approximately 4 hours prior to administration and the test article was administered once orally, by gavage, adjusting the dose volume according to each dose level. Three male and female animals were dosed with 1000, 2000 or 5000 mg/kg body weight. The animals were fed again after administration, and had free access to water throughout the experimental period.

Main Study:
In the preliminary study, no deaths were observed in either sex in any of the dose groups. Based on these results, the dose level of 5000 mg/kg body weight was selected for the main test. The animals were ranked by individual body weights and randomly assigned to groups so as to ensure the homogeneity of group means as far as possible. The animals were fasted for approximately 4 hours prior to administration and the test article was administered once orally, by gavage. The animals in the 5000 mg/kg body weight group and control group were given 0.041 mL/10 g body weight of test article and 'water for injection', respectively. Each group consisted of 5 animals per sex. Animals were fed again after administration, and had free access to water throughout the experimental period.

The animals were observed frequently for the first 6 hours after administration, and then once daily for 14 days for mortality, signs of toxicity and abnormalities. Body weights were recorded prior to fasting, immediately before dosing, and on days 1, 2, 3, 7, 10 and 14 after dosing. A gross necropsy was performed on all animals at the time of terminal sacrifice at the end of the 14-day observation period.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: No clinical signs of toxicity were observed.
Body weight: In males, a slight tendency toward retardation of body weight gain as compared with the control group was observed in the 5000 mg/kg body weight group from 7 days after administration (see Table B.6.2-5). In females, no compound-related changes were observed in the 5000 mg/kg body weight group.

Table B.6.2-5: Summary of male body weights

MON 0139 Dose (mg/kg body weight)	Days After Administration							
	0[a]	0	1	2	3	7	10	14
0	34.4	32.6	34.5	35.2	35.4	37.3	38.1	38.8
5000	34.8	32.8	34.2	34.1	34.4	35.1	35.9	37.3

[a] Prior to fasting

Necropsy: No abnormalities were observed.

Conclusion by the Notifiers
The oral LD_{50} of the test material (MON 0139) in mice was >5000 mg/kg body weight. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, MON 0139 is not to be classified for acute oral toxicity.

Comment by RMS:
The study is considered acceptable and the oral LD_{50} > 5000 mg/kg bw in mice is agreed.

Reference: IIA, 5.2.1/17
Report: [redacted] (1999) NUP5a99 62 % glyphosate MUP: Acute oral toxicity study in rats – Limit test
[redacted]
Study No.: 7907
Date: 1999-09-16, unpublished
ASB2012-11394
Guidelines: US EPA Health Effects Test Guidelines, OPPTS 870.1100 (1998)
Deviations: None
GLP: yes
Acceptability: *Please see comment by RMS*

Materials and methods

Test material:
Identification: NUP5a99 62 % glyphosate MOP
Description: clear viscous amber liquid
Lot/Batch #: Drum Sample E
Purity: 62 %
Stability of test compound: No data available
Vehicle and/
or positive control: None
Test animals:
Species: Rat
Strain: Sprague-Dawley derived, albino
Source: [redacted]
Age: Not specified
Sex: 5 males and 5 females

Weight at dosing:	Young adult/males 227-254 grams and females 178-200 grams at experimental startg
Acclimation period:	14 days
Diet/Food:	Purina Rodent Chow #5012
Water:	Tap water, ad libitum
Housing:	singly housed in suspended stainless steel caging with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:	Temperature: 22-24 °C Humidity: not specified Air changes: not specified 12-hour light/dark cycle

Study design and methods
In life dates: 1999-08-03 – 1999-08-17

Animal assignment and treatment:
Prior to dosing, a group of animals was fasted for approximately 17.25 hours by removing feed from their cages. After weighing and clinical examination, ten (five male and five female) healthy rats were selected for test. Individual doses were calculated based on the initial bodyweights, taking into account the specific gravity (determined by PSL) of the test substance. Each animal received 5000 mg/kg of the test substance via gavage. Feed was replaced approximately 3.5 hours after dosing. The day of administration was considered Day zero of the study. Animals were weighed prior to test substance administration (initial) and again on Days 7 and 14 (termination). Clinical signs were recorded at 1,3 and 22 hours post-dosing and at least once daily thereafter for 14 days. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and product safety labs central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea and coma. All rats were euthanized via CO_2 inhalation on Day 14. Gross necropsies were performed on all animals.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: Most females exhibited anogenital staining and two females exhibited soft feces or diarrhea, but recovered by Day 2.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers
The single dose acute oral LD_{50} of NUP5a99 62 % glyphosate MUP is greater than 5000 mg/kg of bodyweight.
Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, NUP5a99 62 % glyphosate MOP is not to be classified for this endpoint.

Comment by RMS:

The study is considered acceptable and the estimated oral LD_{50} is greater than 5000 mg/kg bw.

Comment by GTF on the first draft of the RAR (July 2013):
The Rat LD_{50}oral and Rat LD_{50}dermal are listed as >2000 mg/kg bw whereas the data presented concludes LD50-values above 5000 mg/kg. This would also be consistent with the values presented in summary Table B.6.13-2 in Volume 3 B.6.

RMS comment (August 2013):
To conclude on the LD50 value >2000 mg/kg bw seems to be more appropriate, because not all different batches were tested up to 5000 mg/kg bw. In any case Glyphosate is considered not to be classified as acute oral or dermal toxic according to GHS categories.

B.6.2.2 Acute percutaneous toxicity

Similarly, for the previous EU evaluation, a multitude of dermal toxicity studies in rats and rabbits were provided using glyphosate acid and its salts. Additional studies in rats with glyphosate acid were submitted for the current re-evaluation.

Table B.6.2-6: Summary of acute dermal toxicity studies with glyphosate acid

Reference (Data owner)		Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
Studies from the 2001 evaluation	Annex B-5.2.2.1.1, Glyphosate Monograph [redacted] 1995 (Sanachem) TOX9650910	Rat, Sprague Dawley	5/sex/2000	97.6	Cotton seed oil	> 2000 (limit test)	splenomegaly, Liver: centri-lobular congestion
	Annex B-5.2.2.1.1, Glyphosate Monograph [redacted], 1994 (SIN) TOX9500378	Rat, Sprague Dawley	5/sex/2000	95	Suspen-ded (50% w/w) in natrosol (1% w/w in water)	> 2000 (limit test)	No findings
	Annex B-5.2.2.1.1, Glyphosate Monograph [redacted] 1994 (Herbex) TOX9500246	Rat, Sprague Dawley	5/sex/2000	95	none	> 2000 (limit test)	No findings
Studies from the 2001 evaluation	Annex B-5.2.2.1.1, Glyphosate Monograph [redacted] 1994 (ALkaloida) TOX9650143	Rat, Wistar	2/sex/0 5/sex/2000	97.2	water	> 2000 (limit test)	No findings

Reference (Data owner)		Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
	Annex B-5.2.2.1.1, Glyphosate Monograph [redacted] 1992 (Barclay) TOX9551813	Rat, Sprague-Dawley	5/sex/2000	> 97	none	> 2000 (limit test)	No findings
	Annex B-5.2.2.1.1, Glyphosate Monograph [redacted] 1991 (ADM TOX9551090	Rat, Wistar	5/sex/2500 5/sex/5000	96.8	Water (slurry)	> 5000	body weight loss
	Annex B-5.2.2.1.1, Glyphosate Monograph [redacted] 1990 (AGC) TOX9551793	Rat, CD	5/sex/0 5/sex/3000 5/sex/5000 5/sex/8000	98.1	0.9 % saline	> 8000	No findings
Studies not reviewed in the 2001 evaluation	IIA 5.2.2/01 [redacted] 1989 (CHE) TOX9300328	Rat, Sprague Dawley	5/sex/2000	98.6	Water for moiste-ning	> 2000 (limit test)	No mortalities, body weight loss in one female, scab formation at application site; 0.5 h-1d after dosing reduced activity and piloerection
	IIA 5.2.2/02 [redacted], 2009 (HAG) ASB2012-11395	Rat, Sprague Dawley	5/sex/5050	96.4	water	> 5050	body weight loss in 1 male and 1 female
	IIA 5.2.2/03 [redacted] 1995 (ALS) ASB2012-11396	Rat, SD	5/sex/2000	95.68	water	> 2000 (limit test)	No findings
	IIA 5.2.2/04 [redacted] 2009 (EXC) ASB 2012-11397	Rat, HanRcc: WIST	5/sex/2000	96.66	water	> 2000	No mortalities, no signs of systemic toxicity; in 4 females slight local signs (erythema, scaling and scabs) at the application sites

	Reference (Data owner)	**Species Strain**	**Number of animals / Dose levels (mg/kg bw)**	**Purity (%)**	**Vehicle**	**LD_{50} (mg/kg bw)**	**Main effects**
Studies not reviewed in the 2001 evaluation	IIA 5.2.2/05 [redacted] 2009 (HAG) ASB2012-11398	Rat, CD	5/sex/2000	98.8	water	> 2000	No findings
	IIA 5.2.2/06 [redacted] 2010 (HAG) ASB2012-11399	Rat, CD	5/sex/2000	96.4	water	> 2000	No findings
	IIA 5.2.2/07 [redacted] 2010 (HAG) ASB2012-11400	Rat, CD	5/sex/2000	97.3	water	> 2000	No findings
	IIA 5.2.2/08 [redacted] 2005 (HAG) ASB2012-11401	Rat, Sprague Dawley	5/sex/5000	97.23	water	> 5000	No findings
	IIA 5.2.2/09 [redacted] 2008 (HAG) ASB2012-11402	Rat, Wistar Hannover	5/sex/2000	98.05	water (for moisto-ning)	> 2000	No findings
	IIA 5.2.2/10 [redacted] 2007 (NUF) ASB2012-11403	Rat, HanRcc: WIST	5/sex/2000	95.1	PEG 300	> 2000 (limit test)	No findings
	IIA 5.2.2/11 [redacted] 1996 (SYN) TOX2000-1983	Rat	5/sex/2000	95.6	Moiste-ned with deionised water	> 2000	Slight erythema in 1♂, small scabs in 1 ♀
	IIA 5.2.2/12 [redacted] 2007 (SYN) ASB2012-11404	Rat	5/sex/5000	96.1	Moiste-ned with purified water	> 5000	No findings
	IIA 5.2.2/13 [redacted] 2011 (SYN) ASB2012-11405	Rat	5/sex/5000	96.3	Moiste-ned with purified water	> 5000	No findings

Reference (Data owner)		Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
Studies from the 2001 evaluation	Annex B-5.2.2.1.2, Glyphosate Monograph [redacted], 1988 (MON) TOX9552325	Rabbit, NZW	5/sex/5000	97.8	Moiste-ned with saline	> 5000	Mortality (1 ♀); anorexia, diarrhea, soft stool

Table B.6.2-7: Summary of acute dermal toxicity studies with glyphosate salts

Reference (Data owner)		Species Strain	Number of animals / Dose levels (mg/kg bw)	Salt type Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
Studies from the 2001 evaluation	Annex B-5.2.2.2.1, Glyphosate Monograph [redacted] 1995 (Sanachem) TOX9650910	Rat, Sprague Dawley	5/sex/2000	IPA 61.8	none	> 2000 (limit test)	severely congested lungs, splenomegaly, hepatomegaly with centrilobular congestion, subcapsuloar renal petechiae
	Annex B-5.2.2.2.1, Glyphosate Monograph [redacted] 1989 (I.Pi.Ci.) TOX9551625	Rat, Wistar	5/sex/2000	IPA 62%	none	> 2000 (limit test)	Erythema maculate (1♂), scales (1 ♀)
	Annex B-5.2.2.2.2, Glyphosate Monograph [redacted] 1987 (MON / CHE) TOX9552327	Rabbit NZW	5/sex/5000	Ammo nium salt 90.8	Moiste ned with saline	> 5000 (limit test)	Mortality (1♀); diarrhea, soft stool
Studies from the 2001 evaluation	Annex B-5.2.2.2.2, Glyphosate Monograph [redacted] 1987 (MON / CHE) TOX9552328	Rabbit NZW	5/sex/5000	Sodiu m salt 80.3	Saline (1mL/g)	> 5000 (limit test)	Soft stool
	Annex B-5.2.2.2.2, Glyphosate Monograph [redacted] 1981 (MON / CHE) TOX9552326	Rabbit NZW	5/sex/5000	IPA 65	none	> 5000 (limit test)	No findings

Tier II summaries are presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.
For details regarding studies reviewed during the 2001 EU evaluation please refer to the monograph.

Reference:	IIA, 5.2.2/01
Report:	[redacted] (1989) Acute dermal toxicity (limit) test in rats [redacted] Data owner: Cheminova A/S Report No.: 5884 Date: 1989-06-21, not published TOX9300328
Guidelines:	OECD, EEC, EPA[1]
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	206-Jak-25-1
Purity:	No data given in the report.
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	Water
Test animals:	
Species:	Rats
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	8-10 weeks
Sex:	Males and females (nulliparous and non-pregnant)
Weight at dosing:	♂ 212 - 240 g; ♀ 188 - 234 g
Acclimation period:	6 days
Diet/Food:	Expanded Rat and Mouse Maintenance Diet, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	polypropylene cages with mesh floors in groups of 5 animals/cage.

[1] Guideline numbers are not specified in the report, however the study is compliant with OECD 402, EEC B3 and EPA 81-2 with the exception of a slightly different test item application procedure.

Environmental conditions: Temperature: 19 - 22 °C
Humidity: 49 %
12-hour light/dark cycle

Study design and methods
In life dates: 1989-06-06 to 1989-06-21

Animal assignment and treatment:
A group of 5 male and 5 female rats was prepared by clipping the backs free of hair, approximately 24 hours before application of the test material. Care was taken to avoid abrading the skin. Glyphosate technical was administered dermally in a single application under occlusion at a dose level of 2000 mg/kg bw.
The test material was applied evenly onto gauze dressing, which was applied to the shaved back of each rat. The trunk of the rat was then encircled with a strip of non-irritating tape. After 24 hours the tape was removed and the skin was wiped with a water-dampened tissue to remove excess test material.
The rats were observed frequently on the day of dosing and once daily for 14 days following dosing. They were weighed immediately prior to dosing, 7 days after dosing and at sacrifice at the end of the 14-day observation period.
At the end of the observation period and sacrifice by carbon dioxide asphyxiation, each animal was subjected to a gross *post mortem* examination.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: Clinical signs noted 30 minutes to 1 day after dosing included piloerection and reduced activity. Scab formation was noted at test sites 2 to 14 days after dosing.
Body weight: Body weight gains with the exception of one animal, which lost weight, were acceptable.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers
The dermal LD_{50} of the test material, glyphosate technical, in Sprague-Dawley Rats was estimated to be greater than 2000 mg/kg bw.

Comment by RMS:
In the study report no specific Test Guideline was mentioned, however, the study was considered acceptable. Under the condition of this study, the estimated dermal LD_{50} > 2000 mg/kg bw is agreed.

Reference: IIA, 5.2.2/02
Report: [redacted] (2009) Glyphosate – Acute Dermal Toxicity Study in Rats.
[redacted]
Data owner: Helm AG
Report No.: 12171-08
Date: 2009-03-11
GLP:
not published

	ASB2012-11395
Guidelines:	US EPA OPPTS 870.1200 (1998)
Deviations:	Humidity was in the range of 30-86% instead of 30-70%. This deviation did not affect the study outcome.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate Tech Grade Mixed 5-Batch
Description:	White powder
Lot/Batch #:	080704-1 thru 5
Purity:	96.71 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	Deionised water
Test animals:	
Species:	Rat albino
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Approx. 8 weeks
Sex:	5 males and 5 females
Weight at dosing:	Males: 299 - 348 g Females: 189 - 207 g
Acclimation period:	5 days
Diet/Food:	Formulab #5008 (PMI Feeds Inc.), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individual housing in suspended, wire bottom, stainless steel cages.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: 10 - 12/hour 12-hour light/dark cycle

Study design and methods

In life dates: 2008-12-04 to 2008-12-18

Animal assignment and treatment:

One dose level group of 5 male and 5 female rats was examined in a limit test. The dose level of 5050 mg/kg bw was applied once for 24 hours on the shaved intact dorsal skin of the rats (ca. 1/10 of body surface). Observations for mortality and clinical/behavioural signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. Observations for evidence of dermal irritation were made at approximately 60 minutes after removal of wrappings, and on Days 4, 7, 11 and 14. On Day 14 after dosing, animals were euthanized by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

Results and discussion

Mortality: There were no mortalities during the study.
Clinical observations: A single dermal administration of 5050 mg/kg bw to 5 male and 5 female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.
Body weight: Body weight gain was unaffected by the administration of the test substance, with the exception of 2 animals that lost or failed to gain weight during the study..
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The dermal LD_{50} of the test material (glyphosate) in rats was estimated to be greater than 5050 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate is not to be classified for acute dermal toxicity.

Comment by RMS:
The study is considered acceptable and the dermal LD_{50} >5000 mg/kg bw is agreed.

Reference:	IIA, 5.2.2/03
Report:	[redacted] 1995, HR-001: Acute dermal toxicity study in rats. [redacted] Data owner: Arysta LifeScience Report No.: 94-0154 Date: 1995-03-14, not published ASB2012-11396
Guidelines:	U.S. EPA FIFRA Guideline Subdivision F 81-2
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate technical. Code HR-001
Description:	White crystal
Lot/Batch #:	Batch n° 940908-1
Purity:	95.68 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	Deionised water
Test animals:	
Species:	Rat
Strain:	Specific pathogen free SD rats (Crj:CD)
Source:	[redacted]
Age:	6 weeks
Sex:	Male and female

Weight at dosing:	248 – 268 g in males and 178 – 198 g in females
Acclimation period:	9 days
Diet/Food:	Certified diet pellet MF (Oriental yeast Co.)
Water:	Tap water, *ad libitum*
Housing:	Individual housing in suspended, wire bottom, stainless steel cages.
Environmental conditions:	Temperature: 24.3 ± 0.3 °C Humidity: 53 - 55 % Air changes: 12 times/hour 12-hour light/dark cycle

Study design and methods
In life dates: 1995-01-31 – 1995-02-23

Animal assignment and treatment:
The test material was prepared as a suspension in deionised water. The suspension was applied on the shave skin of 5 males and 5 females specific pathogen free SD rats (Crj:CD) at a dose level of 2000 mg/kg. Mortality and clinical signs were recorded 1, 3 and 6 hours after administration and at least once daily thereafter until the termination of the 14-day observation period. All animals were weighed on the day of administration and on days 7 and 14 after administration. The surviving animals were euthanized after completion of the observation period (day 14) and examined for gross abnormalities. All animals were subjected to necropsy.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: No clinical signs were noted in any animals of 0 and 2000 mg/kg groups.
Body weight: All animals gained their body weights on days 7 and 14 after administration.
Necropsy**:** There was no macroscopic abnormality in any surviving animals at final necropsy after completion of the observation period.

Conclusion by the Notifiers
The oral LD_{50} of the test material (glyphosate) in rats was estimated to be greater than 2000 mg/kg bw. However, the protocol of this study does not allow to confirm that glyphosate is not to be classified for acute oral toxicity based on the EU and the OECD Globally Harmonized System (GHS) classification criteria.

Comment by RMS:
The study is considered acceptable and the dermal LD_{50} >2000 mg/kg bw is agreed.

Reference:	IIA, 5.2.2/04
Report:	[redacted] 2009, Glyphosate Technical: Acute Dermal Toxicity Study in Rat. [redacted] Data owner: Excel Report No.: C22875 Date: 2009-04-02, not published

	ASB2012-11397
Guidelines:	OECD 402 (1987) Commission Regulation (EC) No 440/2008 (2008); method B.3
Deviations:	Weight of female animals was outside of the range specified in the guideline (200-300 g).
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	GI-1045
Purity:	96.66 %
Stability of test compound:	No data given in the report. (Stable in purified water for 2 days.) Expiry date: July 2010
Vehicle and/or positive control:	Purified water
Test animals:	
Species:	Rat
Strain:	HanRcc: WIST (SPF)
Source:	[redacted]
Age:	Males: 9 weeks Females: 11 weeks
Sex:	Males and female
Weight at dosing:	189.8 – 258.3 g
Acclimation period:	7 days
Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 61/08 (Provimi Kliba AG, 4303 Kaiseraugst / Switzerland) ad libitum.
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation in groups of five per sex in Makrolon type-4 cages with standard softwood bedding. Individually in Makrolon type-3 cages with standard softwood bedding (“Lignocel”, Schill AG, 4132 Muttenz / Switzerland) during treatment and observation.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: 10 - 15/hour 12-hour light/dark cycle

Study design and methods:
In life dates: 2009-01-20 to 2009-02-03

Animal assignment and treatment:
Single dose of 2000 mg/kg bw/day of test substance (glyphosate technical) was applied dermally to an area of clipped skin (approx. 10 % of body surface area) of five male and five female young adult rats. The treatment area was covered with a semi-occlusive dressing. Application volume was 4 mL/kg bw. Twenty-four hours after the application the dressing was removed and the skin was flushed with lukewarm tap water and dapped off with disposable paper towels. The animals were evaluated for effects on the day of dosing (Day 1) at 30 minutes and at 1, 2, 3 and 5 hours after application and once daily during days 2-15. Clinical observations, dermal findings, body weights and gross post mortem examinations were recorded.

Results and discussion
Mortality: No deaths occurred during the study.
Clinical observations: No systemic clinical signs were observed during the course of the study.
Body weight: A slight body weight loss (0.3-0.8 %) was observed in two females between acclimatisation and treatment start. The animals recovered until the end of the study. In spite of this body weight loss, the body weights of all animals were considered to be within the range commonly recorded for this strain and age.
Necropsy: No macroscopic findings were recorded at necropsy.
Skin observations: No local dermal signs were observed in any of the treated male animals. After removal of the patch, a very slight erythema was noted in four females on test day 4 and persisted up to test days 6, 11 or 12. Scaling was observed in the same four females on test day 4 and persisted up to test days 10, 11 and 12. Scabs were recorded in two females on test day 9 that persisted to test day 11.

Conclusion by the Notifiers
The dermal LD_{50} of the test material (glyphosate technical) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate technical is not to be classified for acute dermal toxicity.

Comment by RMS:
According to the study report the body weight of female rats were in the range of 189.8 and 208.8 (mean 199.6) and therefore only slightly outside the suggested weight range. Under the present conditions the study is considered acceptable and the dermal LD_{50} > 2000 mg/kg bw is agreed.

Reference:	IIA, 5.2.2/05
Report:	[redacted] 2009, Acute Dermal Toxicity Study of Glyphosate TC in CD Rats. [redacted] Hamburg, Germany Data owner: Helm AG Report No.: 23912 Date: 2009-06-16, not published ASB2012-11398
Guidelines:	EC method B.3. (92/69/EEC), OECD 402 (1987) and US EPA OPPTS 870.1200 (1998)
Deviations:	A personnel change in the Head of the Quality Assurance Unit did

	not affect the validity of the study outcome.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate TC
Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	20080801
Purity:	98.8 %
Stability of test compound:	2010-08-01
Vehicle and/or positive control:	*Aqua ad iniectabilia*
Test animals:	
Species:	Rat albino
Strain:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Males: 51 days Females: 65 days
Sex:	5 males and 5 females
Weight at dosing:	Males: 224 - 234 g Females: 200 - 217 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before administration
Water:	Tap water, *ad libitum*
Housing:	Individual housing in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Rel. humidity: 40 - 70 % Air changes: 12 - 18/hour 12-hour light/dark cycle

Study design and methods
In life dates: 2009-02-04 to 2009-03-04

Animal assignment and treatment:
One dose level group of 5 male and 5 female rats was examined in a limit test. The dose level of 2000 mg/kg bw was applied once for 24 hours on the shaved intact dorsal skin of the rats (ca. 1/10 of body surface). The administration volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded before administration of the test item and thereafter in weekly intervals up to the end of the study. The skin was observed for the development of erythema and oedema. At the end of the experiments, all animals were sacrificed, dissected and inspected macroscopically, and all abnormalities were recorded.

Results and discussion

Mortality: There were no mortalities during the study.
Clinical observations: A single dermal administration of 2000 mg/kg bw to 5 male and 5 female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The dermal LD_{50} of the test material (glyphosate TC) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate TC is not to be classified for acute dermal toxicity.

Comment by RMS:
The study is considered acceptable and the dermal LD_{50}> 2000 mg/kg bw is agreed.

Reference:	IIA, 5.2.2/06
Report:	[redacted] 2010, Acute Dermal Toxicity Study of Glyphosate TC in CD Rats. [redacted] Data owner: Helm AG Report No.: 24876 Date: 2010-01-06, not published ASB2012-11399
Guidelines:	EC method B.3. (92/69/EEC), OECD 402 (1987) and US EPA OPPTS 870.1200 (1998)
Deviations:	There were no deviations from the study plan.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate TC
Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	2009051501
Purity:	96.4 %
Stability of test compound:	2011-05-15
Vehicle and/or positive control:	*Aqua ad iniectabilia*
Test animals:	
Species:	Rat albino
Strain:	CD / Crl:CD(SD)
Source:	[redacted]

Age:	Males: approx. 7 weeks Females: approx. 9 weeks
Sex:	5 males and 5 females
Weight at dosing:	Males: 233 - 249 g Females: 211 - 229 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before administration
Water:	Tap water, *ad libitum*
Housing:	Individual housing in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Rel. humidity: 40 - 70 % 12-hour light/dark cycle

Study design and methods
In life dates: 2009-10-15 to 2009-11-12

Animal assignment and treatment:
One dose level group of 5 male and 5 female rats was examined in a limit test. The dose level of 2000 mg/kg bw was applied once for 24 hours on the shaved intact dorsal skin of the rats (ca. 1/10 of body surface). The administration volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded before administration of the test item and thereafter in weekly intervals up to the end of the study. The skin was observed for the development of erythema and oedema. At the end of the experiments, all animals were sacrificed, dissected and inspected macroscopically, and all abnormalities were recorded.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: A single dermal administration of 2000 mg/kg bw to 5 male and 5 female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers
The dermal LD_{50} of the test material (glyphosate TC) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate TC is not to be classified for acute dermal toxicity.

Comment by RMS:
The study is considered acceptable and the dermal LD_{50} >2000 mg/kg bw is agreed.

Reference: IIA, 5.2.2/07
Report: [redacted] 2010,
Acute Dermal Toxicity Study of Glyphosate TC in CD Rats.

	[redacted] Data owner: Helm AG Report No.: 24604 Date: 2010-02-19, not published ASB2012-11400
Guidelines:	EC method B.3. (92/69/EEC), OECD 402 (1987) and US EPA OPPTS 870.1200 (1998)
Deviations:	There were no deviations from the study plan.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate TC
Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	20090506
Purity:	97.3 %
Stability of test compound:	May 2011
Vehicle and/or positive control:	*Aqua ad iniectabilia*
Test animals:	
Species:	Rat albino
Strain:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Males: approx. 7 weeks Females: approx. 9 weeks
Sex:	5 males and 5 females
Weight at dosing:	Males: 278 - 292 g Females: 202 - 225 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before administration
Water:	Tap water, *ad libitum*
Housing:	Individual housing in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Rel. humidity: 40 - 70 % 12-hour light/dark cycle

Study design and methods
In life dates: 2009-10-26 to 2009-11-12

Animal assignment and treatment:
One dose level group of 5 male and 5 female rats was examined in a limit test. The dose level of 2000 mg/kg bw was applied once for 24 hours on the shaved intact dorsal skin of the rats

(ca. 1/10 of body surface). The administration volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded before administration of the test item and thereafter in weekly intervals up to the end of the study. The skin was observed for the development of erythema and oedema. At the end of the experiments, all animals were sacrificed, dissected and inspected macroscopically, and all abnormalities were recorded.

Results and discussion

Mortality: There were no mortalities during the study.
Clinical observations: A single dermal administration of 2000 mg/kg bw to 5 male and 5 female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The dermal LD_{50} of the test material (glyphosate TC) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate TC is not to be classified for acute dermal toxicity.

Comment by RMS:
The study is considered acceptable and the dermal LD_{50} >2000 mg/kg bw is agreed.

Reference:	IIA, 5.2.2/08
Report:	[redacted] 2005 Glyphosate Acid Technical: Acute Dermal Toxicity Study in Rats – Limit Test. [redacted] Data owner: Helm AG Report No.: 15275 Date: 2005-04-04, not published ASB2012-11401
Guidelines:	US EPA OPPTS 870.1200 (1998) OECD 402 (1987).
Deviations:	There were no deviations from the study plan.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate Acid Technical
Identification:	Glyphosate Acid Technical
Description:	White crystalline powder
Lot/Batch #:	040205
Purity:	97.23 %
Stability of test compound:	Test substance was expected to be stable for the duration of testing.

Vehicle and/or positive control:	Distilled water
Test animals:	
Species:	Rat albino
Strain:	Sprague-Dawley derived
Source:	[redacted]
Age:	8 weeks
Sex:	5 males and 5 females
Weight at dosing:	Males: 231 - 264 g Females: 193 - 200 g
Acclimation period:	8 days
Diet/Food:	Purina Rodent Chow #5012, *ad libitum*
Water:	Filtered tap water, *ad libitum*
Housing:	Individual housing in suspended stainless steel cages with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:	Temperature: 19-23 °C 12-hour light/dark cycle

Study design and methods
In life dates: 2004-05-05 to 2004-05-19

Animal assignment and treatment:
One dose level group of 5 male and 5 female rats was examined in a limit test. The dose level of 5000 mg/kg bw was applied once for 24 hours on the shaved intact dorsal skin of the rats (ca. 1/10 of body surface). Observations for mortality and clinical/behavioural signs of toxicity were made during the first several hours post-dosing and at least once daily thereafter for 14 days after dosing. Individual body weights were recorded just prior to dosing and on Days 7 and 14.
On Day 14 after dosing, each animal was euthanized by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: A single dermal administration of 5000 mg/kg bw to 5 male and 5 female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers
The dermal LD_{50} of the test material (glyphosate acid technical) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate acid technical is not to be classified for acute dermal toxicity.

Comment by RMS:
The study is considered acceptable and the dermal LD50 > 5000 mg/kg bw is agreed.

Reference:	IIA, 5.2.2/09
Report:	[redacted] 2008, Acute Dermal Toxicity Study in Wistar Hannover Rats for Glyphosate Technical. [redacted] Data owner: Helm AG Report No.: RF-3996.310.456.07 Date: 2008-07-04, not published ASB2012-11402
Guidelines:	OECD guideline 402 (1987)
Deviations:	1. The experimental phase initiation and experimental phase conclusion dates were updated. 2. One female rat weighed less than 200 g on the day of test item application. These deviations did not affect the study outcome.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate Technical
Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	20070606
Purity:	98.05 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	Deionised water
Test animals:	
Species:	Rat albino
Strain:	Wistar Hannover
Source:	[redacted]
Age:	9 - 11 weeks
Sex:	5 males and 5 females
Weight at dosing:	Males: 266 - 298 g Females: 199 - 213 g
Acclimation period:	7 days
Diet/Food:	Autoclaved Nuvilab CR-1 pellet diet type for rodents (Nuvital Nutrients Ltda.), *ad libitum*
Water:	Filtered drinking water, *ad libitum*
Housing:	Individual housing in polypropylene rodents cages with wire mesh tops and bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: min. 10/hour 12-hour light/dark cycle

Study design and methods
In life dates: 2007-09-11 to 2008-06-11
Animal assignment and treatment:
One dose level group of 5 male and 5 female rats was examined in a limit test. The dose level of 2000 mg/kg bw was applied once for 24 hours on the shaved intact dorsal skin of the rats (ca. 1/10 of body surface). Observations for mortality and clinical/behavioural signs of toxicity were made once within the first 30 minutes after dosing, three times more during the first 4 hours after dosing, and daily thereafter for a period of 14 days. Individual body weights were determined before the application of the test item (Day 0) and on days 7 and 14. On Day 14 after dosing, each animal was euthanized by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: A single dermal administration of 2000 mg/kg bw to 5 male and 5 female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.
Body weight: All animals gained the expected body weight, except for two females in the second week of the observation.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers
The dermal LD_{50} of the test material (glyphosate technical) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate technical is not to be classified for acute dermal toxicity.

Comment by RMS:
The study is considered acceptable and the dermal LD_{50} >2000 mg/kg bw is agreed.

Reference:	IIA, 5.2.2/10
Report:	[redacted] 2007, GLYPHOSATE TECHNICAL (NUP05068): Acute dermal toxicity study in rats [redacted] Data owner: Nufarm RCC Study No.: B02283 Date: 2007-03-01, unpublished ASB2012-11403
Guidelines:	Japanese guideline Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Preparation of Study Results, Acute Dermal Toxicity Studies, Guideline 2-1-2. Notification 12 NohSan No. 8147, as partly revised in 16-Shouan-9260, on 16 March 2005. English translation by AGIS on 17 Oct 2005 Directive 92/69/EEC, B.3. "Acute Toxicity-Dermal", July 31, 1992. OECD Guidelines for Testing of Chemicals, Section 4, Number 402 "Acute Dermal Toxicity", adopted February 24,1987.

Deviations: None
GLP: yes
Acceptability: *Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate Technical (NUP 05068)
Description:	Solid
Lot/Batch #:	200609062
Purity:	95.1 %
Stability of test compound:	Stable under storage conditions.
Vehicle and/or positive control:	Polyethylene glycol 300 (PEG 300)
Test animals:	
Species:	Rat
Strain:	HanRcc:WIST (SPF)
Source:	[redacted]
Age:	Males: 8 weeks Females: 11 weeks
Sex:	Male / Female
Weight at dosing:	194.8-254.3g
Acclimation period:	6 days
Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 67/06 (Provimi Kliba AG, CH-4303 Kaiseraugst/ Switzerland) ad libitum
Water:	Tap water, ad libitum
Housing:	During acclimatization in groups of five per sex in Makrolon type-4 cages with standard softwood bedding. Individually in Makrolon type-3 cages with standard softwood bedding ("Lignocel", Schill AG, CH-4132 uttenz) during treatment and observation.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30-70 % Air changes: 10 - 15/hour 12-hour light/dark cycle

Study design and methods
In life dates: 12-DEC-2006 to 02-JAN-2007

Animal assignment and treatment:
One day before treatment, the backs of the animals were clipped with an electric clipper, exposing an area of approximately 10 % of the total body surface. Only those animals without injury or irritation on the skin were used in the test. On test day 1, the test item was applied at a dose of 2000 mg/kg body weight evenly on the intact skin with a syringe and covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and fixed with an

elastic adhesive bandage. Application volume/kg body weight: 6 mL Twenty-four hours after the application the dressing was removed and the skin was flushed with lukewarm tap water and dried with disposable paper towels. Thereafter, the reaction sites were assessed. The fur of all animals was shaved on test days 4 and 9 just after the assessment of the reaction to facilitate the skin reading for the next day. Rationale: Dermal administration was used as this is one possible route of human exposure during manufacture, handling and use of the test item.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: No systemic or local signs of toxicity were observed during the study period.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers
The median lethal dose of glyphosate technical (NUP 05068) after single dermal administration to rats of both sexes, observed over a period of 14 days is: Dermal LD_{50} (rat) > 2000 mg/kg body weight.
Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate is not to be classified for this end point.

Comment by RMS:
The study is considered acceptable and the dermal LD_{50} >2000 mg/kg bw is agreed.

Reference:	IIA, 5.2.2/11
Report:	[redacted] 1996, Glyphosate Acid: Acute Dermal Toxicity Study In The Rat. [redacted] Data owner: Syngenta Report No.: [redacted]/P/4664 Date: 1996-08-23, not published TOX2000-1983
Guidelines:	OECD guideline 402 (1987), OPPTS 870.1200 (1998): 92/69/EEC B.3 (1992)
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate acid
Description:	Technical, white solid
Lot/Batch number:	P24
Purity:	95.6 % w/wi
CAS#:	Not reported
Stability of test compound:	The test substance was used within the expiry date

Vehicle and/or positive control:	Deionised water
Test animals:	
Species:	Rat
Strain:	Alpk:AP_fSD (Wistar-derived)
Age/weight at dosing:	Young adult / 250-274 g (males), 203-216 g (females)
Source:	[redacted]
Housing:	Individually in multiple rat racks suitable for animals of this strain and the weight range expected during the course of the study.
Acclimatisation period:	At least 6 days
Diet:	Diet (PCD), supplied by Special Diet Services Limited, Witham, Essex, UK *ad libitum*.
Water:	Mains water *ad libitum*
Environmental conditions:	Temperature: 21±2 °C Humidity: 40-70 % Air changes: Approximately 25-30/hour Photoperiod: 12 hours dark / 12 hours light

Study design and methods

In-life dates: Start: 16 March 1995 End: 30 March 1995

Animal assignment and treatment: In an acute dermal toxicity study, a group of five male and five female, young adult Alpk:AP_fSD (Wistar-derived) rats were given a single dermal application of 2000 mg/kg bw of glyphosate acid.

Sixteen to thirty-two hours before application, the hair was removed by clipping from an area on the dorso-lumbar region of each rat (approximately 10 cm x 5 cm). The appropriate amount of test substance was moistened to a dry paste with 0.6-0.8 mL of deionised water. Approximately half the application site was covered by test substance (equivalent to 20.0-21.9 mg/cm^2 for males and 16.2-17.3 mg/cm^2 for females). The application site was covered with a 4-ply gauze patch (approximately 7 cm x 7 cm) and kept in place for 24 hours using an occlusive dressing. The gauze patch was covered by a piece of plastic film (7 cm x 7 cm), held in place using adhesive bandage (25 cm x 7.5 cm) secured by two pieces of PVC tape.

At the end of the 24 hour contact period, the dressings were carefully removed and the skin cleansed of any residual test substance using clean warm water.

Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. The animals were observed for signs of systemic toxicity once between one and four hours of dosing. Subsequent observations were made daily, up to day 15.

The animals were weighed immediately before dosing (day 1) and on days 3, 8, 8 and 15.

All animals were subjected to an examination *post mortem*. This involved an external observation and a careful examination of all thoracic and abdominal viscera.

Statistics: The dermal LD_{50} was estimated (limit test, no mortalities).

Results and discussion

Mortality: There were no mortalities.

Clinical observations: There were no significant signs of systemic toxicity (only urinary incontinence due to bandaging). There were practically no signs of skin irritation. One male showed slight erythema on days 2 and 3 and one female had small scabs from day 3 to 8.
Body weight: Two males and three females lost weight initially, but all had exceeded their initial weight by day 5, and except for one female, continued to gain weight throughout the remainder of the study. One female lost weight slightly from day 5.
Necropsy: Red mottled lungs were seen in one female. This is a common spontaneous finding in rats of this age and strain and is considered not to be treatment-related.

Conclusion by the Notifiers:
The acute dermal LD_{50} of glyphosate acid is in excess of 2000 mg/kg to male and female rats (limit test, no mortality).

Comment by RMS:
The study is considered acceptable and the dermal LD_{50} > 2000 mg/kg bw is agreed.

Reference:	IIA, 5.2.2/12
Report:	[redacted] 2007, Glyphosate technical material: Acute dermal toxicity study in rats. [redacted] Data owner: Syngenta Report No.: B02766 Date: 2007-02-08, not published ASB2012-11404
Guidelines:	OECD 402 (1987), JMAFF 12 NohSan 8147 (2000), US EPA Health Effects Test Guidelines, OPPTS 870.1200 (1998)
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate technical material
Description:	Technical, white powder
Lot/Batch number:	0507
Purity:	96.1 % w/w glyphosate acid
CAS#:	Not reported
Stability of test compound:	Re-certification date August 2008. Stable under storage conditions (room temperature range 20±5°C, protected from light and humidity).
Vehicle and/or positive control:	The test item was applied moistened with purified water before application.

Test animals:	
Species:	Rat
Strain:	HanRcc:WIST (SPF)
Age/weight at dosing:	8 weeks (males), 11 weeks (females) / 247.0-222.7 g (males),-191.3-204.2 g (females)

Source:	[redacted]
Housing:	Individually in Makrolon type-3 cages with standard softwood bedding
Acclimatisation period:	7 days
Diet:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet (Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland) *ad libitum*
Water:	Community tap water *ad libitum*
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30-70 % Air changes: 10-15 air changes per hour Photoperiod: 12 hours light / 12 hours dark

Study design and methods

In-life dates: Start: 07 December 2006 End: 22 December 2006

Animal assignment and treatment: In an acute dermal toxicity study, a group of one male and one female and a second group of four male and four female HanRcc:WIST (SPF) rats were treated with glyphosate technical material (96.1 % w/w glyphosate technical) at 5000 mg/kg by dermal application. The test item was moistened with purified water before application. The application period was 24 hours.

One day before treatment, the backs of the animals were clipped with an electric clipper, exposing an area of approximately 10 % of the total body surface. Only those animals without injury or irritation on the skin were used in the test.

A single animal of each sex was treated first. As no deaths and neither severe local effects nor severe systemic symptoms were observed after the 24-hour exposure, the test was completed using the four remaining male and female animals for an exposure period of 24 hours.

On test day 1, the mass of the dose (5000 mg/kg) was calculated for each animal on the basis of its body weight. The appropriate amount of test item was weighed out on a suitable precision balance into a plastic weighing boat and moistened to a dry paste with a minimum amount of purified water (0.5 to 0.6 mL) to allow good skin contact. The dry paste was applied evenly on the intact skin of the clipped area and covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and anchored with tape. The area of skin covered by the test item was approximately 8 cm^2 for the males and females.

Twenty-four hours after the application the dressing was removed and the skin was flushed with lukewarm tap water and dried with disposable paper towels. Thereafter, the reaction sites were assessed.

The fur of all males and females was shaved, on test day 6 (female no. 2), on test days 5 and 9 (male no. 1), on test days 4 and 8 (males nos. 3 - 6 and females nos. 7 - 10) just after the assessment of the reaction to facilitate the skin reading for the next day.

The animals were checked daily for mortality/viability during the acclimatization period, at approximately 30 minutes, 1, 2, 3 and 5 hours after administration on test day 1 (with the clinical signs) and twice daily during days 2-15.

Clinical observations were recorded daily during the acclimatisation period, at approximately 30 minutes, 1, 2, 3 and 5 hours after administration on test day 1 and once daily during days 2-15. The animals were examined daily for local signs at the application site.

Body weights were recorded on test days 1 (prior to administration), 8 and 15.

All animals were killed at the end of the observation period by Carbon dioxide asphyxiation and discarded after macroscopic examinations were performed. No organs or tissues were retained.
Statistics: Not applicable (limit test, no mortalities).

Results and discussion
Mortality: There were no mortalities.
Clinical observations: No systemic signs or local signs of irritation were noted during the course of the study.
Body weight: The body weights of the animals were within the range commonly recorded for this strain and age.
Necropsy: No macroscopic findings were recorded at the scheduled necropsy.

Conclusion by the Notifiers:
The acute dermal LD_{50} of glyphosate technical material after a single dermal administration to male and female HanRcc:WIST (SPF) rats, observed over a period of 14 days was greater than 5000 mg/kg body weight (limit test, no mortalities).

Comment by RMS:
The study is considered acceptable and the dermal LD50 is estimated greater than 5000 mg/kg bw

Reference:	IIA, 5.2.2/13
Report:	[redacted] 2011, Glyphosate technical: Acute dermal toxicity study in rats; Final report amendment 1. [redacted] Data owner: Syngenta Report No.: 10/218-002P Date: 2011-04-13, not published ASB2012-11405
Guidelines:	OECD 402 (1987): OPPTS 870.1200 (1998): EC 440/2008 (2008)
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test Material:	Glyphosate technical
Description:	Technical, dry white powder
Lot/Batch number:	569753 (BX20070911)
Purity:	96.3 % w/w Glyphosate technical
CAS#:	Not reported
Stability of test compound:	Stable under storage conditions (room temperature range <30 °C), recertification date end August 2011
Vehicle and/or positive control:	None (tested as supplied)

Test animals:
Species: Rat

Strain:	RjHan:(WI) Wistar
Age/weight at dosing:	Young adult / 220-259 g
Source:	[redacted]
Housing:	Individually in Type II. polypropylene/polycarbonate cages with Lignocel Bedding for Laboratory Animals
Acclimatisation period:	6 days
Diet:	ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and rats – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494 Soest Germany *ad libitum*
Water:	Tap water *ad libitum*
Environmental conditions:	Temperature: 20.7-24.0 °C Humidity: 39-65 % Air changes: 15-20 air changes per hour Photoperiod: 12 hours light / 12 hours dark

Study design and methods

In-life dates: Start: 06 October 2010 End: 20 October 2010

Animal assignment and treatment: In an acute dermal toxicity study, a group of one male and one female and a second group of four male and four female RjHan:(WI) Wistar rats were treated with a single administration of glyphosate technical (96.3 % w/w glyphosate technical) at 5000 mg/kg by dermal application. The test item was applied as supplied. The application period was 24 hours, followed by a 14-day observation period.

The backs of the animals were shaved (approximately 10 % area of the total body surface) approximately 24 hours prior to the treatment. Only those animals without injury or irritation on the skin were used in the test. On test day 0, the test item was applied at a single dose of 5000 mg/kg body weight applied uniformly over the skin and remained on the skin throughout a 24- hour exposure period. The test item was moistened with water to ensure good contact with the skin. Sterile gauze pads were placed on the skin of rats at the site of application. These gauze pads were kept in contact with the skin by a patch with adhesive hypoallergenic plaster. The entire trunk of the animal was then wrapped with semi occlusive plastic wrap for 24 hours. At the end of the exposure period, residual test item was removed, using body temperature water.

A clinical examination was performed on the day of treatment, at 1 and 5 hours after the application of the test item, and once each day for 14 days thereafter.

Observations included the skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, and somatomotor activity and behaviour pattern. Particular attention was directed to the observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

The body weight of all animals was recorded on Day 0 (beginning of the experiment) and on Days 7 and 14.

All animals were anaesthetised with Euthasol®40 % and exsanguinated. After examination of the external appearance, the cranial, thoracic and abdominal cavities were opened and the appearance of the tissues and organs were observed. Any gross macroscopic findings were recorded.

Statistics: Not applicable (limit test, no mortalities).

Results and discussion

Mortality: There were no mortalities.

Clinical observations: There were no clinical signs noted in any animals throughout the study.
No treatment related skin irritation was observed in any animal throughout the study.
Body weight: There were no effects on body weight and body weight gain during the observation period.
Necropsy: No macroscopic findings were recorded at the scheduled necropsy.

Conclusion by the Notifiers
The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female RjHan:WI Wistar rats, observed over a period of 14 days was greater than 5000 mg/kg body weight (limit test, no mortalities).

Comment by RMS:
The study is considered acceptable and the dermal LD_{50} > 5000 mg/kg bw is agreed.

Comment by GTF on the first draft of the RAR (July 2013):
The Rat LD_{50}oral and Rat LD_{50}dermal are listed as >2000 mg/kg bw whereas the data presented concludes LD_{50}-values above 5000 mg/kg. This would also be consistent with the values presented in summary Table B.6.13-2 in Volume 3 B6.

RMS comment (August 2013):
To conclude on the LD_{50} value >2000 mg/kg bw seems to be more appropriate, because not all different batches were tested up to 5000 mg/kg bw. In any case glyphosate is considered not to be classified as acute oral or dermal toxic according to GHS categories.

B.6.2.3 Acute inhalation toxicity

For the previous EU evaluation, a multitude of acute inhalation toxicity studies in rats were provided using glyphosate acid and its IPA salt. For the current re-evaluation, additonally eleven inhalation studies with glyphosate acid and two with glyphosate salts (one for potassium salt) were submitted.

Table B.6.2-8: Summary of acute inhalation toxicity studies with glyphosate acid

Reference (Data owner)		Species Strain	Number of animals / Dose level (mg/L)	Purity (%)	Vehicle	LC_{50} (mg/L air)	Main effects
Studies from the 2001 evaluation	Annex B-5.2.3.1, Glyphosate Monograph [redacted] 1995 (Herbex) TOX9500247	Rat, Sprague Dawley	5/sex/5.35	95	Compressed air; 4 h nose-only	> 5.35	Wet fur, hunched posture, piloerection, incidents of decreased respiratory rate, ptosis, brown stained fur (head)

Reference (Data owner)		**Species Strain**	**Number of animals / Dose level (mg/L)**	**Purity (%)**	**Vehicle**	**LC_{50} (mg/L air)**	**Main effects**
	Annex B-5.2.3.1, Glyphosate Monograph [redacted] 1994 (Alkaloida) TOX9650144	Rat, Wistar	5/sex/0 5/sex/1.138 5/sex/2.876	97.2	Watery aerosol; 4 h exposure, route not stated	> 2.876	Trachea: lymphoid cell infiltration, mucous lung: congestion, haemorrhages, oedema liver: mononuclear cell infiltrations, congestion kidney: congestion, nephrocalcinosis
	Annex B-5.2.3.1, Glyphosate Monograph [redacted], 1989 (CHE) TOX9552329	Rat, Sprague Dawley	5/sex/4.98	98.6	Dust aerosol; 4 h snout only	> 4.98	No adverse findings
Studies not reviewed in the 2001 evaluation	IIA, 5.2.3/01 [redacted] 2010 (HAG) ASB2012-11406	Rat, CD	5/sex/5.18	97.3	4 h nose only (MMAD: 4.63 µm)	> 5.18 (limit test)	Slight tremor, slight dyspnoea
	IIA 5.2.3/02 [redacted] 1995 (ALS) ASB2012-11407	Rat, Fischer F344	5/sex/5.48	97.56	Dust,4 h whole body (MMAD: 4.8 µm)	> 5.48	Wet and soiled fur (periocular and nasorostral)
	IIA 5.2.3/03 [redacted] 2009 (EXC) ASB2012-11408	Rat	5/sex/5.04	96.66	Dust, 4 h, nose-only, (MMAD 5.25 µm)	> 5.04	Increased respiratory rate, hunched posture, pilo-erection, wet fur
	IIA 5.2.3/04 [redacted] 2009 (HAG) ASB2012-11409	Rat, CD	5/sex/5.12 (dust)	98.8	4h (MMAD: 6.62µm)	> 5.12 (limit test)	Slight dyspnoea and ataxia during exposure
	IIA 5.2.3/05 [redacted] 2010 (HAG) ASB2012-11410	Rat, CD	5/sex/5.02	96.4	4h (MMAD: 4.2µm)	> 5.02	Slight dyspnoea, slight ataxia and slight tremor during exposure until 3 h after exposure
Studies not reviewed in the 2001 evaluation	IIA 5.2.3/06 [redacted] 2009 (HAG) ASB2012-11411	Rat, Sprague-Dawley	5/sex/2.24	96.4	4 h (MMAD: 2.6 µm)	>2.24 (limit test)	No findings
	IIA 5.2.3/07 [redacted] 2005 (HAG) ASB2012-11412	Rat, Sprague-Dawley	5/sex/2.04	97.23	4 h (MMAD: 2.5 µm)	> 2.04 (limit test)	No findings

Reference (Data owner)		Species Strain	Number of animals / Dose level (mg/L)	Purity (%)	Vehicle	LC_{50} (mg/L air)	Main effects
	IIA 5.2.3/08 [redacted] 2008 (HAG) ASB2012-11413	Rat, Wistar Hannover	5/sex/5.21	98.05	4 h (MMAD: 18.2-19.9µm)	> 5.21 (study not accepta-ble)	Wheeze and dyspnoea
	IIA 5.2.3/09 [redacted] 2007 (NUF) ASB2012-11414	Rat, albino	5/sex/3.252 (highest techn. attain.)	95.1	4 h (MMAD: 2.95 – 3.05 µm)	> 3.252	Salivation in males, breathing effects in both sexes, body weight loss
	IIA 5.2.3/10 [redacted] 1996 (SYN) TOX2000-1984	Rat	5/sex/4.43 5/sex/2.47	95.6	4 h, nose-only, (MMAD: 2.91 and 3.41 µm)	> 4.43	Mortality: 2♂ & 2♀ at 4.43 mg/L. Irregular breathing, splayed gait, shaking & reduced righting reflex
	IIA 5.2.11/ [redacted] 2011 (SYN) ASB2012-11415	Rat	5/sex/5.04	96.9	4 h nose-only (MMAD: 3.65 µm)	> 5.04	Mortality: 1♂ on day 4. Laboured and noisy respiration, respiratory rate increase, gasping respiration, sneezing, decreased activity and thin body appearance observed until day 3.

Table B.6.2-9: Summary of acute inhalation toxicity studies with glyphosate salts

Reference (Data owner)		Species Strain	Number of animals / Dose level (mg/L)	Purity (%)	Vehicle	LC_{50} (mg/L air)	Main effects
Studies from the 2001 evaluation	Annex B-5.2.3.2, Glyphosate Monograph [redacted] 1994 (MON/CHE) TOX9552331	Rat, Sprague Dawley	5/sex/4.24 (max. attainable conc.)	IPA 62	aerosol; 4 h nose-only	> 4.24	Lung: dark areas or multiple dark foci in 6 of 10 rats
	Annex B-5.2.3.2, Glyphosate Monograph [redacted], 1989 (I. Pi. Ci.) TOX9551626	Rat, Wistar	5/sex/4.1 5/sex/4.42 5/sex/6.49	IPA 62% in water	Nebu-lised; 4 h nose-only	> 6.49	Nose bleeding, ruffled fur

Reference (Data owner)		Species Strain	Number of animals / Dose level (mg/L)	Purity (%)	Vehicle	LC_{50} (mg/L air)	Main effects
	Annex B-5.2.3.2, Glyphosate Monograph [redacted] 1988 (MON / CHE) TOX9552332	Rat, Sprague Dawley	5/sex/1.9 (max. attainable conc.)	Ammonium salt 85.5	Aerosol / vapor; 4 h whole body	> 1.9	Hyperactivity, perinasal encrustion
	Annex B-5.2.3.2, Glyphosate Monograph [redacted] 1987 (MON / CHE) TOX9552330	Rat, Sprague-Dawley	5/sex/1.3 (max. attainable conc.)	IPA 53.8	Aerosol / vapor; 4 h whole body	> 1.3	Mortality (1♀); yellow/brown nasal discharge, local and/or generalised hairloss, slight decreased body weight
Studies not reviewed in the 2001 evaluation	IIA 5.2.3/12 [redacted] 1999 (NUF) ASB2012-11416	Rat, Sprague Dawley	Isopropylamine glyphosate 5/sex/2.08	IPA 62	air / 4 h nose-only (MMAD 2.6 µm)	> 2.08	During exposure: ocular and nasal discharge, hunched posture and hypoactivity. After exposure no findings
	IIA 5.2.3/13 [redacted] 2004 (MON) ASB2012-11417	Rat. Sprague-Dawley	K-salt of glyphosate 5/sex/2.21 5/sex/5.27	K-salt 57.8% (= 47.2% glyphosate)	Aerosol / 4 h nose-only (MMADs: 2.9 µm, 3.8 µm)	> 5.27	2.21 mg/L: congested breathing, dark material around eyes and nose, few faeces 5.27 mg/L: congested breathing,

Tier II summaries are presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.
For details regarding studies reviewed during the 2001 EU evaluation please refer to the monograph.

Reference: IIA, 5.2.3/01
Report: [redacted] 2010, Acute Inhalation Toxicity Study of Glyphosate TC In Rats
[redacted]
Data owner: Helm AG
Report No.: 24603
Date: 2010-06-03, not published
ASB2012-11406
Guidelines: EC method B.2, OECD 403, EPA Health Effects Test Guidelines, OPPTS 870.1300
Deviations: None
GLP: yes
Acceptability: *Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate TC
Description:	White powder
Lot/Batch #:	20090506
Purity:	97.3 %
Stability of test compound:	At room temperature in the dark stable until May 2011.
Vehicle and/or positive control:	none
Test animals:	
Species:	Rat
Strain:	CD/Crl:CD (SD)
Source:	[redacted]
Age:	approx. 7 - 9 weeks
Sex:	Males and females
Weight at dosing:	♂ 234 - 270 g; ♀ 208 - 244 g
Acclimation period:	At least 5 days
Diet/Food:	ssniff RIM-H V1 534 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum* (except 16 h before exposure)
Water:	tap water, *ad libitum*
Housing:	In groups of 2-3 animals per cage in Makrolon type III plus cages with granulated textured wood bedding.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 55 ± 15 % Air changes: no data 12 hours light/dark cycle

Study design and methods

In life dates: 2010-02-02 to 2010-02-16

Test atmosphere generation:
A dust atmosphere was produced from the test material using a rotating brush dust generator and compressed air.

Exposure chamber conditions:
The cylindrical exposure chamber had a volume of approximately 40 L. The actual dust concentration was measured four times gravimetrically with an air sample filter (Minisart SM 17598; 0.45 pm) and pump (Vacuubrand, MZ 2C, Vacuubrand, Germany) controlled by a rotameter. Dust samples were taken once every hour during the exposure. For that purpose, a probe was placed close to the animals' noses in the inhalation chamber and air was sucked through the air sample filter at a constant flow of air of 5 L/min for 1 minute. The filters were weighed before and after sampling on an analytical balance (accuracy 0.1 mg).
Chamber airflow rates ranged from 800 to 900 L/h, providing ≥ 12 air changes per hour.

Particle size distribution:
A Malvern Spraytec Lasersystem (Malvern Instruments, Germany) was employed for the determination of the particle size distribution of the particle diameter (volume) in the exposure air. The particle size distribution of the test atmospheres was measured using a cascade impactor two times during the exposure period. The results were as follows:

Table B.6.2-10: Details of test atmosphere

Mean achieved actual concentration (HPLC)	**Actual concentration (gravimetric method)**	**MMAD**	**GSD**	**Respirable amount particle size ≤ 4 µm**	
(mg/L air)	(mg/L air)	(µm)		(mg/L air)	(%)
5.18	5.05	4.633	3.02	1.08	20.8

MMAD = mean mass median aerodynamic diameter
GSD = geometric standard deviation

The generated dust had a mass median aerodynamic diameter (MMAD) of 4.633 µm as determined with a cascade impactor. The Geometric Standard Deviation (GSD) of the MMAD was calculated as 3.02. No smaller MMAD and GSD could be obtained with the test item supplied.

Animal assignment and treatment:
A group of five fasted rats per sex received the test material at a dose level of 5.18 mg/L using a dynamic inhalation apparatus (≥ 12 air changes/h) with a nose-only exposure. Observations for mortality and clinical/behavioural signs of toxicity were made at least once per day for 14 days. Individual body weights were recorded just prior to dosing and weekly thereafter. On Day 14 after dosing, each animal was euthanised and all study animals were subjected to gross necropsy.

Results and discussion
Mortality: No deaths occurred.
Clinical observations: Clinical signs of toxicity included slight tremor and slight dyspnoea immediately until 3 hours after end of exposure.
Body weight: All animals gained the expected body weight.
Necropsy: No pathological findings were noted at necropsy.

Conclusion by the Notifiers
The acute inhalation LC_{50} for the test substance glyphosate TC was calculated to be greater than 5.18 mg/L. According to EU and OECD Globally Harmonised System (GHS) classification criteria glyphosate is not to be classified for acute inhalation toxicity.

Comment by RMS:
The MMAD and GSD are greater than recommended in OECD 403, however the explanation given by the authors is acceptable. Under the present conditions the study is considered acceptable and the acute inhalation LC_{50} >5 mg/L air/4 hours is agreed.

Reference: IIA, 5.2.3/02
Report: [redacted] 1995,
HR-001: Acute inhalation toxicity study in rats.
[redacted]
Data owner: Arysta LifeScience
Report No.: 94-0155, not published

	ASB2012-11407
Guidelines:	U.S. EPA FIFRA Guideline Subdivision F
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment RMS*

Materials and methods

Test material:	
Identification:	Glyphosate TC
Description:	Whitish crystals
Lot/Batch #:	T-941209
Purity:	97.56 %
Stability of test compound:	Not mentioned in the report.
Vehicle and/or positive control:	None
Test animals:	
Species:	Rat
Strain:	F344/DuCrj
Source:	[redacted]
Age:	8 weeks
Sex:	Males and females
Weight at dosing:	♂ 176 - 187 g; ♀ 138 - 144 g
Acclimation period:	8 days
Diet/Food:	ssniff RIM-H V1 534 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum* (except 16 h before exposure)
Water:	tap water, *ad libitum*
Housing:	By group of 5 animals of the same sex in stainless steel wire cages during pre- and post-exposure periods. Individually in stainless steel wire cages during exposure.
Environmental conditions:	Temperature: 22 ± 2 °C Humidity: 55 ± 1[redacted] % Air changes: 10/hour 12 hours light/dark cycle

Study design and methods
In life dates: 1995-03-28 to 1995-04-20

Test atmosphere generation:
The dust was generated by a turn-table type dust feeder with compressed air from air compressor. The compressed air was supplied to the dust feeder through an air filter. The air was introduced into the chamber as diluting air after filtering it through a HEPA filter.

Exposure chamber conditions:
The nominal atmospheric concentration of HR-001 was calculating by dividing the total amount of the test substance supplied to the duct feeder during the 4-hour exposure by the total air volume delivered during the exposure.

The actual atmospheric concentration was measured gravimetrically.

Particle size distribution:
The results for the air samples taken for the determination of particle size distribution are given in Table B.6.2-11.

Table B.6.2-11: Particule size distribution

Exposure group	Time of sampling	Analytical concentration	Particule size	
mg/L	(min)	(mg/L)	MMAD[2] (µm)	σg[3]
5.48	60	6.54	5.0	1.6
	120	4.80		
	180	5.11	4.6	1.8
	Mean S.D.[4]	5.48 0.93	4.8 0.3	1.7 0.1

MMAD : Mass median aerodynamic diameter
σg: geometric standard deviation
SD: Standard deviation

The results showed that particles size of HR-001 expressed as the mean value of NMAD during the exposure was 4.8 µm (σg = 1.7). Thus more than 91 % of the test substance dust consisted of particles present consisted of inhalable particles

Animal assignment and treatment:
Groups of 5 male and 5 female specific pathogen free Fisher rats (F344/DuCrj) were exposed (whole-body) continuously for 4 hours to test substance mist containing concentrations of HR-001 at 5.48 mg/l. The flow rate was stable at approximately 100 l/min. Mortality and signs of reaction to treatment were recorded during a subsequent 14-day observation period. All animals were observed for clinical signs at 2 hours after the initiation of exposure, immediately and at 2 hours after the termination of exposure. In addition, animals were observed for lethality at 4 hours after the termination of exposure. All animals were weighed shortly before the exposure and on days 7 and 14. The surviving animals were euthanized on the following day (day 15). All animals were subjected to necropsy.

Results and discussion
Mortality: There were no deaths in either sex at the tested concentration 5.48 mg/L.
Clinical observations: No notable serious changes were observed as clinical signs. Wetted and soiled fur in the periocular and nasorostral regions were not considered to be particularly caused by HR-001 because the changes were slight in degree and are frequently observed in tha acute inhalation toxicity study.
Body weight: All animals gained weights, reflecting their good healthy conditions.
Necropsy: No abnormalities were observed in any animal of either sex at necropsy.

Conclusion by the Notifiers
The acute inhalation LC_{50} for the test substance glyphosate HR-001 was calculated to be greater than 5.48 mg/L. According to EU and OECD Globally Harmonised System (GHS) classification criteria glyphosate is not to be classified for acute inhalation toxicity

[2] Mass median aerodynamic diameter
[3] geometric standard deviation
[4] Standard deviation

Comment by RMS:
The study is considered acceptable and the acute inhalation LC_{50} > 5 mg/L air/4 hours is agreed.

Reference:	IIA, 5.2.3/03
Report:	[redacted] 2009, Glyphosate Tech: Acute Inhalation Toxicity (Nose only) Study in the Rat [redacted] Data owner: Excel Report No.: 2743/0001 Date: 2009-06-22, not published ASB2012-11408
Guidelines:	OECD 403 (1981) Commission Regulation (EC) No 440/2008 (2008), method B.2 (2008)
Deviations:	Particle size diameter larger than required by the test guidelines.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate Tech
Description:	White powder
Lot/Batch #:	GI-1045
Purity:	96.66 %
Stability of test compound:	Expiration in July 2010.
Vehicle and/or positive control:	Not relevant
Test animals:	
Species:	Rat
Strain:	HsdRccHan™ : WIST
Source:	[redacted]
Age:	Approx. 8 - 12 weeks
Sex:	Male and female
Weight at dosing:	178 - 350 g
Acclimation period:	5 days
Diet/Food:	With the exception of the exposure period, free access to food (Harlan 2014 Rodent Diet, Harlan UK Limited, Oxon, UK) was allowed throughout the study.
Water:	With the exception of the exposure period, free access to drinking water was allowed throughout the study.

Housing:	Housed in groups of five by sex in solid-floor polupropylene cages with stainless steel lids, furnished with softwood flakes (Datesand Ltd., Cheshire, UK) and provided with environmental enrichment items: wooden chew blocks and cardboard "fun tunnels" (Datesand Ltd., Cheshire, UK).
Environmental conditions:	Temperature: 19 – 25 °C Humidity: 30 – 70 % Air changes: At least 15/hour 12-hour light/dark cycle

Study design and methods
In life dates: 2009-05-06 to 2009-05-12

Animal assignment and treatment:
Five male and female rats were exposed to one dose level of an aerosol atmosphere of glyphosate. The single 5 mg/L four hour exposure was "nose only" at a mean actual concentration of 5.04 ± 0.37 mg/L (nominal concentration was 27.3 mg/L).
Operational conditions (flow rate, oxygen levels, temperature, and humidity in the inhalation systems) were checked throughout the exposure period. All animals were observed for clinical signs at hourly intervals during exposure, immediately on removal from the restraining tubes at the end of exposure, one hour after termination of exposure and subsequently once daily for 14 days. Individual body weights were recorded prior to treatment on the day of exposure and on Days 7 and 14. At the end of the fourteen day observation period the animals were killed by intravenous overdose of sodium pentobarbitone. All animals were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded. The respiratory tract was subjected to a detailed macroscopic examination for signs of irritancy or local toxicity.
The chamber flow rate was maintained at 45 L/min providing 90 air changes per hour.

Results and discussion
Test atmosphere
The particle size analysis of the atmosphere drawn from the animals' breathing zone, was as follows:

Table B.6.2-12: Details of test atmosphere

Mean Achieved Atmoshpere Concentration (mg/L)	Mean Mass Median Aerodynamic Diameter (µm)	Inhalable Fraction (% <4 µm)	Geometric Standard Deviation
5.04	5.25	41.1	3.35

It is noted that the achieved particle size is larger than required by the test guidelines.
During characterisation, changes were made to the generation system (addition of particle sizes separator) and grinding techniques in an attempt to increase the inhalable portion of the test material. However, this reduced the achieved concentration, and therefore, also reduced the actual concentration of particles <4 µm. It was, therefore, preferable to expose the animals to a higher concentration of test material, even though this also increased the mean mass median aerodynamic diameter, as this resulted in the animals being exposed to the highest possible concentration of particles <4 µm.

Mortality: There were no mortalities during the study.

Clinical observations: Signs of hunched posture and piloerection are commonly seen in animals for short periods on removal from the chamber following 4-hour inhalation studies. Wet fur is commonly recorded both during and for a short period after exposure. These observations are considered to be associated with the restraint procedure and, in isolation, are not indicative of toxicity.
In addition to the observations considered to be due to the restraint procedure, increased respiratory rate was noted in all animals during exposure, on removal from the chamber and one hour post-exposure.
Body weight: Normal bodyweight development was noted during the course of the study.
Necropsy: No macroscopic abnormalities were detected at necropsy.

Conclusion by the Notifiers
The inhalation LC_{50} (4 hours) of the test material (glyphosate tech) in rats was estimated to be greater than 5.04 mg/L. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate tech is not to be classified for acute inhalation toxicity.

Comment by RMS:
Under the present conditions the study is considered acceptable and the acute inhalation LC_{50} >5 mg/L air/4 hours is agreed.

Reference:	IIA, 5.2.3/04
Report:	[redacted] 2009, Acute Inhalation Toxicity Study of Glyphosate TC in Rats. [redacted] Data owner: Helm AG Report No.: 23911 Date: 2009-11-09 not published ASB2012-11409
Guidelines:	EC method B.2. (92/69/EEC), OECD 403 and OPPTS 870.1300.
Deviations:	There were several minor deviations from the Study Plan which did not affect the scientific outcome or the validity of the study.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate TC
Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	20080801
Purity:	98.8 %
Stability of test compound:	2010-08-01
Test animals:	
Species:	Rat albino

Strain / Stock:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Males: 52 days Females: 66 days
Sex:	5 male and 5 female
Weight at dosing:	Males: 240 - 267 g Females: 209 - 216 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Animals were kept by sex in groups of 2-3 animals in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Rel. humidity: 40 - 70 % Air changes: 12/hour 12-hour light/dark cycle

Study design and methods
In life dates: 2009-02-04 to 2009-07-30

Animal assignment and treatment:
The study was carried out using a dynamic inhalation apparatus (≥ 12 air changes/h) with a nose-only exposure of the animals (exposure chamber volume 40 L). The test item was micronized before administration and the dust was generated with a rotating brush dust generator. Exposure started by locating the rats (5 male and 5 female animals) into the exposure chamber after equilibration of the chamber concentration for at least 15 minutes. The dust concentration in the inhalation chamber was determined gravimetrically as well as by HPLC once every hour during exposure. A laser measured the size of the individual particles or individual aerosol drops. Animals were exposed four 4 hours to an actual concentration of 5.12 mg/L air (determined by HPLC).
After completion of exposure, animals were observed for a period of 14 days. Observations for clinical/behavioural signs of toxicity were made at least once daily until symptoms subsided, and thereafter each working day. Observations on mortality were made at least once daily. Individual body weights were determined before the exposure and weekly after exposure. On Day 14 after completion of exposure, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

Results and discussion
Dust concentration and particle size distribution
The actual dust concentration of 5.12 mg glyphosate TC/L air was measured at the animals' nose and was determined by HPLC.

Laser measurement revealed the following particle size distribution during the exposure:

Diameter	Actual concentration

	5.12 mg/L air
$d_{[10]}$	5.64 µm
$d_{[50]}$	6.62 µm
$d_{[90]}$	8.10 µm
[xx] = percentage of cumulative particle size distribution	

No finer dust concentration of the test item could be generated.

Mortality: There were no mortalities during the study.
Clinical observations: A 4-hour exposure to glyphosate TC at the concentration of 5.12 mg/L revealed slight dyspnoea and ataxia in all 5 of 5 male and 5 of 5 female animals immediately until 60 minutes after the end of exposure.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers
The acute inhalation LC_{50} of the test material (glyphosate TC) in rats (males and females combined) was estimated to be greater than 5.12 mg/L air/4 hours. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate TC is not to be classified for acute inhalation toxicity.

Comment by RMS:
Under the present conditions the study is considered acceptable and the acute inhalation LC_{50} >5 mg/L air/4 hours is agreed.

Reference: IIA, 5.2.3/05
Report: [redacted] 2010 Acute Inhalation Toxicity Study of Glyphosate TC in Rats.
[redacted]
Data owner: Helm AG
Report No.: 24875
Date: 2010-06-03, not published
ASB2010-11410
Guidelines: EC method B.2. (92/69/EEC), OECD 403 and OPPTS 870.1300.
Deviations: There were several minor deviations from the Study Plan which did not affect the scientific outcome or the validity of the study.
GLP: yes
Acceptability: *Please see comment by RMS*

Materials and methods

Test material:	Glyphosate TC
Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	20080801
Purity:	96.4%

Stability of test compound:	May 2011
Test animals:	
Species:	Rat albino
Strain / Stock:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Males: approx. 7 weeks Females: approx. 9 weeks
Sex:	5 male and 5 female
Weight at dosing:	Males: 270 - 282 g Females: 220 - 251 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Animals were kept by sex in groups of 2-3 animals in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Rel. humidity: 40 - 70 % Air changes: 12/hour 12-hour light/dark cycle

Study design and methods
In life dates: 2009-10-15 to 2010-02-18

Animal assignment and treatment:
The study was carried out using a dynamic inhalation apparatus (≥ 12 air changes/h) with a nose-only exposure of the animals (exposure chamber volume 40 L). The test item was generated with a rotating brush dust generator. Exposure started by locating the rats (5 male and 5 female animals) into the exposure chamber after equilibration of the chamber concentration for at least 15 minutes. The dust concentration in the inhalation chamber was determined gravimetrically as well as by HPLC once every hour during exposure. Animals were exposed four 4 hours to an actual concentration of 5.02 mg/L air (determined by HPLC).
A laser measured the size of the individual particles or individual aerosol drops. The particle size distribution for the estimation of the Mass Median Aerodynamic Diameter (MMAD) was carried out twice during the exposure period using a cascade impactor. The median particle size distribution of the test item was determined with a Malvern Sizer.
After completion of exposure, animals were observed for a period of 14 days. Observations for clinical/behavioural signs of toxicity were made at least once daily until symptoms subsided, and thereafter each working day. Observations on mortality were made at least once daily. Individual body weights were determined before the exposure and weekly after exposure. On Day 14 after completion of exposure, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

Results and discussion

Dust concentration and particle size distribution

Dust concentration and particle size distribution

The actual dust concentration of 5.02 mg glyphosate TC/L air was measured at the animals' nose and was determined by HPLC. The mean actual exposure concentration of glyphosate TC was as follows:

Table B.6.2-13: Details of test atmosphere

Actual concentration (HPLC) [mg/L air]	**Actual concentration (gravimetric method) [mg/L air]**	**MMAD [µm]**	**Respirable amount particle size ≤4 µm**	
			[mg/L air]	[%]
5.02	4.99	4.197 ± 2.64	1.03	20.5

No smaller MMAD could be obtained with the test item and no higher fraction of respirable particles could be obtained.

Laser measurement revealed the following particle size distribution during the exposure:

Diameter	**Actual concentration 5.02 mg/L air**
$d_{[10]}$	12.51 µm
$d_{[50]}$	37.15 µm
$d_{[90]}$	86.42 µm
[xx] = percentage of cumulative particle size distribution	

The particle size distribution of the delivered test item was d[50] = 14.5 µm.

Mortality: There were no mortalities during the study.

Clinical observations: A 4-hour exposure to glyphosate TC at the concentration of 5.02 mg/L revealed slight ataxia, slight tremor and slight dyspnoea immediately until 3 hours after the end of exposure.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The acute inhalation LC_{50} of the test material (glyphosate TC) in rats (males and females combined) was estimated to be greater than 5.02 mg/L air/4 hours. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate TC is not to be classified for acute inhalation toxicity.

Comment by RMS:

Under the present conditions the study is considered acceptable and the acute inhalation LC_{50} >5 mg/L air/4 hours is agreed.

Reference: IIA, 5.2.3/06

Report: [redacted] 2009
Glyphosate – Acute Inhalation Toxicity Study in Rats.
Stillmeadow, Inc., Sugar Land, TX, US
Data owner: Helm AG

	Report No.: 12107-08 Date: 2009-03-09, not published ASB2012-11411
Guidelines:	US EPA OPPTS 870.1300.
Deviations:	Humidity was in the range of 33-89 % instead of 30-70 %. Female weight was outside the protocol range. These minor deviations did not affect the study outcome.
GLP:	yes
Acceptability:	*Please see comment by RMS.*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate Tech Grade Mixed 5-Batch
Description:	White powder
Lot/Batch #:	080704-1 thru 5
Purity:	96.71 %
Stability of test compound:	No data given in the report.
Test animals:	
Species:	Rat albino
Strain / Stock:	Sprague-Dawley
Source:	[redacted]
Age:	Approx. 7-8 weeks
Sex:	5 male and 5 female
Weight at dosing:	Males: 262 - 289 g Females: 172 - 191 g
Acclimation period:	5 days
Diet/Food:	Formulab #5008 (PMI Feeds Inc.), *ad libitum* except during the exposure period
Water:	Tap water, *ad libitum* except during the exposure period
Housing:	Individual housing in suspended, wire bottom, stainless steel cages.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: 10 - 12/hour 12-hour light/dark cycle

Study design and methods
In life dates: 2008-11-07 to 2008-11-21

Animal assignment and treatment:
The study was carried out using a 500 L nose-only stainless steel, dynamic flow inhalation chamber with 25 ports in 5 rows. Polycarbonate tubes were inserted into 10 designated individual ports. The test substance was ground for 10 hours and dried prior to exposure. The aerosol was generated from the undiluted test substance by a Venturi Aspirator and sprayed directly into the exposure chamber. Exposure started by locating the rats (5 male and 5 female animals) into the exposure chamber. Animals were exposed to the aerosol for a period of 4

hours. The dust concentration in the inhalation chamber was determined gravimetrically twice per hour and nominally at the end of the exposure. Particle size, taken from the breathing zone of the animals, was determined twice during the exposure using a cascade impactor, and the mass median aerodynamic diameter (MMAD) and particle size distribution were calculated.
Observations for mortality and signs of pharmacological and/or toxicological effects were made frequently on the day of exposure and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to the inhalation exposure and on Days 7 and 14. On Day 14 after completion of exposure, all animals were euthanized by an intraperitoneal injection, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

Results and discussion
Dust concentration and particle size distribution: The exposure concentration was determined to be 2.24 mg/L with an average MMAD of 2.6 µm.
Mortality: There were no mortalities during the study.
Clinical observations: The only prominent in-life observations were piloerection and activity decrease. Animals were asymptomatic by Day 4.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers
The acute inhalation LC_{50} of the test material (glyphosate) in rats (males and females combined) was estimated to be greater than 2.24 mg/L air/4 hours.

Comment by RMS:
The study is considered acceptable and the acute inhalation LC_{50} > 2.24 mg/L air/4 hours is agreed.

Reference:	IIA, 5.2.3/07
Report:	[redacted] 2005, Glyphosate Acid Technical: Acute Inhalation Toxicity Study in Rats – Limit Test. [redacted] Data owner: Helm AG Report No.: 15276 Date: 2005-04-04, not published ASB2012-11412
Guidelines:	OPPTS 870.1300 (1998), OECD 403 and JMAFF 59 NohSan No. 4200 (1985).
Deviations:	There were no deviations from the Study Plan.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate acid technical
Identification:	Glyphosate acid technical

Description:	White crystalline powder
Lot/Batch #:	040205
Purity:	97.23 %
Stability of test compound:	Test substance was expected to be stable for the duration of testing.
Test animals:	
Species:	Rat albino
Strain / Stock:	Sprague-Dawley derived
Source:	[redacted]
Age:	9-10 weeks
Sex:	5 male and 5 female
Weight at dosing:	Males: 280 - 318 g Females: 205 - 224 g
Acclimation period:	13 days
Diet/Food:	Purina Rodent Chow #5012, *ad libitum*
Water:	Filtered tap water, *ad libitum*
Housing:	Individual housing in suspended stainless steel cages with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:	Temperature: 19-23 °C 12-hour light/dark cycle

Study design and methods:
In life dates: 2003-05-17 to 2003-05-24

Animal assignment and treatment:
The study was carried out using a nose-only inhalation chamber with an internal volume of approximately 6.7 L and approximately 283 air changes per hour during the study. Animals were individually housed in polycarbonate holding tubes. The test item was micronized before administration and aerosolized using a dust generator which was directly connected to the inhalation chamber. Gravimetric samples were withdrawn at 6 intervals from the breathing zone of the animals to gravimetrically determine the dust concentration in the inhalation chamber. Particle size distribution of the test atmosphere was determined with an Andersen Cascade Impactor. Samples were withdrawn from the breathing zone of the animals at two intervals. Animals were exposed four 4 hours and 1 minute to an actual concentration of 2.04 mg/L air (determined gravimetrically).
Observations for mortality and clinical/behavioural signs of toxicity were made upon removal from the exposure chamber and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to test substance exposure and on Days 7 and 14.
On Day 14 after dosing, each animal was euthanized by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

Results and discussion
Dust concentration and particle size distribution: The gravimetric and nominal chamber concentrations were 2.04 and 8.99 mg/L, respectively. the mass median aerodynamic diameter was estimated to be 2.5 µm based on the particle size distribution as measured with an Andersen Cascade Impactor.

Mortality: There were no mortalities during the study.

Clinical observations: All animals appeared active and healthy upon removal from the exposure chamber and over the entire 14-day observation period.

Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers:
The acute inhalation LC_{50} of the test material (glyphosate acid technical) in rats (males and females combined) was estimated to be greater than 2.04 mg/L air/4 hours.

Comment by RMS:
The study is considered acceptable and the acute inhalation LC_{50} >2.04 mg/L air/4 hours is agreed.

Reference:	IIA, 5.2.3/08
Report:	[redacted] 2008, Acute Inhalation Toxicity Test of Glyphosate Technical in Rats *(Rattus norvegicus)*. [redacted] Data owner: Helm AG Report No.: RF-3996.309.377.07 Date: 2008-09-11, not published ASB2012-11413
Guidelines:	OECD guideline 403
Deviations:	The experimental phase initiation and conclusion dates were updated. This deviation did not affect the study outcome.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate Technical
Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	20070606
Purity:	98.05 %
Stability of test compound:	No data given in the report.
Test animals:	
Species:	Rat albino (*Rattus norvegicus*)
Strain / Stock:	Wistar Hannover
Source:	[redacted]
Age:	Males: 9 weeks Females: 11 weeks
Sex:	5 males and 5 females

Weight at dosing:	Males: 262 - 291 g Females: 178 - 208
Acclimation period:	9 days
Diet/Food:	Nuvilab CR-1 pellet diet type for rodents (Nuvital Nutrients Ltda.), *ad libitum*
Water:	Potable drinking water, *ad libitum*
Housing:	Polypropylene rodents cages with autoclaved wood shavings and stainless steel mesh lids containing five rats of each sex per cage.
Environmental conditions:	Temperature: 19-25 °C Humidity: 30 - 70 % Air changes: 10-15/hour 12-hour light/dark cycle

Study design and methods:
In life dates: 2008-06-06 to 2008-06-20

Animal assignment and treatment:
The study was carried out using an inhalation chamber with a nose-only exposure of the animals. The test item was aerosolised. Exposure started by locating the rats (5 male and 5 female animals) into the exposure chamber. Animals were exposed to the aerosol at the maximum attainable concentration (5.211 mg/L) for a period of 4 hours. The actual concentration in the inhalation chamber was determined gravimetrically by taking eight equally time-spaced air samples from the breathing zone. Aerodynamic particle size distribution was determined two times using a Seven Stage Cascade Impactor.
After completion of exposure, animals were observed for a period of 14 days. Observations for clinical/behavioural signs of toxicity were made right after the exposure, and thereafter each working day. On Day 14 after completion of exposure, all animals were euthanised in a carbon dioxide chamber, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

Results and discussion
Dust concentration and particle size distribution
The mean actual concentration was 5.211 mg/L. The actual concentration of the test item in each sample was within the ±15 % interval from the mean actual concentration, indicating that the test atmosphere was held stable over the 4-hour exposure period.

Analysis of the particle size distribution of samples from the breathing zone indicates that 4.72 to 5.15 % of the mass collected from the aerosol were within the respirable size range. The MMAD ranged from 18.555 to 19.901 µm and the geometric standard deviation (GSD) ranged from 2.869 to 2.914.

Mortality: There were no mortalities during the study.
Clinical observations: Clinical signs observed during the 14-day observation period included wheeze and dyspnoea. These acute respiratory signs started within the first day and reverted within the fourth day of the observation period.
Body weight: The mean body weight increased for both sexes, except for the males on the first post-exposure day. All animals exceeded their initial body weight by the conclusion of the experimental phase.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers
The acute inhalation LC_{50} of the test material (glyphosate Technical) in rats (males and females combined) was estimated to be greater than 5.211 mg/L air/4 hours. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate technical is not to be classified for acute inhalation toxicity.

Comment by RMS:
The study is considered not acceptable: The MMAD ranged from 18.555 to 19.901 µm, which is far above what is recommended in OECD 403 (MMAD ranging from 1 to 4 µm) and therefore, only 4.72 to 5.15 % of the particles were within the respirable size range. No explanation was given by the author why the standard could not be achieved.

Reference:	IIA, 5.2.3/09
Report:	[redacted] 2007, Glyphosate Technical (NUP05068) : 4-Hour acute inhalation toxicity study in rats [redacted] Data owner: Nufarm Report No.: B02327 Date: 2007-04-02, unpublished ASB2012-11414
Guidelines:	European Communities, Directive 92/69/EEC, Part B.2 "Acute Toxicity (Inhalation)", published December 29, 1992. - OECD Guidelines for Testing of Chemicals, Section 4, No. 403: "Acute Inhalation Toxicity", adopted May 12, 1981. - U.S. Environmental Protection Agency, Health Effects Test Guidelines OPPTS 870.1300, Acute Inhalation Toxicity, August 1998. - Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Preparation of Study Results, Acute Inhalation Toxicity Studies Guideline 2-1-3. Notification 12 NohSan No. 8147, as partly revised in 16-Shouan-9260, on 16 March 2005. English translation by ACIS on 17 Oct 2005
Deviations:	The following, minor deviations from the study plan were considered not to have compromised the quality, integrity or outcome of the study. - The reference to the JMAFF inhalation test guideline was altered on request of the Sponsor. However, this did not affect the contents of the JMAFF inhalation test guideline. - In the animal room, on brief occasions (for a total of less than 2 hours) the relative humidity was slightly higher than the upper limit of the target range given in the study plan.

- On the day of inhalation exposure (test day 1), the total aerosol generation period lasted 4 hours and 30 minutes, because a test aerosol was generated also for 30 minutes prior to the beginning of the exposure. This 30-minute pre-exposure aerosol generation period was used for fine-tuning of the settings of the aerosol generation and exposure system for the inhalation exposure. Consequently the nominal test atmosphere concentration was determined for the total of 4 hours and 30 minutes of aerosol generation (30 min pre-exposure aerosol generation without animals being present plus 4 h inhalation exposure of the animals).

GLP: yes

Acceptability: *Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate Technical (NUP 05068)
Description:	White powder
Lot/Batch #:	200609062
Purity:	95.1 %
Stability of test compound:	Stable under storage conditions.
Vehicle and/or positive control:	None
Test animals:	
Species:	Rat
Strain:	HanRcc:WIST (SPF)
Source:	[redacted]
Age:	Males: 9 weeks Females: 10 weeks
Sex:	Male / Female
Weight at dosing:	Males 241.6 – 257.4 g Females 200.6 – 219.8g
Acclimation period:	5 days
Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 67/06 (Provimi Kliba AG, CH-4303 Kaiseraugst/ Switzerland) ad libitum
Water:	Tap water, ad libitum
Housing:	During acclimatization in groups of five per sex in Makrolon type-4 cages with standard softwood bedding.
Environmental conditions:	Temperature: 19-20 °C Humidity: 35-78 % Air changes: 10 - 15/hour 12-hour light/dark cycle

Study design and methods

In life dates: 14-DEC-2006 to 28-DEC-2006

Animal assignment and treatment:
A dust aerosol was generated from the milled and pre-dried test item using a rotating brush aerosol generator (CR 3020, CR Équipements SA, CH-1295 Tannay, Switzerland) connected to a micronising jet mill. No extra diluent air was added. The generated aerosol was discharged into the exposure chamber through a 63Ni charge neutraliser. The achieved mean aerosol concentration of 3.252 mg/L air administered for 4 hours was considered to represent the highest technically achievable concentration suitable for acute inhalation toxicity testing in rodents. An increase in aerosol concentration by an increased supply of test item to the rotating brush of the aerosol generator would have led to complete blockage of the rotating brush (which had happened in a pre-study technical trial not performed under GLP), and consequently to complete blockage of the aerosol generation and exposure system. Two generator cylinders containing test item were needed, in order to generate the highest technically achievable aerosol concentration over a 4-hour and 30-minute aerosol generation period.
The test atmosphere enters the top under slight positive pressure and is distributed to the entrance of each feed tube. It is then delivered through these tubes to the animal's nose. The nhalation exposure system is located inside a ducted extraction cabinet. Test atmosphere samples for the gravimetric measurements of the test item concentration and particle size distribution, and for the measurement of temperature, relative humidity and oxygen concentration, were collected directly from the feed tube in the breathing zone of the animals, at an empty port of the exposure chamber delivering "fresh" test item to the animal's nose. This approach was chosen in order to obtain representative samples of what was delivered to the animals.
The particle size distribution was determined twice during the exposure using a Mercer 7 stage cascade impactor (Model 02-130, In-Tox Products Inc., Albuquerque, New Mexico, U.S.A.).
Representative samples of the test atmosphere were drawn through the impactor with a flow rate of 1.0 L/min and the particles deposited according to their aerodynamic size onto stainless steel
slips and the final filter stage (Type HVLP, Polyvinylidenedifluoride membrane, pore size 0.45 µm), on each stage of the impactor. To obtain the mass deposited on each stage of the impactor, the
steel slips and the final filter stage were carefully weighed before and after sampling using a Mettler MX5 analytical balance (Mettler AG, CH-8604 Volketswil, Switzerland). The total mass (µg) deposited in the impactor was then calculated by adding together the mass deposited on each of the stainless steel slips and the final filter stage. As the Effective Cut-off Diameters (ECD) represent the lower size limit of the particles collected on each stage, the cumulative percent less than the indicated size was tabulated as a function of the ECDB. This data was used to calculate the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) using Microsoft Excel software. The target range for the MMAD was 1 to 4 µm, and was achieved.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: The following clinical signs were recorded during and/or after the inhalation exposure, whereby the whole range of the stated severity grades was not necessarily recorded in each affected animal: Salivation, moderate in degree, and deep respiration in two male animals (nos. 3 & 5), and breath sounds [rales], slight to marked in degree, in three male (nos. 1, 3 & 5) and two female animals (nos. 6 & 10).

The findings of salivation and deep respiration were seen at approximately 3 and 4 hours after exposure start, when the animals were restrained in the exposure tubes. Deep respiration was still evident one hour afterwards, at approximately one hour after the end of the exposure period. Breath sounds [rales] were only noticed at approximately one hour after the end of the exposure period and on the day afterwards (test day 2) after the animals had returned to their housing cages. By two days after the inhalation exposure (test day 3) all clinical signs had cleared, and all animals remained free from clinical signs until the scheduled necropsy day (test day 15).

Body weight: Losses in body weight were evident in three of five male animals (mean loss in the affected males –3.0 %) and three of five female animals (mean loss in the affected females –2.1 %), and retardation in body weight gain in one other male animal (+0.8 % weight gain) over the first three days following the inhalation exposure (test days 1 to 4). The effects on body weight were only transient and were followed by normal body weight gain in all animals.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The LC_{50} of glyphosate technical (NUP 05068) for acute 4-hour inhalation toxicity in male and female rats observed for a period of 15 days, was estimated to be greater than 3.252 mg/L air (gravimetrically determined mean aerosol concentration). This concentration was considered to represent the highest technically achievable aerosol concentration suitable for acute inhalation toxicity testing in rodents.

Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate is not to be classified for this endpoint.

Comment by RMS:

The study is considered acceptable and the acute inhalation LC_{50} >3.252 mg/L air/4 hours is agreed.

Reference:	IIA, 5.2.3/10
Report:	[redacted] 1996, Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats [redacted] Data owner: Syngenta Report No.: [redacted]/P/4882 Date: 1996-04-29, not published TOX2000-1984
Guidelines:	OECD 403 (1981): OPPTS 870.1300 (1998): 92/69/EEC B.2 (1992) + amendment 93/21/EEC (1993)
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Table B.6.2-14: Details of test atmosphere

Target concentration mg/L	**Achieved particulate concentration mg/L**	**MMAD* µm**	**GSD+**
2	2.47- ± 0.15	3.57, 3.03	1.94, 1.90
5	4.43 + 1.297	2.91, 3.41	1.74, 2.04

* Mass Median Aerodynamic Diameter (µm)
\+ Geometric Standard Deviation

Two males and two females exposed to 4.43 mg glyphosate acid/L were found dead or were terminated *in extremis* during the observation period, the remaining animals in this group survived until scheduled termination. Clinical signs indicative of moderate toxicity were seen in this group. All surviving animals had regained their initial bodyweight by the end of the study.
Similar but less severe clinical signs were seen in animals exposed to 2.47 mg/L, all animals survived and showed complete recovery by the end of the study. All animals exposed to 2.47 mg/L survived to scheduled termination
It was concluded that the acute inhalation LC_{50} of glyphosate acid exceeded 2.47 mg/L for male rats and exceeded 4.43 mg/L for female rats.

Materials and methods

Test material:	
Identification:	Glyphosate acid
Description:	Technical; white solid
Lot/Batch #:	P25
Purity:	95.6 % w/w
Stability of test compound:	Confirmed by Sponsor
Vehicle and/ or positive control:	None
Test animals:	
Species:	Rat
Strain:	Alpk:APfSD
Source:	[redacted]
Age:	Young adult; 9 - 12 weeks old at delivery
Sex:	Males and females
Weight at dosing:	243-365 g (males); 210-247 g (females) at the start of exposure
Acclimation period:	At least five days
Diet/Food:	PCD diet (Special Diet Services Limited, Witham, Essex, UK) *ad libitum* except during exposure.
Water:	Mains water *ad libitum* except during exposure.
Housing:	5 per cage, sexes separately, except during exposure, in rat racks suitable for animals of the strain and weight range expected during the study.

Environmental conditions:	Temperature:	19-20 °C
	Humidity:	40-70 %
	Air changes:	at least 15/hour
	Photoperiod:	12-hour light/dark cycle

Study design and methods

In-life dates: Start: 22 November 1995 End: 11 March 1996

Exposure conditions: Trial generations were carried out prior to the start of the study in order to determine the appropriate generation system and conditions, to determine the appropriate target concentration that could be achieved, or if not, what was the maximum stable attainable concentration, to obtain data on the aerodynamic particle size of the atmosphere generated, to determine an appropriate method of analysis of glyphosate acid. Exposure conditions during the study are given later in a table of the test atmosphere characteristics of glyphosate acid.
Animal assignment and treatment: The study consisted of two main study groups of 5rats/sex/group exposed nose-only for a single four-hour period to glyphosate acid at target particulate concentrations of 5 mg/L and 2 mg/L. Prior to the start of the study the rats were examined to ensure that they were physically normal and exhibited normal activity. During exposure they were observed frequently and, at the end of the 4-hour exposure period, each rat was given a detailed clinical examination. They were also subjected to detailed clinical observations, daily during a 14-day observation period. The bodyweight of each rat was recorded on day -1, 1, 8 and prior to termination on day 15. All rats were killed on day 15 and subjected to a gross examination *post mortem* involving external observation and careful internal examination of all thoracic and abdominal viscera.

Generation of the test atmosphere / chamber description: Before exposure of the test animals, the atmosphere was shown to have been acceptably stable. The test atmosphere was generated using a modified Wright's dust-feed mechanism. Clean, dry air was passed through the dust feed at a nominal flow rate of 2.5 L/minute (at normal temperature and pressure) and carried the atmosphere to the exposure chamber, having an internal volume of 27.6 litres. Since diluting air was not employed, the flow rate through the exposure chamber was the same as that employed in the generation of the test atmosphere. Air flows were monitored and recorded at approximately 30 minute intervals using variable area flow-meters and were altered as necessary to maintain target concentration. Animals were exposed nose-only to the atmosphere. They were restrained in polycarbonate tubes (Battelle, Switzerland), which were inserted into the Perspex exposure chamber. The chamber was covered with an aluminium cone and stood on an aluminium base.

Test atmosphere concentration: The particulate concentration of the test atmosphere, close to the animals' breathing zone, was measured gravimetrically at frequent intervals during the exposure period. This was done by drawing the test atmosphere, at a known flow rate, for a known time, through a 25 mm diameter, polyvinyl chloride (PVC) GLA 5000 filter housed in a Delrin open-faced filter holder. The filter was weighed before and after the sample was taken. The concentration was calculated as follows:

$$\text{Concentration (mg/L)} = \frac{\text{post wt (mg)} - \text{pre wt (mg)}}{\text{time (minutes)} \times \text{airflow (L/minute)}}$$

Pre wt = weight of filter prior to sampling
Post wt = weight of filter after sampling

Particle size determination: The aerodynamic particle size distribution of the test atmosphere was measured twice during the exposure period, using a Marple Cascade Impactor, which aerodynamically separates airborne particles into pre-determined size ranges. Using a microcomputer, the data were transformed using a log/probit transform and a linear regression derived from the cumulative data. The linear regression line was then used to calculate the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD).

Table B.6.2-15: Summary of acute study test atmosphere characteristics

Parameter	**Target concentration 5 mg/L**				**Target concentration 2 mg/L**			
Measured particulate concentration	4.43 ± 1.30 mg/L				2.47 ± 0.15 mg/L			
% total particulate	96.9 ± 4.2				98.5 ± 7.7			
Analysed concentration of glyphosate acid(mg/L)	Mean 4.27 ± 1.15				Mean 2.43 ± 0.19			
Particle size MMAD; GSD	2.91, 3.41µm; 1.74, 2.04				3.57, 3.03µm; 1.94, 1.90			
Size range (µm)	% by weight in range				% by weight in range			
	Run 1 (1hr 35min into exposure)		Run 2 (3hr 29min into exposure)		Run 1 (54min into exposure)		Run 2 (2hr 59min into exposure)	
	Analysed	Gravi-metric	Analysed	Gravi-metric	Analysed	Gravi-metric	Analysed	Grav-imetric
Particles > 9.8 µm (% w/w)	0.9	0.7	5.1	4.3	3.1	3.1	2.0	2.0
Particles 9.8-6.0 µm (% w/w)	21.1	20.6	26.3	23.8	20.9	23.0	16.7	16.4
Particles 6.0-3.5 µm (% w/w)	34.4	35.5	34.3	31.3	47.9	46.4	37.7	36.8
Particles 3.5-1.55 µm (% w/w)	32.1	32.3	21.3	28.0	19.5	18.8	38.0	36.9
Particles 1.55-0.93 µm (% w/w)	7.5	7.6	9.1	8.6	5.9	5.4	3.6	4.0
Particles 0.93-0.52 µm (% w/w)	2.2	2.3	2.7	2.3	1.9	2.0	1.3	1.6
Particles ≤0.52 µm (% w/w)	1.5	1.0	1.3	1.8	0.8	1.3	0.8	2.4
Flow rate (whole system)	2.5 L/min							
Temperature	14.7 – 21.7 °C							
Humidity	25 - 65 %							

- Percentages are calculated as follows:

Gravimetric: $\frac{\text{weight trapped at each size range} \times 100}{\text{Total weight trapped}}$

Statistics: The acute inhalation LC_{50} was estimated.

Results and discussion

Mortality: Two males and two females exposed to 4.43 mg/L were found dead or were terminated in extremis on days 5, 6 or 9 of the study, the remaining animals in this group survived until scheduled termination.

There were no mortalities at 2.47 mg/L.

Table B.6.2-16: Mortality / animals treated

Target exposure concentration mg/L	**Day number**	**Cumulative mortality (Number dead / total)**		
		Males	Females	Combined
5	5	1/5	0/5	1/10
	6	2/5	1/5	2/10
	9	2/5	2/5	3/10
	Day 14	2/5	2/5	4/10
2	Day 14	0/5	0/5	0/10

Clinical observations: Abnormalities generally associated with restraint (wet fur) were seen in all animals during exposure. Clinical changes seen were salivation, irregular breathing and auditory hypoaesthesia, these effects were considered to be related to treatment.
Immediately after exposure, abnormalities generally associated with restraint (hunched posture, piloerection and wet fur) were seen in both males and females. At an exposure concentration of 4.43 mg/L the clinical abnormalities seen in both sexes included breathing irregularities, reduced righting reflex, shaking, splayed gait andwere considered to be indicative of moderate toxicity.
At an exposure concentration of 2.47 mg/L the number of adverse clinical changes observed was reduced in both sexes. Those abnormalities observed were similar to those seen in animals exposed to 4.43 mg/L glyphosate acid
The clinical condition of most animals appeared to have improved by day 5 of the study, with the exception of 2 males and 2 females exposed to 4.43 mg/L. There was generally an improvement in clinical condition during the remainder of the study.
Bodyweight: Animals showed a treatment related reduction in bodyweight. At an exposure concentration of 4.43 mg/L all animals had exceeded their initial bodyweight by the end of the study. At an exposure concentration of 2.47 mg/L all animals had exceeded their initial weight by day 8 of the study.
Necropsy: In the animals exposed to 4.43 mg/L that died or were killed prior to termination, the two males found dead had dark lungs (probably a result of agonal congestion), the lungs of the females were normal.
At scheduled termination, the lungs of rats exposed to 4.43 mg/L were normal. One female exposed to 2.47 mg/L had red spots on the lungs and another female had dark lungs. These findings are considered to be incidental to treatment. Changes at necropsy in a variety of tissues in males exposed to 2.47 mg/L were of low incidence and were considered to be unrelated to treatment.

Conclusion by the Notifiers:
It was concluded that the acute inhalation LC_{50} of glyphosate acid exceeded 2.47 and exceeded 4.43 mg/L for male and female rats.

Comment by RMS:
The study is considered acceptable and the acute inhalation LC_{50} >4.43 mg/L air/4 hours is agreed.

Reference:	IIA, 5.2.3/11
Report:	[redacted] 2011, Glyphosate technical: Acute inhalation toxicity study (nose-only) in the rat [redacted] Data owner: Syngenta Report No.: 11/054-004P Date: 2011-06-06 GLP: not published ASB2012-11415
Guidelines:	OECD 403 (1981): OPPTS 870.1300 (1998): 440/2008 B.2 (2008)
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate technical
Description:	Technical; dry white powder
Lot/Batch #:	614034 (20100609\Milled)
Purity:	96.9 % w/w glyphosate technical
Stability of test compound:	Stable under storage conditions (room temperature range <30 °C), recertification date end January 2014
Vehicle and/or positive control:	None
Test animals:	
Species:	Rat
Strain:	RjHan:WI
Source:	[redacted]
Age:	8-10 weeks
Sex:	Males and females
Weight at dosing:	229-386 g
Acclimation period:	At least five days
Diet/Food:	ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and rats – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494 Soest Germany *ad libitum* (except during exposure)
Water:	Tap water *ad libitum* (except during exposure)
Housing:	In groups of 5 (or 2 in the case of sighting exposure), by sex, in solid-floor cages (Type III) with stainless steel mesh lids and softwood flake bedding.

Environmental conditions: Temperature: 22 ± 3 °C
Humidity: 30-70 %
Air changes: 15-20 air changes per hour
Photoperiod: 12 hours light / 12 hours dark

Study design and methods
In-life dates: Start: 14 April 2011 End: 28 April 2011

Exposure conditions: Prior to animal exposures, test material atmospheres were generated within the exposure chamber. During these technical trials, air-flow settings and test material input rates were adjusted to achieve the required atmospheric characteristics. Exposure conditions during the study are given later in a table of the test atmosphere characteristics of Glyphosate Technical.
Exposure system: The animals were exposed, nose-only, to an atmosphere of the test item using a TSE Rodent Exposure System (TSE Systems GmbH, Bad Homburg, Germany). This system comprises of 2, concentric anodised aluminium chambers and a computer control system incorporating pressure detectors and mass flow controllers.
Fresh aerosol from the generation system was constantly supplied to the inner plenum (distribution chamber) of the exposure system from where, under positive pressure, it was distributed to the individual exposure ports. The animals were held in polycarbonate restraint tubes located around the chamber which allowed only the animal's nares to enter the exposure port. After passing through the animal's breathing zone, used aerosol entered the outer cylinder from where it was exhausted through a suitable filter system. Atmosphere generation was therefore dynamic.
Airflows and relative pressures within the system were constantly monitored and controlled by the computer system thus ensuring a uniform distribution and constant flow of fresh aerosol to each exposure port (breathing zone). The flow of air through each port was at least 0.7 L/min. This flow rate was considered adequate to minimise re-breathing of the test atmosphere as it is about twice the respiratory minute volume of a rat.
Homogeneity of the test atmosphere within the test chamber and amongst the exposure ports was not specifically determined during this study. However, chambers of this design have been fully validated and have shown to produce evenly distributed atmospheres in the animals' breathing zones.
Exposure procedure: Each rat was individually held in a tapered, polycarbonate restraining tube fitted onto a single tier of the exposure chamber. Only the nose of each animal was exposed to the test atmosphere. Following an equilibration period of at least the theoretical chamber equilibration time (T99), a group of 10 rats (5 male and 5 female) was exposed to a target atmosphere concentration 5 mg/L for a period of at least 4 hours.
Generation of the test atmosphere / chamber description: The test item was aerosolised using a rotating brush powder disperser (Palas GmbH, Karlsruhe, Germany) located at the top of the exposure chamber. Compressed air was supplied by means of an oil-free compressor and passed through a suitable filter system prior to introduction to the dust generator.
Test atmosphere concentration: The test atmosphere was sampled at regular intervals during each exposure period. Samples were taken from an unoccupied exposure port (representing the animal's breathing zone) by pulling a suitable, known volume of test atmosphere through weighed GF10 glass fibre filters. The difference in the pre and post sampling weights, divided by the volume of atmosphere sampled, was equal to the actual achieved test atmosphere concentration.
The nominal concentration was calculated by dividing the mass of test material disseminated into the chamber by the total volume of air that through the chamber during the same period.

Particle size determination: The particle size of the test atmosphere was determined three times during the exposure period using a 7-stage impactor of Mercer style (which employs an inertial separation technique to isolate particles in the discrete aerodynamic size ranges). Samples were taken from an unoccupied exposure port (representing the animal's breathing zone).
The collection substrates and the backup filter were weighed before and after sampling and the weight of test item, collected at each stage, calculated by this difference.
The total amount collected for each stage was used to determine the cumulative amount below each cut-off point size. In this way, the proportion (%) of aerosol less than 0.55, 0.96, 1.55, 2.11, 3.56, 6.66 and 10.55 µm was calculated.
From these data, using software supplied with the impactor (TSE Systems GmbH, Bad Homburg, Germany), the Mass Median Aerodynamic Diameter (MMAD), and Geometric Standard Deviation were calculated. In addition, the proportion (%) of aerosol less than 4µm (considered to be the inhalable portion) was determined.

Table B.6.2-17: Summary of main study test atmosphere characteristics

Parameter	**Target concentration 5 mg/L**	
Mean achieved concentration (mg/L)	5.04 ± 0.17	
Nominal (mg/L)	7.71	
Particle size MMAD; GSD	3.65 µm; 2,24	
Inhalable fraction (% < 4 µm)	54.4	
	% by weight in range #	
Size range (µm)	Total mass/stage (mg)	Cumulative mass (%)
<0.55	0.35	2.05
0.55 – 0.96	0.30	3.81
0.96 – 1.55	0.91	9.13
1.55 – 2.11	1.90	20.26
2.11 – 3.56	5.43	52.05
3.56 – 6.66	4.69	79.51
6.66 – 10.55	2.06	91.57
>10.55	1.44	100.00
T99 (Minimum Acceptable Equilibration Time)	1 minute	
Chamber volume (inner plenum)	3.85 L	
Air Flow In (Inner Plenum) (L/min)	20.0-20.6	
Air Flow Out (Inner Plenum) (L/min)	19.4 – 38.4	
Temperature	21.6 – 24.7 °C	
Humidity	3.9 – 10.2 % (n = 3)	
Oxygen Concentration (%)	19.6 – 20.3	
Carbon Dioxide	0.1 – 0.8	

Sighting studies: Two sighting exposures using 2 male and 2 female rats were performed before the main study due to insufficient information about the test item's inhalation toxicity.
Animal assignment and treatment: Five male and 5 females were exposed to a target aerosol concentration of 5 mg/L glyphosate technical. The animals were exposed for 4 hours using a nose-only exposure system, followed by a 14 day observation period. The day of exposure was designated Day 0.
Animals were checked hourly during exposure, 1 hour after exposure and twice daily (early and late in the working day) during the 14 days of the observation period for morbidity and/or mortality. All animals were observed for clinical signs at hourly intervals during exposure, as soon as practically possible following removal from restraint at the end of exposure, 1 hour

after exposure and subsequently once daily for 14 days. The body weight of each rat was recorded prior to treatment on the day of exposure (day 0) and on Days 1, 3, 7 and 14.
At the end of the 14 day observation period, the animals were sacrificed by exsanguination under anaesthesia and a gross macroscopic examination was performed, which included a detailed examination of the abdominal and thoracic cavities. Special attention was given to the respiratory tract for macroscopic signs of irritancy or local toxicity.
Statistics: The acute inhalation LC_{50} was calculated from the mortality data.

Results and discussion
Mortality: One male rat died following a 4 hour exposure to 5.04 mg/L glyphosate technical.
Clinical observations: Wet fur and fur staining were commonly recorded on the day of and the day following exposure. These observations were considered to be related to the restraint and exposure procedures and, in isolation, were considered not to be treatment related.
Significant clinical signs were recorded on day of exposure and the following day included laboured and noisy respiration, respiratory rate increased, gasping respiration, sneezing, decreased activity, thin body appearance (weak/wasted).
The majority of animals recovered from Day 3.
Body weight: Normal body weight gain was noted for all surviving animals from Day 1, with the exception of one male where a slight bodyweight loss was recorded during first week of the observation period.
Necropsy: There were no macroscopic abnormalities in animals surviving to scheduled termination. A specific cause of death was not determined for the single male that died in the main study.

Conclusion by the Notifiers:
Under the experimental conditions of this study, a single death occurred in a group of 10 rats exposed to a mean achieved atmosphere of 5.04 mg/L for 4 hours. The acute inhalation LC_{50} of glyphosate technical, in Wistar RjHan: (WI) strain rats is considered to be greater than 5.04 mg/L.

Comment by RMS:
The study is considered acceptable and the acute inhalation LC_{50} >5.04 mg/L air/4 hours is agreed.

Reference:	IIA, 5.2.3/12
Report:	[redacted] 1999, NUP5a99 62% glyphosate MUP: Acute inhalation toxicity study in rats – Limit test [redacted] Data owner: Nufarm Study No.: 7909 Date: 1999-09-16, unpublished ASB2012-11416
Guidelines:	Health Effects Test Guidelines, OPPTS 870.1300 (1998)
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	NUP5a99 62 % glyphosate MUP
Description:	clear viscous amber liquid
Lot/Batch #:	Drum Sample E
Purity:	62 %
Stability of test compound:	No data available
Vehicle and/ or positive control:	None
Test animals:	
Species:	Rat
Strain:	Sprague-Dawley derived, albino
Source:	[redacted]
Age:	Not specified
Sex:	5 males and 5 females
Weight at dosing:	males 224-256 grams and females 179-201 gramsg
Acclimation period:	10 days
Diet/Food:	Purina Rodent Chow #5012
Water:	Tap water, ad libitum
Housing:	singly housed in suspended stainless steel caging with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:	Temperature: 22-24 °C Humidity: not specified Air changes: not specified 12-hour light/dark cycle

Study design and methods
In life dates: August 6-20, 1999

Animal assignment and treatment:
Prior to initiation of the full inhalation study, pre-test trials were conducted to establish generation procedures for achieving as closely as possible the desired chamber concentration (2.0 mg/L) and desired particle size distribution (mass median aerodynamic diameter ≤4 /µm). The animals were placed in a rectangular whole body plexiglass chamber with a volume of 150 liters with prechamber operated under slight negative pressure, and were exposed to the test atmosphere for 4 hours and 15 minutes. The exposure period was extended beyond 4 hours to allow the chamber to reach equilibrium (T99). The times for 90 and 99 % equilibration of the chamber atmosphere were 7.5 and 15.1 minutes, respectively. The gravimetric and nominal chamber concentrations were 2.08 and 18.38 mg/L, respectively. The mass median aerodynamic diameter was estimated to be 2.6 microns based on the particle size distribution as measured with an Andersen Cascade Impactor. At the end of the exposure period, the generation was terminated and the chamber was operated for a further 15 minutes with clean air. At the end of this period the animals were removed from the chamber. Prior to being returned to their cages, excess test substance was removed from the fur of each animal.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: In-chamber animal observations included ocular and nasal discharge, hunched posture and hypoactivity. Apart from test substance noted on the fur, all animals recovered from the above symptoms upon removal from the exposure chamber and appeared active and healthy throughout the study.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers:

The single exposure acute inhalation LC_{50} ofNUP5a99 62 % glyphosate MUP is >2.08 mg/L. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate is not to be classified for this end point.

Comment by RMS:

The study is considered acceptable and the acute inhalation LC_{50} > 2.08 mg/L air/4 hours is agreed.

Reference:	IIA, 5.2.3/13
Report:	[redacted] 2004, An Acute Nose-Only Inhalation Toxicity Study in Rats with MON 78623 [redacted] Data owner: Monsanto Monsanto Report No.: SB-2003-116 Date: 2004-02-06, not published ASB2012-11417
Guidelines:	EC method B.2, OECD 403, EPA Health Effects Test Guidelines, OPPTS 870.1300, JMAFF 12 Nohsan No. 8147
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	MON 78623
Description:	Clear colourless liquid (pipet), light amber liquid (bulk)
Lot/Batch #:	GLP-0306-14124-F
Purity:	47.2 % glyphosate (57.8 % potassium salt of glyphosate)
Stability of test compound:	Expiry June, 2004
Vehicle and/ or positive control:	None
Test animals:	
Species:	Rat
Strain:	Sprague Dawley

Source:	[redacted]
Age:	8-9 weeks
Sex:	Males and females
Weight at dosing:	♂ 276 – 312 g; ♀ 182 – 210 g
Acclimation period:	At least 5 days
Diet/Food:	PMI Certified Rodent Chow #5002 (PMI Nutrition International), *ad libitum* (except during acclimatisation to the exposure tubes and during the exposure)
Water:	Tap water, *ad libitum* (except during acclimatisation to the exposure tubes and during the exposure)
Housing:	Individually housed in suspended stainless steel cages
Environmental conditions:	Temperature: 19 - 23 °C Humidity: 31 - 65 % Air changes: 10-15 per hour Light cycle: 12 hours light/dark cycle

Study design and methods
In life dates: 2003-10-29 to 2003-12-29

Test atmosphere generation:
The test aerosol was generated with a Master Flex Pump and Pump Head (7523-30 and 77200-60) and a Pistol Spraying System. Conditioned high pressure external air was used in generating the test atmosphere. The aerosol was blown through a 5 L Elutriator, the Multi-Stage 10 L nose-only inhalation chamber and then vented from the chamber to an air treatment system which consisted of a prefilter, a HEPA filter, a charcoal bed and a water scrubbing tower.

Exposure chamber conditions:
Air flow readings were recorded at the initiation of the T99 equilibration period, at approximately 30-minute intervals during each aerosol exposure and at the conclusion of the de-equilibration period. The aerosol concentration was measured at the beginning of each aerosol exposure (after equilibration), at approximate 30-minute intervals during the aerosol exposure, and at the conclusion of each aerosol exposure (before de-equilibration). Samples of the test article aerosol were collected in the inhalation chamber by gravimetric technique. Both gravimetric and analytical aerosol concentrations were determined. A 5 L sample of the aerosol was drawn from the breathing zone of the animals in the chamber through a preweighed glass fiber filter. For the analytical concentration, the gravimetrically obtained samples were analysed by liquid chromatography for the non-volatile glyphosate component of the test article. These analyses were performed in order to determine the analytical (actual) concentrations of the aerosol in the chamber for each sampling period. Chamber oxygen content was measured and recorded at approximate 30-minute intervals during each aerosol exposure using a GC-501 Oxygen Detector.

Particle size distribution:
The aerosol aerodynamic particle-size distribution was determined three times during each aerosol exposure using the ITP 7 Stage Cascade Impactor. Each stage of the impactor was fitted with a preweighed glass fiber filter. Five liters per minute of the chamber air were drawn through the impactor and the change in weight of each filter was then determined and recorded. The mean particle-size distribution was subsequently determined using an Excel

computer adaptation of the manual method. The Mass Median Aerodynamic Diameter, Geometric Standard Deviation and percentage of particles ≤ 4.0 µ were then determined. The results were as follows:

Table B.6.2-18: Details of test atmosphere

Mean Achieved Actual Concentration (analytical method)	**MMAD**	**GSD**	**Respirable Amount Particle Size ≤ 4 µm**
(mg/L)	(µm)		(%)
2.21	2.9	2.18	67
5.27	3.8	2.20	53

MMAD = mean mass median aerodynamic diameter
GSD = geometric standard deviation

Animal assignment and treatment:
The animals chosen for study use were randomly selected from healthy stock animals using a computerized random numbers table to avoid potential bias. On day 0, the animals chosen for the limit test were weighed, placed in a nose-only exposure tube and allowed to acclimate to the exposure tube for at least one hour. Animals that appeared to have been acclimated to the exposure tube (i.e., minimal struggling and no inversion) were considered to be acceptable. Animals that did not appear to acclimate to the exposure tube were not acceptable. All animals were removed from the exposure tubes and returned to their cages.

The acceptable animals were then placed in exposure tubes and the tubes inserted into the Multi-Stage 10 L nose-only inhalation chamber and the test article aerosolised at the following levels:

Table B.6.2-19: Dose Levels

Analytical Exposure Level (mg/L)	**No. of Animals**	
	Male	Female
2.21	5	5
5.27	5	5

Each aerosol exposure consisted of a 3-minute T99 equilibration period, a 240-minute exposure period and a 3-minute de-equilibration period equal to the T99 equilibration period. After each aerosol exposure, animals were removed from the exposure tubes and residual test article was removed from the animal's exterior surfaces (where practical) by wiping the haircoat with a towel. The animals were then returned to ad libitum feed and water.

The limit test animals were observed for clinical abnormalities during each aerosol exposure (no positive clinical observations were noted during either exposure), two times on study day 0 (post-exposure) and daily thereafter (days 1-14). Individual body weights were recorded just prior to dosing and weekly thereafter. On Day 14 after dosing, each animal was euthanized and all study animals were subjected to gross necropsy.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: The most notable clinical abnormalities observed for the 2.21 mg/L dose level included transient incidences of congested breathing and dark material around the facial area.

The most notable clinical abnormalities observed for the 5.27 mg/L dose level included transient incidences of congested breathing and few faeces.

Body weight: Body weight gain was noted for all animals for the 2.21 mg/L dose level.

For the 5.27 mg/L dose level, slight body weight loss was noted for two females during the day 0 to 7 body weight interval and for one female during the day 7 to 14 body weight interval. Body weight gain was noted for all other animals and all animals exceeded their initial body weight at study termination.

Necropsy: No gross internal findings were observed at necropsy for the 2.21 mg/L and 5.27 mg/L dose levels on study day 14.

Conclusion by the Notifiers

The acute inhalation LC_{50} for the test substance MON 78623 was estimated to be greater than 5.27 mg/L. According to EU and OECD Globally Harmonised System (GHS) classification criteria glyphosate is not to be classified for acute inhalation toxicity.

Comment by RMS:

The study is considered acceptable and the acute inhalation LC_{50} > 5.25 mg/L air/4 hours is agreed.

B.6.2.4 Skin irritation

According to the previous EU evaluation, glyphosate acid and its salts were considered non-irritant to intact skin and only slightly irritant to abraded skin. For the current re-evaluation, 13 additional studies with glyphosate acid on rabbits were submitted.

Table B.6.2-20: Summary of skin irritation studies with glyphosate acid

Reference (Data owner)		Species Strain	Number and /or sex of animals /	Purity [%]	Amount applied / Exposure conditions	Results
Studies from the 2001 evaluation	Annex B-5.2.5.1, Glyphosate Monograph [redacted] 1994 (Alkaloida) TOX9650145	Rabbit NZW	4	97.2	0.5 g; intact + abraded skin	Very slight irritation
	Annex B-5.2.5.1, Glyphosate Monograph [redacted] 1991 (I. Pi. Ci.) TOX9551627	Rabbit NZW	3 ♂	98	0.5 g moistened with saline; intact skin	Very slight irritation

Reference (Data owner)		**Species Strain**	**Number and /or sex of animals /**	**Purity [%]**	**Amount applied / Exposure conditions**	**Results**
	Annex B-5.2.5.1, Glyphosate Monograph [redacted] 1991 (ADM) TOX9551092	Rabbit NZW	2 ♂, 1 ♀	96.8	0.5 g; intact skin	Non irritant
	Annex B-5.2.5.1, Glyphosate Monograph [redacted] 1990 (AGC) TOX9551794	Rabbit NZW	3 ♂	98.1	0.5 g moistened with saline; intact + abraded skin	Non irritant
Studies from the 2001 evaluation	Annex B-5.2.5.1, Glyphosate Monograph [redacted], 1989 (CHE) TOX9552333	Rabbit NZW	2 ♂, 4 ♀	98.6	0.5 g moistened with water; intact skin	Non irritant
Studies not reviewed in the 2001 evaluation	IIA 5.2.4/01 [redacted] 2007 (NUF) ASB2012-11418	Rabbit NZW	1 ♂, 2 ♀	95.1	0.5 g moistened with 0.5 mL water; intact skin	Non irritant
	IIA 5.2.4/02 [redacted] 2009 (HAG) ASB2012-11419	Rabbit Himalayan	3 ♂	96.4	0.5 g moistened with water; intact skin	Non irritant
	IIA 5.2.4/03 Hideo, 1995 ([redacted] ASB2012-11420	Rabbit NZW	6 ♀	97.56	0.5 g moistened with 0.5 mL water; intact skin	Non irritant
	IIA 5.2.4/04 [redacted] 2009 (HAG) ASB2012-11421	Rabbit Himalayan	3 ♂	98.8	0.5 g moistened with water; intact skin	Non irritant
	IIA 5.2.4/05 [redacted] 2010 (HAG) ASB2012-11422	Rabbit Himalayan	3 ♂	97.3	0.5 g moistened with water; intact skin	Non irritant
	IIA 5.2.4/06 [redacted] 2009 (HAG) ASB2012-11423	Rabbit NZW	1 ♂, 2 ♀	96.4	0.5 g moistened with water; intact skin	Non irritant
	IIA 5.2.4/07 [redacted] 2005 (HAG) ASB2012-11424	Rabbit, NZW	3 ♂	97.23	0.5 g moistened with water; intact skin	Slightly irritating

Reference (Data owner)		Species Strain	Number and /or sex of animals /	Purity [%]	Amount applied / Exposure conditions	Results
Studies not reviewed in the 2001 evaluation	IIA 5.2.4/08 [redacted] 2008 (HAG) ASB2012-11425	Rabbit, NZW	3 ♀	98.05	0.5 g moistened with water; intact skin	Non irritant
	IIA 5.2.4/09 [redacted], 1988 (MON) Z35394	Rabbit NZW	3 ♂, 3 ♀	97.76	0.5 g moistened with saline; intact skin; semi-occluded	Non irritant
	IIA 5.2.4/10 [redacted] 1979 (MON) Z35544	Rabbit	3 ♂, 3 ♀	98.5	0.5 g moistened with water; intact skin; occluded	Primary dermal irritation index 0.1 (study not acceptable)
	IIA 5.2.4/11 [redacted] 1996 (SYN) TOX2000-1985	Rabbit, NZW	6 ♀	95.6	0.5 g moistened with 0.5 mL water; intact skin	Non irritant
Studies not reviewed in the 2001 evaluation	IIA 5.2.4/12 [redacted] 2007 (SYN) ASB2012-11426	Rabbit NZW	1 ♂, 2 ♀	96.1	0.5 g moistened with 0.5 mL water; intact skin	Non irritant
	IIA 5.2.4/13 [redacted] 2011 (SYN) ASB2012-11427	Rabbit NZW	3 ♂	96.3	0.5 g moistened with water; intact skin	Mild Irritant (Primary dermal irritation index 0.11)

NZW = New Zealand White

Table 6.2-21: Summary of skin irritation studies with glyphosate salts

Reference (Data owner)		Species Strain	Number and /or sex of animals /	Purity [%]	Amount applied / Exposure conditions	Results
Studies from the 2001 evaluation	Annex B-5.2.5.1, Glyphosate Monograph Dreher, 1994 (MON / CHE) TOX9552335	Rabbit NZW	1 ♂, 5 ♀	IPA 62%	0.5 mL (pure)	Slight irritation
	Annex B-5.2.5.1, Glyphosate Monograph Snell, 1994 (Herbex) TOX9500248	Rabbit NZW	2 ♂, 1 ♀	IPA 360 g/L	0.5 mL; intact skin	Non irritant

Reference (Data owner)		Species Strain	Number and /or sex of animals /	Purity [%]	Amount applied / Exposure conditions	Results
	Annex B-5.2.5.1, Glyphosate Monograph [redacted] 1989 (I.Pi.Ci.) TOX9551628	Rabbit NZW	3 ♂, 3 ♀	IPA 62	0.5 mL (pure); ♂: intact skin; ♀: abraded skin	Slight irritant
	Annex B-5.2.5.1, Glyphosate Monograph [redacted] 1987 (MON/CHE) TOX9552336	Rabbit NZW	6	NH_4-salt 90.8	0.5 g moistened with saline	Non irritant

NZW = New Zealand White

Tier II summaries are only presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.
For details regarding studies reviewed during the 2001 EU evaluation please refer to the Monograph.

Reference: IIA, 5.2.4/01

Report: [redacted] 2007, Glyphosate Technical (NUP 05068): Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application)
[redacted]
[redacted] Study No.: B02294
Data owner: Nufarm
Date: 2007-03-01, unpublished
ASB2012-11418

Guidelines: OECD 404 (2002); Commission Directive 2004/73/EC B.4 (2004), JMAFF guideline 2-1-4 (2005)

Deviations: Yes
The test patch used had a surface of 16 cm^2 instead of 6 cm^2.

GLP: yes

Acceptability: *Please see comment by RMS*

Materials and methods

Test material:	Glyphosate technical
Identification:	NUP 05068
Description:	Solid
Lot/Batch #:	200609062
Purity:	95.1%
Stability of test compound:	Stable under storage conditions (20 ± 5 °C); Expiry date: 2008-09-14
Vehicle and/ or positive control:	Purified water

Test animals:	
Species:	Rabbit
Strain:	New Zealand White, SPF
Source:	[redacted]
Age:	13 weeks (male); 14 weeks (females)
Sex:	One male and two females
Weight at dosing:	2.662 kg (male), 2.637 kg and 2.97 kg (females)
Acclimation period:	At least five days
Diet/Food:	Pelleted standard Provimi Kliba 3418 rabbit maintenance diet (Provimi Kliba AG, CH-Kaiseraugust), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel cages with feed hoppers and drinking water bowls. Wood blooks and haysticks were provided for gnawing.
Environmental conditions:	Temperature: 17 - 23 °C Humidity: 30 - 70 % Air changes: 10 - 15/hour 12 hours light/dark cycle

Study design and methods
In life dates: 2007-01-04 to 2007-01-15

Animal assignment and treatment:
The test was conducted using young adult New Zealand albino rabbits (1 male, 2 females). The test was performed in a sequential manner, first using one animal. Since no signs of corrosion were observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.5 g of the solid test substance was moistened with approximately 0.5 mL of purified water was applied to the intact skin of the clipped left flank of the rabbits on an approx. 16-cm^2 gauze patch. The patch was covered with a semi-occlusive dressing. After 4 hours of exposure the dressing was removed and the skin was cleaned with lukewarm tap water.
Skin reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC approximately 1, 24, 48 and 72 hours after removal of the patch. The animals were observed for mortality and clinical signs daily. Body weights were determined at beginning of acclimatisation, on the day of application and at termination.

Results and discussion
Mortality: No mortality occurred.
Clinical observations: No clinical signs of systemic toxicity were observed during the study.
Body weight: All rabbits showed the expected body weight gain.
Necropsy: No necropsy was performed.
Skin observations: No skin reactions were observed at the application site of any animal at any observation time point (all scores were 0). The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema. The test substance produced no staining on the treated skin. In addition, neither alterations of the treated skin, nor corrosive effects were observed.

Conclusion by the Notifiers:
Based on the EU classification criteria, glyphosate technical (NUP 05068) is not to be classified for skin irritation. According to the OECD Globally Harmonized System (GHS) classification criteria glyphosate technical (NUP 05068) is also not classified for skin irritation.

Comment by RMS:
The study is considered acceptable and the tested substance not irritant.

Reference:	IIA, 5.2.4/02
Report:	[redacted] 2009, Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC In Rabbits [redacted] Data owner: Helm AG Report No.: 24877 Date: 2009-11-27, unpublished ASB2012-11419
Guidelines:	OECD 404 (2002); Commission Directive 2004/73/EC B.4 (2004), OPPTS 870.2500 (1998)
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate TC
Description:	White powder
Lot/Batch #:	2009051501
Purity:	96.4 %
Stability of test compound:	At room temperature in the dark stable until May 15, 2011.
Vehicle and/or positive control:	Purified water
Test animals:	
Species:	Rabbit
Strain:	Himalayan
Source:	[redacted]
Age:	Approx. 7.5 - 8.5 months
Sex:	Males
Weight at dosing:	2.6 - 3.2 kg
Acclimation period:	At least 20 days.
Diet/Food:	ssniff K-H V2333 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum* before and after the exposure period

Water:	Tap water, *ad libitum* before and after the exposure period
Housing:	Individual housing
Environmental conditions:	Temperature: 20 ± 3 °C Humidity: 30 - 70 % Air changes: no data 12 hours light/dark cycle

Study design and methods
In life dates: 2009-10-15 to 2009-10-23
Animal assignment and treatment:
The test was conducted using three young male adult Himalayan albino rabbits. The test was performed in a sequential manner, first using one animal. Since no signs of corrosion were observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.5 g of the solid test substance was moistened with purified water and applied to the intact skin of the rabbits on an approx. 6-cm^2 gauze patch. The patch was covered with a semi-occlusive dressing. After 4 hours of exposure the dressing was removed. No residual test item had to be removed.
Skin reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC approximately 1, 24, 48 and 72 hours after removal of the patch.

Results and discussion
Mortality: No mortality occurred.
Clinical observations: No clinical signs of systemic toxicity were observed during the study.
Body weight: All rabbits showed the expected body weight gain.
Necropsy: No necropsy was performed.
Skin observations: No skin reactions were observed at the application site of any animal at any observation time point (all scores were 0). The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema. The test substance produced no staining on the treated skin. In addition, neither alterations of the treated skin, nor corrosive effects were observed.

Conclusion by the Notifiers
Based on the scores for erythema and oedema and according to the EU and OECD Globally Harmonized System (GHS) classification criteria, glyphosate TC is not to be classified for skin irritation.

Comment by RMS:
The study is considered acceptable and the tested substance not irritant.

Reference:	IIA, 5.2.4/03
Report:	[redacted] 1995, HR-001: Primary Dermal irritation study in rabbits. [redacted]. Report No.: IET 95-0035 Data owner: Arysta LifeScience Date: 1995-06-28, unpublished ASB2012-11420
Guidelines:	U.S. EPA FIFRA Guideline Subdivision F MAFF 59 NohSan No. 4200 (1985) Draize method

Deviations: None
GLP: yes
Acceptability: *Please see comment by RMS*

Materials and methods

Test material:	Glyphosate technical
Identification:	HR-001
Description:	White crystal
Lot/Batch #:	T-941209
Purity:	97.56 %
Stability of test compound:	Not mentioned in the report
Vehicle and/or positive control:	Deionised water
Test animals:	
Species:	Rabbit
Strain:	New Zealand White, SPF
Source:	[redacted]
Age:	12 weeks
Sex:	Six females
Weight at dosing:	2.512 (2408 – 2686) kg
Acclimation period:	18 days
Diet/Food:	Pellet Diet GC4 (Oriental Yeast Co., Ltd.)
Water:	Water filtrated and sterilized, *ad libitum*
Housing:	Individually in stainless steel cages with feed hoppers and drinking water bowls.
Environmental conditions:	Temperature: 23.9 - 24 °C Humidity: 52.8 – 56.6 % Air changes: 15/hour 12 hours light/dark cycle

Study design and methods
In life dates: 1995-05-16 – 1995-05-19

Animal assignment and treatment:
Glyphosate (0.5 g) moistened with 0.5 mL of deionised water was then applied to the closely-clipped dorso-lumbar region of 6 New Zealand rabbits and covered by a semi-occlusive gauze patch for 4 hours. At the end of the exposure period, the patch was removed and the treatment site was washed with distilled water to remove any residual test substance. All animals were observed for primary dermal irritation 1, 24, 48 and 72 hours after removal of the patch. Degree of erythema and edema relative to treatment were recorded during a subsequent 72-hour observation period. Body weights were measured prior to application, and after the final observation.

Results and discussion
Mortality: No mortality occurred.

Clinical observations: Clinical signs were not observed.

Body weight: All rabbits showed the expected body weight gain.
Necropsy: No necropsy was performed.
Skin observations: : No signs of erythema, eschar, edema and any other evidence of irritation were observed in either the test sunstance treated site or the negative control site at any time during the observation period.
The observation period was therefore completed after 72 hours.

Conclusion by the Notifiers
Based on the EU classification criteria, glyphosate technical (HR-001) is not to be classified for skin irritation. According to the OECD Globally Harmonized System (GHS) classification criteria glyphosate technical (HR-001) is also not classified for skin irritation.

Comment by RMS:
The study is considered acceptable and the tested substance not irritant.

Reference:	IIA, 5.2.4/04
Report:	[redacted] 2009, Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits [redacted] Data owner: Helm AG Report No.: LPT 23913 Date: 2009-04-30, unpublished ASB2012-11421
Guidelines:	OECD 404 (2002) US EPA OPPTS 870.2500
Deviations:	Personnel change of the head of the Quality Assurance Unit: until January 31, 2009: Dipl. Biol. S. Steuer as of February 1, 2009: Dr. med. vet. K. R. habil. Sultan. This minor deviation did not have any effect on the validity and integrity of the scientific results obtained in this study
GLP:	yes
Acceptability:	*Please see comment by RMS.*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate TC
Description:	White powder
Lot/Batch #:	20080801
Purity:	98.8 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	Deionised water
Test animals:	
Species:	Rabbit

Strain:	Himalayan
Source:	[redacted]
Age:	Approx. 4 - 5 months
Sex:	Male animals
Weight at dosing:	Animal no. l : 4.4 kg Animal no. 2: 4.0 kg Animal no. 3: 3.8 kg
Acclimation period:	At least 20 adaptation days
Diet/Food:	Commercial diet, ssniffB K-H V2333 (ssniff Spezialdiäten GmbH) served as food. The food was available *ad libitum* before and after the exposure period.
Water:	Tap water, *ad libitum*
Housing:	The animals were kept singly in cages measuring 380 mm x 425 mm x 600 mm (manufacturer: Dipl. Ing. W. EHRET GmbH, 16352 Schönwalde, Germany).
Environmental conditions:	Temperature: 20 ± 3 °C Humidity: 30 - 70 % 12-hour light/dark cycle

Study design and methods

In life dates: 2009-02-04 to 2009-02-13

Animal assignment and treatment:

Approximately 24 hours before the test, the fur was removed by closely clipping the dorsal area of the trunk of the animals. Care was taken to avoid abrading the skin. Only animals with healthy intact skin were used.

A dose of 500 mg of the test item was applied to the test site (area: approx. 6 cm²).

The test item was applied to the test site and then covered with a gauze patch. The patch was held in contact with the skin with non-irritating tape for the duration of the exposure period. The surrounding untreated skin served as a control.

Exposure time was 4 hours. During the exposure the animals were kept in comfortable restrainers.

At the end of the exposure time no residual test item had to be removed. As it was expected that the test item would not produce any severe irritancy or corrosion, the test was started using at first only one animal, receiving a single patch for an exposure period of 4 hours.

As neither a corrosive effect nor a severe irritant effect was observed after a four hour exposure, the test was completed using two additional animals, each with one patch only, for an exposure period of 4 hours.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: The reactions of the intact skin were evaluated at 60 minutes and then at 24, 48 and 72 hours after patch removal. None of the three rabbits showed any significant test item-related lesions at these examination time points.

Conclusion by the Notifiers

Under the present test conditions, none of three rabbits exposed for 4 hours to 500 mg Glyphosate TC/patch (semi-occlusive conditions) showed any test item-related changes. There were no systemic intolerance reactions.

According to the EC-Commission directive 67/548/EEC and its subsequent amendments on the approximation of the laws, regulations and administrative provision relating to the classification, packaging and labelling of dangerous substances and the results obtained under the present test conditions, Glyphosate TC was non - irritating to skin, hence, no labelling is required.

Comment by RMS:

The study is considered acceptable and the tested substance not irritant.

Reference:	IIA, 5.2.4/05
Report:	[redacted] 2010, Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits [redacted] Data owner: Helm AG Report No.: [redacted] 24605 Date: 2010-01-06, unpublished ASB2012-11422
Guidelines:	OECD 404 (2002) US EPA OPPTS 870.2500
Deviations:	No deviations from the Study Plan
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate TC
Description:	White powder
Lot/Batch #:	20090506
Purity:	97.3 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	Deionised water
Test animals:	
Species:	Rabbit
Strain:	Himalayan
Source:	[redacted]
Age:	Approx. 6 - 7 months
Sex:	Male animals

Weight at dosing:	Animal no. l : 2.9 kg Animal no. 2: 2.4 kg Animal no. 3: 2.5 kg
Acclimation period:	At least 20 adaptation days
Diet/Food:	Commercial diet, ssniffB K-H V2333 (ssniff Spezialdiäten GmbH) served as food. The food was available ad libitum before and after the exposure period.
Water:	Tap water, *ad libitum*
Housing:	The animals were kept singly in cages measuring 380 mm x 425 mm x 600 mm (manufacturer: Dipl. Ing. W. EHRET GmbH, 16352 Schönwalde, Germany).
Environmental conditions:	Temperature: 20 ± 3 °C Humidity: 30 - 70 % 12-hour light/dark cycle

Study design and methods:
In life dates: 2009-10-26 to 2009-11-06

Animal assignment and treatment:
Approximately 24 hours before the test, the fur was removed by closely clipping the dorsal area of the trunk of the animals. Care was taken to avoid abrading the skin. Only animals with healthy intact skin were used.
A dose of 500 mg of the test item was applied to the test site (area: approx. 6 cm²).
The test item was applied to the test site and then covered with a gauze patch. The patch was held in contact with the skin with non-irritating tape for the duration of the exposure period. The surrounding untreated skin served as a control.
Exposure time was 4 hours. During the exposure the animals were kept in comfortable restrainers.
At the end of the exposure time no residual test item had to be removed. As it was expected that the test item would not produce any severe irritancy or corrosion, the test was started using at first only one animal, receiving a single patch for an exposure period of 4 hours.
As neither a corrosive effect nor a severe irritant effect was observed after a four hour exposure, the test was completed using two additional animals, each with one patch only, for an exposure period of 4 hours.

Results and discussion
Mortality: There were no mortalities during the study.

Clinical observations: The reactions of the intact skin were evaluated at 60 minutes and then at 24, 48 and 72 hours after patch removal. None of the three rabbits showed any significant test item-related lesions at these examination time points.

Conclusion by the Notifiers
Under the present test conditions, none of three rabbits exposed for 4 hours to 500 mg Glyphosate TC/patch (semi-occlusive conditions) showed any test item-related changes. There were no systemic intolerance reactions.
According to the EC-Commission directive 67/548/EEC and its subsequent amendments on the approximation of the laws, regulations and administrative provision relating to the classification, packaging and labelling of dangerous substances and the results obtained under

the present test conditions, Glyphosate TC was non - irritating to skin, hence, no labelling is required.

Comment by RMS:
The study is considered acceptable and the tested substance not irritant.

Reference:	IIA, 5.2.4/06
Report:	[redacted] 2009, Glyphosate – Acute Dermal Irritation Study in Rabbits [redacted] Report No..: 12173-08 Date: 2009-03-11, unpublished ASB2012-11423
Guidelines:	US EPA OPPTS 870.2500 Equivalent to OECD 404 (2002).
Deviations:	Humidity was in the range of 43-92% instead of 30-70%. This deviation did not affect the study outcome
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate Tech Grade Mixed 5-Batch
Description:	White powder
Lot/Batch #:	080704-1 thru 5
Purity:	96.4%
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	Deionised water
Test animals:	
Species:	Albino rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Approx. 3 months
Sex:	1 male and 2 females (nulliparous and non-pregnant)
Weight at dosing:	Male: 2.000 kg; Females: 2.600 kg
Acclimation period:	5 days
Diet/Food:	PMI Feeds, Inc.™ Lab Rabbit Diet #5321, 8 oz. daily
Water:	Tap water, *ad libitum*
Housing:	Individual housing in suspended, wire bottom, stainless steel cages.

Environmental conditions:	Temperature:	20 ± 3 °C
	Humidity:	30 - 70 %
	Air changes:	10 - 12/hour
	12-hour light/dark cycle	

Study design and methods:
In life dates: 2008-11-03 to 2008-11-14

Animal assignment and treatment:
Each animal was prepared on the day prior to treatment by clipping the dorsal area of the trunk free of hair to expose an area at least 8 x 8 cm. Only those animals with exposure areas free of pre-existing skin irritation or defects were selected for testing. A single intact exposure site was selected as the test site while the contralateral intact site served as a control site.
On Day 0, 500 mg of test substance moistened with 0.2 mL of deionized water was applied to each test site and covered with a 4 ply, 2.5 x 2.5 cm surgical gauze patch. Each patch was secured in place with a strip of non-irritating adhesive tape. The entire trunk of each animal was loosely wrapped with a semi-permeable dressing (orthopedic stockinette) which was secured on both edges with strips of tape to retard evaporation of volatile substances and to prevent possible ingestion of the test substance. After four hours, the patches and wrappings were removed. The test sites were gently washed with room temperature tap water and a clean cloth to remove as much residual test substance as possible.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: The test sites were observed for erythema and edema formation, and any other dermal defects or irritation at 1, 24, 48 and 72 hours after unwrap.
Body weight: Body weight gain was unaffected by the administration of the test substance.

Conclusion by the Notifiers
The primary irritation index of 0.0 out of a possible 8.0 was obtained from the 1, 24, 48 and 72 hour observations and was used to give Glyphosate a descriptive rating of non-irritating. Based on the 72-hour observations only, Glyphosate is assigned to Toxicity Category IV.

Comment by RMS:
The study is considered acceptable and the tested substance not irritant.

Reference:	IIA, 5.2.4/07
Report:	[redacted] 2005, Glyphosate Acid Technical – Primary Skin Irritation Study in Rabbits [redacted] Report No.: PSL 15278 Date: 2005-04-04, unpublished ASB2012-11424
Guidelines:	US EPA OPPTS 870.2500 Equivalent to OECD 404 (2002).
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate Acid Technical
Description:	White crystalline powder
Lot/Batch #:	040205
Purity:	97.23 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	Distilled water
Test animals:	
Species:	Rabbit
Strain:	New Zealand albino
Source:	[redacted]
Age:	Young adult
Sex:	Male
Weight at dosing:	No data given in the report.
Acclimation period:	21 days
Diet/Food:	Pelleted Purina Rabbit Chow #5326
Water:	Tap water, *ad libitum*
Housing:	The animals were singly housed in suspended stainless steel caging with mesh floors which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals DHEW (NIH)*. Litter paper was placed beneath the cage and was changed at least three times per week
Environmental conditions:	Temperature: 18-22 °C 12-hour light/dark cycle

Study design and methods
In life dates: 2004-05-05 to 2004-05-08

Animal assignment and treatment:
On the day before application, a group of animals was prepared by clipping (Oster model #A5-small)
the dorsal area and the trunk. On the day of dosing, but prior to application, the animals were examined for health and the skin checked for any abnormalities. Three healthy animals without preexisting skin irritation were selected for test. Prior to application, the test substance was moistened with distilled water to achieve a dry paste by preparing a 70 % w/w mixture. Five-tenths of a gram of the test substance (0.71 g of the test mixture) was placed on a l-inch x l-inch, 4-ply gauze pad and applied to one 6-cm² intact dose site on each animal. The pad and entire trunk of each animal were then wrapped with semi-occlusive 3-inch Micropore tape to avoid dislocation of the pad. Elizabethan collars were placed on each rabbit and they were returned to their designated cages.
After 4 hours of exposure to the test substance, the pads and collars were removed and the test sites were gently cleansed of any residual test substance. Individual dose sites were scored

according to the Draize scoring system at approximately 1 , 24, 48, and 72 hours after patch removal.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: All animals appeared active and healthy. Apart from the dermal irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. Two of three sites were free from irritation throughout the study. One hour after patch removal, one animal exhibited very slight erythema. Dermal irritation cleared from this animal by 24 hours.

Conclusion by the Notifiers
Under the conditions of this study, Glyphosate Acid Technical is classified as slightly irritating to the skin.

Comment by RMS:
The study is considered acceptable. The test substance provoke very slight erythema in one animal one hour after patch removal, which was cleared within 24 hours. According to current EU and GHS criteria classification as 'irritating to skin' is not warranted.

Reference:	IIA, 5.2.4/08
Report:	[redacted] 2008, Acute Dermal Irritation/Corrosion Study in Rabbits with Glyphosate Technical [redacted] Report No.: RF-3996.311.476.07 Date: 2008-09-23, unpublished ASB2012-11425
Guidelines:	OECD 404 (2002).
Deviations:	The experimental phase initiation and experimental phase conclusion dates were updated
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	20070606
Purity:	98.05 %
Stability of test compound:	No data given in the report.
Vehicle and/ or positive control:	Deionised water
Test animals:	
Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]

Age:	17 weeks
Sex:	Female
Weight at dosing:	2.907 - 3.145 kg
Acclimation period:	5 to 6 days
Diet/Food:	Pelleted and autoclaved commercial diet for rabbits (*Guabi, Mogiana Alimentos S.A. - Brazil*)
Water:	Tap water, *ad libitum*
Housing:	The animals were housed individually in galvanized steel cages. Autoclaved wood shavings were placed in a tray below the cages to collect excrements.
Environmental conditions:	Temperature: 17 - 22 °C Humidity: 30 - 70 % Air changes: 10 - 15/hour 12-hour light/dark cycle

Study design and methods
In life dates: 2008-05-20 to 2008-05-24

Animal assignment and treatment:
Each animal provisionally selected for the test was prepared by clipping the fur from the back approximately 24-hr prior to the application of the test item, using a small animal clipper (Oster model Golden A5, Electric Razor) with great care taken to avoid abrading the skin during the clipping procedure, so as not to alter its permeability. The clipped area was large enough to allow clear visualisation of the test site. After being clipped, visual examination of the skin confirmed the skin was intact and healthy. 0.5 g of the test item was applied over the skin of each animal. The test item was first placed onto a moistened gauze dressing, which was applied over a small section of the test area (approximately 6 cm^2) in such a manner that there was good contact and uniform distribution of the test item on the skin. After application, the gauze was held in the test site by an adhesive and non-irritating tape. Removal and ingestion of the test item was prevented by placing a suitable adhesive tape (semi-occlusive dressing) around the trunk and test area. Adjacent untreated shaved areas of the skin were used as the negative control. After the 4-hour exposure period, the gauze patches were removed, any residual test item washed using physiological saline and then the treated areas examined for signs of irritation at specified intervals. The test was performed initially using one single animal for evaluation of any irritant/corrosive effect of the test item to the skin. Because no severe dermal reaction was observed in the initial test, two additional animals were tested to confirm the response.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: Animals' skin was examined for signs of erythema, eschars and edema formation at approximately 1, 24, 48 and 72 hours after removal of the patch. No signs of dermal irritation were observed at any of the time points in any of the animals tested.
Body weight: All animals presented gain in body weight during the observation period.

Conclusion by the Notifiers:
Under the test conditions, the test item Glyphosate technical when applied on the skin of the rabbits did not cause any dermal irritation. No treatment-related behavioral or clinical alterations were noted during the observation period.

Comment by RMS:
The study is considered acceptable and the test substance not irritant.

Reference:	IIA, 5.2.4/09
Report:	[redacted] 1988b, Primary Dermal Irritation Study of Glyphosate Batch/Lot/NBR No. XLI-55 in New Zealand White Rabbits [redacted] Data owner: Monsanto Monsanto Report No.: FD-88-29 Date: 1988-06-08, Unpublished Z35394
Guidelines:	US EPA 81-5
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	XLI-55
Purity:	97.76 %
Stability of test compound:	Stored at room temperature
Vehicle and/or positive control:	Physiological saline
Test animals:	
Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adult
Sex:	Three males and three females
Weight at dosing:	Between 2 and 3 kg
Acclimation period:	At least five days
Diet/Food:	NIH 09 Rabbit Ration certified feed (Zeigler Brothers, Inc., Gardners, PA, US), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel mesh cages

Environmental conditions:	Temperature:	20 – 23.9 °C
	Humidity:	40 - 60 %
	Air changes:	Not specified
	Light cycle:	12 hours light/dark cycle

Study design and methods:
In life dates: 1998-04-11 to 1998-04-14

Animal assignment and treatment:
Six healthy animals weighing between two and three kilograms were selected randomly from the acclimated colony and assigned to the test group. Selection suitability was based on health, weight requirement and of dorsal skin for testing. The fur on the back of each rabbit was clipped with an electric clipper on the day prior to dose administration. The test article (0.5 g moistened with 0.5 mL physiological saline) was applied topically to each of two intact dorsal test sites per rabbit. Immediately after dosing, the test sites were semi–occluded with a one–inch square gauze patch held in place with tape. The animals were collared during the exposure period to prevent removal of the patches. The patches and collars were removed four hours after dose administration and the exposure sites gently wiped with gauze to remove as much non-absorbed test article as possible.
Dermal irritation was evaluated at 0.5, 24, 48 and 72 hours after patch removal. Erythema and edema were scored separately according to the Draize method. The animals were observed twice daily for mortality at least five hours apart. Body weights were obtained on study day 1 prior to dose administration. At study termination, the animals were euthanised by intra-cardiac injection of sodium pentobarbital and discarded.
A mean primary irritation score was calculated at each scoring interval from individual scores obtained from the test animals. Six individual animal scores were calculated from dermal irritation readings taken at 0.5, 24, 48 ad 72 hours after patch removal. Individual animal scores were obtained at each scoring interval by adding the total erythema and eschar formation scores from both application sites to the total edema formation scores from both sites and dividing by two. The mean of the six individual animal scores represents the mean primary irritation score at each interval.

Results and discussion
Mortality: No mortality occurred.
Clinical observations: Not reported.
Body weight: Not reported.
Necropsy: No necropsy was performed.
Skin observations: No dermal irritation was noted following test substance application.

Conclusion by the Notifiers:
Based on the EU classification criteria, glyphosate is not to be classified for skin irritation. According to the OECD Globally Harmonized System (GHS) classification criteria glyphosate is also not classified for skin irritation.

Comment by RMS:
The study is considered acceptable and the test substance not irritant.

Reference: IIA, 5.2.4/10
Report: [redacted] 1979, Primary

Dermal Irritation in Rabbits

[redacted]

Data owner: Monsanto
Monsanto Report No.: BND-77-428
Date: 1979-08-06, Unpublished
Z35544

Guidelines: Not specified
Deviations: Not specified
GLP: no (pre-GLP)
Acceptability: *Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate Technical
Description:	Fine white powder
Lot/Batch #:	XLI-180
Purity:	99 %
Stability of test compound:	Not specified
Vehicle and/or positive control:	Distilled water
Test animals:	
Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Not specified
Sex:	Three males and three females
Weight at dosing:	2.25 – 2.80 kg
Acclimation period:	Not specified
Diet/Food:	Not specified
Water:	Not specified
Housing:	Not specified
Environmental conditions:	Temperature: Not specified Humidity: Not specified Air changes: Not specified Light cycle: Not specified

Study design and methods:
In life dates: Not specified.

Animal assignment and treatment:
Six albino rabbits were closely clipped over the back and sides with an electric clipper. There were four test sites per rabbit, each site 1" x 1" in area. Two sites, one on each side of the spinal column were abraded, while the remaining two sites were left intact. The abrasions were sufficiently deep so as to penetrate the stratum corneum, but not so deep as to disturb the derma or produce bleeding.
The test material was administered as a 25 % w/v solution in distilled water. In all cases 0.5 mL of the test substance was applied beneath a surgical gauze square, 1" x 1", eight single layers thick, placed directly on the test site and secured with tape. The animals were then wrapped with plastic sheeting secured with masking tape to help contain the test material. After 24 hours the sheeting and gauze patches were removed.
Observations for signs of dermal irritation or systemic toxicity were recorded at 24 and 72 hours after application. At each observation all treated sites were scored for erythema, edema and eschar formation

Results and discussion
Mortality: No mortality occurred.
Clinical observations: Not reported.
Body weight: Not reported.
Necropsy: No necropsy was performed.
Skin observations: In the intact skin, one animal had very slight erythema (score of 1) at 24 hours following test substance application. There was no other irritation noted for intact skin at 24 or 72 hours.

Conclusion by the Notifiers
Based on the EU classification criteria, glyphosate should not be classified for skin irritation. According to the OECD Globally Harmonized System (GHS) classification criteria glyphosate is also not classified for skin irritation.

Comment by RMS:
The study is considered not acceptable due to several deficiencies (pre-GLP, no guideline, observation period too short, clinical signs & body weight not reported).

Reference:	IIA, 5.2.4/11
Report:	[redacted] 1996, Glyphosate Acid: Skin Irritation to the Rabbit Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK Data owner: Syngenta Report No.: [redacted]/P/4695 Date: 1996-08-23, not published TOX2000-1985
Guidelines:	OECD 404 (2002): OPPTS 870.2500 (1998): 2004/73/EC B.4 (2004)
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test Material:	Glyphosate acid
Description:	Technical, white solid
Lot/Batch number:	P24
Purity:	95.6 % w/wi
CAS#:	Not reported
Stability of test compound:	The test substance was used within the expiry date
Vehicle and/or positive control:	Deionised water (for moistening)
Test Animals:	
Species	Rabbit
Strain	New Zealand White albino
Age/weight at dosing	Young adult / 3001-4386 g
Source	[redacted]. [redacted]
Housing	Individually in aluminium sheet cages in racks suitable for animals of this strain and the weight range expected during the course of the study.
Acclimatisation period	At least 6 days
Diet	STANRAB SQC, (Special Diet Services Limited, Stepfield, Witham, Essex, UK) *ad libitum*
Water	Mains water *ad libitum*
Environmental conditions	Temperature: 17±2 °C Humidity: 40-70 % Air changes: Approximately 25/hour Photoperiod: 12 hours light / 12 hours dark

Study design and methods

In-life dates: Start: 9 March 1995 End: 28 April 1995

Animal assignment and treatment: In a primary skin irritation study, Glyphosate acid (95.6 % w/w) was applied by semi-occlusive application of 500 mg to the intact skin of the left flank of each of six female, young adult New Zealand White rabbits.
Approximately one day before treatment, the left flank was clipped with an electric clipper, exposing an area of approximately 7 cm x 13 cm.
On the day of treatment, 500 mg of glyphosate acid (95.6 % w/w) (moistened with approximately 0.5 mL deionised water) was applied to the test site (approximately 2.5 cm x 2.5 cm) on the left flank of the rabbit. The treated area was covered with a piece of 8-ply surgical gauze (approximate size 2.5cm x 2.5cm), which was secured by two strips of surgical tape (approximate size 1 cm x 8cm). This was covered by a piece of impermeable rubber sheeting (approximate size 35cm x 13cm) wrapped once around the trunk of the animal and secured with adhesive impermeable polyethylene tape (7.5cm wide).
The dressings were left in position for approximately four hours. The application site was gently cleansed free of any residual test substance using clean swabs of absorbent cotton wool soaked in clean warm water and was then dried gently with clean tissue paper.
The Draize scale was used to assess the degree of erythema and oedema at the application sites approximately 30-60 minutes, 1, 2 and 3 days after removal of the dressings. Any other signs of skin irritation were also noted.

Results and discussion

There were no signs of ill-health and no signs of skin irritation in any animal during the study.

Table B.6.2-22: Individual and mean skin irritation scores of glyphosate acid according to the Draize scheme

Time	**Erythema**						**Oedema**					
Animal number	1	2	3	4	5	9	1	2	3	4	5	9
after 1 hour	0	0	0	0	0	0	0	0	0	0	0	0
after 24 hours	0	0	0	0	0	0	0	0	0	0	0	0
after 48 hours	0	0	0	0	0	0	0	0	0	0	0	0
after 72 hours	0	0	0	0	0	0	0	0	0	0	0	0
mean score 24-72 h	0	0	0	0	0	0	0	0	0	0	0	0

Conclusion by the Notifiers:

Glyphosate acid is non-irritant following a single four-hour application to rabbit skin.

Comment by RMS:

The study is considered acceptable and the test substance is considered not irritant.

Reference: IIA, 5.2.4/12

Report: [redacted] 2007, Glyphosate Technical Material: Primary Skin Irritation Study In Rabbits (4-Hour Semi-Occlusive Application)
[redacted]
Data owner: Syngenta
Report No.: R61837/1010
Date: 2007-02-08, not published
ASB2012-11426

Guidelines: OECD 404 (2002): OPPTS 870.2500 (1998): 2004/73/EC B.4 (2004)

Deviations: None

GLP: yes

Acceptability: *Please see comment by RMS*

Materials and methods

Test Material:	Glyphosate Technical Material
Description:	Technical, white powder
Lot/Batch number:	0507
Purity:	96.1 % w/w glyphosate acid
CAS#:	
Stability of test compound:	Stable under storage conditions of room temperature (range of 20 ^{o}C ± 5 ^{o}C), protected from light and humidity.
Vehicle and/or positive control:	The test substance was moistened with purified water before application.
Test Animals:	
Species	Rabbit

Strain	New Zealand White (SPF)
Age/weight at dosing	Male: 10-11 weeks / 2440 g; Females: 15-16 weeks / 2749 and 2815 g
Source	[redacted]
Housing	Individually in stainless steel cages equipped with feed hoppers and drinking water bowls.
Acclimatisation period	5/6 days
Diet	Pelleted standard Provimi Kliba 3418 rabbit maintenance diet *ad libitum.*
Water	Community tap water *ad libitum*
Environmental conditions	Temperature: 17-23 °C Humidity: 30-70 % Air changes: 10-15 per hour. Photoperiod: 12 hours light, 12 hours dark.

Study design and methods

In-life dates: Start: 18 December 2006 End: 22 December 2006

Animal assignment and treatment: Three young adult (1 male and two female) New Zealand White rabbits were used in the study. As it was suspected that the test substance might produce irritancy, a single animal (one female) was treated first. As no corrosive effect was observed after the 4-hour exposure, the test was completed using the two remaining animals for an exposure period of 4 hours.

Four days before treatment, the left flank was clipped, exposing an area of approximately 100 cm^2 (10 cm x 10 cm). The skin of the animals was examined one day before treatment, and regrown fur of all animals was clipped again. Animals with overt signs of skin injury or marked irritation which may have interfered with the interpretation of the results were not used in the test.

On the day of treatment, 0.5 g of Glyphosate Technical Material was placed on a surgical gauze patch (2.5 cm x 2.5 cm). This gauze patch was applied to the intact skin of the clipped area. The patch was covered with a semi-occlusive dressing which was was wrapped around the abdomen and anchored with tape.

The duration of treatment was 4 hours after which the dressing was removed and the skin was flushed with lukewarm tap water to clean the application site so that any reactions (erythema) were clearly visible at that time.

Observations for viability, mortality and clinical signs were carried out daily from acclimatisation of the animals to the termination of the study. Bodyweights of individual animals were recorded at the start of the acclimatisation period, on the day of application and at termination of the observation period.

The skin reaction was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, 29 April 2004, approximately 1, 24, 48 and 72 hours, after removal of the dressing, gauze patch and test substance. To evaluate the irritation potential of the test substance, the mean values of erythema/eschar and oedema formation were calculated for each animal using the scores between 24 and 72 hours. The Primary Irritation Index (P.I.I.) was calculated by adding together the mean cumulative scores at 24, 48 and 72 hours and then dividing by the number of available figures.

Results and discussion

No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred. The test substance did not elicit any skin reactions at the application site of any animal at any of the observation times (all scores 0). The individual mean score for erythema/eschar and oedema for each of the three animals was therefore 0. No staining produced by the test item of the treated skin was observed and no alterations of the treated skin or corrosive effects were evident. The bodyweights of the rabbits were considered to be within the normal range of variability.

Conclusion by the Notifiers:
The application of Glyphosate Technical Material to the intact skin resulted in no signs of irritation. According to Draize classification criteria Glyphosate Technical Material is considered to be "not irritant" to rabbit skin (P.I.I. = 0.00).

Comment by RMS:
Under the present conditions the study is considered acceptable and the tested substance not irritant.

Reference:	IIA, 5.2.4/13
Report:	[redacted] 2011, Glyphosate technical: Primary skin irritation study in rabbits [redacted] Data owner: Syngenta Report No.: 10/218-006N Date: 2011-04-13 not published ASB2012-11427
Guidelines:	OECD 404 (2002): OPPTS 870.2500 (1998): 2004/73/EC B.4 (2004)
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test Material:	Glyphosate technical
Description:	Technical, dry white powder
Lot/Batch number:	569753 (BX20070911)
Purity:	96.3 % w/w Glyphosate technical
CAS#:	Not reported
Stability of test compound:	Stable under storage conditions (room temperature range <30 °C), recertification date end August 2011
Vehicle and/or positive control:	None
Test Animals:	
Species	Rabbit
Strain	New Zealand White
Age/weight at dosing	Approximately 12 weeks / 2995-3095 g
Source	[redacted]
Housing	Individually in metal cages

Acclimatisation period	5 days
Diet	Purina Base – Lap gr. diet (AgribrandsEurope Hungary PLC, H-5300 Karcag, Madarasi út, Hungary) *ad libitum*
Water	Municipal tap water *ad libitum*
Environmental conditions	Temperature: 17-20 °C Humidity: 30-70 % Air changes: 15-20/hour Photoperiod: 12 hours light/12 hours dark

Study design and methods

In-life dates: Start: 02 November 2010 End: 05 November 2010

Animal assignment and treatment: In a primary dermal irritation study, three male, young adult, New Zealand White rabbits were each given a dermal application of 0.5 g of undiluted Glyphosate Technical (96.3 % w/w Glyphosate technical).
Approximately 24 hours prior to the test the hair was clipped from the back and flanks of the animals with an electric clipper, exposing an area approximately 10 cm x 10cm. Animals with overt signs of skin injury or marked irritation which may have interfered with the interpretation of the results were not used in the test.

On the day of treatment, 0.5g of glyphosate technical was placed on a surgical gauze pad (approximately 2.5 cm x 2.5 cm). This gauze pad was applied to the intact skin of the clipped area and was kept in contact with the skin by a patch with a surrounding adhesive hypoallergenic plaster. The entire trunk of the animals was then wrapped with plastic wrap held in place with an elastic stocking.
The dressing was left in place for 4 hours after which it was removed and the skin was flushed with lukewarm tap water to clean the application site so that any reactions (erythema) were clearly visible.
As it was suspected that the test item might produce irritancy, a single animal was treated first. As no corrosive effect was observed after the 4-hour exposure, the test was completed using the two remaining animals.
The animals were checked daily for signs of systemic toxicity and mortality. Body weights were recorded on the day of application and at termination of observations.
The skin reaction was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, April 29, 2004, which was based on the Draize scoring system approximately 1, 24, 48 and 72 hours after the removal of the dressing, gauze patch and test item. The mean score was calculated across 3 scoring times (24, 48 and 72 hours after patch removal) for each animal for erythema/eschar grades and for oedema grades, separately. An animal was positive when the mean score was 2 or greater. The test was positive for irritation when at least 2 animals were positive for the same endpoint (erythema/eschar or oedema).
The Cumulative Scores for the Skin Irritation Scores were calculated and represent the sum of all numerical scores for each animal at each time point. The resulting Mean Cumulative Skin Irritation Score was calculated for all animals at each time point.
The Primary Irritation Index (P.I.I.) was calculated by totalling the mean cumulative scores at 24, 48 and 72 hours and then dividing by the number of available figures.

Results and discussion

No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred. The bodyweights of all rabbits were considered to be within the normal range of variability. There was no staining of the skin, alterations or corrosive effects.
At the observations 1 and 24 hours after patch removal, very slight erythema (score 1) was observed in one animal. No signs of irritation were observed in the other treated animals throughout the study.
As no signs of irritation were observed 72 hours after patch removal, the study was terminated after the 72 hour observation.

Table B.6.2-23: Individual and mean skin irritation scores of glyphosate technical

Time	**Erythema**			**Oedema**		
Animal number	606	622	620	606	622	620
after 1 hour	0	1	0	0	0	0
after 24 hours	0	1	0	0	0	0
after 48 hours	0	0	0	0	0	0
after 72 hours	0	0	0	0	0	0
mean score 24-72 h	0	0.3	0	0	0	0

Conclusion by the Notifiers:
According to the Draize classification criteria, glyphosate technical is considered to be a "mild-irritant" to rabbit skin (P.I.I. = 0.11).

Comment by RMS:
The study is considered acceptable. The test substance provoke slight erythema in one animal one hour after patch removal, which was cleared within 48 hours. According to current EU and GHS criteria classification as 'irritating to skin' is not warranted.

Comment by GTF on the first draft of the RAR (July 2013):
RMS stated: "Three of the new studies revealed mild or slight irritating effects.
GTF: non-irritant to inact skin and only slightly irritant to abraded skin.

RMS comment (August 2013):
It is clearly stated that no classification and labelling is necessary and that in the majority of studies no evidence of skin irritation was obtained. Furthermore, studies conducted by [redacted] (2005, ASB2012-11424) and [redacted] (2011, ASB2012-11427) showed slight or mild effects at intact skin.

B.6.2.5 Eye irritation

Eye irritation was examined in numerous studies in rabbits that were performed either with the acid or its different salts. In additon to the previous evaluation a huge number of further studies were provided for the current re-evaluation. To conclude on all study results, glyphosate acid was tested to be a strong eye irritant and classification and labelling (Xi/R41 or Cat. 1, H318) is needed.

Table B.6.2-24: Summary of eye irritation studies with glyphosate acid

	Reference (Data owner)	Species Strain	Number and /or sex of animals	Purity [%]	Amount applied Exposure conditions	Effects / Result
Studies from the 2001 evaluation	Annex B-5.2.6.1, Glyphosate Monograph [redacted] 1994 (Herbex) TOX9500249	Rabbit NZW	1 ♀	95	0.1 mL (ca. 76 mg)	Due to strong ocular effects the test was stopped for humane reasons after 1 hour irritant
	Annex B-5.2.6.1, Glyphosate Monograph [redacted] 1994 (Alkaloida) TOX9650146	Rabbit NZW	4 ♀	97.2	100 mg (pure)	Significant ocular lesions, especially chemosis irritant
	Annex B-5.2.6.1, Glyphosate Monograph [redacted] 1991 (ADM) TOX9551093	Rabbit NZW	2 ♂, 1 ♀	96.8	100 mg	Mortality (1♀: severe enteritis and opacity); significant ocular lesions that were not reversible within 3 weeks moderately irritating
	Annex B-5.2.6.1, Glyphosate Monograph [redacted] 1991 (I. Pi. Ci.) Z101610	Rabbit NZW	3 ♂	98	100 mg (pure)	Several ocular effects (score 1 & 2) that were reversible within 3 days slightly irritant
Studies from the 2001 evaluation	Annex B-5.2.6.1, Glyphosate Monograph [redacted] 1990 (AGC) TOX9500264	Rabbit NZW	3 ♀	98.1	100 mg	Corneal opacity not reversible within 7 days (2♀), iris lesions not reversible within 6 days (1♀), conjunctivcal redness not reversible within 6 days (3 ♀) slightly irritant
	Annex B-5.2.6.1, Glyphosate Monograph [redacted] J[redacted], 1989 (CHE) TOX9552338	Rabbit NZW	1 ♂	98.6	100 mg (pure)	Due to strong ocular effects the test was stopped for humane reasons after treatment of the first of six animals moderately to severely irritating
	IIA 5.2.5 [redacted] 1996 (CHE) TOX1999-881	Rabbit NZW	6 ♂, 3 ♀	98.2	65 mg one group (3♂, 3 ♀) eyes were not washed; one group (3♂) eyes were washed 30 sec after treatment	Severely irritating (washed eyes) moderately irritating (non-washed eyes)

	Reference (Data owner)	Species Strain	Number and /or sex of animals	Purity [%]	Amount applied Exposure conditions	Effects / Result
Studies not reviewed in the 2001 evaluation	IIA 5.2.5/01 [redacted] 2007 (NUF) ASB2012-11428	Rabbit NZW	1 ♂ 2 ♀	95.1	100 mg	Marked, early-onset and transient ocular changes, like very slight to slight corneal opacity, slight to marked conjunctival redness, conjunctival chemosis, reddening of the sclera and discharge. All eye effects were reversible within 10 days after instillation. No signs of corrosion or staining were observed in any eye. irritating
	IIA 5.2.5/02 [redacted] 2009 (HAG) ASB2012-11429	Rabbit Himalayan	3 ♂	96.4	100 mg (eyes were rinsed 1 h post-application) (study considered supplementary)	slight and fully reversible ocular changes (≤ grade 1); all eye effects were reversible within 8 days after instillation. No signs of corrosion or staining were observed in any eye Non irritant
	IIA 5.2.5/03 [redacted] 1995 (ALS) ASB2012-11430	Rabbit NZW	12 ♀	97.56	100 mg (pure) (one group without irrigation of eyes, two groups with irrigation of eyes at different time intervals after application)	Severely irritating; animals in the irrigation groups showed reduced eye irritation and faster recovery
	IIA 5.2.5/04 [redacted] 2009 (EXC) (Expert statement) ASB2012-11431	Rabbit	Not applicable	96.66	Test solution: 1% in purified water	Not performed since pH of test item solution was < 2 (corrosive)
Studies not reviewed in the 2001 evaluation	IIA 5.2.5/05 [redacted] 2009 (HAG) ASB2012-11432	Rabbit Himalayan	3 ♂	98.8	100 mg (eyes were rinsed 1 h post-application) (study considered supplementary)	Non irritant
	IIA 5.2.5/06 [redacted] 2010 (HAG) ASB2012-11433	Rabbit Himalayan	3 ♂	97.3	100 mg (eyes were rinsed 1 h post-application) (study considered supplementary)	Non irritant

Reference (Data owner)		Species Strain	Number and /or sex of animals	Purity [%]	Amount applied Exposure conditions	Effects / Result
	IIA 5.2.5/07 [redacted] 2009 (HAG) ASB2012-11434	Rabbit NZW	2♂, 1 ♀	96.4	0.1 mL (93.2 mg)	Irritant
	IIA 5.2.5/08 [redacted] 2005 (HAG) ASB2012-11435	Rabbit NZW	3 ♂	97.23	0.1 mL (60 mg)	Irritant
	IIA 5.2.5/09 [redacted] 2008 (HAG) ASB2012-11436	Rabbit NZW	1 ♂ 2 ♀	98.05	100 mg	Severely irritating
	IIA 5.2.5/10 [redacted], 1988 (MON) Z35395	Rabbit NZW		97.76		Produced corneal opacity and conjunctival irritation with blistering, irritation persisted in 4/5 rabbits on day 21
	IIA 5.2.5/11 [redacted] 1997 (SYN) TOX2000-1986	Rabbit NZW	6 ♀	95.6	100 mg	Irritant
	IIA 5.2.5/12 [redacted] 2007 (SYN) ASB2012-11437	Rabbit NZW	1 ♂ 2 ♀	96.1	100 mg	Slight conjunctival redness, conjunctival chemosis, reddening of the sclera and discharge were observed. Effects reversible and no longer evident 7 days after treatment.
	IIA 5.2.5/13 [redacted] 2011 (SYN) ASB2012-11438	Rabbit NZW	1 ♂	96.3	100 mg	Corrosive. Due to strong ocular effects the test was stopped for humane reasons after 24 hours.

NZW = New Zealand White

Table B.6.2-25: Summary of eye irritation studies with glyphosate salts

	Reference (Data owner)	Species Strain	Number and /or sex of animal TOXs /	Purity [%]	Amount applied / Exposure conditions	Results
Studies from the 2001 evaluation	Annex B-5.2.6.2, Glyphosate Monograph [redacted] 1994 (MON / CHE) TOX9552340	Rabbit NZW	1 ♂, 5 ♀	IPA 62%	0.1 mL (pure) (pre-treatement with local anaesthetic)	Iris, conjunctivae: redness & chemosis within 1 h; discharge after 1 h Slightly irritating
	Annex B-5.2.6.2, Glyphosate Monograph [redacted] 1989 (I.Pi.Ci.) TOX9551629	Rabbit NZW	3 ♂, 3 ♀	IPA 62	0.1 mL; ♂: eyes unrinsed; ♀: eyes rinsed	Conjunctival redness (24 h) Slightly irritating
	Annex B-5.2.6.2, Glyphosate Monograph [redacted] 1987 (MON/CHE) TOX9552342	Rabbit NZW	6	NH_4-salt 90.8	0.1 g	conjunctivae: redness & chemosis (48h); discharge in all animals within 48 h mucous membrane appearing blistered in all animals after 1 h Slightly irritating

NZW = New Zealand White

Tier II summaries are only presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.
For details regarding studies reviewed during the 2001 EU evaluation please refer to the Monograph.

Reference: IIA, 5.2.5/01
Report: [redacted] 2007, Glyphosate Technical (NUP 05068): Primary Eye Irritation Study In Rabbits
[redacted] Study No.: B02305
Data owner: Nufarm
Date: 2007-03-06, unpublished
ASB2012-11428
Guidelines: OECD 405 (2002); Commission Directive 2004/73/EC B.5 (2004), JMAFF guideline 2-1-5 (2005)
Deviations: None
GLP: yes
Acceptability: *Please see comment by RMS*

Materials and methods

Test material: Glyphosate technical
Identification: NUP 05068
Description: Solid

Lot/Batch #:	200609062
Purity:	95.1 %
Stability of test compound:	Stable under storage conditions (20 ± 5 °C), light protected; Expiry date: 2008-09-14
Vehicle and/or positive control:	None
Test animals:	
Species:	Rabbit
Strain:	New Zealand White, SPF
Source:	[redacted]
Age:	15 weeks (male); 12 and 15 weeks (females)
Sex:	One male and 2 females
Weight at dosing:	2.969 kg (male), 2.605 kg and 3.416 kg (females)
Acclimation period:	At least five days
Diet/Food:	Pelleted standard Provimi Kliba 3418 rabbit maintenance diet (Provimi Kliba AG, CH-Kaiseraugust), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel cages with feed hoppers and drinking water bowls. Wood blooks and haysticks were provided for gnawing.
Environmental conditions:	Temperature: 17 - 23 °C Humidity: 30 - 70 % Air changes: 10 - 15/hour 12 hours light/dark cycle

Study design and methods:
In life dates: 2007-01-17 to 2007-02-26

Animal assignment and treatment:
The test was conducted using young adult New Zealand albino rabbits (1 male, 2 females). The test was performed in a sequential manner, first using one animal. Since no corrosive or severe eye effects were observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.1 g of the solid test substance was applied into the conjunctival sac of the left eye of the rabbits. The lids were then gently held together for about one second. The treated eyes were not rinsed after instillation. The right eye remained untreated and served as the reference control. Eye reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC approximately 1, 24, 48 and 72 hours, as well as 7, 10 and 14 days after instillation. Scleral reddening and ocular discharge was also assessed. The animals were observed for mortality and clinical signs daily. Body weights were determined at beginning of acclimatisation, on the day of application and at termination.

Results and discussion
Mortality: No mortality occurred.
Clinical observations: No clinical signs of systemic toxicity were observed during the study.
Body weight: All rabbits showed the expected body weight gain.
Necropsy: No necropsy was performed.

Eye observations: Very slight to slight corneal opacity were observed in all rabbits from 1 hour after instillation up to 72 hours. No signs of iritis, corrosion or staining were observed in any animal throughout the study period. One hour after instillation slight to moderate conjunctival redness was observed in the treated eyes of all rabbits. By 24 hours the redness increased to marked in two animals and to moderate in one rabbit. After 48 hours the conjunctival redness decreased in all rabbits. Only slight redness was observed in the rabbits after 7 days. Moderate to marked chemosis of the conjunctivae was observed from 1 hour after instillation up to 24 hours. The swelling decreased with time. 72 hours after treatment slight swelling was still present in two animals. One hour after instillation two rabbits exhibited moderate ocular discharge. Moderate or marked discharge was observed in all animals at the 24-hour reading time point. This persisted at the 48-hour reading as slight or moderate in all rabbits. After 72 hour slight discharge was still present in one rabbit.
Reddening of the sclera was observed in all animals. However, one hour after instillation sclera of one animal was not assessable due to conjunctival swelling. In two animals moderate or marked reddening of the sclera was observed at this time point. After 24 hours all rabbits showed marked reddening of the sclera. This sign persisted in the rabbits as moderate or marked at the 48- and 72-hour readings. In one animal slight reddening was still present after 7 days. All rabbits were free of ocular signs by day 10 after instillation.
The group mean irritation scores (24 to 72 hours) were calculated to be 1.5 for corneal opacity, 0.0 for iris lesions, and 2.2 conjunctival redness, and 1.7 for chemosis of the conjunctiva.
The individual scores for each time point, individual mean and group mean scores (24 to 72 hours) are presented in Table B.6.2-26.

Table B.6.2-26: Results of the eye irritation test

Animal	**Scoring [h]**	**Cornea**		**Iris**	**Conjunctiva**		**Sclera**
		Opacity	Area		Redness	Chemosis	
Rabbit 1 (male)	1	1	1	0	2	3	3
	24	2	2	0	3	3	3
	48	2	2	0	3	2	3
	72	1	1	0	2	1	2
	Day 7	0	0	0	1	0	1
	Day 10	0	0	0	0	0	0
	Day 14	0	0	0	0	0	0
Mean (24, 48, 72 h)		1.67	---	0.0	2.67	2.0	---
Rabbit 2 (female)	1	1	1	0	1	2	2
	24	2	4	0	2	3	3
	48	2	4	0	2	2	3
	72	2	4	0	2	1	2
	Day 7	0	0	0	1	0	0
	Day 10	0	0	0	0	0	0
	Day 14	0	0	0	0	0	0
Mean (24, 48, 72 h)		2.0	---	0.0	2.0	2.0	---
Rabbit 3 (female)	1	1	4	0	2	3	n.a.
	24	1	4	0	3	2	3
	48	1	4	0	2	1	2
	72	0	0	0	1	0	2
	Day 7	0	0	0	1	0	0
	Day 10	0	0	0	0	0	0
	Day 14	0	0	0	0	0	0
Mean (24, 48, 72 h)		0.67	--	0.0	2.0	1.0	---
Group means		1.5	---	0.0	2.2	1.7	---

n.a. = not assessable due to swelling of the conjunctivae

Conclusion by the Notifiers

Based on the study results and on the and according to EU classification criteria the test substance glyphosate technical (NUP 05068) is to be classified as irritating to the eyes (Xi, R36). This corresponds to Category 2A according to the OECD Globally Harmonized System (GHS) classification criteria.

Comment by RMS:

The study is considered acceptable and the results revealed that glyphosate acid is irritant to rabbit eyes..

Reference:	IIA, 5.2.5/02
Report:	[redacted], 2009, Acute Eye Irritation/Corrosion Test Of Glyphosate TC In Rabbits [redacted] Data owner: Helm AG Report No.: 24878 Date: 2009-11-27, unpublished ASB2012-11429
Guidelines:	OECD 405 (2002); Commission Directive 2004/73/EC B.5 (2004), OPPTS 870.2400 (1998)
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate TC
Description:	White powder
Lot/Batch #:	2009051501
Purity:	96.4 %
Stability of test compound:	At room temperature in the dark stable until May 15, 2011.
Vehicle and/or positive control:	None
Test animals:	
Species:	Rabbit
Strain:	Himalayan
Source:	[redacted]
Age:	Approx. 6.5 - 7.5 months
Sex:	Males
Weight at dosing:	2.5 - 2.8 kg
Acclimation period:	At least 20 days.

Diet/Food:	ssniff K-H V2333 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum* before and after the exposure period
Water:	Tap water, *ad libitum* before and after the exposure period
Housing:	Individual housing
Environmental conditions:	Temperature: 20 ± 3 °C Humidity: 30 - 70 % Air changes: no data 12 hours light/dark cycle

Study design and methods
In life dates: 2009-10-15 to 2009-10-29

Animal assignment and treatment:
The test was conducted using three young male adult Himalayan albino rabbits. The test was performed in a sequential manner, first using one animal. Since no corrosive or severe eye effects were observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.1 g of the solid test substance was applied into the conjunctival sac of the right eye of the rabbits. The lids were then gently held together for about one second. 1 hour after instillation the eyes were rinsed with 20 mL NaCl solution. The left eye remained untreated and served as the reference control. Eye reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC approximately 1, 24, 48 and 72 hours, as well as 4, 5, 6, 7 and 8 days after instillation. The animals were observed for mortality and clinical signs daily. Body weights were determined at beginning of the study and at termination.

Results and discussion
Mortality: No mortality occurred.
Clinical observations: No clinical signs of systemic toxicity were observed during the study.
Body weight: There were no effects on body weight noted.
Necropsy: No necropsy was performed.
Eye observations: Corneal opacity (grade 1) was observed in all animals 24 to 72 hours, in animal no. 2 until 4 days and in animal no. 1 until 7 days after instillation. The fluorescein test performed 24 hours after instillation demonstrated corneal staining in all animals. The fluorescein test performed 7 days after instillation demonstrated corneal staining only in animal no. 1.
Irritation of the iris (grade 1) was observed in all animals 24 hours, in animal no. 2 until 48 hours and in animal no. 1 until 72 hours after instillation.
Conjunctival redness (grade 1) was observed in all animals 60 minutes to 72 hours, in animal no. 1 until 4 days and in animal no. 2 until 5 days after instillation.
Chemosis (grade 1) was observed in all animals 60 minutes, in animal no. 2 until 24 hours and in animal no. 1 until 48 hours after instillation.
In addition, secretion was observed in all animals 60 minutes after instillation. There were no systemic intolerance reactions. All rabbits were free of ocular signs by Day 8 after instillation.
The group mean irritation scores (24 to 72 hours) were calculated to be 1.0 for corneal opacity, 0.7 for iris lesions, and 1.0 conjunctival redness and 0.3 for chemosis of the conjunctiva.
The individual scores for each time point, individual mean and group mean scores (24 to 72 hours) are presented in Table B.6.2-27.

Table B.6.2-27: Results of the eye irritation test

Animal	Scoring [h]	Cornea	Iris	Conjunctiva	
				Redness	Chemosis
Rabbit 1	1	0	0	1	1
	24	1	1	1	1
	48	1	1	1	1
	72	1	1	1	0
	Day 4	1	0	1	0
	Day 5	1	0	0	0
	Day 6	1	0	0	0
	Day 7	1	0	0	0
Mean (24, 48, 72 h)		1.0	1.0	1.0	0.7
Rabbit 2	1	0	0	1	1
	24	1	1	1	1
	48	1	1	1	0
	72	1	0	1	0
	Day 4	1	0	1	0
	Day 5	0	0	1	0
	Day 6	0	0	0	0
	Day 7	—	—	—	—
Mean (24, 48, 72 h)		1.0	0.7	1.0	0.3
Rabbit 3	1	0	0	1	1
	24	1	1	1	0
	48	1	0	1	0
	72	1	0	1	0
	Day 4	0	0	0	0
	Day 5	—	—	—	—
	Day 6	—	—	—	—
	Day 7	—	—	—	—
Mean (24, 48, 72 h)		1.0	0.3	1.0	0.0
Group means		1.0	0.7	1.0	0.3

Conclusion by the Notifiers
Based on the scores for cornea, iris and conjunctiva and according to the EU and OECD Globally Harmonized System (GHS) classification criteria, glyphosate TC is not to be classified for eye irritation.

Comment by RMS:
The study is considered supplementary: Based on the study desgin, it is not possible to conclude, that the test substance is not irritating to rabbit eyes, because the instilled test substance was washed out after one hour instead of 24 hours according to the current OECD Guideline 405. The results provided only additonal information.

Reference: IIA, 5.2.5/03
Report: [redacted] 1995, HR-001: Primary Eye irritation study in rabbits.
[redacted].
Report No.: IET 95-0034
Data owner: Arysta LifeScience
Date: 1995-06-29, unpublished
ASB2012-11430
Guidelines: U.S. EPA FIFRA Guideline Subdivision F
MAFF 59 NohSan No. 4200 (1985)

Deviations: None
GLP: yes
Acceptability: *Please see comment by RMS*

Materials and methods

Test material:	Glyphosate technical
Identification:	HR-001
Description:	White crystal
Lot/Batch #:	T-941209
Purity:	97.56 %
Stability of test compound:	Not mentioned in the report
Vehicle and/or positive control:	None
Test animals:	
Species:	Rabbit
Strain:	New Zealand White, Kbl:NZW
Source:	[redacted].
Age:	11 weeks
Sex:	females
Weight at dosing:	2.378 kg (Group A), 2.357 kg (Group B) and 2.426 kg (Group C)
Acclimation period:	Eleven days
Diet/Food:	Pellet Diet GC4 (Oriental Yeast Co., Ltd.)
Water:	Water filtrated and sterilized, *ad libitum*
Housing:	Individually in stainless steel cages.
Environmental conditions:	Temperature: 24 °C Humidity: 52.8 – 57.9 % Air changes: 15/hour 12 hours light/dark cycle

Study design and methods:
In life dates: 1995-04-28 – 1995-05-30

Animal assignment and treatment:
12 female specific pathogen free New Zealand rabbits were given a single ocular instillation of 0.1 g of technical glyphosate. The dose was instilled in the conjunctival sac of left eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of test substance. The left eyes of animals in the eye treated groups were irrigating with water at 30 seconds (3 animals) or 2 minutes (3 animals) after application. The right eye remained untreated. All animals were observed for primary eye irritation 1, 24, 48 and 72 hours, 4, 7, 10, 13, 16, 19 and 21 days after instillation. The cornea, iris and conjunctive were examined with a hand slit-lamp during a subsequent 21-day observation period. Body weights were measured prior to application, and after the final observation.

Results and discussion

Mortality: No mortality occurred.
Clinical observations: No clinical signs of systemic toxicity were observed during the study.
Body weight: All rabbits showed the expected body weight gain.
Necropsy: No necropsy was performed.
Eye observations: Results of the no eye irrigating group are summarised in the Table B.6.2-28.

Without eye irrigation
Irritation of cornea
At 1 hour after application, all animals showed score 2, the easily discernible translucent aera, details of iris slightly obscured. At 24 hours after application, one animal showed score 3, nacreous area, no details of iris visible, size of pupil barely discernible. These opacities remained until Day 21 in three animals.

Irritation of iris
At 1 hour after application, all animals showed score 1, the congestion and/or markedly deepened rugae of iris. The irritation disappeared by Day 10.

Irritation of conjunctivae
At 1 hour after application, all groups showed redness score 1, the definite hyperemia of some blood vessels. At 24 or 48 hours after application, score 2 redness of conjunctivae in all animals. These conjunctival irritations gradually began to decrease thereafter, and disappeared by Day 16.
At 1 hour after application, 4 animals showed chemosis score 2, the obvious swelling with partial eversion of lids. In addition, 2 animals showed score 3, the swelling with lids about half closed. The chemosis disappeared by Day 7.

With eye irrigation (30 seconds or 2 minutes after application)
The irridial and conjunctival irritations observed in irrigation group were almost the same as those in non-irrigating group, while the corneal irritation was slighter.

Animals in the irrigating groups showed reduced eye irritations and faster recovery as compared with animals of the non-irrigating group. Each of irrigation at 30 seconds or 2 minutes after application was effective for reduction of irritation and for recovery.

Table B.6.2-28: Results of the eye irritation without eye irrigation after application*

Animal	**Scoring [h]**	**Cornea**		**Iris**	**Conjunctiva**		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 1 (female)	1	2	4	1	1	2	3
	24	2	4	1	2	2	3
	48	2	4	1	2	2	3
	72	2	4	1	2	2	1
	Day 4	1	3	1	2	1	0
	Day 7	1	2	0	1	0	0
	Day 10	1	1	0	1	0	0
	Day 13	1	1	0	1	0	0
	Day 16	1	1	0	0	0	0
	Day 19	1	1	0	0	0	0
	Day 21	1	1	0	0	0	0
Mean (24, 48, 72 h)		2.0	---	1.0	2.0	2.0	---

Animal	**Scoring [h]**	**Cornea**		**Iris**	**Conjunctiva**		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 2 (female)	1	2	4	1	1	2	3
	24	3	4	1	2	2	3
	48	3	4	1	2	2	3
	72	2	3	1	2	1	1
	Day 4	2	2	1	2	1	0
	Day 7	1	2	0	1	0	0
	Day 10	1	2	0	1	0	0
	Day 13	1	2	0	1	0	0
	Day 16	1	2	0	0	0	0
	Day 19	1	2	0	0	0	0
	Day 21	1	2	0	0	0	0
Mean (24, 48, 72 h)		2.67	---	1.0	2.0	1.67	---
Rabbit 3 (female)	1	2	4	1	1	3	3
	24	2	4	1	2	3	3
	48	2	4	1	2	2	3
	72	2	4	1	2	2	3
	Day 4	2	3	1	2	1	0
	Day 7	1	2	1	1	0	0
	Day 10	1	2	0	1	0	0
	Day 13	1	2	0	1	0	0
	Day 16	1	2	0	0	0	0
	Day 19	1	1	0	0	0	0
	Day 21	1	1	0	0	0	0
Mean (24, 48, 72 h)		2.0	---	1.0	2.0	2.33	---
Rabbit 4 (female)	1	2	4	1	1	3	3
	24	2	4	1	2	3	3
	48	2	4	1	2	2	3
	72	2	3	1	2	2	1
	Day 4	2	2	1	2	1	1
	Day 7	1	2	0	1	0	0
	Day 10	1	2	0	1	0	0
	Day 13	1	2	0	1	0	0
	Day 16	0	0	0	0	0	0
	Day 19	0	0	0	0	0	0
	Day 21	0	0	0	0	0	0
Mean (24, 48, 72 h)		2.0	---	1.0	2.0	2.33	---
Rabbit 5 (female)	1	2	4	1	1	2	3
	24	2	4	1	2	2	3
	48	2	4	1	2	2	3
	72	2	4	1	2	2	1
	Day 4	1	2	1	2	1	0
	Day 7	1	2	0	1	0	0
	Day 10	1	1	0	1	0	0
	Day 13	0	0	0	0	0	0
	Day 16	0	0	0	0	0	0
	Day 19	0	0	0	0	0	0
	Day 21	0	0	0	0	0	0
Mean (24, 48, 72 h)		2.0	---	1.0	2.0	2.0	---

Animal	**Scoring [h]**	**Cornea**		**Iris**	**Conjunctiva**		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 6 (female)	1	2	4	1	1	2	3
	24	2	4	1	2	2	3
	48	2	2	1	2	2	2
	72	1	2	0	2	1	0
	Day 4	1	1	0	1	0	0
	Day 7	0	0	0	0	0	0
	Day 10	0	0	0	0	0	0
	Day 13	0	0	0	0	0	0
	Day 16	0	0	0	0	0	0
	Day 19	0	0	0	0	0	0
	Day 21	0	0	0	0	0	0
Mean (24, 48, 72 h)		1.67	---	0.67	2.0	1.67	---
Group means		2.1	---	1.0	2.0	2.0	---

* - according to the report

Table B.6.2-29: Results of the eye irritation with eye irrigation (30 seconds after application)*

Animal	**Scoring [h]**	**Cornea**		**Iris**	**Conjunctiva**		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 7 (female)	1	0	0	0	1	3	2
	24	1	3	1	1	2	3
	48	1	1	0	2	2	1
	72	1	1	0	1	1	1
	Day 4	0	0	0	1	0	0
	Day 7	0	0	0	0	0	0
Mean (24, 48, 72 h)		1.0	---	0.33	1.33	1.67	---
Rabbit 8 (female)	1	0	0	0	1	2	2
	24	1	2	0	1	2	2
	48	1	1	0	1	1	1
	72	0	0	0	1	0	0
	Day 4	0	0	0	1	0	0
	Day 7	0	0	0	0	0	0
Mean (24, 48, 72 h)		0.67	---	0.0	1.0	1.0	---
Rabbit 9 (female)	1	1	2	1	1	2	2
	24	1	3	1	2	2	3
	48	1	3	0	2	1	1
	72	1	2	0	1	1	1
	Day 4	0	0	0	1	0	0
	Day 7	0	0	0	0	0	0
Mean (24, 48, 72 h)		1.0	---	0.33	1.67	1.33	---
Group means		0.9	---	0.2	1.3	1.3	---

* - according to the report

Conclusion by the Notifiers

Based on the study results and on the and according to EU classification criteria the test substance glyphosate technical (HR-001) is to be classified as irritating to the eyes (Xi, R36). This corresponds to Category 2 according to the OECD Globally Harmonized System (GHS) classification criteria.

Comment by RMS:

The study is considered acceptable. The test substance was irritating to rabbit eyes.

Reference:	IIA, 5.2.5/04
Report:	[redacted] 2009, Expert Statement Glyphosate technical: Primary eye irritation study in rat [redacted] Data owner: Excel Report No.: C22897 Date: 2009-01-23, not published ASB2012-11431
Guidelines:	OECD 405 (2002) Council Regulation (EC) No 440/2008 (2008)
Deviations	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Executive Summary

A pH measurement was performed with the test item in a 1% (w/w) solution in purified water before the study initiation. The pH of the test item was found to be 1.93.
According to the OECD Guidelines 405 and Council Regulation (EC) No 440/2008 B.5: Physicochemical properties and chemical reactivity – Substances exhibiting pH extremes such as ≤ 2,0 may have strong local effects. If extreme pH is the basis for identifying a substance as corrosive or irritant to the eye, then its acid reserve (buffering capacity) may also be taken into consideration.
It is assumed that the test substance item has corrosive properties; therefore, no eye irritation study in rabbits with Glyphosate Technical was performed.
According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate technical is classified for eye irritation:

- Xi R41 (Risk of serious damage to eyes);
- Category 1, H318 (Causes serious eye damage).

Conclusion by the Notifiers

The eye irritation of the test material (glyphosate technical) was concluded to be positive. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate technical is classified for eye irritation: Category 1, H318 (Causes serious eye damage).

Comment by RMS:
The study is considered acceptable. Agreed on the statement provided by the Notifiers.

Reference:	IIA, 5.2.5/05
Report:	[redacted] 2009, Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits [redacted] Data owner: Helm AG Report No.: LPT 23914 Date: 2009-04-30, unpublished ASB2012-11432
Guidelines:	OECD 405 (2002) US EPA OPPTS 870.2400.

Deviations:	Personnel change of the head of the Quality Assurance Unit: until January 31, 2009: Dipl. Biol. S. Steuer as of February 1, 2009: Dr. med. vet. habil. K. R. Sultan. This minor deviation did not have any effect on the validity and integrity of the scientific results obtained in this study.
GLP	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate TC
Description:	White powder
Lot/Batch #:	20080801
Purity:	98.8 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	No vehicle used
Test animals:	
Species:	Rabbit
Strain:	Himalayan
Source:	[redacted]
Age:	Approx. 4 - 32.5 months
Sex:	Male
Weight at dosing:	Animal no. l : 4.0 kg Animal no. 2: 3.9 kg Animal no. 3: 4.1 kg
Acclimation period:	At least 20 adaptation days
Diet/Food:	Commercial diet, ssniffB K-H V2333 (ssniff Spezialdiäten GmbH) served as food. The food was available ad libitum before and after the exposure period.
Water:	Tap water, *ad libitum*
Housing:	The animals were kept singly in cages measuring 380 mm x 425 mm x 600 mm (manufacturer: Dipl. Ing. W. EHRET GmbH, 16352 Schönwalde, Germany).
Environmental conditions:	Temperature: 20 ± 3 °C Humidity: 30 - 70 % 12-hour light/dark cycle

Study design and methods

In life dates: 2009-02-02 to 2009-02-15

Animal assignment and treatment:
100 mg of the test item was administered into one eye each of three animals. The test item was placed into the conjunctival sac of the right eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of the material. The left eye, which remained untreated, served as a control.The test was performed initially using one animal. As no corrosive or severe irritant effects were observed in this animal, 2 further animals were employed 24 hours after start of the initial test.
1 hour after instillation the eyes were rinsed with 20 mL NaCl solution. The eyes were examined ophthalmoscopically with a slit lamp prior to the administration and l , 24, 48, 72 hours and 4 days after the administration. The eye reactions were observed and registered. 24 hours after administration, fluorescein was applied to the eyes before being examined to aid evaluation of the cornea for possible lesions.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: A single instillation of 100 mg glyphosate TC per animal into the conjunctival sac of the right eye of three rabbits caused the following changes:
Corneal opacity (grade 1) was observed in animal no. one 24 t o 72 hours and in animal no. three 24 and 48 hours after instillation.
The fluorescein test performed 24 hours after instillation revealed cornea1 staining in animal no. 1 and 3 (up to 1/4 of the surface).
Conjunctival redness (grade 1) was observed in all animals 60 minutes to 48 hours, in animal no. 1 until 72 hours after instillation.
Chemosis (grade 1) was observed in animal no. one 24 and 48 hours after instillation.
In addition, secretion was observed in all animals 60 minutes after instillation.
The irises were not affected by instillation of the test item.
There were no systemic intolerance reactions.

Conclusion by the Notifiers
According to the EC-Commission directive 67/548/EEC and its subsequent amendments on the approximation of the laws, regulations and administrative provision relating to the classification, packaging and labelling of dangerous substances and the results obtained under the present test conditions Glyphosate TC was non – irritating to eyes, hence, no labelling is required.

Comment by RMS:
The study is considered supplementary: Based on the study desgin, it is not possible to conclude, that the test substance is not irritating to rabbit eyes, because the instilled test substance was washed out after one hour instead of 24 hours according to the current OECD Guideline 405. The results provided only additonal information.

Reference: IIA, 5.2.5/06
Report: [redacted]2010, Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits
[redacted]
Data owner: Helm AG
Report No.: LPT 24606

	Date: 2010-01-06, unpublished ASB2012-11433
Guidelines:	OECD 405 (2002) US EPA OPPTS 870.2400.
Deviations:	No deviations from the Study Plan.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate TC
Description:	White powder
Lot/Batch #:	20090506
Purity:	97.3 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	No vehicle was used
Test animals:	
Species:	Rabbit
Strain:	Himalayan
Source:	[redacted]
Age:	Approx. 6 - 8 months
Sex:	Male
Weight at dosing:	At dosing Animal no. l : 2.5 kg Animal no. 2: 2.5 kg Animal no. 3: 2.7 kg
Acclimation period:	At least 20 adaptation days
Diet/Food:	Commercial diet, ssniffB K-H V2333 (ssniff Spezialdiäten GmbH) served as food. The food was available *ad libitum* before and after the exposure period.
Water:	Tap water, *ad libitum*
Housing:	The animals were kept singly in cages measuring 380 mm x 425 mm x 600 mm (manufacturer: Dipl. Ing. W. EHRET GmbH, 16352 Schönwalde, Germany).
Environmental conditions:	Temperature: 20 ± 3 °C Humidity: 30 - 70 % 12-hour light/dark cycle

Study design and methods

In life dates: 2009-10-26 to 2009-11-12

Animal assignment and treatment:
100 mg of the test item were administered into one eye each of three animals. The test item was placed into the conjunctival sac of the right eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of the material. The left eye, which remained untreated, served as a control. The test was performed initially using one animal. As no corrosive or severe irritant effects were observed in this animal, 2 further animals were employed 24 hours after start of the initial test.
1 hour after instillation the eyes were rinsed with 20 mL NaCl solution. The eyes were examined ophthalmoscopically with a slit lamp prior to the administration and 1, 24, 48, 72 hours and 4 to 7 days after the administration. The eye reactions were observed and registered.
24 hours and 7 days after administration, fluorescein was applied to the eyes before being examined to aid evaluation of the cornea for possible lesions.

Results and discussion
Mortality: There were no mortalities during the study.

Clinical observations: Corneal opacity (grade 1) was observed in all animals 24 to 72 hours, in animal no. 1 until 4 days and in animal no. 3 until 5 days after instillation. The fluorescein test performed 24 hours after instillation revealed corneal staining in all animals (1/2 to 3/4 of the surface). Irritation of the iris (grade 1) was observed in all animals 24 and 48 hours, in animal no. 3 until 72 hours after instillation. Conjunctival redness (grade 1 or 2) was observed in all animals 60 minutes to 4 days, in animal no. 3 until 6 days after instillation. Chemosis (grade 1) was observed in all animals 60 minutes and 24 hours after instillation. In addition, secretion was observed in all animals 60 minutes and 24 hours after instillation.There were no systemic intolerance reactions.

Conclusion by the Notifiers
According to the EC-Commission directive 67/548/EEC and its subsequent amendments on the approximation of the laws, regulations and administrative provision relating to the classification, packaging and labelling of dangerous substances and the results obtained under the present test conditions Glyphosate TC was non - irritating to eyes, hence, no labelling is required.

Comment by RMS:
The study is considered supplementary: Based on the study desgin, it is not possible to conclude, that the test substance is not irritating to rabbit eyes, because the instilled test substance was washed out after one hour instead of 24 hours according to the current OECD Guideline 405. The results provided only additonal information.

Reference: IIA, 5.2.5/07
Report: [redacted] 2009, Glyphosate – Acute Eye Irritation Study in Rabbits
[redacted]
Data owner: Helm AG
Report No.: 12172-08
Date: 2009-03-11, unpublished
ASB2012-11434

Guidelines:	US EPA OPPTS 870.2400 Equivalent to OECD 405 (2002).
Deviations:	Humidity was in the range of 33-92 % instead of 30-70 %. This deviation did not affect the study outcome
GLP:	yes
Acceptability:	*Please see comment by RMS.*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate Tech Grade Mixed 5-Batch
Description:	White powder
Lot/Batch #:	080704-1 thru 5
Purity:	96.4 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	No vehicle was used
Test animals:	
Species:	Albino rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Approx. 3 months
Sex:	2 males and 1 female (nulliparous and non-pregnant)
Weight at dosing:	Males: 2.200-2.400 kg; Female: 2.300 kg
Acclimation period:	5 days
Diet/Food:	PMI Feeds, Inc.™ Lab Rabbit Diet #5321, 8 oz. daily
Water:	Tap water, *ad libitum*
Housing:	Individual housing in suspended, wire bottom, stainless steel cages.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: 10 - 12/hour 12-hour light/dark cycle

Study design and methods
In life dates: 2008-11-10 to 2008-11-27

Animal assignment and treatment:
Healthy albino rabbits were released from quarantine. Both eyes of each animal were carefully examined within 24 hours prior to treatment with a fluorescein sodium ophthalmic solution and cobalt-filtered light. Both eyes of each animal were again carefully examined just prior to treatment, but without the fluorescein sodium ophthalmic solution. Only those animals without eye defects or irritation were selected for testing. On Day 0, a dose of 0.1 mL by volume (93.2 mg) of the undiluted test substance was placed into the conjunctival sac of the right eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which the test substance was dropped. The lids were gently held together for one

second to prevent loss of material. The untreated left eyes served as comparative controls. The grades of ocular reaction were recorded at 1, 24, 48 and 72 hours, and at 4, 7, 10, 14 and 17 days after treatment. The corneas of all treated eyes were examined immediately after the 24 hour observation with a fluorescein sodium ophthalmic solution. All treated eyes were washed with room temperature deionized water for one minute immediately after recording the 24-hour observation.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: The maximum average irritation score of 3 1.7, obtained at 24 hours after treatment, was used to rate
Glyphosate moderately irritating. Fluorescein staining was observed in two of three eyes at 24 hours after treatment and was not observed in any eyes on Day 10 after treatment.Toxicity categories are determined by the presence and duration of corneal involvement, iridic irritation, and positive conjunctival irritation. Any corneal involvement or iridic irritation with a score of 1 or more is considered positive. Any conjunctival irritation (redness or chemosis) with a score of 2 or more is considered positive.

Conclusion by the Notifiers
Based on the maximum average irritation score of 3 1.7, the test substance Glyphosate is rated moderately irritating. Since all positive effects had cleared on Day 10 after dosing, the test substance is assigned to Toxicity Category 11. No irritation was observed in any eyes on Day 17.

Comment by RMS:
The study is considered acceptable and the test substance is irritating to rabbit eyes. The Notifiers conclusion on irritating potential based on US EPA criteria: Toxicity Category II (not 11, obviously typing error) due to average irriation score of 31.7 in rabbit eyes

Reference:	IIA, 5.2.5/08
Report:	[redacted] 2005, Eye Irritation/Corrosion Effects in rabbits (Oryctolagus cuniculus) of Glyphosate 95 TC [redacted] Data owner: Helm AG Report No.: PSL 15277 Date: 2005-04-04, unpublished ASB2012-11435
Guidelines:	US EPA OPPTS 870.2400 OECD 405 (2002).
Deviations:	No deviations from Study Plan.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate Acid Technical
Description:	White crystalline powder

Lot/Batch #:	040205
Purity:	97.23 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	No vehicle was used.
Test animals:	
Species:	Rabbit
Strain:	New Zealand albino
Source:	[redacted]
Age:	Young adult
Sex:	Male
Weight at dosing:	No data given in the report
Acclimation period:	7 days
Diet/Food:	Pelleted Purina Rabbit Chow #5326
Water:	Tap water, *ad libitum*
Housing:	The animals were singly housed in suspended stainless steel caging with mesh floors which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals DHEW (NIH)*. Litter paper was placed beneath the cage and was changed at least three times per week
Environmental conditions:	Temperature: 18-22 °C 12-hour light/dark cycle

Study design and methods:
In life dates: 2004-05-26 to 2004-06-05

Animal assignment and treatment:
A primary eye irritation test was conducted with rabbits to determine the potential for Glyphosate Acid Technical to produce irritation from a single instillation via the ocular route. Under the conditions of this study, the test substance is classified as severely irritating to the eye. Prior to use, the test substance was ground to a powder. One-tenth of a milliliter (0.06 grams) of the ground test substance was instilled into the right eye of three healthy rabbits. The left eye remained untreated and served as a control. Ocular irritation was evaluated by the method of Draize *et al*.. One hour after test substance instillation, all three treated eyes exhibited cornea1 opacity, iritis, and conjunctivitis. The overall incidence and severity of irritation decreased gradually over time. All animals were free of ocular irritation by Day 10 (study termination).

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: All animals appeared active and healthy. Apart from the eye irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects or abnormal behavior. One hour after test substance instillation, all three treated eyes exhibited cornea1 opacity, iritis, and conjunctivitis. The overall incidence and severity of irritation decreased gradually over time. All animals were free of ocular irritation by Day 10 (study termination).

Conclusion by the Notifiers
Under the conditions of this study, Glyphosate Acid Technical is classified as severely irritating to the eye.

Comment by RMS:
The study is considered acceptable and the conclusion by the Notifiers is supported..

Reference:	IIA, 5.2.5/09
Report:	[redacted] 2008, Acute Eye Irritation/Corrosion Study in Rabbits with Glyphosate Technical [redacted] Data owner: Helm AG Report No.: RF-3996.312.599.07 Date: 2008-09-12, unpublished ASB2012-11436
Guidelines:	OECD 405 (2002).
Deviations:	The experimental phase initiation and experimental phase conclusion dates were updated.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate Technical
Description:	White powder
Lot/Batch #:	20070606
Purity:	98.05 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	No vehicle was used
Test animals:	
Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	18 weeks old
Sex:	One male and one female
Weight at dosing:	between 3.346 and 3.624 kg
Acclimation period:	5 to 6 days
Diet/Food:	Pelleted and autoclaved commercial diet for rabbits (*Guabi, Mogiana Alimentos S.A. - Brazil*)
Water:	Tap water, *ad libitum*
Housing:	The animals were housed individually in galvanised steel cages. Autoclaved wood shavings were placed in a tray below the cages to collect excrements.

Environmental conditions:	Temperature:	17 - 22 °C
	Humidity:	30 - 70 %
	Air changes:	10 - 15/hour
	12-hour light/dark cycle	

Study design and methods:
In life dates: 2008-05-26 to 2008-06-17
Animal assignment and treatment:
0.1 g of the test item was applied to the eye of each animal. The test item was applied into the conjunctival sac of the left eye of each animal after gently pulling the lower lid away from the eyeball. Following application, the eyelids were gently held together for about one second in order to prevent test item loss. The right eye that remained untreated was used as a negative control.
The test was performed initially using one animal for evaluation of any irritant/corrosive effect of the test item to the eye. Because some severe ocular reactions were observed in the initial test, only one additional animal was tested to confirm the response.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: The following additional ocular changes were noted to the animals during the observation period:
Rabbit #04: Blepharitis at the 48- and 72-hr time points.
Rabbit #05: Blepharitis at the 24-, 48- 72-hr, and 7- and 14-day time points; and a small raised off area on the corned surface in the right inferior quadrant at the 21-day time point.

Conclusion by the Notifiers:
Based on the "UN GHS / EU CLP Criteria for Classification (UN, 2009; EC, 2008)" the ocular Classification for Glyphosate Technical is "Category l Irreversible effects on the eye".

Comment by RMS:
The study is considered acceptable and the conclusion by the Notifiers is supported.

Reference:	IIA, 5.2.5/10
Report:	[redacted] 1988, Primary Eye Irritation Study of Glyphosate Batch/Lot/NBR No. XLI-55 in New Zealand White Rabbits [redacted] Data owner: Monsanto Monsanto Report No.: FD-88-29 Date: 1988-06-08, unpublished Z35395
Guidelines:	US EPA 81-4
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	XLI-55
Purity:	97.76 %
Stability of test compound:	Stored at room temperature
Vehicle and/or positive control:	None
Test animals:	
Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adult
Sex:	Not specified
Weight at dosing:	Between 2-3 kg
Acclimation period:	At least five days
Diet/Food:	NIH 09 Rabbit Ration certified feed (Zeigler Brothers, Gardners, PA, US), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in wire mesh cages
Environmental conditions:	Temperature: 20 – 23.9 ºC Humidity: 40 – 60 % Air changes: Not specified Light cycle: 12 hours light/dark cycle

Study design and methods

In life dates: 1988-04-11 to 1988-05-02

Animal assignment and treatment:

The test was conducted using six young adult New Zealand albino rabbits. The test substance (0.1 g) was instilled into one eye of each rabbit. The lower eyelid was pulled gently away from the eyeball to form a cup (conjunctival sac) and the test substance inserted therein. The lids were then held together for one second and released. Following scoring at 24 hours after dose administration, any residual material was rinsed from the eye with physiological saline. Treated and untreated eyes were examined at 1, 24, 48, and 72 hours, and 7, 14, and 21 days after test substance instillation. The cornea, iris, and conjunctiva were scored separately according to the Draize system. The animals were observed twice daily for mortality at least five hours apart. Body weights were obtained on study day 1 prior to dose administration and at death. At study termination, surviving animals were euthanized by intracardiac injection of sodium pentobarbital and discarded.

Results and discussion

Mortality: One rabbit was found dead 20 days after dose administration. Prior to death, this animal exhibited anorexia, and gross necropsy revealed a clear gel-like substance in the large intestine. These findings are consistent with mucoid enteropathy, a condition occasionally

noted in stock laboratory rabbits. Therefore, the death was considered spontaneous and unrelated to treatment.
Clinical observations: Not reported.
Body weight: Not reported.
Necropsy: Not reported.
Eye observations: At one hour after test substance instillation, all animals exhibited conjunctival irritation (redness, swelling, blistering and discharge). Corneal opacity was noted one hour after test substance instillation in four of six (4/6) animals. Corneal opacity and conjunctival irritation were noted in all rabbits at the 24, 48 and 72 hour ad 7 day examinations. Three rabbits exhibited pannus on the cornea; two eyes (iris) had sluggish reactions to light; one rabbit had prominent vascularization of the conjunctival and another animal had a blood-like discharge. Corneal opacity persisted through study termination (day 21) in three of five (3/5) rabbits. Of the remaining two rabbits, one exhibited slight conjunctival discharge at study termination and the other rabbit's treated eye appeared normal 14 days after dose administration.
The group mean irritation scores (24 to 72 hours) were calculated to be 2.1 for corneal opacity, 0.2 for iris lesions, 2.0 for conjunctival redness, and 2.6 for conjunctival chemosis. The individual scores for each time point, individual mean and group mean scores (24 to 72 hours) are presented in Table B.6.2-30.

Table B.6.2-30: Results of the Eye Irritation Test [a]

Animal No.	**Scoring [h]**	**Cornea**		**Iris**	**Conjunctivae**		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 1	1	2	1	0	2	2 [b]	2
	24	2	3	0	2	2 [b]	2
	48	3	1	0	2	2 [b]	2 [c]
	72	3 [d]	1	0	2	2 [b]	2
	7 days	3 [d]	1	0	2	1	0
	14 days	2	1	0	1	0	0
	21 days	2	1	0	0	0	0
Mean	24-72 h	2.7		0.0	2.0	2.0	
Rabbit 2	1	2	1	0	2	2 [b]	3
	24	2	2	0	2	4 [b]	3
	48	2	2	0	2	4 [b]	2
	72	1	1	0	2	2 [b]	1
	7 days	2	1	0	1	1	0
	14 days	2	1	0	0	0	1
	21 days	0	0	0	0	0	1
Mean	24-72 h	1.7		0.0	2.0	3.3	
Rabbit 3	1	2	1	0	2	2 [b,e]	2
	24	2	2	1	2	4 [b,e]	2
	48	2	1	1	2	3 [b,e]	2
	72	2	1	1	2	3 [b,e]	2
	7 days	3 [d]	2	1	3	2 [b]	2
	14 days	2	1	1	1	0	0
	21 days	2	1	0	0	0	0
Mean	24-72 h	2.0		1.0	2.0	3.3	

Animal No.	Scoring [h]	Cornea		Iris	Conjunctivae		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 4	1	0	0	0	2	3 [b]	3
	24	1	4	0	2	4 [b]	3
	48	1	3	0	2	2 [b]	2
	72	1	3	0	2	2 [b]	2
	7 days	3	1	0	2	2	1
	14 days	0	0	0	0	0	0
	21 days	0	0	0	0	0	0
Mean	24-72 h	1.0		0.0	2.0	2.7	
Rabbit 5	1	0	0	0	2	2 [b]	2
	24	2	2	0	2	2 [b]	2
	48	2	2	0	2	2 [b]	2
	72	3	1	0	2	2 [b]	1
	7 days	4 [d]	1	1	2	2	1
	14 days	3 [d]	1	1	2	1	0
	21 days	3 [d]	1	0	1	0	0
Mean	24-72 h	2.3		0.0	2.0	2.0	
Rabbit 6	1	2	1	0	2	2 [b]	3
	24	2	2	0	2	2 [b]	2
	48	3	1	0	2	2 [b]	2
	72	3	1	0	2	2 [b]	0
	7 days	2	1	0	1	1	0
	14 days	2	1	0	0	0	0
	21 days	- [f]	-	-	-	-	-
Mean	24-72 h	2.7		0.0	2.0	2.0	
Group mean	24-72 h	2.1		0.2	2.0	2.6	

[a] Scores for treated eyes; untreated eyes appeared normal at all times.
[b] Mucus membrane of the eyelid appeared blistered.
[c] Blood-like discharge noted.
[d] Pannus on the cornea.
[e] Prominent vascularisation of the conjunctiva.
[f] Animal found dead 20 days after dose administration.

Conclusion by the Notifiers

Based on the study results and on the and according to EU classification criteria the test substance glyphosate is to be classified as risk of serious damage to the eyes (Xi, R41). This corresponds to Category 1 according to the OECD Globally Harmonized System (GHS) classification criteria.

Comment by RMS:

The study is considered acceptable and the conclusion by the Notifiers is supported.

Reference: IIA, 5.2.5/11

Report: [redacted] 1997 Glyphosate Acid: Eye Irritation to the Rabbit [redacted], [redacted]
Data owner: Syngenta
Report No.: [redacted]/P/5138
Date: 1997-03-18, not published
TOX2000-1986

Guidelines: OECD 405 (2002): OPPTS 870.2400 (1998): 2004/73/EC B.5 (2004)

Deviations: None.
GLP: yes
Acceptability: *Please see comment by RMS*

Materials and methods

Test Material:	Glyphosate acid
Description:	Technical, white solid
Lot/Batch number:	P24
Purity:	95.6 % w/wi
CAS#:	Not reported
Stability of test compound:	The test substance was used within the expiry date
Vehicle and/or positive control:	None
Test Animals:	
Species	Rabbit
Strain	New Zealand White albino
Age/weight at dosing	Young adult / 2951-3702 g
Source	[redacted].
Housing	Individually in aluminium sheet cages in racks suitable for animals of this strain and the weight range expected during the course of the study.
Acclimatisation period	At least 6 days
Diet	STANRAB SQC, (Special Diet Services Limited, Stepfield, Witham, Essex, UK) *ad libitum*
Water	Mains water *ad libitum*
Environmental conditions	Temperature: 17±2 °C Humidity: 40-70 % Air changes: Approximately 25/hour Photoperiod: 12 hours light / 12 hours dark

Study design and methods
In-life dates: Start: 22 May 1996 End: 6 July 1996

Animal assignment and treatment: Initially, the test substance (approximately 100mg) was applied into the conjunctival sac of the left eye of one rabbit by gently pulling the lower lid away from the eyeball to form a cup into which the test substance was dropped. The lids were then gently held together for 1-2 seconds after which the animal was released. The other eye was untreated (control eye).
When the eye irritation potential had been fully assessed in the first animal, the test substance was applied into the test eye of the remaining five animals, as described previously.
As the initial pain reaction of the first rabbit was moderate and the irritation was less than severe, the eyes of the remaining rabbits were pre-treated with five drops of local anaesthetic (OPHTHAINE, 0.5% proparacaine hydrochloride solution) at three minute intervals between each drop.
Both eyes of each rabbit were examined within the twenty-four hours prior to dosing. The examination consisted of a visual assessment with the aid of fluorescein and only rabbits without any apparent eye defects or ocular irritation were used.
Immediately after the application of the test substance, an assessment of the initial pain reaction of the rabbit was made using a six-point scale.

The eyes were examined and the Draize scale was used to assess the grade of ocular reaction approximately one hour and 1, 2, 3, 4, 7 and 8 days after application where necessary. In addition, as an aid in the assessment of corneal damage, fluorescein staining was used at all readings from 1 day after application. A modified form of the Kay and Calandra system was used to interpret and classify the numerical scores.

Results and discussion

No deaths occurred. No systemic signs of toxicity were noted during the study.

Application into the eye caused moderate initial pain in the first animal dosed, therefore the subsequent five animals were pre-treated with the local anaesthetic OPHTHAINE prior to dosing. The group initial pain reaction was none to moderate (class 0-3 on a 0-5 scale).

Corneal effects, consisting of slight to mild opacity affecting up to the entire cornea, were seen in all animals during the first two days, persisting to day 4 in five rabbits. Slight iritis was seen in all animals during the first two days, persisting to day 3 in two rabbits. Conjunctival, effects consisting of slight to moderate redness, slight to mild chemosis and slight to severe discharge, were seen in all animals up to day 4.

Additional observations included mucoid discharge, eye closed, irregular corneal surface, convoluted eyelids, erythema of the upper and/or lower eyelids, raised corneal opacity, Harderian gland discharge and nictitating membrane partially haemorrhagic.

All signs of irritation had completely regressed in five animals 7 days after application. Slight conjunctival redness was seen in the remaining animal on day 7; the animal had completely recovered by day 8.

Table B.6.2-31: Eye irritation scores of glyphosate acid (95.6 % w/w) according to the Draize scheme

Time	**Cornea**						**Iris**						**Conjunctiva**											
													Redness						Chemosis					
Animal number	17	18	19	7	8	9	17	18	19	7	8	9	17	18	19	7	8	9	17	18	19	7	8	9
after 1 hour	0	0	0	1	1	1	0	0	0	0	0	0	1	2	2	1	2	2	2	2	1	1	1	2
after 24 hours	1	1	2	2	1	2	0	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
after 48 hours	1	1	1	2	1	2	1	1	1	1	1	1	2	2	2	2	2	2	1	1	2	2	2	2
after 72 hours	0	1	1	2	1	2	0	0	0	0	1	1	1	2	2	2	2	2	1	1	1	2	2	2
mean scores 24-72h	1.3						0.7						1.9						1.4					
after 4 days	0	1	1	1	1	1	0	0	0	0	0	0	1	1	1	2	2	2	0	1	1	1	1	2
after 7 days	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	-0	0
after 8 days	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-	0	-

Conclusion by the Notifiers

Glyphosate acid is a moderate irritant (class 5 on a 1-8 scale) to the rabbit eye.

Comment by RMS:

The study is considered acceptable.The test substance revealed irritating properties to rabbit eyes based on Draize scheme as seen in Table B.6.2-31 [Notifiers conclusion based on modified form of Kay and Chalandra system as mentioned above].

Reference:	IIA, 5.2.5/12
Report:	[redacted]2007, Glyphosate Technical Material: Primary Eye Irritation Study In Rabbits [redacted] Data owner: Syngenta Report No.: B02788 Date: 2007-03-26, not published ASB2012-11437
Guidelines:	OECD 405 (2002): OPPTS 870.2400 (1998): 2004/73/EC B.5 (2004)
Deviations:	None.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test Material:	Glyphosate Technical Material
Description:	Technical, white powder
Lot/Batch number:	0507
Purity:	96.1 % w/w Glyphosate acid
CAS#:	
Stability of test compound:	Stable under storage conditions of room temperature (range of 20 ^{o}C ± 5 ^{o}C), protected from light and humidity.
Vehicle and/or positive control:	The test substance was undiluted.
Test Animals:	
Species	Rabbit
Strain	New Zealand White (SPF)
Age/weight at dosing	Male: 11-12 weeks / 2640 g; Females: 14-16 weeks / 2990 and 3001 g
Source	[redacted]
Housing	Individually in stainless steel cages equipped with feed hoppers and drinking water bowls.
Acclimatisation period	5/6 days
Diet	Pelleted standard Provimi Kliba 3418 rabbit maintenance diet *ad libitum.*
Water	Community tap water *ad libitum*
Environmental conditions	Temperature: 17-23 °C Humidity: 30-70 % Air changes: 10-15 per hour. Photoperiod: 12 hours light, 12 hours dark.

Study design and methods

In-life dates: Start: 27 December 2006 End: 4 January 2007

Animal assignment and treatment: On the day of treatment, 0.1 g of Glyphosate Technical Material was placed into the conjunctival sac of the left eye of each animal after gently

pulling the lid away from the eyeball. The lids were then gently held together for about one second to prevent loss of the test substance. The right eye remained untreated and acted as the reference control. The treated eyes were not rinsed after instillation of the test substance.
As it was suspected that the test substance might produce irritancy, a single female was treated first. As neither a corrosive effect nor a severe irritant effect was observed after 1- and 24-hour examinations, the test was completed using the two remaining animals.
The ocular reaction (ie. corneal opacity, iridic effects, conjunctivae and chemosis) was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, 29 April 2004, at approximately 1, 24, 48 and 72 hours, as well as 7 days after instillation. Additionally, ocular discharge, reddening of the sclerae and staining of conjunctivae, sclerae and cornea by the test substance was assessed according to the scheme presented in the guideline.
The animals were observed daily throughout the study for viability, mortality and clinical signs. Bodyweights were measured at the start of acclimatisation, on the day of treatment and at termination of the observation period.

Results and discussion
No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred. No abnormal findings were observed in the cornea or iris of any animal at any of the measurement intervals.
Moderate reddening of the conjunctivae was noted in all animals at the 1-hour reading and persisted in one animal as slight until the 48-hour reading and in two animals as moderate to slight until 72 hours after treatment. Slight to obvious swelling (chemosis) was observed in all three animals at the 1-hour reading and persisted as slight in one animal until the 48-hour reading and in one animal as moderate until 24 hours after instillation. Slight to moderate reddening of the sclerae was noted in all animals at the 1- and 24 hour reading and persisted as slight reddening until the 48-hour reading. Slight to moderate ocular discharge was seen in all animals at the 1-hour reading and persisted as slight to moderate discharge in two animals at the 24-hour reading. No abnormal findings were observed in the treated eye of any animal 7 days after treatment, the end of the observation period for all animals. No staining of the treated eyes produced by the test substance was observed and no corrosion of the cornea was observed at any of the reading times.

Table B.6.2-32: Eye irritation scores of Glyphosate Technical according to the numerical scoring system listed in the Commission Directive 2004/73/EC, 29 April 2004

Time		**Cornea**			**Iris**			**Conjunctiva**					
								Redness			Chemosis		
	Animal number	31M	32F	33F	31M	32F	33F	31M	32F	33F	31M	32F	33F
after 1 hour		0rd	0rd	0rd	0	0	0	2	2	2	1	2	2
after 24 hours		0^{r}	0rd	0rd	0	0	0	1	2	2	0	1	2
after 48 hours		0^{r}	0^{r}	0^{r}	0	0	0	1	2	2	0	0	1
after 72 hours		0	0	0	0	0	0	0	1	1	0	0	0
mean scores 24-72h		0	0	0	0	0	0	0.67	1.67	1.67	0	0.33	1
After 7 days		0	0	0	0	0	0	0	0	0	0	0	0

M = male, F = female, d = discharge, r = reddening of the sclerae.

Conclusion by the Notifiers
The instillation of Glyphosate Technical Material into the eye resulted in mild, early-onset and transient ocular changes. These effects were reversible and were no longer evident 7 days

after treatment. Thus, the test item did not induce significant or irreversible damage to the rabbit eye.

Comment by RMS:
The study is considered acceptable and the test substance is irritating to eyes.

Reference:	IIA, 5.2.5/13
Report:	[redacted] 2011, Glyphosate technical: Acute eye irritation study in rabbits [redacted] Data owner: Syngenta Report No.: 10/218-005N Date: 2011-05-13, not published ASB2012-11438
Guidelines:	OECD 405 (2002): OPPTS 870.2400 (1998): 2004/73/EC B.5 (2008)
Deviations:	None.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test Material:	Glyphosate technical
Description:	Technical, dry white powder
Lot/Batch number:	569753(BX20070911)
Purity:	96.3 % w/w Glyphosate technical
CAS#:	Not reported
Stability of test compound:	Stable under storage conditions (room temperature range <30 °C), recertification date end August 2011
Vehicle and/or positive control:	None
Test Animals:	
Species	Rabbit
Strain	New Zealand White
Age/weight at dosing	Approximately 12 weeks / 3035 g
Source	[redacted]
Housing	Individually in metal cage
Acclimatisation period	13 days
Diet	Purina Base – Lap gr. diet (AgribrandsEurope Hungary PLC, H-5300 Karcag, Madarasi út, Hungary) *ad libitum*
Water	Municipal tap water *ad libitum*
Environmental conditions	Temperature: 20±3 °C Humidity: 24-64 % Air changes: 15-20/hour Photoperiod: 12 hours light/12 hours dark

Study design and methods
In-life dates: Start: 21 December 2011 End: 22 December 2011

Animal assignment and treatment: The primary eye irritation potential of Glyphosate Technical (96.3 % w/w glyphosate technical) was investigated according to OECD test guideline no. 405. Approximately 1 hour before the start of the test, the eyes of the provisionally selected test rabbits were examined for evidence of ocular irritation or defect using a hand-held slit-lamp. The animal used in the study was free of ocular damage. Initially, a single rabbit was treated.
An amount of 0.1 g of the test material was placed into the conjunctival sac of the left eye, formed by gently pulling the lower lid away from the eyeball. The upper and lower eyelids were held together for about 1 second immediately after treatment, to prevent loss of the test material, and then released. The right eye remained untreated and was used for control purposes.
Immediately after administration of the test material, an assessment of the initial pain reaction was made according to 0-5 scale. Following review of the ocular responses produced in the first treated animal, no further animals were treated. The treated eyes were not rinsed after instillation.
The ocular reaction (i.e. corneal opacity, iridic effects, conjunctivae and chemosis) was assessed approximately 1 and 24 hours following treatment, according to the numerical evaluation described by Draize.
The treated eye was further examined using 2 % fluorescein solution before treatment and then 24 hours after treatment.
Additionally, any other signs of eye irritation were recorded.

Results and discussion
No clinical signs of systemic toxicity were observed in the animal during the study and no mortality occurred. The body weight was considered to be within the normal range of variability.
An initial pain reaction score of 3 (on a 0-5 scale) was recorded.
Conjunctival redness, chemosis and conjunctival discharge, as well as corneal opacity, were observed in the rabbit 1 and 24 hours after application. Additionally, corneal erosion, redness of the conjunctiva with pale areas, pink, clean ocular discharge, oedema of the eyelids, a few black points on the conjunctiva and dry surface of the eye were noted one hour after the treatment. Fluorescein staining was positive at the 24 hours observation.
Based on the symptoms, no further animals were dosed and the study was terminated after the 24 hour observation (Regulation (EC) No 440/2008).

Table B.6.2-33: Eye irritation scores of Glyphosate Technical according to the Draize scheme

Time	**Cornea**	**Iris**	**Conjunctiva**		
			Redness	Chemosis	Discharge
after 1 hour	2	0	2	3	3
after 24 hours	3	1	3	4	3

Conclusion by the Notifiers:
Under the conditions of this study, Glyphosate Technical is classified as corrosive to the eye.

Comment by RMS:
The study is considered acceptable. The conclusion by the Notifiers is supported.

B.6.2.6 Skin sensitisation

For the previous evaluation a variety of studies on glyphosate acid and two studies on glyphosate salts (IPA) regarding skin sensitising were available. For the current re-evaluation, several additional studies for skin sensitising effects on glyphosate acid were provided: 8 Magnusson-Kligman Tests and 3 Buehler Tests in guinea pigs and two Local lymph node assays in mice. No further studies on glyphosate salts were provided.

Comment by NL during peer review:
Minor comment: throughout the document the Magnusson & Kligman test for skin sensitisation is inconsequently referred to as "Magnusson & Kligman", Magnusson-Kligman", Magnusson and Kligman", or "Magnusson & Kligman".

RMS' response: Agreement, a consistent nomenclature will be used in a revised RAR. However, this is necessary only in few cases, because the headings of the study reports should not be corrected.

Table B.6.2-34: Summary of skin sensitisation studies with glyphosate acid

Reference (Data owner)		Species Strain	Number and /or sex of animals	Purity [%]	Exposure conditions	Test Method	Results
Studies from the 2001 evaluation	Annex B-5.2.7.1, Glyphosate Monograph [redacted] 1994 (Herbex) TOX9500250	Guinea pig Dunkin Hartley	15 ♀	95	Induction: 1% w/v in arachis oil; challenge: 25% w/w or 50% w/w in arachis oil	MKT	Not sensitising
	Annex B-5.2.7.1, Glyphosate Monograph [redacted] 1993 (Luxan) TOX9650652	Guinea pig English	48 (both sexes)	≥ 95	Intradermal induction: 5% in propylene glycol; topical: 50% in petrolatum	MKT	Not sensitising
	Annex B-5.2.7.1, Glyphosate Monograph [redacted] 1991 (AGC) TOX9551796	Guinea pig Dunkin Hartley	38 ♀	Not stated	Intradermal induction: 0.1% (w/v) in water; topical: 50% (w/v) in water; challenge: 25% (w/w) in water	MKT	Not sensitising
	Annex B-5.2.7.1, Glyphosate Monograph [redacted], 1989e (CHE) TOX9552343	Guinea pig Dunkin Hartley	46 ♀	98.6	induction: 10% in water; challenge: 25% in water	MKT	Not sensitising

	Reference (Data owner)	Species Strain	Number and /or sex of animals	Purity [%]	Exposure conditions	Test Method	Results
Studies not reviewed in the 2001 evaluation	IIA 5.2.6/01 [redacted] 2007 (NUF) ASB2012-11439	Guinea pig	20 ♀/test 10 ♀/control	95.1	Intradermal induction: 3% (w/v) in PEG-300; topical induction: 50% (w/v) in PEG-300; challenge: 25% (w/v) in PEG-300	MKT	Not sensitising
	IIA 5.2.6/02 [redacted] 2010 (HAG) ASB2012-11440	Guinea pig, Dunkin Hartley	15 ♀ (+ 20 for positive control)	96.4	Intradermal induction: 0.01% in water; topical induction: 50%; challenge: 25%	MKT	Not sensitising
	IIA 5.2.6/03 [redacted] 1995 (ALS) ASB2012-11441	Guinea pig Hartley	60 ♀	97.56	Intradermal induction: 5% (w/v) in paraffin oil, topical induction: 25% (w/v) in white petrolatum; challenge: 25% (w/w) in white petrolatum	MKT	Not sensitising
	IIA 5.2.6/04 [redacted] 2009 (EXC) ASB2012-11442	Guinea pig	15 ♂	96.66	Intradernal induction: 10% (w/w) in purified water; topical induction: 50% (w/w) in purified water; challenge: 15% (w/w) in purified water	MKT	Not sensitising
	IIA 5.2.6/05 [redacted] 2009 (HAG) ASB2012-11443	Guinea pig	15 ♂ (+ 20 for positive control)	98.8	Intradermal induction: 0.01% in water, topical induction: 50%; challenge: 50%	MKT	Not sensitising
Studies not reviewed in the 2001 evaluation	IIA 5.2.6/06 [redacted] 2010 (HAG) ASB2012-11444	Guinea pig	15 ♂ (+ 20 for positive control)	97.3	Intradermal induction: 0.5% in water; topical induction: 50%; challenge: 25%	MKT	Not sensitising
	IIA 5.2.6/07 [redacted] 2009 (HAG) ASB2012-11445	Guinea pig	15 ♂ 15 ♀	96.4	400 mg moistened induction and challange	Buehler Test	Not sensitising
	IIA 5.2.6/08 [redacted] 2005 (HAG) ASB2012-11446	Guinea pig	30 ♂ ♀	97.23	70 % in water induction and challange	Buehler Test	Not sensitising
	IIA 5.2.6/09 [redacted] 2008 (HAG) ASB2012-11447	Guinea pig	30 ♂	98.05	50 % inductions and challange	Buehler Test	Not sensitising

	Reference (Data owner)	Species Strain	Number and /or sex of animals	Purity [%]	Exposure conditions	Test Method	Results
Studies not reviewed in the 2001 evaluation	IIA 5.2,6/10 [redacted] 2006 (NUF) ASB2012-11448	Guinea pig	20 ♀/test 10 ♀/control	95.7	Intradermal induction: 0.195% (w/v) in isotonic saline; topical induction: 60% (w/v) in water; challenge: 60% (w/v) & 30% (w/v) in water	MKT	Not sensitising
	IIA 5.2.6/11 [redacted] 1996 (SYN) TOX2000-1987	Guinea pig	20 ♀/test 10 ♀/control	95.6	Intradermal induction: 0.1% (w/v) in water; topical induction: 75% (w/v) in water; challenge: 75% (w/v) & 30% (w/v) in water	MKT	Not sensitising
	IIA 5.2.6/12 [redacted] 2007 (SYN) ASB2012-11449	Mouse CBA	4 ♀/group	96.1	Glyphosate acid dose levels: 0, 10, 25, 45 (% w/v) Hexylcinnamaldehyde positive control demonstrated sensitivity of study	LLNA	Not sensitising
	IIA 5.2.6/13 [redacted]o, 2011 (SYN) ASB2012-11450	Mouse CBA	4 ♀/group	96.3	Glyphosate acid dose levels: 0, 10, 25, 50 (% w/v) Hexylcinnamaldehyde positive control demonstrated sensitivity of study	LLNA	Not sensitising

MKT = Magnusson Kligman Maximisation Test
LLNA = Local Lymph Node Assay

Table B.6.2-35: Summary of skin sensitisation studies with glyphosate salts (provided by the Notifiers)

	Reference (Data owner)	Species Strain	Number and /or sex of animals	Purity [%]	Exposure conditions	Test Method	Result
Studies from the 2001 evaluation	Annex B.5.2.7.2, Glyphosate Monograph [redacted] 1994 (MON / CHE) TOX9552345	Guinea pig, Dunkin Hartley	38 ♀	IPA 62.2 %	Induction: 25 % w/v (injection) and 100 % (topical); challenge: 100 %	MKT	Not sensitizing
	Annex B.5.2.7.2, Glyphosate Monograph [redacted] 1984* (MON) Z35238	Guinea pig	16	IPA 53.8 %	Induction and challange: 100 %	Modified Buehler Test	Not sensitising

* Study was considered supplementary data in the 2001 EU glyphosate evaluation
MKT = Magnusson Kligman Maximisation Test

Tier II summaries are only presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.
For details regarding studies reviewed during the 2001 EU evaluation please refer to the Monograph.

Reference:	IIA, 5.2.6/01
Report:	[redacted] 2007, Glyphosate Technical (NUP 05068): Contact Hypersensitivity in Albino Guinea Pigs, Maximisation Test [redacted] [redacted] Study No.: B02316 Data owner: Nufarm Date: 2007-03-06, unpublished ASB2012-11439
Guidelines:	OECD 406 (1992); Commission Directive 96/54/EC B.6 (1996), JMAFF guideline 2-1-6 (2005)
Deviations:	No
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate technical
Identification:	NUP 05068
Description:	Solid
Lot/Batch #:	200609062
Purity:	95.1 %
Stability of test compound:	Stable under storage conditions (20 ± 5°C), light protected; Expiry date: 2008-09-14
Vehicle and/or positive control:	Polyethylene glycol 300 (PEG 300)
Test animals:	
Species:	Guinea pig
Strain:	Albino Dunkin Hartley, CRL:(HA)BR, SPF
Source:	[redacted]
Age:	5 - 6 weeks
Sex:	female
Weight at dosing:	Pre-tests: 362 – 372 g; main test: 337 – 381 g
Acclimation period:	Main test: at least 10 days
Diet/Food:	Pelleted standard Provimi Kliba 3418 guinea pig breeding / maintenance diet (Provimi Kliba AG, CH-Kaiseraugust), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in Makrolon type 4 cages with standard softwood bedding

Environmental conditions:	Temperature:	22 ± 3 °C
	Humidity:	30 - 70 %
	Air changes:	10 - 15/hour
	12 hours light/dark cycle	

Study design and methods:
In life dates: 2007-01-10 to 2007-02-15

Animal assignment and treatment:
Glyphosate technical (NUP 05068) was tested for its sensitising effect on the skin of the guinea pig using the Maximisation test according to Magnusson and Kligman. Female Dunkin Hartley guinea pigs, young adults with body weights ranging from 337 to 381 g were used. The test substance concentrations for the main study were selected based on the results of the pre-testing performed with three animals. The main study was performed in 20 test animals and 10 control animals.
The induction phase consisted of an intradermal injection at day 1 and an epidermal application on day 8. On day 1 the test substance was injected (0.1 mL/site) into the clipped dorsal skin from the scapular region at a concentration of 3 % either in PEG 300 or in a 1:1 (v/v) mixture of Freund´s Complete Adjuvant and physiological saline. On day 8 the test substance was topically applied at a concentration of 50 % to the clipped and shaved skin of the scapular area and covered with an occlusive dressing, which was left in place for 48 hours. The reaction sites were assessed 24 and 48 hours after removal of the bandage.
The challenge was conducted on day 22 by an occlusive patch containing 0.2 mL of the test material at a concentration of 25 % in PEG 300 that was applied to the clipped and shaved left flank of each animal for 24 h. The clipped and shaved right flank of each animal was treated in the same way with the vehicle only (PEG 300). 24 and 48 hours after removal of the dressing skin reactions were scored according the Magnusson and Kligman grading scale.
Body weights were determined at the first day of treatment of the main study and at termination. Mortality and clinical signs were recorded daily during the study period.
A positive control (reliability check) with a known sensitizer was not included in this study. However, a separate study was performed from June to August 2006 in the laboratory. The positive controls with alpha-hexylcinnamaldehyde (3 % in PEG 300) showed that the chosen guinea pig strain was able to detect sensitizing compounds under the laboratory conditions chosen.
Evaluation criteria for classification as a potential skin sensitizer:
At the 24-hour and/or 48-hour reading, 30 % or more of the test animals exhibit a positive response (scores ≥ 1) in the absence of similar results in the vehicle control group.

Results and discussion
Mortality: No deaths occurred.
Clinical observations: No signs of systemic toxicity were observed.
Body weight: All animals showed the expected gain in body weight with the exception of one of the pre-test animals that did not gain body weight between the day of epidermal application and day of sacrifice one week later.
Necropsy: No necropsy was performed.
Skin reactions: No skin reactions were observed 24 or 48 h after the challenge treatment with glyphosate technical (NUP 05068) in the control or test group.

Conclusion by the Notifiers:

Based on the EU classification criteria, glyphosate technical (NUP 05068) is not to be classified for skin sensitization. According to the OECD Globally Harmonized System (GHS) classification criteria glyphosate technical (NUP 05068) is also not classified for skin sensitization.

Comment by RMS:
The study is considered acceptable and revealed no evidence for skin sensitisation potential of the test substance.

Reference:	IIA, 5.2.6/02
Report:	[redacted] 2010, Examination Of Glyphosate TC In The Skin Sensitisation Test In Guinea Pigs According To Magnusson And Kligman (Maximisation Test) [redacted] Data owner: Helm AG Report No.: 24879 Date: 2010-01-22, unpublished ASB2012-11440
Guidelines:	OECD 406 (1992); Commission Directive 96/54/EC B.6 (1996), OPPTS 870.2600 (1998)
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate TC
Description:	White powder
Lot/Batch #:	2009051501
Purity:	96.4 %
Stability of test compound:	At room temperature in the dark stable until May 15, 2011.
Vehicle and/or positive control:	Purified water
Test animals:	
Species:	Guinea pig
Strain:	Dunkin Hartley
Source:	[redacted]
Age:	32 days
Sex:	Female
Weight at dosing:	312 - 355 g; positive control group: 249 - 317 g
Acclimation period:	At least 5 days.
Diet/Food:	ssniff Ms-H V2333 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum*
Water:	Tap water, *ad libitum*

Housing:	In pairs in Makrolon cages (MZK 80/25) with granulated textured wood bedding
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 55 ± 15 % Air changes: no data 12 hours light/dark cycle

Study design and methods:
In life dates: 2009-10-15 to 2009-11-28

Animal assignment and treatment:
Glyphosate TC was tested for its sensitising effect on the skin of the guinea pig using the Maximisation test according to Magnusson and Kligman. Female Dunkin Hartley guinea pigs, young adults with body weights ranging from 312 to 355 g were used. The test substance concentrations for the main study were selected based on the results of the pre-testing performed with eight animals. The main study was performed in 10 test animals, 5 control animals and 20 positive control animals.
The induction phase consisted of an intradermal injection at Day 0 and an epidermal application on Day 7. On Day 0 the test substance was injected (0.1 mL/site) into the clipped dorsal skin of the shoulder region at a concentration of 0.01% in *aqua ad iniectabilia*, together with injections of Freund´s Complete Adjuvant in physiological saline, or test item in a 1:1 (v/v) mixture of Freund´s Complete Adjuvant and physiological saline.
On Day 6 the skin was shaved and coated with 0.5 mL sodium laurylsulfate 10 % in vaseline in order to induce a local irritation. On Day 7 the test substance was topically applied at a concentration of 50 % to the clipped and shaved skin of the shoulder region using the patch technique. The patch was left occluded in place for 48 hours.
The challenge was conducted on Day 21 by an occlusive patch at a concentration of 25 % in *aqua ad iniectabilia* which was applied to the clipped and shaved left flank of each animal for 24 h. The clipped and shaved right flank of each animal was treated in the same way with the vehicle alone. 24 and 48 hours after removal of the dressing skin reactions were scored according the Magnusson and Kligman grading scale.
The animals of the positive control group were treated with a 2 % benzocaine solution intracutaneously in the induction phase and with a 5 % solution topically in the induction phase and at challenge.
Body weights were determined at the first day of treatment of the main study and at termination. Mortality and clinical signs were recorded daily during the study period.
Evaluation criteria for classification as a potential skin sensitizer:
At the 24-hour and/or 48-hour readings, 30 % or more of the test animals exhibit a positive response (scores ≥ 1) in the absence of similar results in the vehicle control group.

Results and discussion
Mortality: No deaths occurred.
Clinical observations: No signs of systemic toxicity were observed.
Body weight: All animals showed the expected gain in body weight.
Necropsy: No necropsy was performed.
Skin reactions: No skin reactions were observed 24 or 48 h after the challenge treatment with glyphosate TC in the control or test group.
Animals treated with the positive control benzocaine in 40 % ethanolic 0.9 % NaCl solution exhibited a sensitising reaction in all animals in form of a discrete or patchy erythema (grade 1).

Conclusion by the Notifiers
Based on the study results and according to the EU and OECD Globally Harmonized System (GHS) classification criteria, glyphosate TC is not to be classified for skin sensitization.

Comment by RMS:
The study is considered acceptable and revealed no evidence for skin sensitisation potential of the test substance.

Reference:	IIA, 5.2.6/03
Report:	[redacted] 1995, HR-001: Dermal sensitisation study in Guinea pigs. [redacted].
	Study No.: IET 95-0036
	Data owner: Arysta LifeScience
	Date: 1995-06-28, Unpublished
	ASB2012-11441
Guidelines:	U.S. EPA FIFRA Guideline Subdivision F
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate technical
Identification:	HR-001
Description:	White crystal
Lot/Batch #:	T-941209
Purity:	97.56 %
Stability of test compound:	Not mentioned in the report
Vehicle and/or positive control:	Polyethylene glycol 300 (PEG 300)
Test animals:	
Species:	Guinea pig
Strain:	Hartley, Crj:Hartley
Source:	[redacted]
Age:	6 weeks
Sex:	female
Weight at dosing:	332 – 423 g at the time of intradermal injection
Acclimation period:	1 week
Diet/Food:	Pellet diet GC4 (Oriental Yeast Co., Ltd.), *ad libitum*
Water:	Filtered and sterilised water, *ad libitum*
Housing:	Aluminium cage with wire-mesh floor

Environmental conditions:	Temperature:	23.9 °C
	Humidity:	51.8 – 56.3 %
	Air changes:	15/hour
	12 hours light/dark cycle	

Study design and methods:
In life dates: 2007-01-10 to 2007-02-15
Animal assignment and treatment:
The test was carried out according to the maximization method of Magnusson and Kligman.
20 female specific pathogen free Hartley guinea pigs (Crj:Hartley) were exposed to concentrations of 5 %, 25 % and 25 % glyphosate technical for intradermal induction (3 pairs of injection), topical induction (applied with an occlusive dressing) and challenge (applied with an occlusive dressing) respectively. These doses selected for both induction and challenge application in the main study were based on the results of range-finding studies. DCNB (2,4-dichlorobenzene) was used (in a 10 female group) as the positive control substance at concentrations of 0.1 %, 1 % and 0.5 % for intradermal induction, topical induction and challenge, respectively. Groups of 10 and 20 animals were used for the negative control group for DCNB (treated with DCNB at the challenge but not at the induction) and negative control group for technical glyphosate (treated with test substance at the challenge but not at the induction), respectively. Skin reaction to the challenge was observed 24 and 48 hours after temoval of the patch and dermal sensitisation rates were calculated. Body weights were measured at the first induction and 48 hours after the removal of the patch.

Results and discussion
Mortality: One animal died in the DNCB treatment group. At necroscpy of the dead animal, consolidation of lung and hydrothorax were noted. These findings were associated with the hindrance of circulation and the respiratory abnormality, which led to the death. The remaining animals in this groupdid not show any abnormality in the health condition and the skin reactions were clearly observed.
Clinical observations: No signs of systemic toxicity were observed.
Body weight: No abnormal body weight changes were noted in any animal of the four groups.
Necropsy: No necropsy was performed.
Skin reactions: No oedema or erythema were observed in test animals following challenge with 25 % technical glyphosate. The rate of sensitization in the test substance treatment group was therefore 0 %.
On the other hand, the rate of sensitization in the DCNB treatment group was 100 %, which was considered to sufficiently assure the reliability of this study.

Conclusion by the Notifiers
Based on the EU classification criteria, glyphosate technical (HR-001) is not to be classified for skin sensitization. According to the OECD Globally Harmonized System (GHS) classification criteria glyphosate technical (HR-001) is also not classified for skin sensitization.

Comment by RMS:
The study is considered acceptable and revealed no evidence for skin sensitisation potential of the test substance.

Reference: IIA, 5.2.6/04

Report:	[redacted], 2009, Glyphosate Technical: Contact Hypersensitivity in albino guinea pigs – Maximization-Test [redacted] Data owner: Excel Report No.: C22908 Date: 2009-05-15, not published ASB2012-11442
Guidelines:	OECD 406 (1992) Commission Regulation (EC) No 440/2008 (2008); method B.6
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	GI-1045
Purity:	96.66 %
Stability of test compound:	Expiry date: July 2010 Test item dilution: Stable in purified water for 2 days.
Vehicle and/or positive control:	Purified water
Test animals:	
Species:	Guinea pig
Strain:	Albino Dunkin Hartley, CRL:(AH)BR, SPF
Source:	[redacted]
Age:	4 – 6 weeks (at pre-test / at beginning of acclimatization period)
Sex:	Male
Weight at dosing:	348 – 358 g (at pre-test) 335 – 365 g (at beginning of acclimatization period)
Acclimation period:	Approx. 2 weeks (for main study)
Diet/Food:	Pellet standard Provimi Kliba 3418 guinea pig breeding / maintenance diet batch nos. 55/08 and 72/08, containing Vitamin C (Provimi Kliba AG, 4303 Kaiseraugst / Switzerland), *ad libitum.*
Water:	Tap water, *ad libitum*
Housing:	Individually in Makrolon type-4 cages with standard softwood bedding (“Lignocel”, Schill AG, 4132 Muttenz / Switzerland)
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 – 70 % Air changes: 10 - 15/hour 12-hour light/dark cycle

Study design and methods:

In life dates: 2009-01-14 to 2009-02-27

Animal assignment and treatment:
Glyphosate Technical was tested for its sensitizing effect on the skin of guinea pig in the Maximization-Test according to Magnusson –and Kligman. Fifteen (10 test and 5 control) male Albino Dunkin Hartley CRL:(AH)BR, SPF guinea pigs, 4-6 weeks of age, and 335-365 g of weight were employed for this study.
The concentrations of test substance for the main test were selected based on the results of a pre-test (during the acclimatization period of the main animals).
The intradermal induction of sensitisation in the test group was performed in the nuchal region with a 10 % dilution of the test item in purified water and in an emulsion of Freund's Complete Adjuvant (FCA)/physiological saline. The epidermal induction of sensitization was conducted for 48 hours under occlusion with the test item at 50 % in purified water one week after the intradermal induction. The animals of the control group were intradermally induced with purified water and FCA/physiological saline and epidermally induced with purified water under occlusion.
Two weeks after epidermal induction the control and test animals were challenged by epidermal application of the test item at 15 % in purified water and purified water alone under occlusive dressing.
Cutaneous reactions were evaluated at 24 and 48 hours after removal of the dressing (according to the criteria laid down in test guidelines).
A positive control (reliability check) with a known sensitizer was not included in this study. However, a separate study was performed in the laboratory. The positive controls with α-hexylcinnamaldehyde at 3 % in PEG 300 showed that the chosen guinea pig strain was able to detect sensitising compounds under the laboratory conditions chosen.
Body weights were determined at delivery/acclimatization start, at the end of the pretest, at test day 1 (day of treatment), and at the termination of the study. Mortality was checked daily.

Results and discussion
Mortality: There were no treatment related deaths during the course of the study, hence no necropsies were performed.
One pre-test animal was found in bad conditions before the start of pre-test and during the acclimatization period of the main test animals. This animal was sacrificed for ethical reasons and replaced by a new animal.
Clinical observations: No signs of systemic toxicity were observed in the animals.
Body weight: The body weight of the animals was within the range commonly recorded for animals of this strain and age.
One animal lost visible amount of body weight (31 %) before the start of the intradermal pre-test. It was killed for ethical reasons and replaced by another animal.
Necropsy: No necropsies were performed.
Skin reactions: Skin Effects in the Intradermal Induction (Test Day 1)
The expected and common findings were observed in the control and test group after the different applications using FCA intradermally. These findings consisted of erythema, oedema, necrotizing dermatitis, encrustation and exfoliation of encrustation.
Skin Effects in the Epidermal Induction (Test Day 8)
Control group – No erythematous or oedematous reaction was observed in the animals treated with purified water only.
Test group – Discrete/patchy erythema was observed in eight out of ten test animals at the 24-hour observation and persisted in seven animals up to the 48-hour reading after treatment with the test item at 50 % in purified water.

Skin Effects in the Challenge Procedure
Control group and Test group – No positive/skin reactions were observed in the animals when treated with either purified water only or when treated with the test item at 15 % in purified water.

Conclusion by the Notifiers
Based on the above mentioned findings in the Magnusson & Kligman Test in guinea pigs and in accordance to Commission Directive 2001/59/EC, Glyphosate Technical does not have to be classified and labelled as a skin sensitizer.
Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate technical is not to be classified for skin sensitization.

Comment by RMS:
The study is considered acceptable and revealed no evidence for skin sensitisation potential of the test substance.

Reference:	IIA, 5.2.6/05
Report:	[redacted] 2009, Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) [redacted] Data owner: Helm AG Report No.: LPT 23915 Date: 2009-05-19, unpublished ASB2012-11443
Guidelines:	OECD 406 US EPA OPPTS 870.2600.
Deviations:	Personnel change of the head of the Quality Assurance Unit: until January 31, 2009: Dipl. Biol. S. Steuer as of February 1, 2009: Dr. med. vet. habil. K. R. Sultan. This minor deviation did not have any effect on the validity and integrity of the scientific results obtained in this.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate TC
Description:	White powder
Lot/Batch #:	20080801
Purity:	98.8 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	*Aqua ad iniectabilia/* Benzocaine
Test animals:	

Species:	Guinea pig
Strain:	Dunkin-Hartley
Source:	[redacted]
Age:	32 days
Sex:	Male
Weight at dosing:	313 – 358 g (excluding positive control group) Positive control group: 271 - 331 g
Acclimation period:	At least 5 days
Diet/Food:	Commercial diet, ssniffB MS-H V2233 (ssniff Spezialdiäten GmbH) served as food. The food was offered *ad libitum.*
Water:	Tap water, *ad libitum*
Housing:	The animals were kept in pairs in MAKROLON cages (MZK 80/25). Granulated textured wood (Granulat A2, J. BRANDENBURG, 49424 Goldenstedt, Germany) was used as bedding material in the cages. The cages were changed and cleaned twice a week.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 55 ± 15 % 12-hour light/dark cycle

Study design and methods:
In life dates: 2009-02-04 to 2009-03-28

Animal assignment and treatment:
The purpose of this study was to determine the potential of Glyphosate TC to provoke skin sensitisation reactions in guinea pigs. Possible sensitising properties of the test item were evaluated by administration of the test item to the shoulder region, first by intracutaneous application (stage 1) and 7 days later by topical administration (stage 2, exposure time: 48 hours). In a challenge test (stage 3) the test item was again applied topically but to the flank region (exposure time: 24 hours). This area was then examined for reactions which might indicate sensitising properties of the test item.
Induction: The skin reaction results of the first induction exposure were evaluated at 24 and 48 hours, of the second induction at 48 and 72 hours after beginning of exposure.
Challenge:
Days 23 and 24:
21 hours after removing the filter paper the challenge area was cleaned and cleared of hair if necessary
three hours later (at 48 hours from the start of challenge application) the skin reaction was observed and recorded. 24 hours after this observation a second observation (72 hours) was made and recorded.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: Behaviour of the animals remained unchanged. Given the negative response in all treated animals further testing was not considered necessary in order to reduce animal experiments for animal welfare reasons.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Conclusion by the Notifiers

Under the present test conditions Glyphosate TC revealed no sensitising properties in guinea pigs in a test model according to Magnusson and Kligman.

Comment by RMS:

The study is considered acceptable and revealed no evidence for skin sensitisation potential of the test substance.

Reference:	IIA, 5.2.6/06
Report:	[redacted] 2010, Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) [redacted] Data owner: Helm AG Report No.: LPT 24607 Date: 2010-04-19, unpublished ASB2012-11444
Guidelines:	OECD 406 US EPA OPPTS 870.2600.
Deviations:	The following minor deviations were noted: The batch no. of the sodium lauryl sulfate was 081 K0078 and not 121 H0370 as stated in the Study Plan. Freund's complete adjuvant was manufactured by SIGMA-ALDRICH Chemie GmbH, 82024 Taufkirchen, Germany, and not by DIFCO Laboratories Detroit, Michigan, USA, as stated in the Study Plan. These minor deviations from the Study Plan did not affect the validity and scientific results of the study.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate TC
Description:	White powder
Lot/Batch #:	20090506
Purity:	97.3 %
Stability of test compound:	No data given in the report.

Vehicle and/ or positive control:	*Aqua ad iniectabilia*/ Benzocaine
Test animals:	
Species:	Guinea pig
Strain:	Dunkin-Hartley
Source:	[redacted]
Age:	32 days
Sex:	Male
Weight at dosing:	299 - 364 g (excluding positive control group) Positive control group: 319 - 346 g
Acclimation period:	At least 5 days
Diet/Food:	Commercial diet, ssniffB MS-H V2233 (ssniff Spezialdiäten GmbH) served as food. The food was offered ad libitum
Water:	Tap water, *ad libitum*
Housing:	The animals were kept in pairs in MAKROLON cages (MZK 80/25). Granulated textured wood (Granulat A2, J. BRANDENBURG, 49424 Goldenstedt, Germany) was used as bedding material in the cages. The cages were changed and cleaned twice a week.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 55 ± 15 % 12-hour light/dark cycle

Study design and methods:
In life dates: 2009-10-26 to 2010-01-30

Animal assignment and treatment:
The purpose of this study was to determine the potential of Glyphosate TC to provoke skin sensitisation reactions in guinea pigs. Possible sensitising properties of the test item were evaluated by administration of the test item to the shoulder region, first by intracutaneous application (stage 1) and 7 days later by topical administration (stage 2, exposure time: 48 hours). In a challenge test (stage 3) the test item was again applied topically but to the flank region (exposure time: 24 hours). This area was then examined for reactions which might indicate sensitising properties of the test item.
Induction: The skin reaction results of the first induction exposure were evaluated at 24 and 48 hours, of the second induction at 48 and 72 hours after beginning of exposure.
Challenge: Days 23 and 24: 21 hours after removing the filter paper the challenge area was cleaned and cleared of hair if necessary three hours later (at 48 hours from the start of challenge application) the skin reaction was observed and recorded. 24 hours after this observation a second observation (72 hours) was made and recorded.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: Behaviour of the animals remained unchanged. Given the negative response in all treated animals further testing was not considered necessary in order to reduce animal experiments for animal welfare
reasons.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Conclusion by the Notifiers

Under the present test conditions glyphosate TC revealed no sensitising properties in guinea pigs in a test model according to Magnusson and Kligman.

Comment by RMS:

The study is considered acceptable and revealed no evidence for skin sensitisation potential of the test substance.

Reference:	IIA, 5.2.6/07
Report:	[redacted] 2009, Glyphosate – Skin Sensitization Study in Guinea Pigs. Buehler Test. [redacted] Data owner: Helm AG Report No.: 12174-08 Date: 2009-03-11, unpublished ASB2012-11445
Guidelines:	US EPA OPPTS 870.2600 Equivalent to OECD 406.
Deviations:	Humidity was in the range of 25-98% instead of 30-70%. This deviation did not affect the study outcome
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate Tech Grade Mixed 5-Batch
Description:	White powder
Lot/Batch #:	080704-1 thru 5
Purity:	96.4 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	Deionised water/ alpha-Hexylcinnamaldehyde
Test animals:	
Species:	Guinea Pig
Strain:	Hartley-Albino
Source:	[redacted]
Age:	Approx. 4 weeks

Sex:	Males and Females
Weight at dosing:	Males: 359-414 g; Females: 341-387 g
Acclimation period:	5 days
Diet/Food:	PMI Feeds, Inc.™ Guinea Pig Diet #5025; available *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individual housing in suspended, wire bottom, stainless steel cages.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: 10 - 12/hour 12-hour light/dark cycle

Study design and methods:
In life dates: 2008-11-30 to 2009-01-02

Animal assignment and treatment:
Observations for skin reactions at each test site were made approximately 24 hours after each treatment. In addition, observations for skin reactions were made approximately 48 hours after the first induction treatment and 48 hours after the challenge treatment. An average score for each time period was obtained by adding all of the scores for each time period and dividing by the number of test sites scored for that time period. The test substance is considered a sensitizer if the mean irritation scores, the total number of animals with scores, and/or the total number of scores for the virgin test site in the test group after the challenge treatment are appreciably greater than those for the naive challenge group. The average skin reaction score of this study was 0.0.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: The test substance, glyphosate, produced neither irritation in the test animals (Group II) nor the naive control animals (Group I) after the challenge treatment, and therefore did not elicit a sensitising reaction in guinea pigs.

Conclusion by the Notifiers
The test substance, glyphosate, produced neither irritation in the test animals (Group II) nor the naive control animals (Group I) after the challenge treatment, and therefore did not elicit a sensitizing reaction in guinea pigs and is therefore not sensitising.

Comment by RMS:
The study is considered acceptable and revealed no evidence for skin sensitisation potential of the test substance.

Reference:	IIA, 5.2.6/08
Report:	[redacted] 2005, Glyphosate acid technical – Dermal Sensitization in Guinea Pigs (Buehler Method) [redacted] Data owner: Helm AG Report No.: [redacted] 15279

	Date: 2005-04-04, unpublished ASB2012-11446
Guidelines:	US EPA OPPTS 870.2600 OECD 406.
Deviations:	No deviations from the Study Plan
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate Acid Technical
Description:	White crystalline powder
Lot/Batch #:	040205
Purity:	97.23 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	Distilled water/ alpha-Hexylcinnamaldehyde Technical
Test animals:	
Species:	Guinea pig
Strain:	Hartley albino
Source:	[redacted]
Age:	Young adult
Sex:	Male and Female
Weight at dosing:	327-391 g
Acclimation period:	5 or 38 days
Diet/Food:	Pelleted Purina Guinea Pig Chow #5025
Water:	Tap water, *ad libitum*
Housing:	The animals were group housed in suspended stainless steel caging with mesh floors or plastic perforated bottom caging.
Environmental conditions:	Temperature: 18-22 °C 12-hour light/dark cycle

Study design and methods:
In life dates: 2004-05-03 to 2004-06-03

Animal assignment and treatment:
In order to evaluate the sensitisation response at challenge, two indices were used; one for incidence and one for severity in the test and vehicle control animals. The incidence index was the ratio of animals with erythema scores greater than 0.5 per number of animals evaluated, and is presented for both the 24 and 48 hour intervals after challenge evaluation intervals as follows: Incidence Index =Number of erythema scores greater than 0.5 l/ Number of animals evaluated The severity index is the mean erythema score, and is calculated for both the 24 and 48 hour after challenge evaluation intervals according to the following formula: Severity Index = Sum of erythema scores/Number of animals evaluated. The following

criteria were used to classify the test substance as a potential contact sensitizer: At the 24-hour andlor 48-hour scoring interval, 15 % or more of the test animals exhibit a positive response (scores > 0.5) in the absence of similar results in the vehicle control group. The positive reaction at the 24-hour interval must persist to 48 hours in at least one test animal.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: Induction Phase:
Test Animals (70 % w/w mixture of the test substance in distilled water): Very faint erythema (0.5) was observed for most test sites during the induction phase.
Test Animals (70 % w/w mixture of the test substance in distilled water): Very faint erythema (0.5) was observed at six of twenty test sites 24 hours following the challenge application. Similar irritation persisted at one affected site through 48 hours.

Conclusion by the Notifiers
Based on these findings and on the evaluation system used, Glyphosate Acid Technical is not considered to be a contact sensitizer.

Comment by RMS:
The study is considered acceptable. Based on the study conditions no evidence for skin sensitisation potential of the test substance was observed.

Reference:	IIA, 5.2.6/09
Report:	[redacted] 2008, Skin Sensitisation Test for Glyphosate Technical in Guinea Pigs. Buehler Test. [redacted] Data owner: Helm AG Report No.: RF-3996.318.431.07 Date: 2008-09-30, unpublished ASB2012-11447
Guidelines:	OECD 406.
Deviations:	1. The experimental phase initiation and experimental phase conclusion dates were updated, 2. The test item was appIied using DMSO as vehicle. The deviations listed above had no negative impact on the outcome of the study.
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate Techical
Description:	White powder
Lot/Batch #:	20070606
Purity:	98.05 %
Stability of test compound:	No data given in the report.

Vehicle and/or positive control:	DMSO
Test animals:	
Species:	Guinea Pig
Strain:	Hartley
Source:	[redacted]
Age:	Eight to nine weeks old
Sex:	Male
Weight at dosing:	444 - 556 g
Acclimation period:	7 days
Diet/Food:	Pelleted commercial diet - "Nuvilab Cobaias 6001"
Water:	Tap water, *ad libitum*
Housing:	88 x55 x 28 cm polypropylene cages with autoclaved wood shavings containing five animals per cage during the experimental phase were used
Environmental conditions:	Temperature: 18 - 23 °C Humidity: 30 - 70 % Air changes: 10 - 15/hour 12-hour light/dark cycle

Study design and methods:
In life dates: 2008-06-12 to 2008-07-12

Animal assignment and treatment**:**
The contact sites to the skin (left flank for inductions and right flank for challenge) at each application day were mechanically and closely clipped free of hair using an electrical razor. Skin was observed for lesions after clipping the fur. According to the Buehler's application method, animals were exposed to cotton lint patches with an approximated 6 cm² surface area. Treatment animals were exposed to patches loaded with 1 mL of 50 % (w/v) test solutions in DMSO (equivalent 0.5 g of the test item) for the induction and challenge applications. Control animals were exposed to patches loaded with 1 mL of vehicle on inductions and loaded with 1 mL of test solution on challenge, therefore being submitted to the same procedures as treatment animals, except on inductions. Since control animals were not exposed to the test item on inductions, a hypersensitive state could not be induced in these animals, which then constituted a negative control in order to allow the differentiation between skin irritation and skin sensitisation at challenge. Patches were held in contact with the skin by an occlusive dressing during an approximated 6-hour exposure period in each application, after which patches were carefully removed from the skin and any residue cleaned up using DMSO. Four applications were carried out, with a seven-day interval between inductions and a fourteen-day interval between third induction and challenge. Animals were clinically examined approximately 30 and 54 hours after each application. Skin reactions were evaluated in agreement with Magnusson & Kligman's grading scale after challenge application.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: Neither compound-related clinical signs nor behavioral alterations were observed during inductions. No animal from control group was positive for the test item after

challenge application. One animal from treatment group was positive for the test item after challenge application.

Conclusion by the Notifiers
The epidermal application of glyphosate technical using DMSO as vehicle did not cause skin sensitisation in guinea pigs, according to the Buehler Test Method.

Comment by RMS:
Despite the deviations in the study design, no evidence for skin sensitisation potential of the test substance was observed. The study is considered acceptable.

Reference:	IIA, 5.2.6/10
Report:	[redacted], 2006, Glyphosate Technical: Skin Sensitisation in the Guinea Pig – Magnusson and Kligman Maximisation method Data owner: Nufarm Study No.: SMK-PH-05/2018, Report No.: 2060/009 Date: 2006-01-13 GLP: Unpublished ASB2012-11448
Guidelines:	§ OECD Guidelines for the Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992) § Method B6 Acute Toxicity (Skin Sensitisation) of Commission Directive 96/54/EC § Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Testing Guidelines for Toxicology Studies, 12 NohSan No. 8147, Guideline No. 2-1-6, revised on 24 November 2004 and 13 SeiSan No. 3986 of October 10, 2001
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test material:	
Identification:	Glyphosate Technical
Description:	white powder
Lot/Batch #:	H05H016A
Purity:	95.7 %
Stability of test compound:	No data available
Vehicle and/or positive control:	Distilled water
Test animals:	
Species:	Guinea Pigs

Strain:	albino Dunkin Hartley
Source:	[redacted]
Age:	Not specified
Sex:	Female
Weight at dosing:	295 to 370 gg
Acclimation period:	5 days
Diet/Food:	Not specified. The diet, drinking water and bedding were routinely analysed and were considered not to contain any contaminant that could reasonably be expected to affect the purpose or integrity of the study.
Water:	Tap water, ad libitum
Housing:	in groups of two or three in makrolon cages furnished with woodflakes
Environmental conditions:	Temperature: 19 to 25 °C Humidity: 30 to 70 % Air changes: not specified 12-hour light/dark cycle

Study design and methods:
In life dates: 05 September 2005 to 13 October 2005

Animal assignment and treatment:
The method used for assessing the sensitising properties of the test material was based on the Guinea Pig Maximisation Test of Magnusson &and Kligman. A group of thirty guinea pigs was used for the main study, twenty test and ten control. Two phases were involved in the main study; (a) an induction of a response and (b) a challenge of that response. Induction of the Test Animals: A row of three injections (0.1 ml each) was made on each side of the spine, consisting of a) Freund's Complete Adjuvant plus isotonic sodium chloride in the ratio 1:1 b) a 0.195 % (v/v) formulation of the test material in isotonic sodium chloride c) a 0.195 % (v/v) formulation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus isotonic sodium chloride. On Day 6, the scapular region of all test and control animals was shaved and sodium lauryl sulphate (10 % in petroleum jelly) was spread evenly over the area to create local irritation. On Day 7 the same area on the shoulder region used previously for intradermal injections was treated with a topical application of the test material formulation (60 % (w/w) in distilled water) under occlusive dressing for 48 hours. The intradermal induction on the control animals was performed using an identical procedure without the test material. Injection b) was therefore the vehicle alone, injection c) was a 50 % formulation of the vehicle in a 1:1 preparation of Freund’s Complete Adjuvant plus isotonic sodium chloride. Similarly, the topical induction procedure was identical to that used for the test animals except that the test material was omitted. For the challenge phase, test material formulation at the maximum non- irritant concentration (60 % (w/w) in distilled water) was applied to one side of the shorn flank of each animal under an occlusive dressing. To ensure that the maximum non- irritant concentration was used at challenge, the test material at a concentration of 30 % (v/v) in distilled water was similarly applied under an occlusive dressing to the opposite skin site on the shorn flank. After 24 hours, the dressing was carefully removed and discarded. The topical challenge sites were cleaned if required. Prior to the 24-hour observation the flanks were clipped to remove regrown hair. Approximately 24 and 48 hours after challenge dressing removal, the degree of erythema and oedema was quantified. Any other reactions were also recorded.
None of the animals exposed to test material showed any signs of sensitisation after the challenge phase.

Results and discussion
Mortality: One test group animal was found dead on Day 3 and one other test group animal was found dead on Day 5. The cause of death was not determined but was considered not to be treatment related. The absence of these animals was considered not to affect the purpose or integrity of the study.
Clinical observations: The concentration chosen for use in the Intradermal Induction phase of the main test was 0.195 % (v/v) in isotonic sodium chloride solution (maximal non necrotising concentration (MNNC)). The concentration chosen for use in the Topical Induction phase of the main test was 60% (w/w) in distilled water (maximal non irritant concentration (MNIC)). The concentrations chosen for use in the Topical Challenge phase of the main test were 60 % (w/w) (maximal non irritant concentration (MNIC)) and 30 % (v/v) in distilled water (1/2 MNIC).
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The test material produced a 0 % (0/18) sensitisation rate and was classified as a non-sensitiser to guinea pig skin under the conditions of the test. The test material did not meet the criteria for classification as a sensitiser according to EU labelling regulations Commission Directive 2001/59/EC. No symbol and risk phrase are required.
Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate is not to be classified for this endpoint.

Comment by RMS:
The study is considered acceptable. Based on the study conditions no evidence for skin sensitisation potential of the test substance was observed.

Reference:	IIA, 5.2.6/11
Report:	[redacted] 1996, Glyphosate Acid: Skin Sensitisation To The Guinea Pig [redacted] Data owner: Syngenta Report No.: [redacted]/P/4699 Date: 1996-08-23, not published TOX2000-1987
Guidelines:	OECD 406 (1992): OPPTS 870.2600 (2003): 96/54/EC B.6 (1996)
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test Material:	Glyphosate acid
Description:	Technical, white solid
Lot/Batch number:	P24
Purity:	As given in report 95.6 % a.i
CAS#:	Not reported
Stability of test compound:	Not reported
Vehicle and/or positive control:	Deionised water
Test Animals:	
Species	Guinea pig
Strain	Albino Cr1 (HA) BR
Age/weight at dosing	Young adult females / 250-317 g
Source	[redacted]
Housing	Individually in suspended cages in racks suitable for animals of this strain and the weight range expected during the course of the study.
Acclimatisation period	At least 6 days
Diet	RGP), supplied by Labsure, Manea, Cambridgeshire, UK *ad libitum*
Water	Mains water *ad libitum*

Environmental conditions	Temperature: 17 ± 2 °C Humidity: 40-70 % Air changes: Approximately 25 changes/hour Photoperiod: 12 hours light, 12 hours dark

Study design and methods
In-life dates: Start: 25 April 1995 End: 19 May 1995

Animal assignment and treatment: In a dermal sensitisation study with glyphosate acid (95.6 % w/w a.i.) young adult, female, Albino Cr1 (HA) BR guinea pigs were tested using the method of Magnusson and Kligman. The study involved the treatment of guinea pigs using two procedures: the potential induction of an immune response and a challenge of that response.

Induction: An area approximately 5 x 5 cm on the scapular region of each animal was clipped free of hair and a row of three injections (0.05-0.1 mL each) was made on each side of the mid-line. The injections were:
i) Top: Freund's Complete Adjuvant plus deionised water in the ratio 1:1;
ii) Middle: a 0.1 % w/v preparation of the test substance in deionised water;
iii) Bottom: a 0.1 % w/v preparation of the test substance in a 1: 1 preparation of Freund's Complete Adjuvant plus deionised water.

Control animals were treated the same as the test animals, except that they were treated with deionised water in place of the test substance.
One day prior to topical induction, the application site was clipped and 0.5 mL of a 10 % w/v preparation of sodium lauryl sulphate in paraffin wax was applied in order to provoke a mild inflammatory response.
One week after intradermal injection, the scapular area was treated with a topical application of the test substance as a 75 % w/v preparation in deionised water. This preparation (0.2-0.3 mL) was applied on filter paper (approximate size 4 cm x 2 cm) which was held in place by a piece of surgical tape. The tape was covered by a strip of adhesive bandage (approximate size 20-30 cm x 5 cm) and secured by a piece of self-adhesive PVC tape. This occlusive dressing was kept in place for approximately 2 days.
Deionised water only was applied to the filter paper for control animals.
The application sites were checked approximately 1 day after removal of the dressings.

Challenge: Two weeks after the topical inductions, an area, approximately 15 cm x 5 cm, on both flanks of all the test and control animals, was clipped free of hair. An occlusive dressing was prepared which consisted of two pieces of filter paper (approximate size 1 cm x 1.5-2.0 cm) stitched to a piece of rubber sheeting (approximate size 12 cm x 5 cm).
A 75 % w/v preparation of the test substance in deionised water (0.05-0.1 mL) was applied to one of the pieces of filter paper and a 30 % w/v preparation in deionised water (0.05-0.1 mL) was applied to the second piece of filter paper. The dressing was placed on the shorn flank of the guinea pig so that the 75 % w/v preparation was on the left and the 30 % w/v preparation was on the right. It was then covered with a strip of adhesive bandage (approximate size 25-40 cm x 7.5 cm) which was secured by a self-adhesive PVC tape.

After approximately 1 day, the dressings were carefully removed. Skin sites were examined approximately 1 and 2 days after removal of the dressings and any erythematous reactions were quantified and recorded, using a four-point scale.

Positive Controls: The sensitising potential of hexylcinnamaaldehyde (HCA) was assessed essentially as described above to demonstrate the sensitivity of the strain of animals used and the reliability of the experimental technique. A concentration of 0.3 % w/v HCA in corn oil was used for the intradermal injections and HCA was used undiluted for the topical induction and challenge applications.

Results and discussion

Bodyweights: There were no treatment-related effects on bodyweight during the study.
Induction reactions and duration: Not reported.
Challenge reactions and duration: Following challenge of previously-induced guinea pigs with a 75 % w/v preparation of the glyphosate acid in deionised water, scattered mild redness was seen in three of the twenty test animals and one of the ten control animals. This response is considered to be due to skin irritation following topical challenge. The basis for this conclusion is that an equivalent reaction was seen in one of the ten control animals and the reaction was restricted to the 24 hour clinical observation only, which is characteristic of a mild skin irritation reaction rather than skin sensitisation.
Following challenge of previously-induced guinea pigs with a 30 % w/v preparation of the glyphosate acid in deionised water, no reaction was seen in any of the test or control animals. The net percentage response was calculated to be 0 %.
Positive control: Following challenge of previously induced guinea pigs, scattered mild redness or moderate diffuse redness was observed in 14/20 test animals. Scattered mild redness was seen in two of the ten control animals. The net % response was 50 % and, therefore, HCA was classified as a moderate skin sensitiser which demonstrated the sensitivity of the strain of animals used and the reliability of the experimental technique.

Table B.6.2-36: Number of animals with positive signs following challenge

	Test flank			
	Challenge at 75 %		Challenge at 30 %	
Scored after:	24 hours	48 hours	24 hours	48 hours
Main test – test group	3/20	0/20	0/20	0/20
Main test – negative vehicle control	1/10	0/10	0/10	0/10
	Challenge at 100%			
	24 hours		48 hours	
Positive control – test group	14/20		13/20	
Positive control – vehicle control	2/10		0/10	

Conclusion by the Notifiers:

Glyphosate acid is not a skin sensitiser under the conditions of the test.

Comment by RMS:

Despite reporting deficiencies the study is considered acceptable. Based on the study conditions no evidence of skin sensitising potential was observed.

Reference:	IIA, 5.2.6/12
Report:	[redacted] 2007, Glyphosate Technical Material: Skin Sensitisation (Local Lymph Node Assay In The Mouse). [redacted], Macclesfield, Cheshire, UK Data owner: Syngenta Report No.: R61837/1004 Date: 2007-02-09, not published ASB2012-11449
Guidelines:	OECD 429 (2002): OPPTS 870.2600 (2003): 2004/73/EC B.42 (2004)
Deviations:	None
GLP:	yes
Acceptability:	*Please see comment by RMS*

Materials and methods

Test Material:	Glyphosate Technical Material
Description:	Technical, white solid
Lot/Batch number:	0507
Purity:	96.1 %
Stability of test compound:	Stable under storage conditions – ambient temperature in the dark.
Vehicle and/or positive control:	The vehicle for the test substance was dimethyl sulphoxide. The vehicle for the positive control substance (hexylcinnamaldehyde) was acetone in olive oil.
Test Animals:	
Species	Mouse
Strain	CBA/Ca/Ola/Hsd
Age/weight at dosing	8-12 weeks / 16.5-20.8 g
Source	[redacted]
Housing	Maximum 4 per cage, in cages suitable for animals of this strain and weight range.
Acclimatisation period	At least 5 days
Diet	Diet (RM1), supplied by Special Diets Services Limited, Witham, Essex, UK. *ad libitum*
Water	Mains water supplied by an automatic system *ad libitum*
Environmental conditions	Temperature: 22 ± 3 °C Humidity: 30-70 % Air changes: A minimum of 15 changes/hour Photoperiod: Artificial, 12 hours light / 12 hours dark.

Study design and methods

In-life dates: Start: 10th January 2007 End: 16th January 2007

Animal assignment and treatment: A sample of glyphosate technical material was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay. The assay determines the level of T lymphocyte proliferation in the lymph nodes draining the site of chemical application by measuring the amount of radiolabelled thymidine incorporated into

the dividing cells. The test substance was applied as 10, 25 or 45 % w/v preparations in dimethyl sulphoxide. Groups of four female CBA/Ca/Ola/Hsd mice were used for this study.
Dose selection rationale: Approximately 25µL of a 10, 25 or 45 % w/v preparation of the test substance was used in this study as 45 % w/v was the limit of solubility.
Treatment preparation and administration: Approximately 25µL of a 10, 25 or 45 % w/v preparation of the test substance in dimethyl sulphoxide was applied, using a variable volume micro-pipette, to the dorsal surface of each ear. A vehicle control group was similarly treated using dimethyl sulphoxide alone. The procedure was repeated daily for 3 consecutive days. Three days after the third application, all the animals were injected, via the tail vein, with approximately 250µL of phosphate buffered saline (PBS) containing 20µCi of a 2.0Ci/mmol specific activity ^{3}H-methyl thymidine. Approximately 5 hours later, the animals were humanely killed by inhalation of halothane vapour followed by cervical dislocation. The draining auricular lymph nodes were removed from each animal and, together with the nodes from the other animals in the group, were placed in a container of PBS.
A single cell suspension was prepared by mechanical disaggregation of lymph nodes through a 200-mesh stainless steel gauze. The cell suspensions were then washed three times by centrifugation with approximately 10 ml of PBS. Approximately 3ml of 5 % w/v trichloroacetic acid (TCA) was added and, after overnight precipitation at 4 °C, the samples were pelleted by centrifugation and the supernatant was discarded. The cells were then resuspended in approximately 1mL of TCA.
The lymph node suspensions were transferred to scintillation vials and 10 mL of scintillant (Optiphase) was added prior to β-scintillation counting using a Packard Tri-Carb 3100TR Liquid Scintillation Counter.
The reliability of the test system was assessed in a positive control study using the same method with a known sensitiser (hexylcinnamaldehyde) applied as 5 %, 10 % or 25 % w/v preparations in acetone in olive oil.
Statistics / Data Evaluation: The results are expressed as a disintegrations per minute (dpm) value per lymph node for each group. The activity of each test group is then divided by the activity of the vehicle control group to give a test:control ratio known as the stimulation index (SI), for each concentration.

The criterion for a positive response is that one or more concentrations of the test substance should elicit a 3-fold or greater increase in isotope incorporation relative to the vehicle control group. The assay is able to identify those materials that elicit responses in standard guinea pig tests for skin sensitisation. Consequently, a test substance which does not fulfil the above criterion is designated as unlikely to be a skin sensitiser.

Results and discussion
Group Mean Counts per Minute: The application of the test substance at concentrations of 10, 25 and 45 % w/v in dimethyl sulphoxide resulted in an isotope incorporation which was less than 3-fold at all concentrations. Consequently, the test substance is considered not to be a skin sensitiser under the conditions of the test.

Table B.6.2-37: Radiolabel incorporation into lymph-nodes of mice treated with glyphosate technical material

Concentration of NOA446510 (%w/v)	Number of lymph nodes assayed	Disintegrations per minute (dpm)	dpm per lymph node	Test control ratio (SI)
0 (vehicle only)	8	3912	489	N/A
10	8	2394	299	0.6
25	8	3292	412	0.8
45	8	4067	508	1.0

N/A = not applicable

In the positive control study, the application of hexylcinnamaldehyde at concentrations of 5 %, 10 % and 25 % w/v in acetone in olive oil (4:1) resulted in a greater than 3-fold increase in isotope incorporation at the 25 % w/v concentration. Therefore, hexylcinnamaldehyde was shown to be a skin sensitiser, confirming the validity of the protocol used for the study.

Table B.6.2-38: Radiolabel incorporation into lymph-nodes of mice treated with the positive control substance (hexylcinnamaldehyde)

Concentration of hexylcinnamaldehyde (%w/v)	Number of lynph nodes assayed	Disintegrations per minute (dpm)	dpm per lymph node	Test control ratio (SI)
0 (vehicle only)	8	5939	742	N/A
5	8	10111	1264	1.7
10	8	13747	1718	2.3
25	8	38015	4752	6.4

Conclusion by the Notifiers
Glyphosate technical material is considered not to be a skin sensitiser under the conditions of the test.

Comment by RMS:
The study is considered acceptable and no evidence for skin sensitisation potential of the test substance was observed.

Reference: IIA, 5.2.6/13
Report: [redacted] 2011, Glyphosate technical: Local lymph node assay in the mouse
[redacted]
Data owner: Syngenta
Report No.: 10/218-037E
Date: 2011-04-21, not published
ASB2012-11450
Guidelines: OECD 429 (2010)
Deviations: None
GLP: yes
Acceptability: *Please see comment by RMS*

Materials and methods

Test Material:	Glyphosate technical
Description:	Technical, dry white powder
Lot/Batch number:	569753 9 (BX20070911)
Purity:	96.3 % w/w glyphosate technical
CAS#:	Not reported
Stability of test compound:	Stable under storage conditions (room temperature range 2-8°C), recertification date end August 2011
Vehicle and/or positive control:	The vehicle for the test substance was propylene glycol
Test Animals:	
Species	Mice
Strain	CBA/J Rj
Age/weight at dosing	9-10 weeks / 20.1-21.6 g
Source	[redacted]
Housing	Group housed in Type II. polypropylene/polycarbonate cages
Acclimatisation period	13 days
Diet	ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and rats – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494 Soest Germany *ad libitum*
Water	Tap water *ad libitum*
Environmental conditions	Temperature: 22±3 °C Humidity: 30-70 % Air changes: 15-20 air changes per hour Photoperiod: 12 hours light / 12 hours dark

Study design and methods

In-life dates: Start: 20 October 2010 End: 26 October 2010

A sample of glyphosate Technical (96.3 % w/w Glyphosate technical) was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay, following dermal exposure when administered topically to young adult, female CBA/J Rj mice.

Animal assignment and treatment:

Dose selection rationale**:** A Preliminary Irritation/Toxicity Test was performed on CBA/J Rj mice using two doses, at test item concentrations of 50 and 25 (w/v)%, respectively. This preliminary experiment was conducted in a similar experimental manner to the main study, but it was terminated on Day 6 without radioactive proliferation assay.

During the Preliminary Irritation/Toxicity Test no mortality, systemic toxicity or local irritation were observed. No treatment related effect on body weights was observed. The observations recorded in this preliminary test suggest that the formulations, the application of the material and the local effects on the animal are acceptable for a valid LLNA.

Based on the results of the preliminary experiments the following dose levels were selected for the main assay: 0 (negative control), 10, 25 and 50 w/v% Glyphosate Technical, and positive control (25 % HCA in PG). Each group comprised four mice.

Treatment and observations: Each animal was topically dosed once a day for 3 consecutive days (Days 1, 2 and 3) on the dorsal surface of each ear with 25 µL of the appropriate formulation, applied using a pipette. There was no treatment on Days 4, 5 and 6.

All animals were observed at least once daily (Days 1-6) for any clinical signs, including local irritation and systemic toxicity. Individual body weights were recorded on Day 1 (beginning of the assay) and at Day 6 (prior to ^{3}HTdR injection).
Proliferation assay: On Day 6 each mouse was intravenously injected via the tail vein with 250 µL of sterile PBS (phosphate buffered saline) containing approximately 20 µCi of ^{3}HTdR using a gauge 25G1" hypodermic needle with 1 mL sterile syringe. Once injected, the mice were left for 5 hours.
Five hours after intravenous injection, the mice were killed by CO_2 asphyxiation. The draining auricular lymph nodes were excised by making a small incision on the skin between the jaw and sternum, pulling the skin gently back towards the ears and exposing the lymph nodes. The nodes were then removed using forceps and the carcasses discarded. The nodes of mice from each test group was pooled and collected in separate Petri dishes containing a small amount (1-2 mL) of PBS to keep the nodes wet before processing.
A single cell suspension (SCS) of pooled lymph node cells (LNCs) were prepared and collected in disposable tubes by gentle mechanical disaggregating of the lymph nodes through a cell strainer using the plunger of a disposable syringe. The cell strainer was washed with PBS (up to 10 mL). Pooled LNCs were pelleted with a relative centrifugal force (RCF) of 190 x g (approximately) for 10 minutes at 4 °C. After centrifugation supernatants were discarded. Pellets were gently resuspended and 10 mL of PBS was added to the tubes. The washing step was repeated twice. This procedure was repeated for each group of pooled lymph nodes.
After the final washing step, the suspensions were centrifuged and the supernatants were removed leaving a small volume (<0.5 mL) of supernatant above each pellet. Each pellet was gently agitated before suspending the LCNs in 3 mL of 5 % TCA (trichloroacetic acid) for precipitation of macromolecules. After incubation with 5 % TCA at 2-8 °C overnight (approximately 18 hours) precipitate was recovered by centrifugation at 190 x g for 10 minutes at 4 °C), and supernatants were removed and pellets were resuspended in 1 mL of 5 % TCA solution and dispersed using an ultrasonic water bath. Each precipitate was transferred to a suitable sized scintillation vial with 10 mL of scintillation liquid and thoroughly mixed. The vials were loaded into a β-scintillation counter and ^{3}HTdR incorporation was measured for up to 10 minutes per sample. The β-counter expressed the ^{3}HTdR incorporation as the number of radioactive disintegrations per minute (DPM). Similarly, background radiation levels were also measured in two 1 mL aliquots of 5 % TCA.
Statistics / Data evaluation: In the absence of any positive results, the statistical analysis of the data was not performed.
DPM was measured for each pooled group of nodes. The measured DPM values were corrected with the background DPM value ("DPM"). The results were expressed as "DPN" (DPM divided by the number of lymph nodes) following the industry standard for data presentation.
A stimulation index of 3 or greater is the criteria for defining a positive result.
The test item is regarded as a sensitiser if both of the following criteria are fulfilled:

- That exposure to at least one concentration resulted in an incorporation of ^{3}HTdR at least 3-fold or greater than recorded in control mice, as indicated by the stimulation index.
- The data are compatible with a conventional dose response, although allowance must be made (especially at high topical concentrations) for either local toxicity or immunological suppression.

Results and discussion

Clinical observation: No mortality or signs of systemic toxicity were observed during the Main Study. No cutaneous reactions were observed at the site of the treatment in any treatment groups.

Body weight: No treatment related effects were observed on body weight.

Proliferation assay: Appearance of the lymph nodes was normal in the negative control group and in the test item treated groups. Larger than normal lymph nodes was observed in the positive control group.

No mortality, cutaneous reactions or signs of toxicity were observed in the positive control group. A significant lymphoproliferative response (stimulation index value of 12.2) was noted for α-Hexylcinnamaldehyde in this experiment. The results of the positive control group demonstrated the appropriate performance of the assay.

Conclusion by the Notifiers

In conclusion, under the conditions of the present assay, glyphosate technical tested in a suitable vehicle, was shown to have no skin sensitisation potential (non-sensitiser) in the Local Lymph Node Assay.

Comment by RMS:

The study is considered acceptable and no evidence for skin sensitisation potential of the test substance was observed.

B.6.3 Short-term toxicity (Annex IIA 5.3)

Introduction into this chapter by the RMS
Those short-term studies are reported in detail in this section that were not mentioned in the original DAR (1998, ASB2010-10302) because they either had not been submitted for previous EU evaluation or were conducted more recently. Using the GTF dossier as basis, study decriptons were amended where necessary and each of these "new" studies was commented by RMS. Redundant parts were deleted. The structure of the document was changed. Some summary tables have been included and, where available, studies with formulations are also mentioned now in separate sub-sections.
Most of the previously known studies from the 1998 DAR may be still used for risk assessment purposes. They were all subject to re-evaluation with regard to quality and reliability. For the study design and their results, however, we refer to the old DAR.
Overall evaluation is presented in Volume 1, including tables in which the (new and previously known) valid oral subchronic studies in rats and dogs are summarised.

B.6.3.1 Subacute studies (all routes)

B.6.3.1.1 Subacute oral studies in rats and dogs

No new sub-acute oral toxicity studies with glyphosate have been run since the previous evaluation was performed in the late 1990ies. The five studies mentioned in the old DAR were re-evaluated by the RMS. The studies by [redacted] (1989, TOX9552351) and by [redacted] (1991, TOX9551095, Z102035, Z102043) in rats were still considered acceptable although the latter one had the apparent deficiency that sexes were combined for reporting of hematological and clinical chemistry parameters and organ weights in the summary tables. However, raw data that may be easily found in the study report allowed separate assessment. Of a third study by [redacted] (1989, TOX9552352), at least "part B" in which a limit dose of 1000 mg glyphosate/kg bw was adminstered daily for two weeks to dogs was also considered acceptable whereas part A was difficult to interpret due to a very unusual design with increasing doses.

The pilot studies by [redacted] (1978, TOX9552348) in mice and of [redacted] (1989, TOX9552350) in rats are not acceptable due to serious reporting deficiencies.

The rather small number of valid subacute studies by the oral route is not considered a data gap because there is quite a lot of fully acceptable 90-day studies in all relevant species.

Studies with formulations
A study by [redacted] (1982, TOX9552349) in dogs does also not completely comply to modern standards and may not be used for risk assessment of glyphosate but is of interest because higher toxicity of a formulation as compared to the active ingredient was elucidated. Strong gastrointestinal irritation became apparent when the isopropylamine (IPA) salt of glyphosate (MON 0139) was administered to Beagle dogs. Vomiting and diarrhea together with a reduction in body weight gain were observed at a dose level of 625 mg/kg bw and above. A dose of 312.5 mg/kg bw given for five days still caused a slight decrease in food consumption. Toxicity of isopropylamine alone was also tested in this experiment revealing strong irritation of gastric and esophageal mucosa at a much lower dose of 72 mg/kg bw upon

single and repeated administration suggesting that these effects were most probably not or only partly attributable to glyphosate itself.

B.6.3.1.2 Subacute inhalation studies (rat)

Only one study on subacute inhalative toxicity (14 days) in rodents ([redacted] 1985, TOX9551952) is available and was reviewed in the 2001 EU evaluation. The previous review concluded no treatment-related effects were observed and the NOEL (more correct NOEC) was 3.8 mg/L. However, from a todays regulatory point of view, the study must be considered unacceptable due to serious reporting deficiencies, e.g., absence of information on batch and purity of the test material.

<u>Studies with formulations</u>
A four-week inhalation study with the formulation MON 2139 ([redacted] 1983, TOX2002-694) suggesting some effects at rather low concentrations is reported and evaluated in the Appendix on formulations containing [redacted] as surfactants (see B.6.13).

B.6.3.1.3 Subacute percutaneous toxicity studies (rabbits and rats)

There was only one new study submitted for this evaluation, *i.e.*, a 21-day dermal study on rats by [redacted] (1996, ASB2012-11461) that is reported in the following in detail. A second study not mentioned in the previous DAR ([redacted] 2012, ASB2012-11459) was originally allocated to this section by GTF but is now reported under B.6.12 since its focus was on dermal absorption (*in vitro*, rabbit skin) and not on toxicity.

For details regarding the studies that were reviewed during the 2001 EU evaluation, we refer to the original DAR (1998, ASB2010-10302). Re-evaluation by the RMS revealed that the study on rats by [redacted] (1993, TOX9552367) and those by [redacted] (1982, TOX9552366) and [redacted] (1994, TOX9650151) on rabbits may be still considered acceptable. In contrast, quality assessment of a further study in rabbits ([redacted] 1985, TOX9551951) was downgraded to "not acceptable" (from "supplementary" in the old DAR) because of reporting deficiencies (batch and purity lacking). The absence of statistical analysis may be explained by the very low number of animals (3 per sex and dose) but this small group size is one more reason for doubts on the reliability of this study. .

<u>New subacute dermal toxicity study on rats ([redacted] 1996)</u>

Reference:	IIA, 5.3.7/04
Report:	[redacted]. 1996 Glyphosate Acid: 21 Day Dermal Toxicity Study In Rats [redacted] Data owner: Syngenta Report No.: [redacted]/P/4985 Date: 1996-06-24 not published, ASB2012-11461
Guidelines:	OECD 410 (1981): OPPTS 870.3200 (1998): 87/302/EEC B.28 (1988)
Deviations:	None

GLP: Yes
Acceptability: *See RMS comment*

Dates of experimental work: 1996-01-09 to 1996-06-24

Materials and methods

Test Material:	Glyphosate acid
Description:	Technical, white solid
Lot/Batch number:	P24
Purity:	95.6 % w/w a.i
CAS#:	Not reported
Stability of test compound:	Not reported
Vehicle and/or positive control:	Deionised water
Test Animals:	
Species	Rat
Strain	Alpk:APfSD
Age/weight at dosing	6 - 8.5 weeks / males 214-249 g, females 193-227 g
Source	[redacted]
Housing	Individually, in cages on multiple rat racks suitable for animals of this strain and weight range expected during the course of the study.
Acclimatisation period	At least 5 days
Diet	Diet (PCD) supplied by Special Diet Services Limited, Witham, Essex, UK *ad libitum*
Water	Mains water *ad libitum*
Environmental conditions	Temperature: 21 ± 2 °C Humidity: 40-70 % Air changes: At least 15 changes/hour Photoperiod: 12 hours light/12 hours dark

Study design and methods
In-life dates: Start: 10 January 1996 End: 1 February 1996

Animal assignment: The study was divided into ten (randomised blocks), each containing one cage per treatment group. The animals were randomly allocated to groups as shown below:

Table B.6.3-1: Study design (Pinto, 1996 ASB2012-11461)

Test group	Dose level of glyphosate acid (mg/kg/day)	# male	# female
Control	0	5	5
Low	250	5	5
Mid	500	5	5
High	1000	5	5

Preparation and treatment of animal skin:

Sixteen to twenty-four hours before application of the test substance, the hair was removed with a pair of veterinary clippers from an area, approximately 10 cm x 5 cm, on the dorso-lumbar region of each animal. The rats were dosed dermally and the amount applied was calculated for each animal according to its weight at the time of dosing. The paste covered by a gauze patch (approximately 7 cm x 7 cm x 4-ply) was applied to the shorn back of each animal and was kept in contact with the skin for approximately 6 hours using an occlusive dressing. The gauze patch was covered by a patch of plastic film (7 cm x 7 cm) and was held in position using adhesive bandage (25 cm x 7.5 cm). This was secured by two pieces of PVC tape (approximately 2.5 cm x 20 cm) wrapped around the animal. The control animals were treated in a similar manner except that deionised water only was used. The rats were dosed sequentially in group order at approximately the same time each day.

At the end of each 6-hour contact period, the dressings were carefully removed. The skin, at the site of application, was cleansed using clean swabs of absorbent cotton wool soaked in clean warm water and was then dried gently with clean tissue paper.

A total of 15 six-hour applications was made during a period of 21 days. During this time there were three two-day periods when the animals were not dosed. Following each application there was an 18-hour 'rest' period during which the animals were fitted with plastic collars to prevent oral contamination.

Observations:
Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. Detailed clinical observations were recorded daily and after decontamination. Cage-side observations were also made as soon as possible after dosing, and towards the end of the working day.

Body weight: The body weight of each rat was recorded daily, immediately prior to application of the test substance where applicable and prior to termination on day 22.

Food consumption and test substance intake: Food consumption was recorded continuously throughout the study for each rat and calculated as a weekly mean (g food/rat/day).

Haematology and clinical chemistry: Blood was collected at termination, by cardiac puncture and the following parameters were examined:

Haemoglobin
Haematocrit
red blood cell count
mean cell volume
mean cell haemoglobin
red cell distribution width
activated partial thromboplastin time
mean cell haemoglobin concentration
platelet count
total white cell count
differential white cell count
blood cell morphology
prothrombin time

Clinical chemistry: Blood was collected at termination, by cardiac puncture and the following parameters were examined:

Urea	alkaline phosphatase activity
Creatinine	aspartate aminotransferase activity
Glucose	alanine aminotransferase activity
Albumin	gamma-glutamyl transferase activity
total protein	calcium
Cholesterol	phosphorus (as phosphate)
Triglycerides	sodium
total bilirubin	potassium
creatine kinase activity	chloride

Investigations *post mortem:*
Macroscopic examination: All animals were examined *post mortem*. This involved an external observation and an internal examination of all organs and structures.

Organ weights: From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed:

adrenal glands	liver
Kidneys	testes

Paired organs were weighed together.
Tissue submission: The following tissues were examined *in situ*, removed and examined and fixed in an appropriate fixative:

gross lesions including masses	adrenal gland*
testis*	epididymis*
Kidney	treated skin
Liver	untreated skin

* Tissues marked were stored but not examined microscopically

Microscopic examination: All selected tissues (see above) processed from the control and the group receiving 1000 mg glyphosate acid/kg/day, together with macroscopic abnormalities from these groups, were examined by light microscopy.

Statistics:
Haematology, clinical chemistry, organ weights and weekly food consumption were analysed using Analysis of variance. Body weights, on initial (day 1) body weight, organ weights on final body weight were analysed using analysis of covariance. All data were analysed using *SAS (1989*).

Results and discussion
Mortality: There were no mortalities.
Clinical observations: There were no significant signs of toxicity at any dose level of glyphosate acid. Generally the clinical findings observed were consistent with those

commonly seen in dermal studies as a consequence of bandaging and were considered not to be related to treatment with glyphosate acid.
Bodyweight and weight gain: There were no effects due to treatment with glyphosate acid on bodyweight at any dose level.

Food consumption: There were no effects due to treatment with glyphosate acid on bodyweight at any dose level.
Haematology: A minimal statistically significant increase in haemoglobin levels was observed in females dosed at 1000 mg glyphosate acid/kg/day. A statistically significant decrease compared with control was seen in red cell distribution width in females dosed at 250 and 1000 mg glyphosate acid/kg/day. In the absence of any adverse effects on the red cell parameters, these minor changes are considered not to be of toxicological significance.

Table B.6.3-2: Intergroup comparison of selected haematology parameters (Pinto, 1996 ASB2012-11461)

	Dose level of glyphosate acid (mg/kg/day)							
	Males				Females			
Parameter	0	250	500	1000	0	250	500	1000
Haemoglobin	15.2	15.3	15.3	15.0	13.9	13.7	14.1	14.6*
Red cell distribution width	13.1	12.9	12.6	13.4	13.8	12.4**	13.0	12.6*

* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided)
** Statistically significant difference from control group mean, p<0.01 (Student's t-test, 2-sided)

Blood clinical chemistry: Females dosed at 1000 mg glyphosate acid/kg/day showed a minimal but statistically significant increase in plasma urea levels, but there were no differences seen in the plasma creatinine levels. This minimal change in urea was considered not to be of toxicological significance. A minimal but statistically significant decrease in plasma triglycerides was observed in males dosed at 500 mg glyphosate acid/kg/day and as this did not form part of a dose response relationship was considered not to be treatment related.

Table B.6.3-3: Intergroup comparison of selected clinical chemistry parameters (Pinto, 1996 ASB2012-11461)

	Dose level of glyphosate acid (mg/kg/day)							
	Males				Females			
Parameter	0	250	500	1000	0	250	500	1000
plasma urea	8.4	8.2	8.5	8.1	7.6	7.7	6.9	8.6*
plasma trigycerides	1.27	1.01	0.87*	1.27	0.70	0.66	0.69	0.76

* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided)

Sacrifice and pathology:
Organ weights: Testes weights were slightly but statistically significantly decreased at 500 mg glyphosate acid/kg/day, due to one animal having a very low weight recorded. There were no effects due to treatment with glyphosate acid in the other organs weighed.

Macroscopic findings: A small number of lesions were observed, none of which was related to treatment.

Microscopic findings: A small number of common spontaneous lesions were observed, none of which was related to treatment.

Conclusion by the Notifiers
There was no evidence of systemic toxicity or dermal irritation following 15 dermal applications over a 21 day period of up to 1000 mg glyphosate acid/kg/day.
The no observed adverse effect level (NOAEL) for systemic toxicity and dermal irritation was considered to be 1000 mg glyphosate acid/kg/day in both sexes.

RMS comments:
The study is considered acceptable even though most dermal studies in rats include a treatment period of four weeks while rabbits are administered a test substance via the skin usually for 21 days. We agree with the conclusions including setting of the NOAEL for both systemic effects and dermal irritation at the limit dose of 1000 mg/kg bw.

Studies with formulations
Not available.

B.6.3.2 Subchronic toxicity (oral)

B.6.3.2.1 90-day studies in rats

A total of eight valid studies of this type were used for this evaluation of which 5 had been subject to the previous one (DAR, 1998, ASB2010-10302) yet. Re-evaluation of the studies by [redacted] (1987, TOX9552362), [redacted] (1989; TOX9552364) and Eadie (1989, TOX9551821) from the old DAR revealed that all these may be still considered fully acceptable even though in the latter one the actual mean dietary intake was not calculated.
In contrast, for the study by [redacted] (1993; TOX9650149), former assessment as "acceptable" was changed by the RMS into "supplementary" because the batch number but no purity of the test material was given. This same holds true for a study by [redacted] (1992, TOX9551096) in which reporting deficiencies were noted. Mean dietary intake was calculated in an amendment but not for the recovery group and no information was given which of the salivary gland had been examined histologically. Apparently, no additional control group was employed for the recovery part.
Two more supplementary studies mentiond in the original DAR were not included by the RMS into the re-evalution. The study by [redacted] (1981, TOX9650152, in the former DAR erroneously cited as "anonymous") may be considered as sufficiently replaced by a more recent one ([redacted] 1993, TOX9650149) that had been commissioned by the same company. The study by [redacted] (1990, TOX9500266) was run soon after the more comprehensive by [redacted] (1989, TOX9551821) in the same laboratory and the same rat strain. The same dose levels were used and no effects were noted up to the highest dose of 7500 ppm. Thus, this study would not contribute any new information to overall assessment.

One further study ([redacted] 1985, TOX9551822) was briefly described in the previous DAR and regarded therein as "supplementary" but is now considered not acceptable due to major reporting deficiencies. In addition, absence of statistical analysis was noted.

In addition, three new feeding studies in rats ([redacted] 1996, TOX2000-1990; [redacted] 1996, ASB2012-11451; [redacted] 1995, ASB2012-11452) were submitted that had not been reviewed on EU level before. Subsequent to the summary table, they are reported in detail,

with comments of the RMS on bottom of the respective study. For details regarding studies reviewed during the 2001 EU evaluation, we refer to the old monograph (DAR, 1998, ASB2010-10302).

1st new subchronic study in rats ([redacted] 1996)

Reference:	IIA, 5.3.2/01
Report:	[redacted] 1996 First Revision To Glyphosate Acid: 90 Day Feeding Study In Rats [redacted] Laboratory Report No.: CTL/P/1599 Data owner: Syngenta Date: 1996-11-07, not published, TOX2000-1990
Guidelines:	No guideline statement, but in accordance with OECD 408 (1998), OPPTS 870.3100 (1998), 2001/59/EC B.26 (2001)
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Materials and methods

Test material:	
Identification:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P15
Purity:	97.4 %
Stability of test compound:	Not reported
Vehicle:	Plain diet / none
Test animals:	
Species:	Rat
Strain:	Alpk:APfSD
Source:	[redacted]
Age:	36-38 days
Sex:	Male and female
Weight at dosing:	♂ 98-170 g; ♀ 96-140 g
Acclimation period:	Approximately 1 week
Diet/Food:	CTI diet, *ad libitum*, (except during collection of urine samples)
Water:	Mains water, *ad libitum*, (except during collection of urine samples)
Housing:	4/cage, sexes separately in stainless steel cages 34.0 x 37.5 x 20.3 cm giving a floor area of 1275cm^2

Environmental conditions: Temperature: 21 ± 2 °C
Humidity: 36 - 60 %
Air changes: ≥ 15/hour
12 hours light/dark cycle

Study design and methods:
In life dates: 1996-02-25 to 1996-05-25

Animal assignment and treatment:
The study consisted of one control and three treatment groups each containing twelve male and twelve female rats.

Table B.6.3-4: Study design

Test group	Dietary concentration (ppm)	Males	Females
Control	0	12	12
Low	1000	12	12
Mid	5000	12	12
High	20000	12	12

The study was divided into six single-sex replicates (randomised blocks). Each replicate consisted of four cages, one per treatment group. The animals were randomly allocated to cages.

The experimental diets were made in 60 kg batches by adding the appropriate amount of glyphosate acid to the diet using dry premixes.
Samples from all dietary levels (including controls) were taken from both batches prepared for the study and analysed quantitatively for glyphosate acid. The homogeneity of glyphosate acid in CT1 diet was determined by analysing samples from the low and high dose levels from the first batch of diet. The chemical stability of glyphosate acid in diet was determined at the highest and lowest dose levels at 1, 4, 6 and 10 weeks after preparation. Analysis was by high performance liquid chromatography (HPLC).

Mortality: Each animal was checked for mortality or signs of morbidity at least once daily during the treatment period.
Clinical observations: A check for clinical signs of toxicity was made once daily on all animals. In addition, a detailed clinical examination was performed at least once before of the beginning of the treatment period and then once a week until the end of the study.
Body weight: The bodyweight of each animal was recorded immediately before feeding of the experimental diets commenced and then on the same day, where practicable, of each subsequent week until termination. The body weight determination was done on the same day on which the detailed clinical examination was performed.
Food consumption and untilisation: Food consumption was recorded continuously throughout the study for each cage of rats and calculated as a weekly mean (g food/rat/day) for each cage. The food utilisation value per cage was calculated as the bodyweight gained by the rats in the cage per 100 g of food eaten.
Ophthalmoscopic examination: The eyes of all animals from the control group and the 20000 ppm glyphosate acid dose level group were examined in the week prior to termination, using an indirect ophthalmoscope and a mydriate to dilate the pupil.

Haematology and clinical chemistry: At termination, all surviving rats were bled by cardiac puncture and the blood samples were collected both in tubes containing EDTA as anticoagulant and also in tubes containing 0.11M trisodium citrate. These samples were submitted for haematological examination and the following parameters measured: haemoglobin, haematocrit, red blood cell count, MVC, MCHC, MCH, kaolin-cephalin times, thrombocytes, leucocytes, differential white cell count, red blood cell morphology, prothrombin time.
For clinical chemistry analysis blood samples were collected by tail vein bleeding at week 4 of the study and by cardiac puncture at termination (week 13). The blood was collected in lithium heparinised tubes and the following parameters measured: glucose, urea, total protein, albumin, total cholesterol, triglycerides, alkaline phosphatase (ALP), aspartate aminotransferase (ASAT), and alanine aminotransferase (ALAT).
Urinalysis: Urine samples were collected over an 18 hour (approximately) period from all rats during week 13 (the week prior to termination). During urine collection, the rats were individually housed in metabolism cages and denied access to food and water. The following parameters were measured:volume, pH, specific gravity, proteins, glucose, ketones, and urobilinogen.
Sacrifice and pathology: On completion of the treatment period, all surviving animals were sacrificed and subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined: adrenals, brain, epididymides, heart, kidneys, liver, and testes. Paired organs were weight together.
Tissue samples were taken from the following organs and preserved in buffered formalin: all gross lesions, adrenals, aorta, bone marrow (femur), brain, caecum, colon, duodenum, epididymides, eyes (stored), Harderian gland (stored), heart, ileum, jejunum, kidneys, larynx (stored), liver, lungs, lymph nodes (cervical and mesenteric), mammary gland, ovaries, oviducts, pancreas, pituitary gland, prostrate, rectum, salivary glands, seminal vesicles, spinal cord, sciatic nerve, skin, spleen, stomach, testes, thymus, thyroid gland, trachea, urinary bladder, uterus (with cervix), voluntary muscle and nasal cavity.
Following fixation, all tissues from the control and 20000 ppm glyphosate acid groups (except those stored) were processed by standard methods, embedded in paraffin wax, sectioned at 5µm, stained with haematoxylin and eosin and examined by light microscopy. Liver, kidney, adrenals, lungs and abnormal tissues from animals fed 1000 ppm or 5000 ppm glyphosate acid were also processed to blocks and were examined histologically.
Statistics: All data were evaluated using analysis of variance (bodyweight gain from start of study, final bodyweight, haematology, clinical chemisty – blood and urine, total food consumption and utilisation, organ weights) and covariance (organ weights on terminal bodyweights) for each specified parameter using the GLM procedure in SAS (1982).

Results and discussion
Mortality: There were no mortalities.
Clinical observations: The incidence of clinical findings was low and none was unequivocally related to treatment. There was a low incidence of diarrhoea (during the second week of the study) in the group receiving 20000 ppm glyphosate acid. The faeces of both sexes at this dose level were observed to be paler than those of control or other test groups.
Body weight: No relevant differences in the mean body weight gain were noted between controls and animals given 1000 or 5000 ppm.
Body weight gain was reduced in male rats fed 20000 ppm glyphosate acid from the first week of the study. The body weights continued to diverge from control values as the study

progressed, and final body weights were approximately 8 % lower than those of controls (see Table B.6.3-5).

Table B.6.3-5: Intergroup comparison of bodyweight gain – selected timepoints from start of study

	Mean cumulative bodyweight gain (g)							
Timepoint	Initial	Week 1	Week 2	Week 4	Week 7	Week 10	Week 13	Final weight
Dose (ppm)	Males							
0	135	51.8	104.0	185.5	254.6	305.1	333.3	468.3
1000	140.3	54.3	106.3	187.5	253.4	304.1	327.0	467.3
5000	136.3	51.8	103.4	186.1	255.1	306.3	331.9	468.3
20000	134.5	45.1**	94.0*	166.9**	226.0**	272.00**	295.8**	430.3**
	Females							
0	121.3	26.6	47.3	81.6	112.8	130.8	143.3	264.6
1000	122.2	27.7	51.4	82.7	113.1	132.1	146.0	268.2
5000	121.3	25.9	50.2	82.9	110.0	129.5	138.4	259.8
20000	118.6	24.3	53.5*	83.3	115.1	132.7	142.5	261.1

* Statistically significant from controls, p<0.05 (Student´s t-test, 2-sided)
** Statistically significant from controls, p<0.01 (Student´s t-test, 2-sided)

Food consumption and test substance intake: The food consumption of males fed 20000 ppm glyphosate acid was reduced from the fifth week of the study compared to control values but the reduction was small and did not attain statistical significance in any week. The food utilisation efficiency of males at this dose level was reduced throughout the study. The food consumption and food utilisation efficiency of males fed 1000 or 5000 ppm glyphosate acid and of females at all dose levels were similar to those of controls.

Table B.6.3-6: Intergroup comparison of food utilisation (g growth/100 g food)– selected timepoints from start of study

	Dietary concentration of glyphosate acid (ppm)							
	Males				Females			
Weeks	0	1000	5000	20000	0	1000	5000	20000
1-4	25.15	24.85	24.99	22.89	15.73	15.42	15.78	15.91
5-8	11.25	11.09	11.36	10.31	7.49	6.74	6.71	7.29
9-13	6.45	5.76	6.05	5.88	3.08	3.95	3.10	2.96
Overall (1-13)	13.59	13.30	13.44	12.54	8.28	8.34	8.14	8.25
* Statistically significant from controls, p<0.05 (Student´s t-test, 2-sided)								

Calculated mean test compound intakes are presented in the following table.

Table B.6.3-7: Overall mean test compound intake

	Dietary concentration of glyphosate acid (ppm)							
	Males				Females			
Weeks	0	1000	5000	20000	0	1000	5000	20000
1-13	0	81.33	413.5	1612	0	90.42	446.9	1821
* Statistically significant from controls, p<0.05 (Student´s t-test, 2-sided)								

Ophthalmoscopic examination: There were no test substance-related ophthalmological findings at the end of the treatment period. The small incidence of findings recorded was within the normal background incidence for rats of this age and strain.

Haematology and clinical chemistry
Haematology: There were no treatment-related effects noted in any dose group.
Blood clinical chemistry: The plasma activities of alanine transaminase (ALAT) and alkaline phosphatase (ALP) were increased in both sexes fed 20000 ppm glyphosate acid throughout the study. Plasma aspartate transaminase activity was increased in females fed 20000 ppm glyphosate acid at week 4 only.
Plasma ALT activity was also increased in males receiving 5000 ppm glyphosate acid at weeks 4 and 13 and in females at week 4 only.
The plasma ALP activities of males receiving 5000 or 1000 ppm glyphosate acid were marginally increased. These increases were not dose-related and for the 1000 ppm glyphosate acid group were attributed to the high values in 3 out of 12 males. These marginal differences from the control group are considered to be of doubtful significance and not to be treatment-related.
Plasma urea levels were marginally decreased in both sexes at week 13 and in males at week 4 in the 20000 ppm glyphosate acid group.
Males receiving glyphosate acid showed marginal reductions in plasma glucose levels at week 4 but not at week 13. Females at 20000ppm glyphosate acid showed a slight increase in this parameter at week 13 only.
Plasma cholesterol levels were unaffected by treatment with glyphosate acid. Plasma triglyceride levels were slightly reduced in males receiving 20000 ppm glyphosate acid at both weeks 4 and 13, the effect being greater at week 13.
Both males and females receiving glyphosate acid showed marginal reductions in plasma albumin and total protein. The changes were not consistent, showed no dose-response relationship and are therefore considered to be of dubious significance.

Table B.6.3-8: Intergroup comparison of selected clinical chemistry parameters

		Dietary concentration of glyphosate acid (ppm)							
		Males				Females			
Parameter	Week	0	1000	5000	20000	0	1000	5000	20000
ALAT	4	61.0	66.8	76.0**	83.8**	47.3	50.8	57.7*	73.8**
	13	51.9	52.3	62.3*	65.2**	45.0	45.2	46.2	55.0**
ALP	4	273	326**	320*	411**	188	199	212	309**
	13	148	159	176*	215**	91	94	99	140**
ASAT	4	62.8	67.0	69.1	68.5	56.0	57.0	57.5	64.8**
Urea	4	47.0	45.8	44.6	43.6*	45.8	45.3	46.8	44.2
	13	41.9	39.9	40.0	37.7*	40.6	40.1	42.1	35.9*
Glucose	4	143	133*	132*	128**	141	146	142	136
	13	191	186	208	197	182	183	183	208**
Triglycerides	4	151	145	147	136	80	75	88	84
	13	153	157	144	120**	72	74	77	77

* Statistically significant from controls, p<0.05 (Student´s t-test, 2-sided)
** . Statistically significant from controls, p<0.01 (Student´s t-test, 2-sided)

Urine analysis: There were no treatment-related findings.

Necropsy
Organ weights: Absolute heart weight of top dose males was reduced compared to controls but the reduction reflected the reduced bodyweight. There were no other differences in organ weights which were considered to be related to treatment
Gross pathology: A small number of lesions were observed, none of which was related to treatment.
Histopathology: There were no histopathological findings related to treatment. The incidence of findings was low and, with one exception, of a type commonly found in rats of this strain and age. An uterine leiomyosarcoma was seen in a female fed 5000 ppm glyphosate acid. Whilst the occurrence of a malignant tumour of smooth muscle in the uterus of a young rat is unusual, this isolated finding in an intermediate dose group is considered not to be related to treatment.

Conclusion by the Notifiers
Under the experimental conditions of the study and taking into account the reduced growth (males only) and biologically insignificant changes in clinical chemistry parameters at the high dose-level, the No Observed Adverse Effect Level (NOAEL) is considered to be 5000 ppm (equivalent to 414 and 447 mg/kg bw/day for amles and females, respectively).

RMS comments
This study is considered acceptable and the conclusions, including setting of the NOAEL, are agreed with. The significant changes in clinical chemistry parameters at the mid dose level in males might be indicative of a weak effect on the liver, however, since they were not accompanied by histological lesions and/or liver weight increase, are not regarded as adverse. In addition to the study description above, it should be noted that, as in previous studies with glyphosate, urine pH was significantly decreased in top dose males and in mid and high dose females. From the study report it seems that salivary glands were taken but neither weighed nor examined microscopically although they were a target organ in other studies.

2nd new subchronic study in rats ([redacted], 1996)

Reference:	IIA, 5.3.2/03
Report:	Coles, L.J., Thomas, O.N., Bartlett, A.J., Brooks, P.N. 1996, Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study In The Rat [redacted] [redacted] Project No.: 434/016 Date: 1996-07-16, unpublished, ASB2012-11451
Guidelines:	JMMAF 59 NohSan No. 4200 (Data from the study report is equivalent to OECD 408.)
Deviations:	FOB was not conducted. Some mandatory organs were not weighed/examined.
GLP:	yes
Acceptability:	*See RMS comment*

Materials and methods

Test material:	
Identification:	Technical Glyphosate
Description:	White powder
Lot/Batch #:	H95D 161 A
Purity:	95.3 %
Stability of test compound:	No data given in the report.
Vehicle:	Plain diet
Test animals:	
Species:	Rats
Strain:	Sprague-Dawley (CD)
Source:	[redacted]
Age:	6-7 weeks
Sex:	male and female
Weight at dosing:	♂ 175 - 218 g; ♀ 145 - 195 g
Acclimation period:	7 days
Diet/Food:	Rat and Mouse SQC Ground Diet No.1 (Special Diets Services Limited, Witham, Essex, UK), *ad libitum*
Water:	tap water, *ad libitum*
Housing:	In groups of up to four by sex in polypropylene grid-floor cages.
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 55 ± 15 % Air changes: 15/hour 12 hours light/dark cycle

Study design and methods:
In life dates: 1995-08-11 to 1996-01-30

Animal assignment and treatment:
In a 90 day feeding study, groups of 10 Sprague Dawley rats per sex received daily dietary doses of 0, 1000, 10000 and 50000 ppm (equivalent to mean achieved dose levels of male; 0, 79, 730 and 3706 mg/kg bw/day, female; 90, 844, 4188 mg/kg bw/day respectively) technical glyphosate in the diet.
Test diets were prepared prior to start of treatment and then twice during the three month study period by mixing a known amount of the test substance with a small amount of basal diet and blending for 19 minutes. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes.
The stability and homogeneity of the test material in diet were determined. Samples of each dietary admixture were analysed for homogeneity and achieved concentration.

Clinical observations: A check for clinical signs of toxicity, ill health and behavioural changes was made once daily on all animals. All observations were recorded.
Body weight: Individual body weights were recorded on Day 0 (prior to treatment) and at weekly intervals thereafter. Body weights were also determined at necropsy.

Food consumption and compound intake: Food consumption was recorded once weekly for each cage group throughout the study.
Water consumption: Water intake was observed daily, for each cage group, by visual inspection of the water bottles for any overt changes.
Ophthalmoscopic examination: The eyes of all control and high dose animals were examined before administration of the test and control diets and before termination of treatment (during Week 12). Examinations included observation of the anterior structures of the eye, pupillary and corneal blink reflex and, following pupil dilation with 0.5 % Tropicamide solution ("Mydriacyl" - Alcon Laboratories Ltd., Watford, Hertfordshire, UK), detailed examination of the internal structure of each eye using a direct ophthalmoscope.
Haematology and clinical chemistry: Haematological and blood chemical investigation were performed on all animals from each test and control group at the end of the study (Day 90).
Urinalysis: Urinalytical investigations were performed on all animals during Week 12. Urine samples were collected overnight by housing the rats in metabolism cages. Animals were maintained under conditions of normal hydration during collection but without access to food.

Sacrifice and pathology: All animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined: adrenals, brain, gonads, heart, kidneys, liver, pituitary and spleen.
Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta (thoracic), bone & bone marrow (sternum and femur (incl. stifle joint)), brain (at three levels), caecum, colon, duodenum, eyes, gross lesions, heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, lymph nodes (cervical and mesenteric), muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin (hind limb), spinal cord (cervical), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus and vagina.

Statistics: Absolute and relative organ weights, haematological and blood chemical data were analysed by one way analysis of variance incorporating ‘F-max’ test for homogeneity of variance. Data showing heterogeneous variances were analysed using Kruskal-Wallis non-parametric analysis of variance and Mann Whitney U-Test.
The levels of probability chosen as significant were $p < 0.001$***, $p < 0.01$** and $p < 0.05$*.
Histopathology data were analysed using the following methods to determine significant differences between control and treatment groups for the individual sexes:
1. Chi squared analysis for differences in the incidence of lesions occurring with an overall frequency of 1 or greater.
2. Kruskal-Wallis one way non-parametric analysis of variance for the comparison of severity grades for the more frequently observed graded conditions.
The levels of probability chosen as significant were $p < 0.001$***, $p < 0.01$**, $p < 0.05$* and $p < 0.1$ (*).

Results and discussion
Mortality: No deaths occurred during the study.
Clinical observations: Animals of both sexes treated with 50,000 ppm showed soft faeces and diarrhoea from Day 4 which continued throughout the study period.
The remaining observable sign of generalised fur loss was noted in one male and two females treated with 10,000 and 1,000 ppm respectively. This is a commonly reported incidental finding in laboratory maintained rats that, in the absence of any dose-related response, is of no toxicological significance and unrelated to treatment with the test material.

Body weight: Animals of both sexes treated with 50,000 ppm showed a reduction in bodyweight gain over the first four weeks of treatment when compared with controls (see Table B.6.3-9). Female bodyweight development recovered as the study progressed and was comparable with the control group by the end of the treatment period. Male individuals showed only a partial recovery with body weight gain remaining slightly lower than the control group values during the subsequent weeks of treatment.
Body weight development was unaffected by treatment with the test material at the remaining dose levels.

Table B.6.3-9: Group mean weekly bodyweights and standard deviations (sd)

Dietary concentration		**Bodyweight (g) at Day**													
(ppm)		0	7	14	21	28	35	42	49	56	63	70	77	84	90
		Males													
0	mean	206	269	315	354	382	411	444	457	488	508	523	537	536	551
	sd	8	12	17	24	33	38	45	44	49	52	55	58	56	58
1000	mean	199	260	309	350	377	400	427	446	470	485	497	513	516	528
	sd	11	14	19	21	24	26	30	31	32	32	35	37	36	37
10000	mean	200	257	303	338	364	393	414	429	454	470	483	494	495	506
	sd	12	12	15	21	25	30	35	35	38	38	38	40	39	43
50000	mean	198	215	247	268	283	306	329	335	356	369	382	394	395	408
	sd	8	8	15	21	26	31	33	38	41	43	43	44	42	44
		Females													
0	mean	173	197	214	232	243	256	269	276	284	291	295	306	304	307
	sd	9	11	12	15	16	18	20	19	20	21	24	25	25	27
1000	mean	173	199	218	238	249	261	272	280	286	292	300	308	304	313
	sd	10	13	14	16	16	17	18	19	18	18	19	21	20	20
10000	mean	166	184	201	217	226	237	246	256	262	267	272	277	276	282
	sd	14	18	21	25	24	26	27	27	27	27	27	29	28	29
50000	mean	173	183	197	214	219	231	240	246	251	260	265	271	267	273
	Sd	11	12	14	15	14	18	21	21	20	23	23	26	22	25

Food consumption: Animals of both sexes treated with 50,000 ppm showed a reduction in both dietary intake and food efficiency over the first four weeks of treatment when compared with controls (see Table B.6.3-10). Female food consumption and efficiency recovered as the study progressed and was comparable with control values by the end of the treatment period. Male food consumption however, remained adversely affected during the subsequent weeks of treatment. A similar prolonged effect on food efficiency was not evident during the same period as male body weight gain demonstrated a partial recovery over the corresponding weeks.
Dietary intake and food efficiency were unaffected by treatment with the test material at the remaining dose levels and were comparable with controls.

Table B.6.3-10: Group mean weekly food consumption

Dietary concentration (ppm)	Mean food consumption (g/rat/week)												
	1	2	3	4	5	6	7	8	9	10	11	12	13*
	Males												
0	201	199	204	212	208	218	208	222	224	223	24	192	185
1000	200	205	213	211	205	210	211	217	204	219	214	191	180
	(0)	(3)	(4)	(0)	(-1)	(-4)	(1)	(-2)	(-9)	(-2)	(0)	(-1)	(-3)
10000	187	193	199	204	202	198	201	205	211	211	201	185	179
	(-7)	(-3)	(-2)	(-4)	(-3)	(-9)	(-3)	(-8)	(-6)	(-5)	(-6)	(-4)	(-3)
50000	122	183	178	177	183	182	168	187	189	193	188	174	171
	(-39)	(-8)	(-13)	(-17)	(-12)	(-17)	(-19)	(-16)	(-16)	(-13)	(-12)	(-9)	(-8)
	Females												
0	140	131	171	153	149	149	152	152	151	147	155	139	128
1000	143	146	152	156	158	163	157	159	152	154	161	141	137
	(2)	(+11)	(-11)	(2)	(6)	(9)	(3)	(5)	(1)	(5)	(4)	(1)	(7)
10000	123	135	142	144	143	140	143	146	143	143	142	133	131
	(-12)	(3)	(-17)	(-6)	(-4)	(-6)	(-6)	(-4)	(-5)	(-3)	(-8)	(-4)	(2)
50000	128	143	131	148	167	157	148	151	151	151	161	139	139
	(-9)	(9)	(-23)	(-3)	(23)	(5)	(-3)	(-1)	(0)	(3)	(4)	(0)	(9)

( ) - % change compared to control group * - Week 13 comprises six days only

Water consumption: There were no treatment-related effects on water consumption for either sex noted during the study.
Ophthalmoscopic examination: No treatment-related ocular effects for either sex noted were detected during the study.

Haematology: No treatment-related effects were detected in the haematological parameters measured.
Blood chemistry: Animals of both sexes treated with 50,000 or 10,000 ppm showed a statistically significant reduction in plasma calcium concentration and an increase in alkaline phosphatase (AP) when compared with controls (see Table B.6.3-11). A statistically significant increase in inorganic phosphorus and reduction in plasma creatinine were also evident amongst animals of both sexes treated with 50,000 ppm whilst females at this dose level showed statistically significant reductions in total plasma protein and albumin in comparison with controls.
There were no further treatment-related effects.

Table B.6.3-11: Group mean blood chemical values and standard deviations (sd)

Dietary concentration		Ca^{2+}	AP	P	Creatinine	Total protein	Albumin
(ppm)		(mmol/L)	(IU/L)	(mmol/L)	(mg/dL)	(g/dL)	(g/dL)
		Males					
0	mean	2.74	373	2.23	0.61	–	–
	sd	0.06	101	0.22	0.03	–	–
1000	mean	2.77	404	2.22	0.62	–	–
	sd	0.07	115	0.16	0.05	–	–
10000	mean	2.66*	514*	2.32	0.59	–	–
	sd	0.09	106	0.28	0.04	–	–
50000	mean	2.64*	597***	2.46*	0.57*	–	–
	sd	0.10	150	0.22	0.04	–	–

Dietary concentration		**Ca^{2+}**	**AP**	**P**	**Creatinine**	**Total protein**	**Albumin**
(ppm)		(mmol/L)	(IU/L)	(mmol/L)	(mg/dL)	(g/dL)	(g/dL)
		Females					
0	mean	2.78	230	1.70	0.69	7.63	3.90
	sd	0.11	38	0.33	0.07	0.45	0.23
1000	mean	2.76	261	1.65	0.69	7.64	3.87
	sd	0.05	71	0.21	0.04	0.29	0.13
10000	mean	2.70*	408***	1.76	0.65	7.41	3.82
	sd	0.07	123	0.23	0.04	0.45	0.20
50000	mean	2.56***	358**	2.12***	0.61**	6.86**	3.47***
	sd	0.10	90	0.15	0.05	0.82	0.39

– no significant changes
* significantly different from control group ($p < 0.05$)
** significantly different from control group ($p < 0.01$)
*** - significantly different from control group ($p < 0.001$)

Urinalysis
Animals of both sexes treated with 50,000 ppm showed an increased level of haemoglobin in the urine when compared with controls (see Table B.6.3-12). Microscopic examination of sediment revealed unidentified particulate matter in the samples obtained from males treated at 50,000 ppm. This probably represents external contamination, possibly of faecal origin. There were no treatment-related changes detected at the remaining dose levels.

Table B.6.3-12: Urine findings

Dietary concentration (ppm)	**Blood (haemoglobin)**							
	Males				Females			
	-	+	++	+++	-	+	++	+++
0	8	0	1	1	10	0	0	—
1000	10	0	0	0	10	0	0	—
10000	7	2	1	0	10	0	0	—
50000	1	5	2	2	4	3	3	—

– - negative
+ - ca. 5-10 × 10^6 ery/L; ++ - ca. 50 × 10^6 ery/L; +++ - ca. 250 × 10^6 ery/L

Organ weights
Animals of both sexes treated with 50,000 ppm showed statistically significant increases in both relative liver and kidney weight when compared with controls (see Table B.6.3-13). There were no further direct effects of treatment.

Table B.6.3-13: Group mean organ weights and standard variations (sd)

Dietary concentration (ppm)		**Relative organ weight (%)**			
		Liver		Kidney	
		♂	♀	♂	♀
0	mean	2.9749	2.9734	0.5861	0.6516
	sd	0.2629	0.1558	0.0575	0.0523
1000	mean	2.8868	2.9093	0.5901	0.6257
	sd	0.2552	0.2146	0.0804	0.0375
10000	mean	2.8853	2.9801	0.6070	0.6454
	sd	0.3758	0.1556	0.0552	0.0532
50000	mean	3.2433*	3.1989*	0.6963***	0.7180*
	sd	0.2452	0.2098	0.0436	0.0707

* - significantly different from control group ($p < 0.05$)
*** - significantly different from control group ($p < 0.001$)

Necropsy
Macroscopic abnormalities were detected in the 50,000 ppm dose group with all animals showing enlarged and fluid-filled caecums whilst one female treated with 50000 ppm showed gaseous distension of the stomach at terminal kill.
There were no treatment-related macroscopic abnormalities detected at 10,000 or 1,000 ppm.

Histopathology
Treatment-related changes were observed in the caecum. Atrophy, characterised by flattening of the intestinal mucosa, was observed for five rats of both sexes dosed at 50,000 ppm ($p < 0.05$ for male rats) and for one male and two female rats receiving 10,000 ppm of the test material. The aetiology of this change is uncertain and may represent no more than a stretch atrophy of the mucosa resulting from caecal distension.
There were no further treatment-related changes.

Conclusion by the Notifiers
Dietary administration of the test material, technical glyphosate, to rats for a period of ninety consecutive days at dietary concentrations of up to 50,000 ppm resulted in treatment-related changes at 50,000 and 10,000 ppm. No such effects were demonstrated in the 1,000 ppm treatment group and the "No Observed Effect Level" was, therefore, considered to be 1000 ppm (equivalent to 79 mg/kg bw/day for males, and 90 mg/kg bw/day for females).

RMS comments
The study is considered acceptable and the conclusions drawn are supported. The data submitter/owner was not mentioned in the study description but is presumed to be Nufarm. According to the study report itself, the sponsor was Mastra Industries (Malaysia) that gave Nufarm access to this study.
The NOAEL of 79 mg/kg bw/day is agreed with. It must be kept in mind that, due to dose spacing, the margin between the low and mid dose level was very large. The LOAEL of 730 mg/kg bw/day was well above the NOAELs in other 90-day studies. Thus, despite the (relatively) low NOAEL, this study is not suitable to prove that toxicity of glyphosate was in fact higher than previously assumed. It must be also emphasised that the top dose level of 3700 mg/kg bw/day was indeed extremely high, even for this rather non-toxic compound.
Unfortunately, it is not clear from the report whether or not salivary glands (and if, which of them) were subject to histopathology.
Organ weight changes (including higher relative testis weight in addition to liver and kidney) at the top dose level are considered secondary to reduced body weight.

3d new subchronic study in rats ([redacted] 1995)

Reference: IIA, 5.3.2/04
Report: [redacted] (1995) HR-001 : 13-week Subchronic Oral Toxicity Study in Rats.
[redacted]
Laboratory Report No.: [redacted] 94-0138
Data owner: Arysta LifeScience
Date: 1995-07-20
not published, ASB2012-11452
Guidelines: Japan MAFF Guidelines 59 NohSan No.4200, 1985; U.S. EPA FIFRA Guidelines Subdivision F, 1984; OECD 408 (1981)

Deviations: None
GLP: Yes
Acceptability: *See RMS comment*

Materials and methods

Test material:	Glyphosate technical		
Identification:	HR-001		
Description:	White crystal		
Lot/Batch #:	940908-1	941209	T-941209
Purity:	95.68 %	95.0 %	97.56 %
Stability of test compound:	12/12/1994	19/12/1994	26/12/1994
Vehicle:	Plain diet / none		
Test animals:			
Species:	Rat		
Strain:	Sprague-Dawley Crj:CD		
Source:	[redacted]		
Age:	5 weeks		
Sex:	Male and female		
Weight at dosing:	♂ 136-150 g; ♀ 109-121 g		
Acclimation period:	1 week		
Diet/Food:	MF Mash (Oriental Yeast Co., Ltd.		
Water:	Filtered and sterilized tap water, *ad libitum*		
Housing:	3/cage, sexes separately in stainless steel cages 31.0 x 44.0 x 20.3 cm		
Environmental conditions:	Temperature: 24 ± 2 °C Humidity: 55 ± 15 % Air changes: 15/hour 12 hours light/dark cycle		

Study design and methods:
In life dates: 1994-12-06 to 1995-03-22

Animal assignment and treatment:
The test substance was incorporated into the basal effect diet and administered on a continuous basis in the basal diet to groups of 24 Sprague-Dawley rats (12 males + 12 females) for a period of 13 weeks. Dietary concentrations were 0, 3000, 10000 and 30000 ppm.

Chemical analysis for homogeneity and concentration of the test substance in the diet were performed on samples (about 50 g each) of each does level takent from top, middle and bottom portions of the mixer at the first diet preparation. The control diet was also sampled (50 g each) and analysed to confirm that there was no contamination with the test substance. Concentrations of the test substance in test diets at all dose levels were monitored on the same amount of samples (50 g each) every 3 weeks during the study.

Mortality
Each animal was checked for mortality or signs of morbidity at least once daily during the treatment period.

Clinical observations
Cage-side observation was performed daily on all animals to detect moribund or dead animals and abnormal clinical signs, and all findings were recorded. In addition, a detailed examination including palpation for masses was performed at least once a week.

Body weight
Body weights of all animals were recorded at initiation of treatment and weekly during the study. Group mean body weight was calculated for each dose group at each measurement. Final body weight were recorded for all animals before necropsy.

Food consumption and utilisation
Food consumption for each cage was measured weekly for a period of 3 consecutive days. Mean daily food consumption per animal in each cage was calculated by dividing the weekly food consumptionby the number of animals per cage and by the number of days for measurement. Group mean food consumption (g/rat/day) was calculated at each measurement from the mean daily food consumption per animal in each cage.
Group mean chemical intake (mg/kg/day) was calculated from nominal dietary concentrations of the test substance, food consumption and body weight.
Group mean food efficiency for each dose group was calculated weekly from the ratio of the group body weight gain to group mean food consumption and expressed as percentage. Overall group mean efficiency throughout the treatment period was also calculated for all dose groups.

Ophthalmoscopic examination
Ophthalmological examinations including observation with a haloen ophtalmoscope were performed on all animals during acclimatization period and on all surviving animals in the control and the highest dose groups from the main group at week 13.
The following parts of the eye were inspected: eyeball, cornea, anterior chamber, pupil, iris, lens/vitreous body, fundus.

Haematology and clinical chemistry
After 13 weeks of treatment, all surviving animals were subjected to haematological examinations. The animals were laparotomized under anesthesia following overnight fasting, and blood samples were withdrawn from the posterior vena cava using heparinised syringes. A part of each sample was poured into a cup treated with EDTA and subjected to the examinations.
The following parameters were determined with a fully automated hematology analyzer: Hematocrit (Ht), hemoglobin (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), total leukocyte count (WBC) and differential leukocyte count.

After 13 weeks of treatment, all surviving animals were subjected to blood biochemical examinations. Plasma samples obtained from the heparinised blood were used for examinations.
The following parameters were determined: alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transpeptidase

(GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca), inorganic phosphorus (P), sodium (Na), potassium (K) and chloride (Cl).

Urinalysis
At 13 week of treatment, all surviving animals were subjected to urinalysis. Fresg urine samples were collected by pressing the lumbodorsal region of the animals. Specific gravity was detmerined with a handy refractometer. Glucose, ketones, occult blood, pH, protein, and urobilinogen were semiquantitatively analyzed by Uro-labstix. Then animals were housed individually in metabolic cages overnight, and urine samples collected were examined for volume and appearance. Urinary sediments were also examined microscopically on these samples.

Sacrifice and pathology
Selected organs were weighed at the scheduled necropsy. Histopathological examinations were performed on selected tissues from all animals.
The following organs/tissues were taken: brain, spinal cord, sciatic nerve, pituitary, thyroids with parathyroids, thymus, adrenals, spleen, bone with marrow, tibio-femoral joint, lymph nodes, heart, aorta, pharynx, buccal mucosa of oral cavity, salivary glands, esophagus, stomach, liver, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, head, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus, vagina, harderian glands, eyes, skeletal muscle, skin, mammary gland, all gross lesions.

Statistics
All data were evaluated using variance analysis (bodyweight, food consumption, urine specific gravity, urine volume, hematologicla parameters, blood chemical parameters, and organ weights).
Data on clinical sign, mortality, ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability.

Results and discussion
Mortality:
No deaths were noted in the control and treated groups of either sex.

Clinical observations:
There were no abnormalities related to the treatment in the treated groups of either sex. In the 30 000 ppm group, one female showed a poor general condition including emaciation and decreased spontaneous motor activity. The poor general condition seemed to be caused by elongated incisor, malocclusion, or hepatorenal genetic lesions revealed by histopathology. Thus it was not considered to be treatment related.

Body weight:
In the 30 000 ppm group, body weights of males and females were slightly lower (about 5-10 % decrease in males and 5 % in females) than those in the control throughout the treatment period. Statistically significant decreases in their body weights were sporadically observed during the treatment period (weeks 3, 4 and 11 in males and weeks 10 and 11 in females) when compared to the control.

In the 10 000 and 3 000 ppm groups, body weight changes in males and females were comparable to the control throughout the treatment period.

Food consumption and test substance intake:
In the 30 000 ppm group, males and females showed significant decreases in food consumption at week 1 which were 9 and 14 % lower than that of the control, respectively. However, their food consumption were comparable to the control at week 2 and thereafter.
In the 10 000 no significant change was observed while in the 3000 ppm group, significant changes were sporadically observed during the treatment period in females during the weeks 6 and 7. The food consumption recovered from the week 8 up to the end of the study.
The overall food consumption by males and females was comparable to the control and there were no abnormalities considered treatment related.

The overall group mean chemical intakes averages, calculated from food consumption and nominal concentrations of the test substance, through the treatment period, were:

Table B.6.3-14: Mean daily intake of glyphosate in the study by Kinoshita (1995, ASB2012-11452)

Dose (ppm)	**Chemical Intake (mg/kg bw/day)**	
	Male	Female
3 000	168.4	195.2
10 000	569	637
30 000	1735	1892

Ophthalmoscopic examination
In the ophtalmological examination performed on all animals before the start of the treatment and on the animals of the control and 30 000 ppm groups at 13 weeks of treatment, no abnormalities were observed in either sex.

Haematology: Tere were no abnormalities in any group of either sex.

Blood clinical chemistry: In the 30 000 ppm group, females showed a significant increase in alkaline phosphatase (ALP) activity and a significant decrease in albumin (Alb). There were no abnormalities in males.
In the 10 000 and 3 000 ppm groups, there were no abnormalities in either sex.

Urine analysis: In the 30 000 ppm group, urine pH in males and females was significantly lower than that in the control. Urine protein showed a significant decrease in males and a decreasing trend in females. In addition, females showed a significantly higher urine volume than that of the control, but males showed a decreasing trend in urine volume as compared with the control.
In the 10 000 ppm group, urine, pH and protein in males were lower than those in the control. In females, no statistically significant change was observed in any parameter.
In the 3 000 ppm group, no statistically significant changes was observed in either sex.

Organ weights: In the 30 000 ppm group, both sexes showed significant increases in absolute and relative weights of the cecum (containing contents). In addition, females in this group also showed significant increases in relative weights of the brain and liver.
In the 10 000 ppm group, the absolute and relative weight of the cecum showed a statistically significant increase in males and increasing trend in females.

In the 3 000 ppm group, there were no abnormalities attributable to the treatment in either sex.

Gross pathology: In the 30 000 ppm group, distention of the cecum was observed in 9/12 males and 7/12 females with statistical significance. There were no other macroscopic abnormalities attributable to the treatment.
In the 10 000 ppm group, 3/12 males showed distention of the cecum, but there were no macroscopic abnormalities in females.
In the 3 000 ppm group, there were no macroscopic abnormalities attributable to the treatment in either sex.

Histopathology: Although histopathological examinations revealed various histological changes in each treatment group of both sexes, treatment-related changes were not observed. One male in the 10 000 ppm group and one female in the 30 000 ppm group showed renal lesion (polycystic kidney) and hepatic lesions (bile ductal proliferation and cholangiectasis). It is generally regarded that these lesions were caused by genetic disorder and were not considered to be treatment-related.

Conclusion by the Notifiers
Under the experimental conditions of the study, the No Observed Adverse Effect Level (NOAEL) is considered to be 3000 ppm (equivalent to 168.4 and 195.2 mg/kg bw/day for males and females, respectively).

RMS comments:
The study is considered acceptable and the proposed NOAEL is agreed with. Toxicity became apparent by the caecum findings and alterations in few clinical chemistry and urine parameters. The LOAEL of 569 mg/kg bw/day (10000 ppm) is higher than the NOAEL as established in other studies and, thus, does not contradict the previous assessment. From the study report, it became clear that submaxillary and sublingual salivary glands were histologically examined, without evidence of pathological changes. These glands were not weighed and parotid gland was not taken.

Published information
Glyphosate was tested in the 1980ies in U.S. National Toxicology Program (NTP) for oral subchronic toxicity (Chan and Mahler, 1992, TOX9551954). The following paragraph was partly copied from the previous DAR (1998, ASB2010-10302):

20 F344/N rats per sex and dose were fed glyphosate (supplied by Monsanto, approximately 99% pure) for 13 weeks at dietary levels of 0, 3125, 6250, 12500, 25000 or 50000 ppm. Ten rats/sex and group were used for evaluation of haematological and clinical chemistry parameters. All rats survived until the end of the study and there were no clinical signs of toxicity apart from diarrhea at the top dose level in both sexes. Body weight gain was markedly reduced in high dose males and slightly decreased in high dose females. There were some minor alterations in haematological and clinical chemistry parameters at least at the upper dose levels. Morphologic changes at necropsy were confined to parotid and submandibular (submaxillary) salivary glands in both sexes. This “cytoplasmatic alteration“ consisted of basophilic change and hypertrophy of acinar cells. The parotid gland was more affected. Here, the normal granular, eosinophilic staining cytoplasm of the acinar epithelial cells was replaced by basophilic and finely vacuolated cytoplasm. A NOEL could not be established since these lesions were observed already at the lowest dose level but not in the control groups. The degree of change showed a clear dose response. The outcome of this

study shows that glyphosate is of low toxicity when administered orally over a period of 3 months to rats since the animals tolerated daily doses as high as 50000 ppm (more than 3000 mg/kg bw/day) without mortality or clinical signs of overt toxicity and without pathological changes other than the rather equivocal salivary gland findings.

B.6.3.2.2 90-day studies in mice

There are only two acceptable studies of which one (Perry *et al.*, 1991, TOX9552363) was already available for the previous evaluation and is described in the old DAR (1998, ASB2010-10302). A further, rather old study (Tierney and Rinehart, 1979, TOX9552360) does not comply to modern standards, is now considered not acceptable by the RMS and was deleted from current new assessment. A main deficiency of this study was that hematological and clinical chemistry evaluations were not included.

Below, the new study by Kuwahara (1995, ASB2012-11453) is described in detail and commented by the RMS.

New subchronic feeding study in mice ([redacted] 1995)

Reference:	IIA, 5.3.2/05
Report:	[redacted] 1995, HR-001: 13-week Subchronic Oral Toxicity Study in Mice. [redacted] Laboratory Report No.: [redacted] 94-0136 Data owner: Arysta LifeScience Date: 1995-07-24 not published, ASB2012-12453
Guidelines:	Japan MAFF Guidelines 59 NohSan No.4200, 1985; U.S. EPA FIFRA Guidelines Subdivision F, 1984 OECD 408 (1981)
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: 1994-12-15 to 1995-07-24

Materials and methods

Test material:	Glyphosate technical
Identification:	HR-001
Description:	White crystal
Lot/Batch #:	T-941209
Purity:	97.56 %
Stability of test compound:	26/12/1994
Vehicle:	Plain diet / none
Test animals:	

Species:	Mouse
Strain:	Crj:CD-1
Source:	[redacted]
Age:	5 weeks
Sex:	Male and female
Weight at dosing:	♂ 27.3-32.7 g; ♀ 22.4-25.8 g
Acclimation period:	9 days
Diet/Food:	MF Mash (Oriental Yeast Co., Ltd.)
Water:	Filtered and sterilized tap water, *ad libitum*
Housing:	3/cage, sexes separately in stainless steel cages 21.5 x 33.0 x 18.0 cm
Environmental conditions:	Temperature: 24 ± 2 °C Humidity: 55 ± 15 % Air changes: 15/hour 12 hours light/dark cycle

In life dates: 1995-01-10 to 1995-04-27

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 24 SPF ICR mice (Crj : CD-1) (12 males + 12 females) for a minimum of 90 days. Dietary concentrations were 0, 5 000, 10 000 and 50 000 ppm.

Chemical analysis for homogeneity and concentration of the test substance in the diet were performed on samples (about 50 g each) of each does level takent from top, middle and bottom portions of the mixer at the first diet preparation. The control diet was also sampled (50 g each) and analysed to confirm that there was no contamination with the test substance. Concentrations of the test substance in test diets at all dose levels were monitored on the same amount of samples (50 g each) every 3 weeks during the study.

Mortality: Each animal was checked for mortality or signs of morbidity at least once daily during the treatment period.

Clinical observations: Cage-side observation was performed daily on all animals to detect moribund or dead animals and abnormal clinical signs, and all findings were recorded. In addition, a detailed examination including palpation for masses was performed at least once a week.

Body weight: Body weights of all animals were recorded at initiation of treatment and weekly during the study. Group mean body weight was calculated for each dose group at each measurement. Final body weights were recorded for all animals before necropsy.

Food consumption and utilisation: Food consumption for each cage was measured weekly for a period of 3 consecutive days. Mean daily food consumption per animal in each cage was calculated by dividing the weekly food consumptionby the number of animals per cage and by the number of days for measurement. Group mean food consumption (g/rat/day) was calculated at each measurement from the mean daily food consumption per animal in each cage.

Group mean food efficiency for each dose group was calculated weekly from the ratio of the group body weight gain to group mean food consumption and expressed as percentage. Overall group mean efficiency throughout the treatment period was also calculated for all dose groups.

Group mean chemical intake (mg/kg/day) was calculated from nominal dietary concentrations of the test substance, food consumption and body weight.

Ophthalmoscopic examination: Ophthalmological examinations including observation with a haloen ophtalmoscope were performed on all animals during acclimatization period and on all surviving animals in the control and the highest dose groups from the main group at week 13.
The following parts of the eye were inspected: Eyeball, cornea, anterior chamber, pupil, iris.

Haematology and clinical chemistry: After 13 weeks of treatment, all surviving animals were subjected to haematological examinations. The animals were laparotomized under anesthesia following overnight fasting, and blood samples were withdrawn from the posterior vena cava using heparinised syringes. A part of each sample was poured into a cup treated with EDTA and subjected to the examinations.
The following parameters were determined with a fully automated hematology analyzer: Hematocrit (Ht), hemoglobin (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), total leukocyte count (WBC).

After 13 weeks of treatment, all surviving animals were subjected to blood biochemical examinations. Plasma samples obtained from the heparinised blood were used for examinations.
The following parameters were determined: alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca), inorganic phosphorus (P).

Urinalysis: At 13 week of treatment, all surviving animals were subjected to urinalysis. Fresh urine samples were collected by pressing the lumbodorsal region of the animals. Specific gravity was determined with a handy refractometer. Glucose, ketones, occult blood, pH, protein, and urobilinogen were semiquantitatively analyzed by Uro-labstix. Then animals were housed individually in metabolic cages overnight, and urine samples collected were examined for volume and appearance. Urinary sediments were also examined microscopically on these samples.

Sacrifice and pathology: Clinical pathology evaluations were also conducted. Selected organs were weighed at the scheduled necropsy. Histopathological examinations were performed on selected tissues from all animals.
The following organs/tissues were taken and preserved: brain, spinal cord, sciatic nerve, pituitary, thyroids with parathyroids, thymus, adrenals, spleen, bone with marrow, tibio-femoral joint, lymph nodes, heart, aorta, pharynx, salivary glands, esophagus, stomach, liver, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, head, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands,

ovaries, uterus, vagina, harderian glands, eyes, skeletal muscle, skin, mammary gland, all gross lesions.

Statistics: All data were evaluated using variance analysis (bodyweight, food consumption, urine specific gravity, urine volume, hematologicla parameters, blood chemical parameters, and organ weights).
Data on clinical sign, mortality, ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability.

Results and discussion
Mortality: There were no animals found dead or had to be killed *in extremis* in any groups during the treatment period.
Clinical observations: There were no treatment-related abnormalities in clinical signs in the control and treated groups during the treatment period.
Body weight: In the 50 000 ppm group, mean body weights of males were lower than those of the control form week 2 to the end of the treatment period. Mean body weight of males at week 13 was 91% of that of control. Body weights of females were comparable to the control during the treatment period.
In the groups treated at 10 000 ppm or less, body weights of males and females were comparable to the controls during the treatment period.
Food consumption and test substance intake: In males of the 50 000 ppm group, a significant depression of food consumption was recorded at week 1. Average food consumption of males during the treatment period was 94 % of the control value. Food consumption of females were comparable to the control.
In the groups treated at 10 000 and 5000 ppm, food consumption of males and females was comparable to that of the controls.
The average daily chemical intakes during the treatment are shown in the following Table B.6.3-15:

Table B.6.3-15: Mean daily glyphosate intake in the study by [redacted] (1995, ASB2012-11453)

Dose level (ppm)	**Average chemical intake (mg/kg bw / day)**	
	Male	Female
5 000	600.2	765.0
10 000	1221	1486
50 000	6295	7435

In the 50 000 ppm group, food efficiency of males and females was lower than that of the controls at almost all measuring points during the treatment. Average food efficiency of males and females was calculated to reach only 79 % and 88 % of the respective control value.
In the groups treated at 10 000 and 5 000 ppm, food efficiency in the treated groups of both sexes was comparable to that in the controls though some significant fluctuations were recorded sporadically.
Ophthalmoscopic examination: There were no ophtalmological abnormalities in the animals of both sexes in the highest dose group and the control group.
Haematology: In the 50 000 ppm group, females showed significant decreases in hematocrit (Ht), hemoglobin concentration (Hb) and erythrocyte count (RBC) by up to 92 % while males showed no significant differences from the control in any parameters.

There were no significant differences in any parameters between the treated groups of 10 000 ppm or less and the control of either sex..
Blood clinical chemistry: The statistically significant changes observed in the treated groups are summarized in the following table (Table B.6.3-16):

Table B.6.3-16: Alterations in clinical chemistry parameters in mice fed glyphosate for 90 days ([redacted] 1995, ASB2012-11453)

Parameter	Sex	Dose group (ppm)		
		5 000	10 000	50 000
Alkaline phosphatase (ALP)	Male	No change	No change	↗ 184*
	Female	No change	No change	↗ 150
Glutamic pyrvic transaminase (GPT)	Female	No change	No change	↘ 69
Creatine phosphokinase (CPK)	Female	↗ 361	No change	↗ 943
Blood urea nitrogen (BUN)	Female	No change	↗ 119	No change
Inorganic phosphorus (P)	Female	No change	No change	↗ 128

* The figures represent the mean percentage of change as compared to the respective control.

In the 50 000 ppm group, males and females showed a significant increase in alkaline phosphatase (ALP). In females, creatine phosphokinase (CPK) and inorganic phosphorus (P) were significantly increased, while a significant decrease in glutamic pyrvic transaminase (GPT) was noted.
In the 10 000 ppm group, females exhibited a significant increase in blood urea nitrogen (BUN). There were no significant change in any parameters in males.
In the 5 000 ppm group, females showed a significant increase in CPK, while there were no significant change in any parameters in males
Urine analysis: In all treated groups, males showed a significant decrease in urinary pH. There were no abnormalities in females of any treated groups.
Organ weights: In the 50 000 ppm group, males and females showed significant increases in both absolute and relative weights of the cecum. The absolute weights of the cecum of males and females were 238 % and 187 % of that of the respective control. For relative weight, the ratio of the value to the respective control was 263 % or 195 % in males or females.
In the 10 000 ppm group, absolute and relative weights of the cecum showed increasing tendencies in males and females. The absolute weight of the cecum of males and females were 115 % and 122 % of that of the respective control. For relative weight, the ratio of the value to the respective control was 111 % or 117 % in males or females.
In the 5 000 ppm, there were no significant changes in any organ weights of males and females.
Gross pathology: In the 50 000 ppm group, males and females showed a significant increase in incidence of distention of the cecum (12/12 in males and 10/12 in females, in contrast to 0/12 in males and females of the control group).
In the 10 000 ppm group, distention of the cecum was observed in one female. There were no significant changes in incidence of any macroscopic lesions in males.
In the 5 000 ppm group, there were no treatment-related abnormalities in males and females.
Histopathology: In the 50 000 ppm group, males showed significant increases in incidence of cystitis of the urinary bladder (4/12 as compared to 0/12 in the control group). There were no

significant changes in incidence in females. Although significant increases in incidence of distention of the cecum were noted for males and females at necropsy, histopathological examinations failed to reveal any abnormalities in the cecum.
In the 10 000 and 5 000 ppm groups, there were no significant differences in incidence of histopathological lesions from the control in either sex.

Conclusion by the Notifier
Under the experimental conditions of the study, the No Observed Effect Level (NOEL) is considered to be 5000 ppm (equivalent to 600.2 and 765.0 mg/kg bw/day for males and females, respectively).

RMS comments:
The study is considered acceptable. (It was noted that the study director was in fact [redacted] as in a 90-day rat study from the same laboratory. [redacted] performed the histopathological examinations and was apparently the report writer.)
Because of the only minor effects (slightly higher blood urea nitrogen, slight caecal distention) at the mid dose level of 10000 ppm (equal to 1221 mg/kg bw/day in males) that were not accompanied by any histopathological findings, this dose is considered the NOAEL. Target organs at the very high dose of ca 6300 mg/kg bw/d (50000 ppm) were the caecum and the bladder. Clinical chemistry findings also suggest a weak effect on the liver. This dose level was clearly toxic as additionally proven by effects on body weight gain, food consumption and efficiency and on red blood cell parameters. Thus, the outcome of this study is in line or at least not in contradiction to the previous study by [redacted]. (1991, TOX9552363) on CD mice in which no effects were observed up to the top dose level of 4500 mg/kg bw/day.
The lower urinary pH in males in all dose groups is due to acidic properties of the test subtance and cannot be considered a toxic effect.
In contrast to the publication by [redacted] (1992, TOX9551954, reported below), no histological changes of the salivary glands were observed in this study as they had occurred in another strain from dietary concentrations of 6250 ppm (1065 mg/kg bw/day) onwards. Beside possible strain differences, another explanation might be that [redacted] (1995, ASB2012-11453) examined the sublingual and submaxillary glands histologically but not the parotis. In the study by [redacted] (1991, TOX9552363), histopathological examination of salivary glands was confined to the submaxillary.

Published information
Glyphosate was tested in the 1980ies in U.S. National Toxicology Program (NTP) for oral subchronic toxicity (Chan and Mahler, 1992, TOX9551954). The following paragraph was partly copied from the previous DAR (1998, ASB2010-10302):

Groups of ten B6C3F1 mice per sex and dose were fed glyphosate (supplied by Monsanto, approximately 99 % pure) for 13 weeks at dietary levels of 0, 3125, 6250, 12500, 25000 or 50000 ppm. Clinical pathology investigations were not performed but all animals were necropsied at study termination. Mice of the highest dose group and of the control group were subjected to complete histopathological examination. In addition, salivary glands were examined microscopically in all dose groups. There were no clinical signs of toxicity. However, one high dose female died from undetermined causes. Body weight gain was depressed at the two upper dose levels in both sexes. From the dose level of 6250 ppm onwards, a dose-related increase in occurrence and severity of cellular alteration of the parotid salivary gland was noted. This change consisted of basophilia of the acinar cells and in more severely affected glands, the cells and acini also appeared enlarged with an associated relative

reduction in the number of ducts. A dose of 3125 ppm (ca 507 mg/kg bw/day seems to represent the NOEL for substance-related effects. However, the extent of investigations performed was rather limited.

B.6.3.2.3 Oral 90-day and 1-yr studies in dogs

For the previous EU evaluation, two 90-day studies in dogs had been submitted ([redacted] 1991, TOX9650150; [redacted] 1985, TOX9551823) that do not comply with modern standards and were assessed now by the RMS as "not acceptable". The same holds true for a 12-month study by [redacted] (1992, TOX9650153). Therefore, these studies were excluded from current re-evaluation of glyphosate, as well as a one-year study by [redacted] (1985, Z35385) that was briefly described in the 1998 DAR (ASB2010-10302) but had never been submitted as part of an EU dossier, neither in the 1990ies nor in 2012. It is available in Germany but, without effects up to the highest dose level of 500 mg/kg bw/day, this study would not alter overall assessment.

In contrast, the 12-month study of [redacted] (1990, TOX9552384) is still considered acceptable from a todays point of view and is included in the respective table in Volume 1 (2.6.3) and in this chapter. It is reported in detail in the old DAR (1998, ASB2010-10302).

In addition, there is an acceptable study in which the formulation MON0139 (62.49 % IPA salt of glyphosate) had been administered for six months in gelatine capsules to Beagle dogs [redacted], 1983, TOX9552361). This study is still included since it was found acceptable upon re-evaluation by RMS. At least, it is suitable to show that this salt proved to be of no higher toxicity in dogs than the acid.

Thus, to an even larger extent than in other fields of toxicological testing of glyphosate, evaluation of its toxicity to dogs is based on new studies that are all reported in detail below.

<u>1st new 90-day study in dogs ([redacted] 2007)</u>

Reference:	IIA, 5.3.3/01
Report:	[redacted] 2007 Glyphosate Technical: 13-Week Toxicity Study By Oral Route (Capsule) In Beagle Dogs [redacted] Laboratory Study No.: 29646 TCC Data owner: Nufarm Date: 2007-07-15 not published, ASB2012-11454
Guidelines:	OECD 409 JMMAF 12 NohSan No. 8147
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Materials and methods

Test material:	
Identification:	Glyphosate technical
Description:	White crystalline powder
Lot/Batch #:	H05H016A
Purity:	95.7 %
Stability of test compound:	Stable under storage conditions (< 30 °C), light protected; Expiry date: 2008-03-25
Vehicle:	Empty gelatine capsules, size 12 (Torpac, NY, US)
Test animals:	
Species:	Dogs
Strain:	Beagle
Source:	[redacted]
Age:	Approx. 6 months
Sex	Male and female
Weight at dosing:	♂ 6.5 – 8.0 kg; ♀ 6.6 – 7.7 kg
Acclimation period:	14 days
Diet/Food:	125 C3 pelleted diet (SAFE, Villemoisson, Epinary-sur-Orge, France), 300 g per day (Following reduced food consumption among some animals standard tinned dog food was distributed instead or in addition.)
Water:	tap water, *ad libitum*
Housing:	Individual housing in pens containing wood shavings.
Environmental conditions:	Temperature: 20 ± 5 °C Humidity: 50 ± 20 % Air changes: 12/hour 12 hours light/dark cycle

In life dates: 2005-06-08 to 2005-09-22

Animal assignment and treatment:
In a 13-week oral toxicity study groups of four Beagle dogs per sex received daily doses of 0, 30, 300 and 1000 mg/kg bw/day glyphosate technical by capsule application. The test item capsules were prepared weekly and delivered daily to the animal room, protected from light. As the test item was put into the capsules, no chemical analysis was performed during the study. The purity, characteristics and identification of the test item were indicated on the certificate of analysis that accompanied the test item.

Mortality: Each animal was checked for mortality or signs of morbidity twice a day during the treatment period, including weekends and public holidays.

Clinical observations: A check for clinical signs of toxicity was made once daily on all animals. In addition, a detailed clinical examination was performed at least once before of the beginning of the treatment period and then once a week until the end of the study.

Body weight: The body weight of each animal was recorded twice before group allocation, on the first day of treatment, and then once a week until the end of the study. In addition, the group 4 animals were weighed before final sacrifice on day 75.

Food consumption: The quantity of food consumed was recorded for each animal. Food intake per animal and per day was calculated for 7 days before the beginning of the treatment period and then throughout the study.

Ophthalmoscopic examination: Ophthalmological examinations were performed on all the animals before the beginning and at the end of the treatment period.

Haematology and clinical chemistry: Haematological and blood chemical and urinalytical investigation were performed on all animals from each test and control group before the beginning of the treatment period, in Week 7 and at the end of the treatment period (Week 11 for Group 4 and Week 13 for Groups 1 to 3).
Prior to blood sampling the animals were deprived of food for an overnight period of at least 14 hours.
The following parameters were determined: erythrocytes, haemoglobin, MVC, MCHC, MCH, thrombocytes, leucocytes, differential white cell count including morphology, reticulocytes, prothrombin time, activated partial thromboplastin time, sodium, potassium, chloride, calcium, inorganic phosphorous, glucose, urea, creatinine, total bilirubin, total protein, albumin, albumin/globulin ratio, total cholesterol, triglycerides, alkaline phosphatase, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), and gamma-glutamyl transferase (GGT).

Urinalysis: Urine samples were collected from all animals of the test and control groups before the beginning of the treatment period, in Week 7 and at the end of the treatment period (Week 11 for Group 4 and Week 13 for Groups 1 to 3). During urine collection, the animals were deprived of food for an overnight period of at least 14 hours. The following parameters were assessed: appearance, colour, volume, pH, specific gravity, proteins, glucose, ketones, bilirubin, nitrites, blood, urobilinogen, and sediment.

Sacrifice and pathology: On completion of the treatment period (Week 11 or 13), after at least 14 hours fasting, all surviving animals (as well as moribund dogs before) were sacrificed and subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, pituitary gland, prostate, spleen, testes, thymus, thyroids with parathyroid and uterus.
Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta, bone & bone marrow (sternum and femur), brain (at three levels), caecum, colon, duodenum, epididymides, oesophagus, eyes, gall bladder, heart, ileum (with Peyers patches), jejunum, kidneys, larynx, liver, lungs (with bronchi), lymph nodes (mandibular and mesenteric), mammary gland, muscle (skeletal), optic nerve, ovaries, oviducts, pancreas, pituitary gland, prostrate, rectum, salivary glands (parotid and submandibular), sciatic nerve, skin, spinal cord (cervical, thoracic and lumbar), spleen, stomach, testes, thymus, thyroid with parathyroid, tongue, trachea, ureters, urinary bladder, uterus (horns and cervix) and vagina.

Statistics: Statistical analysis of body weight, haematology, blood biochemistry, urinalysis and organ weight data was done according to the statistical decision tree shown in "*Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies*" (OECD,

2002, ASB2013-3754), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.

Results and discussion

Mortality: Two unscheduled sacrifices (one male and one female) were necessary in animals given 1000 mg/kg bw/day:

One male was sacrificed on Day 61 on humane grounds. Vomiting was seen once in Week 7 (before dosing) and liquid faeces were noted on many occasions in Weeks 8 and 9. Prior to sacrifice, signs of poor clinical condition including thin appearance, dehydration, and pallor of lip mucosa, coldness to the touch, hypothermia (34 to 35 °C) and hypoactivity were observed. These signs were associated with a body weight loss between Weeks 7 and 9 (-34 %) and reduced food consumption from Week 7 (generally only 25 to 50 % of this animal's daily ration was consumed), followed by an absence of food intake on the day before death. Medical care (Smecta® and Lactate Ringer®) was given in order to stop the diarrhoea and rehydrate the animal.

One female was sacrificed on Day 72 for humane reasons. This animal showed liquid or soft faeces on many occasions from Week 4 and dehydration from Week 9. Vomiting was observed once in Week 10. These signs were accompanied by a body weight loss between Weeks 8 and 11 (-22 %) and decreased food consumption from Week 8 (generally only 25 to 50 % of this animal's daily ration was consumed), followed by an absence of food intake on the two days prior to sacrifice. Medical care (Smecta® and lactate Ringer®) was given in many occasions.

Clinical observations: No treatment-related clinical signs were noted in control animals or those given 30 or 300 mg/kg bw/day.

The following treatment-related clinical signs were reported in animals given 1000 mg/kg/day (excluding those killed in extremis, which are discussed separately): liquid or soft faeces on several occasions in all animals, vomiting in 2/3 females on one occasion within 30 minutes or 3 to 5 hours after treatment, thin appearance in 1/3 males and all females, dehydration in 1/3 males and 2/3 females, pallor of ears and mouth in 1/3 females.

Body weight: No relevant differences in the mean body weight gain were noted between controls and animals given 30 or 300 mg/kg bw/day during the treatment period.

Due to numerous individual body weight losses recorded from Week 4 in males and from Week 1 in females, a marked lower mean body weight was noted in animals given 1000 mg/kg/day at termination.

At the end of the treatment period this resulted in only a slight mean body weight gain in males (+4 % vs. +31 % in controls) and a mean body weight loss in females (-7 % vs. +14 % in controls) when compared to their body weight on Day 1. This effect on body weight was considered treatment-related (see Table B.6.3-17).

Table B.6.3-17: Group mean weekly bodyweights and standard deviations (sd)

	Mean bodyweight and body weight change (kg)						
Time point	Day 1	Week 5	Week 9	Week 11	Change week 1-11	Week 13	Change week 1-13
Dose (mg/kg bw/day)	Males						
0	7.4	9.0	9.5	9.7	+2.3	10.4	+3.0
30	7.2	8.5	8.9	9.1	+1.9	9.5	+2.3
300	7.3	8.5	9.0	9.2	+1.9	9.7	+2.4
1000	7.3	8.3	7.7*	7.6*	+0.3	na	na
	Females						
0	7.3	7.8	8.2	8.2	+1.0	8.8	+1.5
30	7.3	8.3	8.7	8.7	+1.6	9.2	+1.9
300	7.4	8.2	8.6	8.6	+1.3	9.2	+1.8
1000	7.2	7.0	6.9	6.9	-0.5	Na	na

* Statistically significant from controls ($p<0.05$).
na not applicable due to premature sacrifice

Food consumption: The food consumption was not affected by the test article treatment in animals given 30 and 300 mg/kg bw/day. Reduced food consumption, varying from 25 to 75 % of the amount given, was observed on many occasions in animals given 1000 mg/kg bw/day. From Day 62, when tinned dog food was distributed instead of pelleted diet, all animals consumed their full daily ration.
Ophthalmoscopic examination: There were no ophthalmological findings at the end of the treatment period.
Heamatology and clinical chemistry: The laboratory investigations of the moribund sacrificed male showed the following changes among haematological and blood biochemical parameters when compared to pre-treatment values:
increase in leucocyte count mainly due to an increase in the neutrophil count,
increase in haemoglobin level, erythrocyte count and packed cell volume,
decrease in platelet count,
decrease in sodium and chloride levels, as well as an increase in potassium and inorganic phosphorus levels,
increase in glucose, protein, albumin, cholesterol, triglycerides, urea and creatinine levels.
Some of the abnormalities found in the laboratory investigations (such as the increase in red blood cell parameters and in protein and albumin levels) were indicative of haemoconcentration, which was probably secondary to the dehydratation caused by the diarrhoea.
The laboratory investigations performed before sacrifice of the moribund female dog showed the following changes among the blood biochemical parameters when compared to pre-treatment values:
decrease in sodium, potassium, chloride and inorganic phosphorus levels,
decrease in urea, protein and albumin levels and increase in total bilirubin level and alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase activities.
The abnormalities reported in blood electrolyte levels were not attributed directly to the test item treatment but were related to the poor clinical condition of the animal (diarrhoea, dehydration).
In the following the results of the laboratory investigations of the surviving animals are summarised.
Haematology: When compared to both pre-dose and control values, no biologically relevant differences were noted in surviving animals of the test item groups in Weeks 7 and 11/13.

Blood chemistry: When compared to control values in Week 13, the following test-substance related differences were noted in animals given 1000 mg/kg bw/day in Week 11 (see Table B.6.3-18): higher alanine aminotransferase (ALAT) activity in 2/3 males and 1/3 females, lower alkaline phosphatase (ALP) activity in 3/3 females, lower protein and albumin levels in 3/3 females.
Other changes were not attributed to the test item-treatment.

Table B.6.3-18: Group mean blood chemical values and standard deviations (sd) in Week 11/13

Dose		**ALAT**	**ALP**	**Total protein**	**Albumin**
(mg/kg bw/d)		(IU/L)	(IU/L)	(g/L)	(g/L)
		Males			
0	mean	31	–	–	–
(Week 13)	sd	4.8	–	–	–
30	mean	34	–	–	–
(Week 13)	sd	5.32	–	–	–
300	mean	30	–	–	–
(Week 13)	sd	8.9	–	–	–
1000	mean	91	–	–	–
(Week 11)	sd	42.5	–	–	–
		Females			
0	mean	29	388	61	35
(Week 13)	sd	6.0	168.0	2.1	1.6
30	mean	31	281	62	34
(Week 13)	sd	10.4	91.5	2.1	1.0
300	mean	29	332	59	35
(Week 13)	sd	4.1	142.6	2.5	0.6
1000	mean	122	321	55	30
(Week 11)	sd	163.9	322.0	5.5	2.5

– - no relevant changes

Urine analysis: When compared to both pre-dose and control values, the following findings were noted at 1000 mg/kg bw/day in Week 11:
decrease in mean specific gravity in 1/3 males and 3/3 females,
increase in mean urinary volume accompanied by less marked colour of urine in 3/3 females.
As these changes were only noted at the highest dose-level, they were attributed to the test item treatment.

Organ weights: Treatment-related, statistically significant effects (reduction) were restricted to the prostate.

Gross pathology: Macroscopic pathological examination of the male that was killed moribund demonstrated a reddish mucosa of the colon and rectum appeared, enlarged adrenal glands and thyroids, and reduced size of the spleen and thymus.
In the high-dose female that was killed moribund, the oesophagus, jejunum and ileum presented many greyish/white areas and the colon mucosa showed reddish/purplish foci. The gall bladder was dilated with blackish deposits and the liver was yellowish, enlarged and firm. The kidneys were pale.

All the macroscopic changes noted in surviving animals at termination were considered to be normal variations, when compared to background data, which may be seen in untreated beagle

dogs of this age, except for changes in the uterus (reduced in size) for females given 1000 mg/kg bw/day.

Histopathology: The major histopathological findings in the male dog sacrificed moribund were bilateral hyaline degeneration of the cortical tubules in the kidneys with pigment deposits, diffuse acute inflammation in the liver with pigment deposits, acute inflammation of the lamina propria of the oesophagus, bilateral hypertrophy of cortex of the adrenals, diffuse lymphoid atrophy in the spleen, acute inflammation in the lungs with alveolar spaces containing blood and increased number of adipocytes in the sternum.
The bilateral hyaline degeneration of the cortical tubules in the kidneys was considered to be test item treatment-related. However, it is not possible to determine if this lesion, which was associated with increase in urea and creatinine levels, was directly due to the test item action or the result of the dehydration caused by a severe intestinal irritation. The inflammation noted in the liver, oesophagus and lungs was considered to be test item related and was associated with change in leucocyte count. The increased number of adipocytes in the sternum seen also in the schedule killed animals was considered treatment-related. The abnormalities reported in blood electrolyte levels, glucose, triglycerides and cholesterol levels were not directly attributed to the test item treatment but were considered to be secondary to the poor clinical condition of the animal (diarrhoea, dehydration, changes in the kidneys). The modifications reported in spleen and adrenal glands were not attributed to the test item treatment, as they were non-specific changes that could be found in treated animals housed in laboratories.

At microscopic level, the major findings in the sacrificed female were bilateral vacuolation of the cortical tubules in the kidneys, macrovesicular vacuolation in the liver, diffuse hypoplasia of langerhans islet in the pancreas, severe atrophy of cortex of the thymus, increased number of adipocytes in the sternum and uterine atrophy.
The liver histopathological modification was considered to have resulted from the test item treatment and was correlated with changes in the blood biochemical parameters (i.e. urea, protein, albumin and bilirubin levels as well as liver enzyme activities). The abnormalities reported in blood electrolyte levels were not attributed directly to the test item treatment but were related to the poor clinical condition of the animal (diarrhoea, dehydration). The uterine atrophy and increased number of adipocytes in the sternum, seen also in the schedule killed top dose animals, were considered treatment-related.
The atrophy noted in the thymus is a non specific change that could be found in laboratory housed animals; therefore a relationship to the test treatment was excluded. The other lesions noted (i.e. in the kidneys and pancreas) can be spontaneously observed in untreated beagle dogs of this age and sex. Therefore a relationship to the test treatment was considered unlikely.

No test-substance related histopathological changes were observed in animals of both sexes at and below 300 mg/kg bw/day.

Treatment-related changes observed in surviving animals given 1,000 mg/kg bw/day consisted of increased number of adipocytes in the sternum of 2/3 males and 3/3 females, prostate atrophy in 2/3 males and uterine atrophy in 2/3 females.
These lesions, also noted among the moribund sacrificed animals, could be related to the low body weight of these high-dose animals caused by the test item.
All the other microscopic findings observed in the organs of both male and female animals of the high-dose group were judged to be unrelated to treatment or normal background findings.

Conclusion by the Notifiers

Under the experimental conditions of the study and taking into account the slight effects on organ weights at the mid dose-level, the No Observed Adverse Effect Level (NOAEL) is considered to be 300 mg/kg bw/day.

RMS comments:

The study is considered acceptable and the NOAEL is agreed. At the top dose level, the MTD was clearly exceeded. It was noticed that high dose effects of glyphosate administration in this study were particularly severe, much more pronounced and rather different from what was seen in other dog studies or other species. Thus, because of the clinical signs and pathological changes, its results do not fit into the toxicity profile of glyphosate as it was established in the majority of studies. In the study by [redacted] (1990, TOX9552384) that is decribed in detail in the original DAR (1998, ASB2010-10302), the same high dose of 1000 mg/kg bw/day was administered also in capsules causing only minor effects. There is no explanation for this apparent difference although it is known from long-term studies in rats and mice that high-dose effects of glyphosate may differ considerably. In any case, it should be taken into consideration that this dose level is by 2000 times higher than the proposed ADI.

2nd new 90-day study in dogs ([redacted] 1999)

Reference:	IIA, 5.3.3/02
Report:	[redacted] 1999 Subchronic (90 Day) Oral Toxicity Study With Glyphosate Technical In Beagle Dogs AND Test compound stability in experimental diet (dog feed) [redacted] Data owner: ADAMA Study No.: 1816 AND 1817-R.FST Date: 1999-04-17 AND 1997-02-21 not published, ASB2012-11455
Guidelines:	OECD 409
Deviations:	Several organ weights missing: epididymis, ovaries, uterus, thymus, spleen, brain, heart; several organs were not sampled (gross, lesions. Spinal cord, eyes with optic nerve, traches and mammary gland.
GLP:	Yes
Acceptability:	*See RMS comment*

Materials and methods

Test material:	
Identification:	Glyphosate technical
Description:	Crystalline solid
Lot/Batch #:	01.12.1997 & 01.06.97
Purity:	> 95 %
Stability of test compound:	Expiry dates: 2000-06-01 and 2000-12-01
Vehicle:	Plain diet

Test animals:	
Species:	Dogs
Strain:	Beagle
Source:	[redacted]
Age:	6 - 8 months
Sex:	Male and female
Weight at dosing:	♂ 10.0 – 12.2 kg; ♀ 8.8 – 11.0 kg
Acclimation period:	6 days
Diet/Food:	Nutripet Pet meal (Tetragon Chemie Pvt.Ltd., Bangalore, India), was offered daily for one hour *ad libitum*
Water:	Deep borewell water passed through activated charcoal filter and exposed to UV rays, *ad libitum*
Housing:	Individual housing in floor pens.
Environmental conditions:	Temperature: 23 - 29 °C Humidity: 40 - 70 % Air changes: no data Natural daylight plus fluorescent light from 9 am to 5 pm

In life dates: 1998-03-18 to 1998-06-26

Animal assignment and treatment:
In a 90 day feeding study groups of four Beagle dogs per sex received daily doses of 0, 200, 2000 and 10,000 ppm Glyphosate technical in the diet (corresponding to 5.3, 53.5 and 252.6 mg/kg bw/day).
Test diets were prepared prior to start of treatment and then twice during the three month study period by mixing a known amount of the test substance with a small amount of basal diet and blending. This pre-mix was then added to larger amount of basal diet and blended for 20 minutes. The feed was fortified with test compound at weekly intervals.
The stability of the test compound was examined in an additional study (No. 1817-R.FST). The homogeneity of the test material in diet was determined at start of the study. Three samples from the food fortified with the test compound were taken and analyzed.

Mortality: Each animal was checked for mortality or signs of morbidity daily during the treatment period.

Clinical observations: Each animal was daily checked for signs of toxicity. A more detailed veterinary investigation was performed before start of exposure, monthly throughout the study and before termination.

Body weight: The body weight of each animal was recorded before allocation and start of treatment, weekly throughout the study and before termination.

Food consumption: The quantity of food consumed was recorded for each animal on a weekly basis.

Ophthalmoscopic examination: Ophthalmological examinations were performed on all the animals before the beginning and at the end of the treatment period.

Haematology and clinical chemistry: Haematological and blood chemical investigation were performed on all animals from each test and control group before the beginning of the treatment period, after 45 days of exposure and at termination from animals fasted since the last feeding.
The following parameters were determined: erythrocytes (RBC), haemoglobin (HB), hematocrit (HCT), MVC, MCHC, MCH, leucocytes (WBC), differential white cell count (Neut, Lymp,Eosi,Mono, Retic), clotting time, glucose, urea, total protein, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), creatinine, total bilirubin, albumin, calcium, inorganic phosphorous, total cholesterol, triglycerides, chloride, sodium, potassium.

Urinalysis: Urine was collected from all animals at termination during autopsy. Urinalyis was performed for control and high-dose group animals,
The following parameters were determined: pH, specific gravity, leucocytes, proteins, glucose, ketones, blood, and urobilinogen.

Sacrifice and pathology: On completion of the treatment period, after an overnight fasting, all surviving animals were killed and subjected to a gross pathological examination. The moribund animals were sacrificed in the same way. Any macroscopic findings were recorded.
The following organ weights were determined: adrenals, kidneys, liver (with gall bladder), testes and thyroids with parathyroids.
Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta, bone & bone marrow (sternum), brain, caecum, colon, duodenum, gall bladder, gonads, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mesenteric), oesophagus, pancreas, pituitary gland, rectum, salivary glands, sciatic nerve, spleen, stomach, thymus, thyroids with parathyroids, urinary bladder, uterus. These tissues (plus parathyroids) were microscopically investigated for all animals of the control and high dose group

Statistics: Body weights, net body weight gain, food intake, laboratory investigations (haematology and clinical chemistry values of days 0, 45 and 90), organ weights data and organ weight ratios were compared by Bartlett's test for homogeneity of intra group Variances. When the Variances proved to be heterogeneous, the data were transformed using appropriate transformation.
The data with homogeneous intra group variances were subjected to one-way analysis of variance. Following ANOVA, when F was found to be significant, Dunnett's pair wise comparison of means of treated groups with control mean was done individually. Following a significant difference of a test group with the control group, the Dose Response correlation was estimated including the control and all treated groups and tested by 't'-test. All analyses and comparisons are evaluated at 5 % probability level.

Results and discussion
Mortality: All animals survived until scheduled necropsy.
Clinical observations: No clinical signs of toxicity were observed.
Body weight: Body weights remain essentially unaffected from treatment. A slight initial depression of body weight gain in the high dose male and female groups might be concluded (and would be in accordance with the food consumption).

Table B.6.3-19: Group mean body weights

	Body weight [kg]													
week	-1	1	2	3	4	5	6	7	8	9	10	11	12	13
Test item [ppm]	Males													
0	11.1	11.7	12.2	12.3	12.5	12.7	12.9	13.2	13.3	13.4	13.5	13.6	13.7	13.8
200	11.2	11.6	12.1	12.3	12.8	12.7	13.2	13.5	13.5	13.5	13.6	13.7	13.6	13.5
2000	11.1	11.7	12.1	12.0	12.6	12.7	12.9	13.2	13.4	13.4	13.3	13.3	13.2	13.2
10000	11.1	11.4	11.2	11.5	12.4	12.4	12.7	12.8	13.0	13.3	13.3	13.5	13.3	13.6
Test item [ppm]	Females													
0	10.1	10.4	10.9	10.7	11.0	11.0	11.2	11.4	11.7	11.9	11.7	12.0	11.7	11.8
200	10.2	10.7	11.1	10.9	11.3	11.4	11.5	11.7	11.8	11.5	11.6	11.6	11.6	11.4
2’000	9.8	10.2	10.3	10.2	10.8	10.9	11.0	11.2	11.4	11.3	11.4	11.5	11.6	11.5
10’000	10.0	9.9	10.1	10.1	10.6	10.6	10.7	10.9	11.2	11.3	11.3	11.3	11.3	11.2

Food consumption: The food intake of the high dose group (10,000 ppm) was significantly lower during the second week of treatment only. Except this finding the food consumption of all the treatment groups were comparable to the control group during the study period.

Table B.6.3-20: Average weekly food intake

	Food consumption [g/animal/day]												
week	1	2	3	4	5	6	7	8	9	10	11	12	13
Test item [ppm]	Males												
0	254	336	342	336	320	346	345	325	312	332	356	368	369
200	287	354	366	376	326	346	350	342	325	343	318	347	312
2’000	305	373	406	347	334	363	358	366	339	321	330	329	339
10’000	262	177*	380	368	342	332	330	324	328	303	341	333	330
Test item [ppm]	Females												
0	247	263	278	302	295	298	283	297	275	290	295	292	285
200	285	332	324	352	323	321	292	294	294	325	317	290	271
2’000	212	306	338	309	298	286	290	298	284	278	285	303	283
10’000	212	166*	348	327	303	261	288	294	298	262	268	262	256

The calculated mean daily test substance intake is summarised in Table B.6.3-21 below.

Table B.6.3-21: Group mean compound intake levels

Dose group	**Dietary concentration (ppm)**	**Mean daily test substance intake (mg/kg bw/day)***		
		Males	Females	Combined
1 (control)	0	0.0	0.0	0.0
2 (low)	200	5.2	5.4	5.3
3 (mid)	2000	54.2	52.8	53.5
4 (high)	10000	252.4	252.7	252.6

* based on actual food intake and body weight data

Ophthalmoscopic examination: There were no ophthalmological findings at the beginning and at the end of the treatment period.

Haematology and clinical chemistry: A significant increase in clotting time and GGT-activity was observed in both sexes at the 45-day interim bleed; however, in absence of any corresponding changes at terminal bleed or any histopathological correlate in the liver, this observation is considered to rather reflect a systemic error during determination than a real effect of the test item.

Table B.6.3-22: Summary of results for clotting time

	Clotting time [s]							
[ppm]	0	200	2’000	10’000	0	200	2’000	10’000
	males				Females			
Pre-exposure bleed	145	150	147	144	154	162	149	131
45 day interim bleed	131	153*	172*	183*	141	161*	173*	182*
90 d final bleed	134	134	136	139	142	142	134	138

Table B.6.3-23: Summary of results for gamma-GT activity

	GGT [U/L]							
[ppm]	0	200	2’000	10’000	0	200	2’000	10’000
	males				Females			
Pre-exposure bleed	9	10	8	7	9	7	7	11
45 day interim bleed	13	13	16	19*	14	14	14	21*
90 d final bleed	11	12	16	18	17	16	16	29

Total bilirubin was highered; however, in absence of a histopathological correlate on the liver, the effect was not considered adverse.

Table B.6.3-24: Summary of results for total bilirubin

	Total bilirubin [µmol/L]							
[ppm]	0	200	2’000	10’000	0	200	2’000	10’000
	Males				females			
Pre-exposure bleed	3.71	3.99	3.71	3.14	3.67	3.51	3.96	4.02
45 day interim bleed	5.25	5.10	5.93	5.97	5.22	5.23	6.49*	6.54*
90 d final bleed	4.21	5.65*	5.95*	6.21*	4.00	6.57*	7.08*	7.18*

Urinalysis: All parameters were in the normal range and comparable between control and treated animals.
Organ weights: No treatment-related effects were observed.
Necropsy: No treatment-related gross pathological effects were observed.
Histopathology: There were a few incidental findings with equal distribution across control and treated groups – no relation to treatment was observed.

Conclusion by the Notifiers
Under the experimental conditions of the study, the No Observed Adverse Effect Level (NOAEL) of glyphosate Technical in Beagle dogs is considered to be 10,000 ppm (252.6 mg/kg bw/day).

RMS comments:
The study is considered acceptable. It is agreed to consider the highest dose the NOAEL because the minor effects were indeed not adverse. The lower body weight gain at the beginning of treatment is very probably a result of impaired food consumption. Lower food intake might be due to a palatability problem or might simply result from the need of the animals to adapt to a diet with a new and perhaps strange taste. The higher bilirubin levels might be due to treatment but were not accompanied by any pathological change.
It was noted that the highest dose choosen, as compared to other studies with dietary administration to dogs, was rather low.

3d new 90-day study in dogs ([redacted] 1996)

Reference:	IIA, 5.3.3/03
Report:	[redacted] 1996 HR-001: 13-week Subchronic Oral Toxicity Study in Dogs. [redacted] Laboratory Report No.: [redacted] 94-0158 Data owner: Arysta LifeScience Date: 1996-09-05 not published, ASB2012-11456
Guidelines:	Japan MAFF Guidelines 59 NohSan No.4200, 1985, U.S. EPA 2012-11456)FIFRA Guidelines Subdivision F, 1984 OECD 409 (1981)
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Materials and methods

Test material:	Glyphosate technical
Identification:	HR-001
Description:	White crystal
Lot/Batch #:	T-940308
Purity:	94.61 %
Stability of test compound:	Not mentioned in the report
Vehicle:	Plain diet / none

Test animals:	
Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	♂ 5 months; ♀ 6 months
Sex:	Male and female
Weight at dosing:	♂ 27.3-32.7 g; ♀ 22.4-25.8 g
Acclimation period:	♂ 21 days; ♀50 days
Diet/Food:	Solid diet DS (Oriental Yeast, Co.) restricted at 250 g/dog/day
Water:	Filtered and sterilized tap water, *ad libitum*
Housing:	Individually in stainless steel cages 83.5 x 90.0 x 80.0 cm
Environmental conditions:	Temperature: 24 ± 2 °C Humidity: 55 ± 10 % Air changes: 15/hour 12 hours light/dark cycle

In life dates: 1995-09-20 to 1996-02-08

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 4 males and 4 females Beagle dogs for a minimum of 90 days. Dietary concentrations were 0, 1 600, 8 000 and 40000 ppm.

Homogeneity of the test substance in diet was ascertained for all dose levels using the samples taken from the top, middle and bottom portions of the mixer at the first diet preparation (before initiation of the study). The coefficient of variation of the concentrations of technical glyphosate was 2.3 % or less for all test diets and confirmed that the test substance was mixed in the basal diet at good homogeneity.
Concentrations of technical glyphosate in test diets were monitored for all batches of test diets of all dose levels during the study. The overall mean concentrations found in test diets were within a range of 94–101 % to the nominal levels and confirmed that the test substance was mixed in the test diets at acceptable concentrations.

Mortality: Mortality was expressed weekly as a ratio of the cumulative number of animals found dead or killed in extremis to the effective number of animals per dose group.

Clinical observations: Cage-side observation was performed daily on all animals to detect moribund or dead animals and abnormal clinical signs, and all findings were recorded. In addition, a detailed examination including palpation for masses was performed at least once a week.

Body weight: Body weights of all animals were recorded at initiation of treatment and weekly during the study. In addition, final body weight of each animal was measured before necropsy.

Food consumption and utilisation: Food residues, if any, were collected and weighted every morning. Daily food consumption by each animal was calculated as follows:

Chemical intake (in mg/kg bw/day) was calculated weekly from food consumption and body weight data and the nominal level.

Ophthalmoscopic examination: Prior to initiation of treatment and at week 13, all animals were subjected to ophthalmological examinations with a direct ophthalmoscope.
The following parameters were determined: Eyeball, eyelid, conjunctiva, cornea, anterior chamber, pupil, iris, lens, vitreous body, and fundus.

Haematology and clinical chemistry: Prior to initiation of treatment and at weeks 7 and 13, all animals were subjected to haematological examinations. Blood samples were withdrawn with heparinised syringes from the cephalic vein of the animals following overnight starvation. A part of each sample was transferred to a cup of treated with EDTA and subjected to the haematological examination.
The following parameters were determined with a fully automated hematology analyzer: Hematocrit (Ht), hemoglobin (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), total leukocyte count (WBC).

Prior to initiation of treatment and at weeks 7 and 13, all animals were subjected to biochemical examinations. Plasma from heparinised blood sample from haematological tests were used.
The following parameters were determined: alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca), inorganic phosphorus (P).

Urinalysis: Prior to initiation of treatment and at week 13 of treatment, all animals were subjected to urinalysis.
Volume and sediments were determined on urine samples collected for 24 hours using trays. The other parameters were determined on fresh urine samples.
The following parameters were determined: specific gravity, pH, protein, glucose, ketones, accult blood, urobilinogen, bilirubin, appearance urine volume, urinary sediments.

Sacrifice and pathology: All animals were subjected to a complete necropsy and all gross findings were recorded. After 13 weeks of treatment, all animals were anesthetized and euthanized by exsanguinations from the carotid artery before necropsy. At necropsy the organs and tissues except eyes were removed and preserved in neutral-buffered 10 % formalin. The eyes were fixed in a phosphate-buffered mixed solution of formalin and glutaraldehyde for about 3 days and transferred to neutral-buffered 10 % formalin.

Weights of the following organs were recorded for all animals and the ratios to the final body weight were calculated: brain, heart, adrenals, thyroids with parathyroids, liver, ovaries, kidneys, prostate, spleen.

The following organs and tissues from all animals were histopathologically examined: brain, spinal cord, peripheral nerve, pituitary, thyroids with parathyroids, thymus, adrenals, tonsil, spleen, bone with marrow, lymph nodes, hearth, aorta, tongue, pharynx, buccal mucosa of

oral cavity, salivary glands, esophagus, stomach, liver, gallblader, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, nasal cavity, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, epididymides, penis, ovaries, oviducts, uterus, vagina, diaphragm, eyes, femoral muscle, skin, mammary gland, all gross lesions

Statistics: All data were evaluated using variance analysis (bodyweight, food consumption, urine specific gravity, urine volume, hematologicla parameters, blood chemical parameters, and organ weights).
Data on clinical sign, mortality, ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability.

Results and discussion
Mortality: There were no animals found dead or killed *in extremis* in any groups during the treatment period.
Clinical observations: Statistically significant differences in incidence of clinical signs were not observed between the control and treated goups in either sex.
Body weight: Statistically significant differences in body weights were not observed between the control and treated groups in either sex throughout the treatment.
Food consumption and test substance intake: There were no significant changes in food consumption and chemical intake in either sex of the treated groups.
The overall group mean chemical intakes (mg/kg bw/day) over the whole treatment period were calculated from food consumption, body weights, and the nominal dose levels. The results are shown in the table below:

Table B.6.3-25: Summary of compound intake

Dose level (ppm)	**Overall group mean chemical intake (mg/kg bw/day)**	
	Male	Female
1 600	39.7	39.8
8 000	198	201
40 000	1015	1014

Ophthalmoscopic examination: No ocular changes were detected in any dose groups of both sexes.
Haematology:_Statistically significant changes in haematology parameters were observed in the treated groups as shown in the following table:

Table B.6.3-26: Results of haematological examinations

		Dose group (ppm)								
		1 600			8 000			40 000		
Week of treatment		0	7	13	0	7	13	0	7	13
Erythrocyte count (RBC)	Male	-	-	↗ (112)	-	-	↑ (115)	-	-	-
Mean corpuscular volume (MCV)	Male	↘ (89)	-	-	-	-	-	-	-	-
Mean corpuscular hemoglobin concentration (MCHC)	Female	-	-	-	-	-	-	↓ (96)	-	↘ (97)
Lymphocytes (Lym)	Female	↘ (148)	-	-	-	-	-	-	-	-

Statistically evaluated by Dunnett's multiples comparison method
Value in parenthesis means percentage of group mean value against control mean value.

Although there were statistically significant differences in some parameters in the treated groups of both sexes, no dose dependency was conceived in the changes. A significant decrease in mean corpuscular hemoglobin concentration (MCHC) observed in females of the 40 000 ppm group was considered to be incidental, because the change was also noted for the pre-treatment measurement and was not accompanied with significant abnormalities of erythrocyte count (RBC), Hematocrit (Ht), and hemoglobin (Hb).

Blood clinical chemistry: Statistically significant changes in blood biochemistry parameters were observed in the treated groups and are shown in the following table:

Table B.6.3-27: Results of clinical chemistry examinations

		Dose level (ppm)								
		1600			8000			40000		
Week of treatment		0	7	13	0	7	13	0	7	13
Glucose (Gluc)	Male	↓ (92)	-	-	-	-	-	↘ (93)	-	-
Chloride (Cl)	Male	-	-	↗ (102)	-	-	-	-	-	↗ (102)
Albumin (Alb)	Female	-	-	↗ (107)	-	-	-		-	-

Statistically evaluated by Dunnett's multiples comparison method
Value in parenthesis means percentage of group mean value against control mean value.

Although there were statistically significant differences in some parameters in the treated group of both sexes, no dose dependency was conceived in the changes. Although significant increases in chloride (Cl) were observed in males of the 1 600 and 40 000 ppm groups at week 13, the changes were considered to be incidental because of no dose dependency and their small degrees of alteration.
Urinalysis: In the 40 000 ppm group, 3 of 4 females showed decrease in urine pH at week 13, although there were no statistically significant differences between the control and treated groups of both sexes in any parameters of urinalysis.
There were no significant changes in urinalysis in males and females treated at 16 000 ppm or less.

Necropsy: There were no gross findings with statistically significant differences in incidence and relationship to the treatment in the treated groups of either sex.
Organ weights: Although a statistically significant increase was noted for the relative weight of the adrenals in females of the 1 600 ppm group, the change was considered to be incidental due to the lack of dose-dependency.
Histopathology: There were no histopathological changes related to the treatment in the treated groups of either sex. A female in the 40 000 ppm group showed cutaneous histiocytoma which is a non-specific lesion in young dogs.

Conclusion by the Notifiers
Under the experimental conditions of the study, the No Observed Effect Level (NOEL) is considered to be 40,000 ppm (equivalent to 1015 and 1014 mg/kg bw/day for males and females, respectively).

RMS comments:
The study is considered acceptable. The highest dose level of 40 000 ppm is considered the NOAEL because there were no adverse effects of treatment observed. The decrease in urine pH in some high dose females is most likely due to the acidic properties of the test substances and was measured in other toxicological studies before and after, too. Occurring in isolation, without concomitant signs of renal or bladder toxicity, this is not considered an adverse finding.

4th new 90-day study in dogs ([redacted] 1996)

Reference:	IIA, 5.3.3/04
Report:	[redacted] 1996 First Revision To Glyphosate Acid: 90 Day Oral Toxicity Study in Dogs [redacted] Data owner: Syngenta Report No.: [redacted]/P/1802 Date: 1996-11-14 not published, TOX2000-1991
Guidelines:	OECD 409 (1998): OPPTS 870.3150 (1998): 2001/59/EC B.27 (2001)
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Materials and methods

Test Material:	Glyphosate acid
Description:	Technical, white solid (passed through a 75 µm mesh)
Lot/Batch number:	D4490/1, P18
Purity:	99.1 % w/w a.i
CAS#:	Not reported
Stability of test compound:	Not reported
Vehicle:	Glyphosate acid was administered in diet.

Test Animals:	
Species	Dog
Strain	Beagle
Age/weight at dosing	22 - 26 weeks
Source	[redacted]
Housing	Individually in indoor pens, with a floor area of 345 x 115 cm. Each pen consisted of an exercise area and separate sleeping quarters with a heated floor.
Acclimatisation period	4 – 5 weeks
Diet	Laboratory Diet A (Special Diet Services Ltd., Witham, Essex, UK) *ad libitum*
Water	Mains water *ad libitum*
Environmental conditions	Temperature: 19 - 22 °C Humidity: Not reported Air changes: Approximately 12 changes / hour Photoperiod: 11 hours light / 13 hours dark

In-life dates: Start: 12 August 1986 End: 19 November 1986

Animal assignment: The study consisted of one control and three treatment groups each containing 4 male and 4 female dogs. The randomisation procedure employed ensured the even distribution of animals across replicates (randomised blocks) and treatment groups, by bodyweight, placing litter mates in different treatment groups. The sexes were randomised separately.

Male dogs received 400 g and females 350 g of the appropriate diet, in the morning between 9 am and 12 noon each day. During the pre-study period, the food was removed 2-5 hours after presentation in an attempt to ensure that the dogs ate the diet rapidly. Several batches of test diets were prepared so that no one batch was fed for longer than 5 weeks.

The clinical condition and body weights of the dogs were monitored during the study, as was their biochemical and haematological status. At the end of the study the dogs were subjected to an examination *post mortem*. The major organs were fixed, processed and examined.

Diet preparation and analysis: All experimental diets were based on expanded, ground Laboratory Diet A.

The glyphosate acid concentration was determined for each occasion diet was mixed. The homogeneity of diets containing glyphosate acid was established by analysis of aliquots of diet taken from each mix of the low and high dose diet on the first occasion on which diets were prepared. The stability of the low and high dose diets was determined over a 39 day period on one mix from the first occasion on which diets were prepared.

Observations: A detailed clinical examination, which included cardiac and pulmonary auscultation was made on all dogs pre-experimentally and in week 13. In the treatment period, the dogs were observed at least twice during the working day for gross clinical and behavioural abnormalities.

A daily record of faecal consistency was made during the pre-experimental and dosing periods.

Body weight: All dogs were weighed weekly, before feeding, throughout the pre-study period, on day 1 and thereafter at weekly intervals, until termination.

Food consumption: Food residues were recorded daily and were then discarded. These measurements were made usually 4 hours (between 2-5 hours) after presentation of the diet during the pre-experimental period and approximately 24 hours after presentation of the diet during the dosing period.

Ophthalmoscopic examination: The eyes of all dogs were examined by indirect ophthalmoscopy pre-experimentally and in week 13.

Haematology: Jugular vein blood samples were taken before feeding from all dogs in weeks -1, 4, 8 and 13 and the following parameters measured:

Haemoglobin
Haematocrit
red blood cell count
mean cell volume
mean cell haemoglobin
kaolin-cephalin time
mean cell haemoglobin concentration
platelet count
total white cell count
differential white cell count
blood cell morphology
prothrombin time

Bone marrow smears were taken from a femur of all dogs at necropsy, air dried, fixed in absolute methanol and stored but not examined.

Clinical chemistry: Jugular vein blood samples were taken before feeding from all dogs in weeks -1, 4, 8 and 13 and the following parameters assessed:.

Urea
Glucose
Albumin
total protein
Cholesterol
Triglycerides
creatine kinase activity
alkaline phosphatase activity
aspartate aminotransferase activity
alanine aminotransferase activity
gamma-glutamyl transferase activity
calcium
sodium
potassium

Urinalysis: Urine was collected by catheterisation from all dogs, once pre-experimentally and in week 13. Microscopic examination of the centrifuged deposits, from all dogs, was made pre-experimentally and in week 13 on the samples taken for biochemical analysis.

Urobilinogen
specific gravity
pH
Bilirubin
glucose
ketones
protein
blood

Macroscopic examination: At the end of the 90 day dosing period, all animals were killed and examined *post mortem*. This involved an external observation and an internal examination of all organs and structures.

Organ weights: From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed:

adrenal glands
Brain
Epididymides
Kidneys
ovaries
liver
testes
thyroid glands (with parathyroids)

The left and right components of paired organs were weighed separately.

Tissue submission: The following tissues were examined *in situ*, removed and examined and fixed in an appropriate fixative:

gross lesions including masses
adrenal gland
aorta
brain
bone and bone marrow (rib)
caecum
colon
duodenum
gall bladder
epididymis
eyes
femur (including stifle joint)
heart
ileum
jejunum
kidney
liver
lung
lymph node - prescapular
lymph node - mesenteric
mammary gland (females only)
oesophagus
ovary
pancreas
pituitary gland
prostate gland
rectum
salivary gland
spinal cord (lumbar)
skin
spleen
sternum
stomach
testis
thymus
thyroid/parathyroid gland
trachea
urinary bladder
uterus
voluntary muscle
cervix
nerve - sciatic

Microscopic examination: All processed tissues were examined by light microscopy.

Statistics: Body weight gains from the start of the study to each week and final body weights were considered by analysis of variance, separately for males and females.
Haematology, blood and urine biochemistry data were considered, at each sampling time after the start of the study, by analysis of co-variance on pre-experimental values. Male and female data were analysed together and the results examined to determine whether differences between control and treated groups were consistent between sexes.
Organ weights at termination were considered by analysis of variance and analysis of co-variance on the last measured body weight, separately for males and females. Left and right components of paired organs were considered separately and combined to investigate for any differential effects.
All analyses allowed for the replicate design of the study and were carried out using *SAS (1982)*. Unbiased estimates of the treatment group means were provided by least square

means (LSMEANS option in SAS). Each treatment group was compared to the control group mean using a two-sided Student's t-test, based on the error mean square from the appropriate analysis. Where male and female data were analysed together, these comparisons were made separately.
All data were checked for atypical values and where such values were detected the analyses were repeated omitting these values to determine their influence on the conclusions.

Results and discussion
Concentration analysis results*:* The achieved dietary concentrations of glyphosate acid were all within ± 9 % of the target concentrations.
Homogeneity results: The homogeneity was considered to be satisfactory with all the mean values from the analysis at the different sampling points being within 6 % of the overall mean.
Stability results: Over a period of 39 days, no significant change was seen in the chemical stability at 2000 and 50000 ppm glyphosate acid.
Mortality: There were no mortalities.
Clinical observations: The clinical observations noted were of a minor nature, often seen in studies of this duration using this strain of dog, and are considered to be unrelated to treatment with glyphosate acid.
Body weight and weight gain: Body weight gain of males given 50000 ppm glyphosate acid showed a slight depression throughout the study, but the differences were not statistically significant.

Females given 50000 ppm glyphosate acid showed slightly reduced bodyweight gains throughout the study and these were occasionally statistically significantly different from the controls.
There was no effect on growth in dogs given 2000 or 10000 ppm glyphosate acid.

Table B.6.3-28: Intergroup comparison of body weight gain (g) (selected timepoints)

	Dietary concentration of glyphosate acid (ppm)							
	Males				Females			
week	0	2000	10000	50000	0	2000	10000	50000
Initial wt	10.97	10.60	11.00	10.90	9.70	9.40	9.47	9.47
4	1.00	1.13	1.07	0.65	0.64	0.75	0.85	0.38*
9	2.07	1.92	2.07	1.65	1.31	1.42	1.52	0.97*
Final wt	13.03	13.00	13.37	12.50	11.31	11.13	11.40	10.95
* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided)								

Food consumption and utilisation: All dogs ate all the diet presented during the dosing period. The dose received (in mg glyphosate acid/kg/day) was similar for both males and females. During the study, there was the expected decrease in the dose received, due to the increasing weight of the dogs.
One dog fed 10000 ppm glyphosate acid was given cubed diet for two days in week 5 to prevent it scooping up powdered diet and thereby allowing healing to a wound in its front paw. No glyphosate acid was received by this dog on these two days.
Dose rates (based on nominal dietary levels of glyphosate acid) were calculated in terms of mg/kg body weight. Mean values are shown below:

Table B.6.3-29: Mean Dose Received (mg/kg bw/day)

Glyphosate acid (ppm)	**2000**	**10000**	**50000**
Males	68	323	1680
Females	68	334	1750

Ophthalmoscopic examination: There were no treatment-related ophthalmological findings.
Haematology: There were no differences in haematological parameters which were considered to be related to treatment.
Blood clinical chemistry: Male dogs fed 50000 ppm glyphosate acid showed slightly reduced plasma albumin and total protein concentrations, possibly representing the start of an expected effect of feeding an inert substance at a sufficiently high level to reduce the intake of nutrients. Plasma calcium levels were also minimally reduced in these animals, possibly a result of calcium sequestration which occurs with compounds structurally-related to glyphosate acid.
Female dogs given 50000 ppm glyphosate acid had slightly elevated plasma alkaline phosphatase activities throughout the study.
There were no treatment-related changes in dogs fed 2000 or 10000 ppm glyphosate acid. There were other isolated instances where results were statistically significantly different from control, but these were considered to be unrelated to treatment.

Table B.6.3-30: Intergroup comparison of clinical chemistry – selected parameters, selected weeks

		Dose Level of glyphosate acid (ppm)							
		Males				Females			
Parameter	Wk	0	2000	10000	50000	0	2000	10000	50000
Albumin	4	3.70	3.70	3.73	3.43*	3.76	3.65	3.89	3.51*
	8	3.77	3.74	3.69	3.53*	3.72	3.71	3.92	3.63
	13	3.92	3.97	3.77	3.66**	3.84	3.70	3.94	3.78
Total protein	4	5.57	5.42	5.34	5.14**	5.36	5.40	5.42	5.22
	8	5.44	5.49	5.32	5.22*	5.32	5.30	5.52*	5.19
	13	5.60	5.70	5.45	5.38	5.39	5.34	5.65*	5.30
Calcium	4	11.2	11.2	11.1	10.5**	10.9	11.1	11.2	10.7
	8	11.2	11.1	10.9*	10.8**	10.9	11.0	11.2*	10.9
	13	10.7	10.5	10.8	10.0**	10.4	10.3	10.6	10.4
plasma alkaline phosphatase	4	182	190	188	193	176	181	182	220**
	8	155	168	164	177	152	155	155	181*
	13	149	165	160	161	140	143	145	166*

* Statistically significant difference from control group mean, $p<0.05$ (Student's t-test, 2-sided)
** Statistically significant difference from control group mean, $p<0.01$ (Student's t-test, 2-sided) Wk – week number

Urinalysis: There were no differences in urine clinical chemistry parameters, nor in urinary sediment examinations, which were considered to be related to treatment.
Organ weights: Kidney weights of males given 10000 or 50000 ppm glyphosate acid were slightly increased above control values, but the increase was not proportional to dose. There was also a small increase in liver weight at these dose levels, but in male dogs only.

Table B.6.3-31: Intergroup comparison of liver weight (g) in male dogs (adjusted for body weight)

0 ppm	2000 ppm	10000 ppm	50000 ppm
385	409	427*	436**

* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided)
** Statistically significant difference from control group mean, p<0.01 (Student's t-test, 2-sided)

Thyroid weights, adjusted for bodyweight, of females given 2000 or 10000 ppm glyphosate acid were statistically significantly reduced from control values. In the absence of any dose response relationship across all groups this is considered not to be of toxicological significance.
Macroscopic findings: No macroscopic findings were observed attributable to the administration of glyphosate acid.
Microscopic findings: There was no microscopic pathology attributable to the administration of glyphosate acid.
Incidental findings included minor granulomatous/inflammatory lesions in lung, alimentary tract and lymph node associated with ascarid migration. Imperfect spermatogenesis and minimal secretory activity of the prostate were observed in several sexually immature males. Minimal cystitis manifest as infiltration of the mucosa by inflammatory cells and small haemorrhages were found in several animals and were consistent with a subclinical bacterial infection of the lower urinary tract.

Conclusion by the Notifiers
Minimal toxicity was seen when glyphosate acid was administered in the diet for 90 days at the limit dose of 50000 ppm. The toxicological no effect level for glyphosate acid from this study was 10000 ppm in the diet, equivalent to a dose of more than 300 mg glyphosate acid/kg/day.

RMS comments:
The study is considered acceptable and the NOAEL of 10000 ppm is agreed with. It was noticed that test material of very high purity was used.

1st new one-year study in dogs ([redacted] 2008)

Reference: IIA, 5.3.4/01
Report: [redacted] 2007 Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule)in Beagle Dogs
[redacted]
Data owner: Nufarm
Study No.: 29647 TCC
Date: 2007-07-23
not published, ASB2012-11457
Guidelines: OECD 452 (1981); JMAFF 2-1-14 (2001)
Deviations: None
GLP: Yes
Acceptability: *See RMS comment*

Materials and methods

Test material:	Glyphosate technical
Identification:	Glyphosate tech
Description:	White crystalline powder
Lot/Batch #:	H05H016A
Purity:	95.7 %
Stability of test compound:	Expiry date: 2008-03-25
Vehicle:	Gelatine capsules size 12 (Torpac, New York, USA)
Test animals:	
Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	Approx. 6 month
Sex:	Males and females
Weight at dosing:	7.8 – 8.9 kg (males); 7.2 – 7.9 kg (females)
Acclimation period:	13 days + 20 days pre-treatment period
Diet/Food:	125 C3 pelleted diet (SAFE, Villemoisson, Epinay-sur-Orge, France), approx. 300 g per day. Due to weight loss in three animals the amount for these dogs was increased to 350 g/day from day 149, 180, and 185, respectively. From day 191 onwards all animals received 350 g/day. One male received 400 g from day 221 onwards.
Water:	Tap water, *ad libitum*
Housing:	Individually in pens containing wood shavings for bedding, except when a urine sample was required. The dogs were group-housed once a week, by sex and dose group, after the last recording of clinical signs in the afternoon, until the next morning.
Environmental conditions:	Temperature: 20 ± 5 °C Humidity: 50 ± 20 % Air changes: approx. 12/hour 12 hours light/dark cycle

In life dates: 2005-09-27 to 2006-10-17

Animal assignment and treatment: In a chronic oral toxicity study groups of four beagle dogs per sex received daily doses of 0, 30, 125 and 500 mg/kg bw/day glyphosate technical in gelatine capsules for 52 consecutive weeks. The dose levels were selected based on results of a 13-week oral (capsule) toxicity study in dogs. Dose formulations were prepared weekly by adding the required amount to the capsules. The dosages were calculated based on minimum nominal active substance content of 950 g/kg glyphosate in the test item. Analyses of the test item showed a glyphosate content consistently above 95 %. Thus, no adjustment was considered necessary. Since the test item was added under GLP conditions, no additional analyses of dose formulations were deemed necessary.

Administrations of dose capsules were done approximately the same daily time each day. The low and mid-dose animals received one capsule per day, the high-dose and control dogs received three capsules per day. The quantity of dosage form applied to each animal was adjusted weekly based on the most recently recorded body weight.

Clinical observations: Observations for morbidity, and mortality were made twice daily. A check for clinical signs of toxicity was made at least once daily on all animals. In addition, a detailed clinical examination was performed once before start of treatment and weekly thereafter until termination.

Body weight: Individual body weights were recorded three times before group allocation, on Day 1 (prior to treatment) and at weekly thereafter during the conduct of study and at termination.

Food consumption and compound intake: Food consumption of each animal was estimated daily by noting the difference between the amount provided and the remaining amount on the next morning. Food consumption was expressed as percentage of quantity provided. Whenever fasting was required, food was removed at the end of the day and estimation of food consumption as made at that time.

Ophthalmological examination: Ophthalmological examinations were performed on all dogs prior to start and at the end of the treatment period. Pupillary light and blink reflexes were evaluated first. Mydriasis was then induced by adding Tropicamide solution into the eyes and the appendages, optic media and fundus were examined by indirect ophthalmoscopy.

Haematology and clinical chemistry: Blood samples were collected from all dogs prior to treatment, in week 25 and at the end of the treatment period in week 51. For sampling dogs were fasted overnight for at least 14 hours. The following haematological parameters were examined: haemoglobin concentration (HB), erythrocyte count (RBC), mean cell volume (MCV), packed cell volume (PCV), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), thrombocytes (PLAT), leukocytes (WBC), differential white cell count with cell morphology, neutrophils (N), eosinophils (E), basophils (B), lymphocytes (L), monocytes ♂, reticulocytes (RETIC), prothrombin time (PT), and activated partial thromboplastin time (APTT). The following clinical chemistry parameters were examined: alkaline phosphatase (ALP), alanine aminotransferase activity (ALAT), aspartate amino transferase (ASAT), albumin, albumin/globulin ratio, total bilirubin, glucose, urea, calcium, chloride, total cholesterol, creatinine, γ-glutamyl-transferase (GGT), inorganic phosphorus, total protein, sodium, potassium, and triglycerides.

Urinalysis: Individual urine samples were collected from all dogs prior to treatment, in week 25 and at the end of the treatment period in week 51. For sampling dogs were fasted overnight for at least 14 hours. Urine was collected in the presence of thymol crystals. The following examinations were made: appearance, colour, specific gravity, pH, volume, proteins, glucose, ketones, bilirubin, nitrites, blood, urobilinogen. The sediment was examined microscopically for leukocytes, erythrocytes, cylinders, magnesium ammonium phosphate crystals, calcium phosphate crystals, calcium oxalate crystals and cells.

Sacrifice and pathology: All surviving dogs were killed after completion of 52 weeks treatment and were subjected to a gross pathological examination. The following organs were weight: adrenals, brain, epididymides, heart, kidneys, liver, spleen, thymus, uterus, pituitary,

prostate, ovaries, testes, thyroids with parathyroid. Organ to body weight ratios were calculated.
Tissue samples were taken from the following organs of all dogs and preserved in 10 % buffered formalin (except for the eyes with the optic nerve which were fixed in Davidson´s fixative, and testes and epididymides which were preserved in Bouin´s fluid): adrenals, aorta, brain, caecum, colon, duodenum, oesophagus, eyes and optic nerve, epididymides, femur with articulation, gall bladder, heart, ileum, jejunum, kidneys, larynx, liver, lungs with bronchi, mammary gland, mandibular lymph node, mesenteric lymph node, skeletal muscle, ovaries, oviducts, parathyroid, pancreas, pituitary, prostate, rectum, salivary glands (parotid and submandibular), skin, spinal cord (cervical, thoracic and lumbar), spleen, sternum with bone marrow, stomach, sciatic nerve, testes, thymus, thyroids with parathyroid, tongue, trachea, urinary bladder, ureters, and uterus (horns and cervix).
A detailed histopathological examination was performed on all sampled tissues of all dogs, except for femur, larynx, oviducts, tongue, ureter and vagina.

Statistics: Statistical analysis of body weight, haematology, blood biochemistry, urinalysis and organ weight data was done according to the statistical decision tree shown in "*Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies*" (OECD, 2002, ASB2013-3754), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.

Results and discussion
Mortality: No mortalities or premature sacrifices occurred during the treatment period.
Clinical observations: There were no treatment-related clinical signs observed during the study period.
Observed clinical signs consisted of vomiting or soft faeces, thin appearance, hyperactivity, ptyalism, skin findings (scabs and erythema, generally localised on the ear(s)) and nodules on the ears. These clinical observations were seen transiently, and were encountered with a similar incidence in both control and treated animals and/or were independent to the administered dose-level and/or are commonly noted when a test item is given by gavage and/or were already present before the beginning of the treatment period.
Body weight: There was no treatment-related effect on body weight development. The lower mean body weight recorded in high dose males at the end of the treatment period was due to the lower mean body weight gain during the first month of the study (see Table B.6.3-32). Individual body weight changes were within the range of physiological variations. In addition, such body weight changes were observed in both control and treated dogs.

Table B.6.3-32: Mean body weight and body weight changes (kg)

	Males				**Females**			
Dose level (mg/kg bw/day)	0	30	125	500	0	30	125	500
Mean bw prior to start (day -1)	8.2	8.3	8.3	8.3	7.4	7.4	7.6	7.4
Weeks 1 – 4	+0.6	+0.3	+0.5	+0.2*	+0.3	+0.3	+0.3	+0.3
Weeks 4 – 26	+1.4	+0.9	+1.4	+1.1	+1.2	+1.1	+1.5	+1.6
Weeks 26 – 52	+0.9	+1.4	+1.1	+0.8	+0.6	+0.2	+0.5	+1.1
Weeks 1 – 52/53	+2.8	+2.6	+2.9	+2.0	+2.1	+1.6	+2.3	+3.0
Mean bw in week 52/53	11.2	11.0	11.2	10.5	9.6	9.2	10.0	10.6

*statistically significant from control ($p < 0.05$)

The weight loss of some dogs observed in the control, and low-dose group during some periods of the study were resolved when the daily food quantity was increased. Therefore, these changes were considered not test substance related.
Food consumption
There was no treatment-related effect on food consumption noted during the study.
The reduced food consumptions noted during the study was not considered test substance related, since they occurred only on some occasions and in control and treated dogs.
Due to weight loss one male each of the low and mid dose group, and one control female received 350 g/day from day 149, 180, and 185, respectively. From day 191 onwards all animals received 350 g/day. One male received 400 g from day 221 onwards.
Ophthalmology: There were no ophthalmological findings observed at the end of the study period.
Haematology: There were no treatment-related effects noted in the haematological parameters.
The significant differences observed for the activated partial thromboplastin time (↓), MCHC (↓) and eosinophil counts (↓) in the treated animals when compared to control dogs were only slight and not dose-related.

Clinical chemistry: There were no treatment-related effects noted in the clinical chemistry parameters.
The significant differences observed for the inorganic phosphorous (↓), calcium (↓), protein (↓), glucose (↑), albumin/globulin ratio (↑) and AP values (↑) in treated animals when compared to control dogs were only slight and not dose-related.
Urine analysis: There were no findings among the quantitative or semi-quantitative and qualitative parameters during the treatment period.
Organ weights: There were no statistically significant differences in organ weights and organ to body weight ratios between control and treated dogs.
The statistically significant lower mean brain weight observed in males at 125 mg/kg bw/day was confined to this dose and, thus, no dose response was apparent. In addition, there were no macroscopic or histopathological findings noted in this organ. Thus, this finding is considered incidental.

Table B.6.3-33: Body/Brain weights and statistics

Dose group (mg/kg bw/day)	**0**	**30**	**125**	**500**
No of animals	4	4	4	4
Mean final body weight (g)	11165.0	10830.0	11090.0	10255.0
Mean brain weight (g)	87.41	80.06	73.96**	84.09
Mean % of bodyweight	0.78978	0.74484	0.67578	0.82550

**: DUNNETT'S TEST based on pooled variances at 1 % (**) level
Assigned control group(s): 1.

Gross pathology: There were no test substance related macroscopic findings observed in any animal of all dose groups.
Histopathology: There were no test substance related microscopic findings observed in any tissue sample of any dose group.

Conclusion by the Notifiers
Based on the study results the NOEL and NOAEL in beagle dogs after 1-year oral exposure to glyphosate technical is 500 mg/kg bw/day.

RMS comments:
This study is considered acceptable. It is agreed to set the NOAEL at the highest dose level of 500 mg/kg bw/day. It can be confirmed that the alterations in clinical chemistry parameters were mostly not statitically significant and, if so, did not show a dose response. The only possible exception is a lower blood calcium level in high dose males that was observed in other studies with glyphosate, too. However, without any concomitant findings, e.g, on bones, this perhaps treatment-related effects is not considered adverse.
This study was run in the same laboratory and under similar conditions as the 90-day study by [redacted] (2007, ASB2012-11454) in which severe adverse effects were seen upon treatment of Beagle dogs with glyphosate at a high dose level of 1000 mg/kg bw/day. It is clear now that these adverse reaction to treatment was in fact confined to an exaggerated dose level and that the NOAEL is higher than 300 mg/kg bw/day as established in that previous study.

2nd new one-year study in dogs ([redacted] 1997)

Reference:	IIA, 5.3.4/02
Report:	[redacted] 1997 HR-001: 12-Month Oral Chronic Toxicity Study in Dogs. [redacted] Data owner: Arysta LifeScience Study No.: IET 94-0157 Date: 1997-03-20 not published, ASB2012-11458
Guidelines:	Japan MAFF Guidelines 59 NohSan No.4200, 1985 U.S. EPA FIFRA Guidelines Subdivision F, 1984 OECD 409 (1981)
Deviations:	None
GLP:	yes
Acceptability:	*See RMS comment*

Materials and methods

Test material:	Glyphosate technical
Identification:	HR-001
Description:	White crystals
Lot/Batch #:	T-950308
Purity:	94.61 %
Stability of test compound:	Not mentioned in the report
Vehicle and/ or positive control:	None
Test animals:	
Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	5 months
Sex:	Males and females

Weight at dosing:	7.8 – 8.9 kg (males); 7.2 – 7.9 kg (females)
Acclimation period:	23 and 31 days for males and females, respectively
Diet/Food:	Solid diet DS (Oriental Yeast, Co.) restricted at 250 g/dog/day
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel cages 83.5 x 90.0 x 80.0 cm
Environmental conditions:	Temperature: 24 ± 2 °C Humidity: 55 ± 10 % Air changes: 15/hour 12 hours light/dark cycle

In life dates: 1996-03-05 to 1997-03-04

Animal assignment and treatment: Groups of 4 males and 4 females Beagle dogs received the test material by incorporating it into the basal diet at a level of 0, 1 600, 8 000 or 50 000 ppm for a period of 12 months.

Clinical observations: All animals were observed daily for clinical signs.

Body weight: Individual body weights were recorded at initiation of treatment, weekly from weeks 1 to 13, and every 4 weeks from weeks 16 to 52. In addition, final body weight was measured before necropsy.

Food consumption and compound intake: Food consumption of each animal was recorded weekly from week 1 to 13 and every 4 weeks from week 16 to 52. Food residues, if any, were collected and weighted every morning. Daily food consumption by each animal was calculated as follows:

$$Food\ consumption = [Feeding\ amount\ (250g\ diet + 250g\ water) - food\ residue] + 2$$

Chemical intake (mg/kg bw/day) was calculated weekly from food consumption and body weight data and the nominal level.

Ophthalmological examination: Ophthalmological examinations were performed on all dogs prior to start of the treatment period. The following items were examined: eyeball, eyelid, conjunctiva, cornea, anterior chamber, pupil, iris, lens, vitreous body, fundus.

Haematology and clinical chemistry: Blood samples were collected from all dogs prior to treatment, in weeks 25 and 52. The following haematological parameters were examined: Hematocrit, Hemoglobin concentration, Erythrocyte count, Mean corpuscular volume, Mean corpuscular hemoglobnin, Mean corpuscular hemoglobin concentration, platelet count, total leukocyte count.

All animals were subjected to blood biochemical examinations at weeks 26 and 52.
The following clinical chemistry parameters were examined alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio

(A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca), inorganic phosphorus (P), sodium (Na), Potassium (K), chloride (Cl).

Urine analysis: Prior to initiation of treatment and at weeks 25 and 51, all animals were subjected to urinalysis on the following parameters: appearance, colour, specific gravity, pH, volume, proteins, glucose, ketones, bilirubin, nitrites, blood, urobilinogen.

Sacrifice and pathology: All surviving dogs were killed after completion of 52 weeks treatment and were subjected to a gross pathological examination. The following organs were weight: adrenals, brain, epididymides, heart, kidneys, liver, spleen, thymus, uterus, pituitary, prostate, ovaries, testes, thyroids with parathyroid. Organ to body weight ratios were calculated.
Tissue samples were taken from the following organs of all dogs and preserved in 10 % buffered formalin (except for the eyes with the optic nerve which were fixed in Davidson´s fixative, and testes and epididymides which were preserved in Bouin´s fluid): brain, spinal cord, peripheral nerve, pituitary, thymus, thyroids with parathyroids, adrenals, tonsil, spleen, bone with marrow, lymph nodes, heart, aorta, tongue, buccal mucosa of oral cavity, pharynx, salivary glands, esophagus, stomach, liver with gallblader , pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, nasal cavity, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, penis, epididymides, ovaries, oviducts, uterus, vagina, diaphragm, eyes, femoral muscle, skin, mammary gland, all gross lesions.

A detailed histopathological examination was performed on all sampled tissues of all dogs, except for femur, larynx, oviducts, tongue, ureter and vagina.

Statistics: Statistical analysis of body weight, haematology, blood biochemistry, urinalysis and organ weight data was done according to the statistical decision tree shown in "*Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies*" (OECD, 2002, ASB2013-3754), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.

Results and discussion
Mortality: There were no deaths in any dose groups of either sex.
Clinical observations: In the 50 000 ppm group, loose stool was observed in 3 of 4 males and 4 of 4 females. The animals in the 8 000 and 1 600 ppm groups did not show the clinical sign at all. In the control group, only one animal in each sex showed it. Most of the animals in the 50 000 ppm group frequently showed the sign throughout the treatment period, whereas the occurrence in the suffering animals of the control group was restricted to a limited period.
For other clinical signs observed, the occurrence was sporadic in all dose groups, or the incidence was almost comparable among the dose groups.
Body weight: In the 50000 ppm groups of both sexes, retarded body weight gain became evident gradually as the study progressed. Consequently, the mean body weights in this group at termination of treatment were 6 % in males and 11 % in females lower than those in the controls. However, statistically significant differences in mean body weights were not observed throughout the treatment between the control and treated groups including the groups receiving 50000 ppm.
Food consumption: Decreased food consumption was noted for one female in the 1 600 ppm group at weeks 24, 28, and 52 and for another female in the same group at week 32. Consequently, group mean food consumption in this group was decreased at those weeks. However, food consumption in this group recorded at other weeks was comparable to that of

the controls. Moreover, the averaged group mean food consumption through the treatment period was almost coparable between the 1 600 ppm and control groups of females.
All males in all dose groups and females except the above 2 animals in the 1 600 ppm group consumed whole amount of diet offered every day.
Group mean chemical intakes were calculated from group mean values of food consumption and body weight, and the nominal dose levels. The overall group mean chemical intakes (mg/kg/day) through the whole treatment period are presented in the table below:

Table B.6.3-34: Mean test substance intake

	Test substance intake (mg/kg bw/day)	
Dose level (ppm)	Male	Female
1 600	34.1	37.1
8 000	182	184
50 000	1203	1259

Ophthalmology: No remarkable ocular changes were detected in animals in any dose groups at week 52.

Haematology: Statistically significant changes in haematology that were observed in treated groups are presented in the following table:

Table B.6.3-35: Results of haematological examination

	Dose level (ppm)	**1 600**			**8 000**			**50 000**		
	Week of treatment	0	26	52	0	26	52	0	26	52
Sex	Female									
Parameter	Hematocrit (Ht)	-	-	-	-	-	-	↓	-	↓
	Hemoglobin concentration (Hb)	-	-	-	-	-	-	-	↓	↓
	Erythrocyte count (RBC)	-	-	-	-	-	-	↓	-	↓

↓: P<0.05 ; - : not significant, statistically evaluated by Dunnett's multiple comparison method.

Male groups showed no significant changes in any parameters.

Females in the 50 000 ppm group showed significantly decreased values of hematocrit (Ht), hemoglobin concentration (Hb), and erythrocyte count (RBC) at week 52. Hemoglobin concentration in this group was also significantly lower at week 26. This group had already showed lower values for these 3 parameters than the controls before initiation of treatment (at week 0). In particular, the differences from the control values in hematocrit and erythrocyte count at week 0 were statistically significant. However, the rates of deviation from the control values were, though slightly, augmented in the treatment period when compared to those at week 0.
Females in the 8 000 and 1 600 ppm groups showed no significant changes in hematological examinations

Clinical chemistry
Statistically significant changes in blood biochemistry that were observed in treated groups are presented in the table hereafter:

Table B.6.3-36: Results of clinical chemistry examination

Dose level (ppm)	**1 600**			**8 000**			**50 000**		
Week of treatment	0	26	52	0	26	52	0	26	52
Male									
Creatine phosphokinase (CPK)	-	-	-	-	-	↓	-	-	-
Female									
Albumin (Alb)	-	-	-	-	-	-	-	-	↓
Calcium (Ca)	-	-	↓	-	-	-	-	-	↓
Inorganic phosphorus (P)	-	-	-	-	-	-	-	-	↓
Chloride (Cl)	-	-	-	-	-	-	-	↑	-

↓: P<0.05 ; ↑: P<0.01 ; - : not significant, statistically evaluated by Dunnett's multiple comparison method.

Females in the 50000 ppm group showed a significant increase in chloride (Cl) at week 26 and significant decreases in albumin (Alb), calcium (Ca), a,d inorganic phosphorous (P) at week 52. A significant decrease in calcium was also noted for females in the 1 600 ppm group at 52 weeks.
For male groups, the 8000 ppm group showed a significant decrease in creatine phosphokinase (CPK) at week 52. But this change was not observed in the 50 000 ppm group.

Urine analysis: There were no findings among the quantitative or semi-quantitative and qualitative parameters during the treatment period.

Organ weights: Males in the 1 600 ppm group showed statistically significant increases in both absolute and relative weights of the pituitary. However, these changes were not observed in the 50 000 or 8 000 ppm groups of males.
In the 50 000 or 8 000 ppm groups, neither males nor females showed statistically significant changes in any organ weights

Gross pathology: The macroscopic lesions observed in the present study were all sporadic in nature and there were no statistically significant differences in the incidence between the control and treated groups.

Histopathology: In the 50 000 ppm group, focal pneumonia / focal granulomatous pneumonia in the lung was observed in all females. In the other female groups including the control group, the lesion was observed in only one of 4 animals each. However, the extent of the lesions was slight in all cases including those of the 50 000 ppm group. Statistically, no significant differences between the control and dose groups were found in incidence of any histological lesions, including the pulmonary lesion.

Conclusion by the Notifiers
Based on the study results the NOEL in beagle dogs after 1-year oral exposure to HR-001 is 8000 ppm (equivalent to 182 and 184 mg/kg/day for males and females, respectively).

RMS comments:
The study is considered acceptable although there was an uncertainty about the dose levels that were actually tested. In the original study summary (p. 17), dose levels of 2000, 10000, and 30000 ppm are mentioned. According to a different information on the same page and in the following part of the report, dose levels were 1600, 8000, and 50000 ppm. It is assumed that the latter is correct but this error might provoke some doubts about the quality assurance

system of the performing laboratory. The NOAEL (assumed to be 8000 ppm), however, is agreed with.

3d new one-year study in dogs ([redacted] 1996)

Reference:	IIA, 5.3.4/03
Report:	Brammer, A. 1996 Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs [redacted] Data owner: Syngenta Report No.: CTL/P/5079 Date: 1996-09-24 not published, TOX2000-1992
Guidelines:	OECD 452 (1981): OPPTS 870.4100 (1998): 87/302/EEC B.30 (1988)
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Materials and methods

Test Material:	Glyphosate acid
Description:	Technical, white solid
Lot/Batch number:	P24
Purity:	As given in report 95.6 % a.i
CAS#:	If available
Stability of test compound:	Confirmed by the Sponsor
Vehicle:	The test substance was administered in the diet.
Test Animals:	
Species	Dog
Strain	Beagle
Age/weight at dosing	20 – 29 weeks
Source	[redacted].
Housing	Housed by treatment group (sexes separately) in indoor pens. The pens had a sleeping platform with heated floor underneath and interlinking gates which enable the dogs to be separated for feeding and dosing.
Acclimatisation period	4 – 5 weeks
Diet	Laboratory Diet A (Special Diet Services Ltd., Stepfield, Witham, Essex, UK) *ad libitum*
Water	Mains water *ad libitum*
Environmental conditions	Temperature: 19 ± 2 °C Humidity: 40-70 % Air changes: Approximately 15 changes / hour Photoperiod: 12 hours light / 12 hours dark

In-life dates: Start: 11 April 1995 End: 12 April 1996

Animal assignment: In a chronic toxicity study, groups of four male and four female beagle dogs were fed diets containing 0 (control), 3000, 15000, or 30000 ppm glyphosate acid, for a period of at least 1 year. A randomisation procedure was used which resulted in the even distribution of dogs (16 males and 16 females) to treatment groups according to body weight ensuring that litter mates were in different groups. Each morning, male dogs received 400 g and female dogs received 350 g of their appropriate experimental diet.

Table B.6.3-37: Study design

Test group	Dietary concentration (ppm)	Dose to animal (mg/kg) Males / females	# male	# female
Control	0		1 – 4	5 – 8
Low	3000	90.9 / 91.1	9 – 12	13 – 16
Mid	15000	440.3 / 447.8	17 – 20	21 – 24
High	30000	906.5 / 926.2	25 – 28	29 – 32

Diet preparation and analysis: The experimental diets were made in 60 kg batches, by direct addition of glyphosate acid (allowing for purity) to ground Laboratory A diet, and mixed thoroughly. Water was then added to each batch and mixed prior to pelleting. The pellets were dried in the residual heat of an autoclave, allowed to cool and were then stored in bins at room temperature.

Samples from all dietary levels (including controls) were taken at approximately two-monthly intervals throughout the study and analysed quantitatively for glyphosate acid. The homogeneity of glyphosate acid in Lab diet A was determined by analysing samples from the low and high dose levels. The chemical stability of glyphosate acid in diet was determined over a period of up to 10 weeks (69 days) for these same diets.

Samples were extracted with water, portions of the supernatant were diluted with water to give sample solution concentrations within the range of the calibration standards. These were derivitised using 9-fluorenylmethylchloroformate (FMOCCL) and analysed by High Performance Liquid Chromatography (HPLC).

Observations: All dogs were observed at least three times daily for clinical behavioural abnormalities (at dosing, after dosing and at the end of the working day) and, on a weekly basis, they were given a thorough examination. Individual, daily assessments of gastro-intestinal findings were made for up to 5 hours post dosing: any subsequent assessments were made on a group basis. All dogs were also given a full clinical examination by a veterinarian pre-study, during weeks 13, 26, 39 and prior to termination. The examination included cardiac and pulmonary auscultation.

Body weight: All dogs were weighed weekly, before feeding, throughout the pre-study period, on day 1 and thereafter at weekly intervals until termination.

Food consumption and test substance intake: Food residues were recorded daily, approximately 4 hours after feeding and any residual food was discarded. These measurements were made for at least 2 weeks pre-study and throughout the treatment period.

Ophthalmoscopic examination: The eyes of all dogs were examined pre-study, during weeks 13, 26, 39 and prior to termination.

Haematology: Blood was collected from all dogs in weeks -1, 4, 13, 26 and prior to termination into tubes containing EDTA or trisodium citrate and the following parameters measured.

haemoglobin
haematocrit
red blood cell count
mean cell volume
mean cell haemoglobin
prothrombin time
blood cell morphology
mean cell haemoglobin concentration
platelet count
total white cell count
differential white cell count
red cell distribution width
activated partial thromboplastin time
bone marrow smears (taken but not examined)

Clinical chemistry: Blood was collected from all dogs in weeks -1, 4, 13, 26 and prior to termination into tubes containing lithium heparin and the following parameters measured.

urea
creatinine
glucose
albumin
total protein
cholesterol
triglycerides
total bilirubin
creatine kinase activity
alkaline phosphatase activity
aspartate aminotransferase activity
alanine aminotransferase activity
gamma-glutamyl transferase activity
Calcium
phosphorus (as phosphate)
Sodium
Potassium
Chloride

Urinalysis: Urine was collected by catheterisation, pre-experimentally, in week 26 and during the week prior to termination. The following parameters were measured and recorded on each urine sample:

volume
colour (if abnormal)
specific gravity
pH
Glucose
Ketones
Protein
Bilirubin
Blood

In addition, each urine sample was centrifuged and the sediment stained and examined microscopically to identify the components.

Macroscopic examination: All animals were were killed by exsanguination under terminal anaesthesia induced by intravenous administration of sodium pentobarbitone and examined *post mortem*.

Organ weights: From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed:

adrenal glands
Brain
Epididymides
thyroid glands
Kidney
Liver
Testes

The left and right components of paired organs were weighed separately.

Tissue submission: The following tissues were examined *in situ*, removed and examined and fixed in an appropriate fixative:

gross lesions including masses
adrenal gland
aorta
brain (cerebrum, cerebellum and brainstem)
bone marrow (sternum)
caecum
colon
duodenum
epididymis
eyes (retina, optic nerve)
femur (including stifle joint, stored not examined)
Gall bladder
heart
ileum
jejunum
kidney
Larynx
liver
lung
lymph node - prescapular
lymph node - mesenteric
mammary gland (females only)
oesophagus
ovary
Pancreas
parathyroid gland
pituitary gland
prostate gland
Rectum
salivary gland
spinal cord (cervical, thoracic, lumbar)
Skin
Spleen
Sternum
Stomach
Testis
Thymus
thyroid gland
Trachea
urinary bladder
uterus (with cervix)
voluntary muscle

Microscopic examination: All processed tissues were examined by light microscopy.

Statistics: All data were evaluated using analysis of variance and / or covariance for each specified parameter using the GLM procedure in SAS (1989).

Results and discussion

Concentration analysis results: The mean achieved concentrations of glyphosate acid in analysed dietary preparation were typically within 12 % of nominal concentration.
The overall mean concentrations were within 9 % of target.
Homogeneity results: The homogeneity of glyphosate acid in diet at concentrations of 3000 ppm and 30000 ppm for a batch size of 60 kg was determined and considered satisfactory; percentage deviations from the overall mean where within 11 %.
Stability results: The chemical stability of glyphosate acid in experimental diets (determined at concentrations of 3000 ppm and 30000 ppm) when stored at room temperature, was shown to be satisfactory for 69 days. This covered the period of usage on the present study.
Mortality: None of the dogs died.
Clinical observations: There were no toxicologically significant findings. Salivation at dosing was observed in individual dogs in all treatment groups throughout the study. The apparent increased incidence in two top dose males and one female, was considered to be related to anticipation of feeding and not to treatment with glyphosate acid. There was also a low

incidence of scrotal skin reddening seen in one male in each treatment group; this was considered to be incidental to treatment with glyphosate acid.
There was no increased incidence of faecal abnormalities in dogs treated with glyphosate acid.
Bodyweight and weight gain: There was a slight bodyweight effect evident in females fed 30000 ppm glyphosate acid with a maximum reduction of 11 % (compared to controls) in week 51. These dogs showed a gradual reduction in growth rate, compared to the controls, which was consistently statistically significant from week 23 onwards. One female lost 0.6 kg during week 32 but this was related to a loss of appetite during this time. There were no effects in males at any dose level or in females at 15000 ppm but females fed 3000 ppm glyphosate acid also showed slightly poorer growth than the controls, with a maximum reduction of 8 % in week 51. However, this effect only achieved statistical significance on occasions during the study and is considered attributable to the poorer growth of two females and not an effect of glyphosate acid, since there was no effect at 15000 ppm.

Table B.6.3-38: Intergroup comparison of body weights (selected timepoints; adjusted mean values shown for weeks 2-14)

	Dietary Concentration of Glyphosate acid (ppm)							
	Males				Females			
week	0	3000	15000	30000	0	3000	15000	30000
1	11.40	11.53	11.33	11.45	9.60	9.55	9.48	9.58
8	12.66	12.40	12.48	12.37	10.74	10.40*	10.68	10.42*
16	13.35	12.97	13.28	12.95	11.46	11.03*	11.50	10.99*
32	14.19	13.69	13.93	13.69	12.28	11.63*	12.59	11.46**
53	14.57	14.24	14.24	13.85	13.10	12.25	12.94	11.76**
* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided) ** Statistically significant difference from control group mean, p<0.01 (Student's t-test, 2-sided)								

Food consumption: There was no effect on food consumption but 3 dogs left food on occasions which affected the group mean values:
Dose rates (based on nominal dietary levels of glyphosate acid) were calculated in terms of mg/kg body weight. Mean values are shown below:

Table B.6.3-39: Mean Dose Received (mg/kg bw/day)

Glyphosate acid (ppm)	**3000**	**15000**	**30000**
Males	90.9	440.3	906.5
Females	91.1	447.8	926.2

Ophthalmoscopic examination: There was a very low incidence of corneal or lenticular opacities but these were seen in control animals as well as those fed glyphosate acid. There were no treatment related abnormalities.
Haematology: There were no differences in haematological parameters which were considered to be related to treatment.
Blood clinical chemistry: There were no toxicologically significant findings
Plasma cholesterol levels were increased slightly in the treated groups of both sexes at weeks 26 and 52 but there was no evidence of any dose relationship.
Plasma phosphorus levels were lower in the male treated groups at week 52 but this was due, in part, to slightly higher individual control values. Similarly the reduced sodium value in males fed 30000 ppm at week 52 was due solely to one male.

Various animals in all groups (including controls) showed evidence of higher plasma alanine aminotransferase, aspartate aminotransferase and creatine kinase activities throughout the study as well as pre-experimentally, but there was little evidence of any conclusive group effects.
Other statistically significant differences were minor and/or not dose related and were considered to be of no toxicological significance.
Urinalysis: There were no differences in urine clinical chemistry parameters which were considered to be related to treatment.
Organ weights: There were no treatment related effects on any organ weights. Adrenal weights were slightly raised in the male 3000 ppm group but this was exaggerated by a low value for one male in the control group.
Macroscopic findings: Several treated females showed red areas in or diffuse reddening of the urinary bladder mucosa. The incidence was not clearly related to dose and in the absence of a similar effect in males it was considered unlikely that the lesion is related to the administration of glyphosate acid.

Microscopic findings: It was considered unlikely that any of the lesions confined to the treated groups were related to the administration of glyphosate acid as they were either of low incidence or the incidence was not related to dose. The pathological no-effect level for glyphosate acid was 30000 ppm.

Conclusion by the Notifiers
Oral administration of 0, 3000, 15000 or 30000 ppm glyphosate acid in the diet for 52 weeks caused minimal toxicity at 30000 ppm, evident as a slight reduction in bodyweight in females. This dose level was equivalent to an overall mean dose of 906 mg/kg/day for males and 926 mg/kg/day for females.

There were no other treatment related findings and the pathological no-effect level was 30000 ppm glyphosate acid.

The no-observed effect level for toxicity over 1 year for females was 15000 ppm glyphosate acid (equivalent to an overall mean dose of 447 mg/kg/day). The no-observed effect level for toxicity over 1 year for males was 30000 ppm glyphosate acid (equivalent to an overall mean dose of 906 mg/kg/day).

RMS comments:
The study is considered acceptable. Based on the reductions in body weight gain in high dose females, the NOAEL was the mid dose level of 15000 ppm, i.e., 447 mg/kg bw/day.

Published information
Not available for dogs.

B.6.4 Genotoxicity (Annex IIA 5.4)

Introduction into this chapter by the RMS
In this section, only genotoxicity studies are reported in detail that were not contained in the original DAR (1998, ASB2010-10302) because they either had not been submitted for previous EU evaluation or were conducted more recently. The study decriptions, evaluations and tables as submitted for the new studies were amended where necessary and each of these studies was commented by the RMS. Redundant parts were deleted. The previously known studies from the 1998 DAR were re-evaluated and used only if considered still acceptable or at least supplementary. A detailed descripton of these studies and their results may be found in the old DAR. If studies were regarded now as "not acceptable", they were only briefly mentioned and deleted from the summary tables.
A sub-section on mutagenicity of formulations was included that is mainly based on an Addendum to the original DAR that was prepared in 2000 (ASB2013-2748). In the last sub-section of this chapter, more recent publications dealing with mutagenicity of glyphosate or its formulations are discussed
Overall evaluation of genotoxicity is presented in Volume 1.

B.6.4.1 *In vitro* genotoxicity testing – Bacterial assays for gene mutation

In Table B.6.4-1, the available Ames tests of acceptable quality are summarised that had been submitted either for first EU evaluation in the 1990ies or, for the first time, for this re-evaluation. The "new" studies are reported below in detail and commented by the RMS. With regard to the "old" studies from the 1998 DAR (ASB2010-10302), most of them (in fact all but two) were deleted from current evaluation because, *e.g.*, purity or batch number were not given, the concentrations were too low as compared to other studies or if they had been assessed as "supplementary" in the previous evaluation for other reasons.

Table B.6.4-1: Summary of valid *in vitro* genotoxicity tests with glyphosate acid in bacteria (Ames test)

Reference; Study identification; Owner		Type of study	Test organism / test system	Dose levels; batch/lot number[$], purity; metabolic activation	Results
	Jensen, 1991; TOX9552371; Cheminova[#]	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537	- S9: 160 – 2500 µg/plate; + S9: 310 – 5000 (plate-incorporation and pre-incubation test); Batch 206-JaK-25-1, 98.6%	Negative
	Shirasu *et al.*, 1978; TOX9552368; Monsanto*[#]	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537, 1538 and *E. coli* WP2 hcr	10 – 5000 µg/plate (plate-incorporation assay); Lot XHJ-46, 98.4%; +/- S9	Negative (supplementary study)
	Akanuma, 1995; ASB2012-11462: Arysta	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	156-5000 µg/plate (pre-incubation test); 95.68%; +/- S9	Negative (supplementary study)
	Sokolowski, 2007; ASB2012-11463; Nufarm	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate–incorporation), 33 – 5000 µg/plate (pre-incubation test); 95.1%; +/- S9	Negative

Reference; Study identification; Owner		Type of study	Test organism / test system	Dose levels; batch/lot number[$], purity; metabolic activation	Results
	Sokolowski, 2007; ASB2012-11464; Nufarm	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate–incorporation) 33 – 5000 µg/plate (pre-incubation test); 97.7%; +/- S9	Negative
	Sokolowski, 2007; ASB2012-11465; Nufarm	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate–incorporation) 33 – 5000 µg/plate (pre-incubation test); 95.0%; +/- S9	Negative
	Riberri do Val, 2007; ASB2012-11466; Helm	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	648 – 5000 µg/plate (plate–incorporation); 98.01%; +/- S9	Negative (supplementary study)
	Flügge, 2009; ASB2012-11468; Helm	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	31.6 – 3160 µg/plate (plate-incorporation and pre-incubation test); 98.8%; +/- S9	Negative
	Flügge, 2010; ASB2012-11469; Helm	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	31.6 – 3160 µg/plate (plate incorporation and pre-incubation test); 96.4%; +/- S9	Negative
	Sokolowski, 2010; ASB2012-11470; Helm	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate incorporation and pre-incubation test); 97.16% technical a.i. containing 0.63% glyphosine; +/- S9	Negative
	Wallner, 2010; ASB2012-11471; Helm	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	31.6 – 5000 µg/plate (plate incorporation and pre-incubation test); 98.2%; +/- S9	Negative
	Thompson, 1996; ASB2012-11472; Nufarm	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	0 – 5000 µg/plate (plate–incorporation); 95.3%; +/- S9	Negative (supplementary study)
	Callander, 1996; ASB2012-11473; Syngenta	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP2P *uvrA* and WP2P	100 – 5000 µg/plate (plate-incorporation and pre-incubation assays); 95.6%; +/- S9 (for pre-incubation test only with S9 mix)	Negative
	Sokolowski, 2009; ASB2012-11474; Syngenta	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP2 *uvrA* pKM 101and WP2 pKM 101	3 – 5000 µg/plate (plate-incorporation and pre-incubation assays); 96.3%; +/- S9	Negative
	Schreib, 2012; ASB2014-9133; Industria Afrasa	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	10 – 5000 µg/plate (plate-incorporation and pre-incubation assays); 97%; +/- S9	Negative
	Thompson, 2014; ASB2014-9148; Albaugh	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP2 *uvrA*	1.5 or 5 – 5000 µg/plate (plate-incorporation and pre-incubation assays); 85.79%; +/- S9	Negative

$ Batch/lot numbers are only given for the "old" studies. For the "new" studies, they are mentioned in the comprehensive study descriptions below.
* Results were also published by Li & Long, 1988 (TOX9500253).
# The study was reported in the 1998 DAR (ASB2010-10302) and, accordingly, was subject to previous EU evaluation yet.

Some of the studies mentioned in Table B.6.4-1 are considered supplementary because either the plate-incorporation method or the pre-incubation method was performed but not both. This assessment is in line with current guideline requirements but does not affect validity of the results obtained with the method used. For overall assessment of this end point, the available database is sufficient.

The studies by Thompson (1995, TOX9500251), Fassio (1995, TOX9551631), Suresh (1993, TOX9551098), Jenkinson (1990, TOX9500268) and Bhide (1986, TOX9551955) as well as the stuy with the IPA salt by Wang *et al.* (1993, TOX9500381) as reported in the 1998 DAR (ASB2010-10302) but were not considered acceptable by the RMS upon re-evaluation. Likewise, the more recent study by Miyaji (2008; ASB2012-11467) was considered not acceptable because the highest concentration level was much too low. However, it should be noted that also these less reliable tests of lower quality did not reveal any indications of genotoxicity.

1st new Ames test (Akanuma, 1995)

Reference:	IIA, 5.4.1/01
Report:	Akanuma, M., 1995a HR-001: Reverse mutation test. The Institute of Environmental Toxicology, Tokyo, Japan Data owner: Arysta LifeScience Study No.: IET 94-0142 Date: 1995-04-03 not published, ASB2012-11462
Guidelines:	U.S. EPA FIFRA Guidelines, Subdivision F
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: 1995-02-21 to 1995-03-09

Materials and methods

Test material:	Glyphosate technical
Identification:	HR-001
Description:	Solid crystals
Lot/Batch #:	940908-1
Purity:	95.68 %
Stability of test compound:	Not mentioned in the report
Solvent used:	Sterile water
control materials:	

Negative: Sterile water

Solvent/final concentration: Water / > 12 mg/mL

Positive: non-activation and activation

Strain	**Positive controls**	
	Without S9 (µg/plate)	With S9 (µg/plate)
TA100	AF–2 (0.01)	2-AA (1)
TA1535	NaN_3 (0.5)	2-AA (2)
WP2 uvra	AF-2 (0.01)	2-AA (10)
TA98	AF-2 (0.1)	2-AA (0.5)
TA1537	9-AA (80)	2-AA (2)

AF-2: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide dissolved inDMSO; NaN_3: sodium azide dissolved in sterile water
2-AA: 2-aminoanthracene dissolved in DMSO; 9-AA: 9-aminoacridine hydrochloride dissolved in sterile water

activation: The enzyme activity measured by mutagenicity was good. S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factor. The component of S9 mix were 10 % (v/v) S9 fraction, 8 mM MgCl2, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADH, 4 mM NADPH and 100 mM sodium phosphate buffer.

test organisms: *Escherichia coli* WP2 uvrA
Salmonella typhimurium (TA100, TA1535, TA98 and TA1537)

Preliminary cytotoxicity assay:
Plate incorporation assay and pre-incubation assay: Concentrations up to 5000 µg/plate were evaluated with and without S9 activation in strain TA1535, TA1537, TA98, TA100 and WP2 uvrA. A single plate was used, per dose, per condition.

Mutation assays:
Plate incorporation assay: 156, 313, 625, 1250, 2500 and 5000 µg/plate were evaluated in triplicate in the presence and absence of S9 activation; all test strains were used.
Pre-incubation assay: As above for the plate incorporation assay.

The independently repeated mutation assay was conducted using the pre-incubation modification to the standard plate incorporation test only. The pre-incubation assay was carried out as described above with the following two exceptions: 0.5 mL of buffer were added to cultures prepared for testing under non-activated conditions; prior to the addition of top agar, reaction mixtures were incubated for 20 minutes at 37 ± 1 °C.

Statistics
Results were judged without statistical analysis.

Reproducibility of results was confirmed by two independent experiments.
Results were judged positive without statistical analysis when the following criteria are all satisfied:
A two-fold or greater increase above solvent control in the mean number of revertants is observed

This increase in the number of revertants is accompanied by a dose-response relationship
This increase in the number of revertants is reproducible.

Results and discussion
Analytical determinations: Not performed.
The results of the preliminary cytotoxicity assay are given in Table B.6.4-2.

Table B.6.4-2: Preliminary dose range finding test

		Dose	**Revertants (n° colonies/plate)**				
			Base-change type			Frameshift type	
		(µg/plate)	TA100	TA1535	WP2uvr A	TA98	TA1537
- S9 mix		Solvent control (H_2O)	124 111 (118)	14 18 (16)	25 21 (23)	17 25 (21)	7 5 (6)
		200	113	16	24	20	7
		500	116	15	23	10	3
		1000	116	16	20	14	4
		2000	82	10	15	5	5
		5000	79	3	19	8	3
+ S9 mix		Solvent control (H_2O)	83 86 (85)	11 9 (10)	21 25 (23)	29 28 (29)	6 10 (8)
		200	99	11	20	28	9
		500	82	7	12	30	6
		1000	97	8	28	25	6
		2000	96	9	18	38	7
		5000	33	4	17	20	5
Positive controls	- S9 mix	Compound	AF-2	NaN_3	AF-2	AF-2	9-AA
		µg/plate	0.01	0.5	0.01	0.1	80
		Revertants/plate	648 724 (686)	583 559 (571)	312 344 (328)	669 708 (689)	798 775 (787)
	+ S9 mix	Compound	2-AA	2-AA	2-AA	2-AA	2-AA
		µg/plate	1	2	10	0.5	2
		Revertants/plate	640 658 (649)	371 372 (372)	610 645 (628)	285 304 (295)	71 81 (76)

( ): average

HR-001 did not show any toxicity to any strain up to the highest dose of 5000 µg/plate with and without S9 Mix.

Mutation assays
Results are shown in tables hereafter.

Table B.6.4-3: Summary data – experiment 1

	Dose	**Revertants (n° colonies/plate)***				
		Base-change type			Frameshift type	
	(µg/plate)	TA100	TA1535	WP2uvr A	TA98	TA1537
- S9 mix	Solvent control (H_2O)	117	12	21	37	3
	156	119	11	12	40	3
	313	117	11	16	42	4
	625	139	9	15	39	2
	1250	125	9	22	43	5

		Dose	**Revertants (n° colonies/plate)***				
			Base-change type			Frameshift type	
		(µg/plate)	TA100	TA1535	WP2uvr A	TA98	TA1537
		2500	106	3	15	38	3
		5000	105	4	20	39	2
+ S9 mix		Solvent control (H_2O)	78	9	21	35	7
		156	83	6	19	36	9
		313	77	7	19	31	5
		625	99	6	19	30	8
		1250	93	6	22	37	6
		2500	73	7	16	39	7
		5000	56	3	16	25	4
Positive controls	- S9 mix	Compound	AF-2	NaN_3	AF-2	AF-2	9-AA
		µg/plate	0.01	0.5	0.01	0.1	80
		Revertants/plate	510	524	305	621	786
	+ S9 mix	Compound	2-AA	2-AA	2-AA	2-AA	2-AA
		µg/plate	1	2	10	0.5	2
		Revertants/plate	606	392	522	360	75

* values are the mean of three plate

Table B.6.4-4: Summary data – experiment 2

		Dose	**Revertants (n° colonies/plate)***				
			Base-change type			Frameshift type	
		(µg/plate)	TA100	TA1535	WP2uvr A	TA98	TA1537
- S9 mix		Solvent control (H_2O)	146	9	16	24	5
		156	137	10	18	18	7
		313	140	7	19	20	4
		625	136	8	17	18	3
		1250	136	7	15	15	3
		2500	144	6	18	10	3
		5000	117	10	14	9	4
+ S9 mix		Solvent control (H_2O)	123	8	17	37	7
		156	112	7	15	32	10
		313	125	7	13	29	9
		625	113	8	20	35	9
		1250	107	7	14	28	9
		2500	89	7	19	20	8
		5000	67	4	17	17	4
Positive controls	- S9 mix	Compound	AF-2	NaN_3	AF-2	AF-2	9-AA
		µg/plate	0.01	0.5	0.01	0.1	80
		Revertants/plate	595	527	252	742	909
	+ S9 mix	Compound	2-AA	2-AA	2-AA	2-AA	2-AA
		µg/plate	1	2	10	0.5	2
		Revertants/plate	768	322	605	327	87

* values are the mean of three plates

Conclusion by the Notifiers

A two-fold or greater increase in the mean number of revertant colonies was not observed in any strain at any dose of HR-001 in the reverse mutation tests with or without metabolic activation. It is concluded that HR-001 is non mutagenic for bacteria under the conditions used with this experiment.

RMS comments:
The study is considered acceptable. No evidence of genotoxicity was obtained. However, it must be clarified that, according to the study report, only the pre-incubation method was used whereas the plate-incorporation assay is not described and was apparently not performed. At least, the results given in Table B.6.4-3 *and* Table B.6.4-4 *were obviously obtained by means of the pre-incubation method.*
When the study description in the dossier was compared to the original study report, it was noted that the study director was Mie Akanuma. Erroneously, the first name had been mentioned in the dossier instead of the authors surname.

2nd new Ames test (Sokolowski, 2007)

Reference:	IIA, 5.4.1/02
Report:	Sokolowski, A. 2007 *Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with glyphosate technical (NUP-05068) RCC Ltd., Itingen, Switzerland Data owner: Nufarm RCC Study No.: 1061401 Date: 2007-03-16 Unpublished, ASB2012-11463
Guidelines:	The OECD Guidelines for Testing of Chemicals No. 471: "Bacterial Reverse Mutation Test", adopted July 21, 1997 referenced as Method B13/14 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC). Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Study Results, Reverse mutation studies. Guideline NO.2-1-19-1. >Notification 12NohSan No. 8147, as partly revised in 16-Shouan-9260, on March 16, 2005. English translation by ACIS on October 17, 2005.
Deviations:	None
GLP:	yes
Acceptability:	*See RMS comment*

Dates of experimental work: January 15, 2007 to January 25,2007

Materials and methods

Test material:	
Identification:	Glyphosate technical (NUP-05068)
Description:	Crystalline powder, White
Lot/Batch #:	200609062
Purity:	95.1 %
Stability of test compound:	Not specified
Vehicle/Controls	Vehicle = water
Negative/solvent control:	Concurrent untreated and solvent controls were performed.
Positive control:	without metabolic activation: Sodium azide, NaN3 with metabolic activation: 2-aminoanthracene, 2-AA

Activation:	Phenobarbital/J3-Naphthoflavone induced rat liver S9 is used as the metabolic activation system. The S9 is prepared from 8 - 12 weeks old male Wistar Hanlbm rats
Test organisms::	Histidine auxotrophic strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, TA100) to prototrophy.
Culture medium:	8 9 Merck Nutrient Broth (MERCK, 0-64293 Darmstadt) 5 9 NaCI (MERCK, 0-64293 Darmstadt)
Test concentrations::	0, 33, 100, 333, 667, 1000, or 5000 µg/plate with or without metabolic activation.g

Study conduct:
For each strain and dose level including the controls, three plates were used.
The following materials were mixed in a test tube and poured onto the selective agar plates:
100 µL Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
500 µL 89 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
100 µL Bacteria suspension (cf. test system, pre-culture of the strains),
2000 µL overlay agar
In the pre-incubation assay 100 µL test solution, 500 µL S9 mix and S9 mix substitution buffer and 100 µL bacterial suspension were mixed in a test tube and shaken at 37°C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45 °C) was added to each tube. The mixture was poured on selective agar plates. After solidification the plates were incubated upside down for at least 48 hours at 37 °C in the dark.

Results and discussion
The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation, with the exception of strain TA 1537, where a minor recuction in the number of revertants was observed at 5000 Iµg/plate without metabolic activation in experiment II. No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Glyphosate technical (NUP-05068) at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies. The laboratory's historical control range was slightly exceeded in the solvent control of strain WP2 uvrA with metabolic activation in experiment I. This minor deviation is judged to be based on biologically irrelevant fluctuations and has no impact on the outcome of the study. In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Conclusion by the Notifiers
Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate is not to be classified for this end point.

RMS comments:

The study is considered acceptable. No evidence of a mutagenic response was obtained. The lower number of revertants in one experiment with TA 1537 might point to a weak cytotoxic effect of the test substance to this strain at a high concentration. In the past, similar observations were occasionally made with glyphosate from different sources at high concentrations (see DAR, 1998, ASB2010-10302).

3d new Ames test (Sokolowski, 2007)

Reference:	IIA, 5.4.1/03
Report:	Sokolowski, A. 2007 *Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with glyphosate technical (NUP-05070) RCC Ltd., Itingen, Switzerland Data owner: Nufarm RCC Study No.: 1061402 Date: 2007-03-16 Unpublished, ASB2012-11464
Guidelines:	The OECD Guidelines for Testing of Chemicals No. 471: "Bacterial Reverse Mutation Test", adopted July 21, 1997 referenced as Method B13/14 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC). Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Study Results, Reverse mutation studies. Guideline NO.2-1-19-1. >Notification 12NohSan No. 8147, as partly revised in 16-Shouan-9260, on March 16, 2005. English translation by ACIS on October 17, 2005.
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: January 15, 2007 to January 25,2007

Materials and methods

Test material:	
Identification:	Glyphosate technical (NUP-05070)
Description:	Crystalline powder White
Lot/Batch #:	20060901
Purity:	97.7 %
Stability of test compound:	Not specified
Vehicle/Controls	Vehicle = water
Negative/solvent control:	Concurrent untreated and solvent controls were performed.
Positive control:	without metabolic activation: Sodium azide, NaN3 with metabolic activation: 2-aminoanthracene, 2-AA
Activation:	Phenobarbital/J3-Naphthoflavone induced rat liver S9 is used as the metabolic activation system. The S9 is prepared from 8 - 12 weeks old male Wistar Hanlbm rats

Test organisms::	Histidine auxotrophic strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, TA100) to prototrophy.
Culture medium:	8 9 Merck Nutrient Broth (MERCK, 0-64293 Darmstadt) 5 9 NaCI (MERCK, 0-64293 Darmstadt)
Test concentrations::	0, 33, 100, 333, 667, 1000, or 5000 µg / plate with or without metabolic activation.g

Study conduct:
See Sokolowski, 2007, (ASB2012-11463) above.

Results and discussion
The plates incubated with the test item showed reduced background growth at 333 - 5000 and 2500 - 5000 in strains TA 1537 and TA 100, respectively. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation. Minor toxic effects occurred at 5000 µg/plate in strain WP2 uvrA in the absence of metabolic activation in experiment I and in strain TA 98 with metabolic activation in experiment II. No substantial increase in revertant colony numbers of any of the five tester strains was bserved following treatment with glyphosate technical (NUP-05070) at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies. The laboratory's historical control range was not quite reached in the untreated control of strain TA 1535 with and without metabolic activation in experiment II. These minor deviations (10 versus 11 colonies and 9 versus 10 colonies, respectively) are judged to be based on biologically irrelevant fluctuations in the number of colonies and have no impact on the outcome of the study. In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Conclusion by the Notifier
Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate is not to be classified for this end point.

RMS comments:
The study is considered acceptable. No evidence of a mutagenic response was obtained. Cytotoxic effects, if occurring, were confined to high concentrations and certain bacterial strains.

4th new Ames test (Sokolowski, 2007)

Reference:	IIA, 5.4.1/04
Report:	Sokolowski, A. 2007 *Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with Glyphosate technical (NUP-05067) RCC Ltd., Itingen, Switzerland Data owner: Nufarm RCC Study No.: 1061403 Date: 2007-03-16

	unpublished, ASB2012-11465
Guidelines:	The OECD Guidelines for Testing of Chemicals No. 471: "Bacterial Reverse Mutation Test", adopted July 21, 1997 referenced as Method B13/14 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC). Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Study Results, Reverse mutation studies. Guideline NO.2-1-19-1. >Notification 12NohSan No. 8147, as partly revised in 16-Shouan-9260, on March 16, 2005. English translation by ACIS on October 17, 2005.
Deviations:	None
GLP:	yes
Acceptability:	See RMS comment

Dates of experimental work: January 16, 2007 to January 25,2007

Materials and methods

Test material:	
Identification:	Glyphosate technical (NUP-05067)
Description:	Crystalline powder White
Lot/Batch #:	0609-1
Purity:	95 %
Stability of test compound:	Not specified
Vehicle/Controls	Vehicle = water
Negative/solvent control:	Concurrent untreated and solvent controls were performed.
Positive control:	without metabolic activation: Sodium azide, NaN3 with metabolic activation: 2-aminoanthracene, 2-AA
Activation:	Phenobarbital/J3-Naphthoflavone induced rat liver S9 is used as the metabolic activation system. The S9 is prepared from 8 - 12 weeks old male Wistar Hanlbm rats
Test organisms::	Histidine auxotrophic strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, TA100) to prototrophy.
Culture medium:	8 9 Merck Nutrient Broth (MERCK, 0-64293 Darmstadt) 5 9 NaCI (MERCK, 0-64293 Darmstadt)
Test concentrations::	0, 33, 100, 333, 667, 1000, or 5000 µg / plate with or without metabolic activation.g

Study conduct:
See Sokolowski, 2007a (ASB2012-11463), above.

Results and discussion
The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation, with the exception of strain TA 1537, where a minor recuction in the number of revertants was observed at 5000 Iµg/plate without metabolic

activation in experiment II. No substantial increase in revertant colony numbers of any of the five tester strains was observed follOowing treatment with Glyphosate technical (NUP-05068) at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies. The laboratory's historical control range was slightly exceeded in the solvent control of strain WP2 uvrA with metabolic activation in experiment I. This minor deviation is judged to be based on biologically irrelevant fluctuations and has no impact on the outcome of the study. In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Conclusion by the Notifiers
Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate is not to be classified for this endpoint.

RMS comments:
The study is considered acceptable. No evidence of a mutagenic response was obtained. Previously known evidence of cytotoxicity was confirmed but findings were confined to high concentrations and certain bacterial strains.

5th new Ames test (Ribeiro do Val, 2007,)

Reference:	IIA, 5.4.1/05
Report:	Ribeiro do Val, R. 2007 Bacterial reverse mutation test (Ames Test) for Glifosato Téchnico Helm TECAM Technologia Ambiental Ltda., Brazil Data owner: HAG Report No.: 3393/2007-2.0AM-B Date: 2007-12-13 Unpublished, ASB2012-11466
Guidelines:	OECD 471
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: 30/11/2007 – 03/12/2007

Materials and methods

Test material:

Identification:	Glifosato Téchnico Helm
Description:	Solid
Lot/Batch #:	2007091801
Purity:	980.1 g/kg
Stability of test compound:	Stable (CIPAC MT 46, 54 °C, 14 days)

Vehicle and/
or positive control:

Negative control: vehicle (DMSO, 100 µL/plate)
Positive controls:

Assay	Strain	Compound
S9-	TA98	2-Nitrofluorene
S9-	TA100; TA1535	Sodium azide
S9-	TA1537	ICR 191 – Acridine
S9-	TA102	Mitomycin C
S9+	TA98; TA100; TA102; TA1535; TA1537	2-aminoanthracene

S9 = metabolic activation

Test organisms/cells:

Species: *S. typhimurium*

Strain: TA98; TA100; TA102; TA1535; TA1537

Source: Moltox Inc. (Annapolis, MD, USA)

Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Aroclor 1254)

Test concentrations:

Preliminary cytotoxicity test 8, 40, 200, 1000, 5000 µg/plate

Mutation assay: 648, 1080, 1800, 3000, 5000 µg/plate

Preliminary cytotoxicity assay: A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 8.0 and 5000.0 µg/plate.

Mutation study: The mutation study was performed with and without metabolic activation. Each sample was prepared by mixing 0.1 mL of test substance, 0.1 mL of a fresh bacterial suspension grown overnight, 0.5 mL S9 mix or substitution buffer (with / without metabolic activation) and 3.0 mL of top agar. Each suspension was incubated on selective agar plates for 72 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 648 and 5000 µg/plate. The protein concentration of the S9 fraction was 34.9 mg/mL.

Liability check: The acceptance criteria of the assay were as follows:
The presence of background lawn in the test plates.
Spontaneous revertant colonies of the negative control were in the range reported in the literature (MARON. D.M, & AMES. B.N. Revised methods for the *Salmonella* mutagenicity test, Mutation Research. 113: 173-215, 1983) and established in the laboratory by historical control values.
Positive controls showed mutagenic activity in all tested strains.

Results and discussion

Preliminary cytotoxicicity assay: None of the concentrations tested showed cytotoxic effects.
Mutation assays: No significant mutation rate was observed up to a concentration of 5000 µg of test item per plate.
Liability check: All acceptance criteria were met.

Conclusion by the Notifiers
The test material glyphosate technical was non-genotoxic.

RMS comments:
The study is considered ~~supplementary because~~ acceptable although only the plate-incorporation assay was performed but not the pre-incubation method and ~~Furthermore,~~ E. coli strains were not included. No evidence of mutagenicity was obtained. According to the study report, some cytotoxicity occurred that became obvious by a lower number of revertants when the strains TA1537 (with metabolic activiation) and TA102 (without) were treated at the highest concentration level of 5000 µg/plate.

6th new Ames test (Miyaji, 2008)

Reference:	IIA, 5.4.1/06
Report:	Miyaji, C. K. 2008 Evaluation of the mutagenic potential of the test substance glyphosate technical by reverse mutation assay in *Salmonella typhimurium* (Ames Test) Bioagri Laboratorios Ltda., Brazil Data owner: HAG Report No.: RF-3996.401.391.07 Date: 2008-09-15 Unpublished, ASB2012-11467
Guidelines:	OECD 471
Deviations:	None
GLP:	yes
Acceptability:	See RMS comment

Dates of experimental work: 05/06/2008 – 30/06/2008

Materials and methods

Test material:

Identification:	GLYPHOSATE TECHNICAL
Description:	Solid
Lot/Batch #:	20070606
Purity:	980.5 g/kg
Stability of test compound:	Stable to hydrolysis at pH 3, 6 and 9 (5-35 °C)
Vehicle and/ or positive control:	Vehicle: DMSO

Positive controls:

Assay	Strain	Compound
S9-	TA98	2-Nitrofluorene
S9-	TA100; TA1535	Sodium azide
S9-	TA97a	9-Aminoacridine
S9-	TA102	Cumene hydroperoxide
S9+	TA97a; TA98; TA100; TA102; TA1535	2-Aminoanthracene

S9 = metabolic activation

Test organisms/cells:

Species: *S. typhimurium*

Strain: TA97a; TA98; TA100; TA102; TA1535

Source: Moltox Toxicology, Inc.

Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Aroclor 1254)

Test concentrations:

Preliminary cytotoxicity test 0.001, 0.01, 0.1, 1.0, 2.5 mg/plate

Mutation assay: 0.001, 0.01, 0.1,0.5, 1.0 mg/plate

Preliminary cytotoxicity assay:
A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 0.001 and 2.5 µg/plate.

Mutation study:
The mutation study was performed with and without metabolic activation. Each sample was prepared by mixing the corresponding volume of test stock solution, of test substance, 0.1 mL of a fresh bacterial suspension grown overnight, 0.5 mL S9 mix or phosphate buffer (with / without metabolic activation) and 2.0 mL of top agar. Each suspension was incubated on selective agar plates for 72 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 0.001 and 1.0 µg/plate.

Liability check: The concentration of test item in the lowest and highest concentrated sample were determined by HPLC-UV

Results and discussion
Preliminary cytotoxicity assay: Only the highest concentration of 2.5 mg/plate showed cytotoxic effects. Therefore, the mutation assay was carried out with a maximum concentration of 1.0 mg/plate.
Mutation assays: No significant mutation rate was observed up to a concentration of 1.0 mg of test item per plate.
Liability check: The concentrations of the lowest and highest concentrations were confirmed.

Conclusion by the Notifier
The test material glyphosate technical was non-genotoxic.

RMS comments:
No evidence of mutagenicity was obtained in the plate-incorporation assay. However, since the highest concentration of 1000 µg/plate was much lower than in most other studies and since no apparent reason for not using higher dose levels was given, the study is considered not acceptable.

7th new Ames test (Flügge, 2009)

Reference: IIA, 5.4.1/07
Report: Flügge, C. 2009 Mutagenicity study of Glyphosate TC in the *Salmonella typhimurium* Reverse Mutation Assay (*in vitro*)
LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG, Hamburg, Germany
Data owner: HAG
Report No.: LPT 23916
Date: 2009-04-30
Unpublished, ASB2012-11468
Guidelines: OECD 471
Deviations: None
GLP: Yes
Acceptability: See RMS comment

Dates of experimental work: 04/02/2009 – 27/02/2009

Materials and methods

Test material:
Identification: Glyphosate TC
Description: Solid, white powder
Lot/Batch #: 20080801
Purity: 988.0 g/kg
Stability of test compound: Stable for two years at ambient temperature
Vehicle and/
or positive control: Negative control: Vehicle (*aqua ad iniectabilia*)
Positive controls:

Assay	Strain	Compound
S9-	TA98	2-Nitrofluorene
S9-	TA100; TA1535	Sodium azide
S9-	TA1537	9-Aminoacridine
S9-	TA102	Methyl methane sulfonate
S9+	TA98; TA102; TA1537	2-Aminoanthracene
S9+	TA100; TA1535	Cyclophosphamide

S9 = metabolic activation

Test organisms/cells:
Species: *S. typhimurium*

Strain:	TA98; TA100; TA102; TA1535, TA1537
Source:	Dr. Bruce N. Ames
Metabolic activation system:	
	S9 (microsomal fraction of rat liver induced with Aroclor 1254)
Test concentrations:	
Preliminary cytotoxicity test	0.316, 1.0, 3.16, 10.0, 31.6, 100.0, 316.0, 1000.0, 3160.0, 5000.0 µg/plate
Mutation assay:	31.6, 100.0, 316.0, 1000.0, 3160.0 µg/plate

Preliminary cytotoxicity assay:
A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 0.316 and 5000.0 µg/plate. Toxicity was defined as appearance of scarce background lawn and / or reduction of revertants by more than 50 %.

Mutation study: Each sample was prepared by mixing 0.1 mL of test item, 0.1 mL of a fresh bacterial suspension, 0.5 mL S9 mix or phosphate buffer (with / without metabolic activation) and 2.0 mL of top agar. Each suspension was incubated on selective agar plates for 48-72 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 31.6 and 3160.0 µg/plate. The protein concentration of the S9 fraction was 31.55 mg/mL.

The mutation study was performed as two independent experiments each with and without metabolic activation. The first experiment was a plate incorporation method where all components were mixed and directly plated. The second experiment was a preincubation method, where test strain, test item and S9 mix were incubated at 37 °C for 20 min prior to mixing with agar and plating as described above.

Liability check: As quality criteria the genotypes, i.e. histidine and biotin requirement (his^-) (bio^-), deep rough character (rfa^-), UV-sensitivity (uvr B^-), Ampicillin resistance (pKM 101) and Ampicillin / Tetracycline resistance (pAQ1) (only strain TA102) of the test strains were regularly confirmed.

Results and discussion
Preliminary cytotoxicity assay: Cytotoxicity was noted at concentrations of 3160.0 and 5000.0 µg/plate. Therefore, the mutation assay was carried out with a maximum concentration of 3160.0 µg/plate.
Mutation assays: No mutagenic effect was observed for glyphosate TC up to the cytotoxic concentration of 3160.0 µg/plate in the two independent experiments with and without metabolic activation.
Liability check: The genotypes of the 5 strains used were confirmed regularly.

Conclusion by the Notifier
The test material glyphosate technical was non-genotoxic.

RMS comments:
The study is considered acceptable. The highest concentration in the mutagenicity assays was choosen because there was evidence of cytotoxicity at this and above dose levels demonstrated at least for the strain TA100. This approach is reasonable and dose selection is supported.

8th new Ames test (Flügge, 2010)

Reference: IIA, 5.4.1/08
Report: Flügge, C. 2010 Mutagenicity study of glyphosate TC in the *Salmonella typhimurium* Reverse Mutation Assay (*in vitro*)
LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG, Hamburg, Germany
Data owner: HAG
Report No.: LPT 24880
Date: 2010-01-25
Unpublished, ASB2012-11469
Guidelines: OECD 471
Deviations: None
GLP: Yes
Acceptability: *See RMS comment*

Dates of experimental work: 15/10/2009 – 23/11/2009

Materials and methods

Test material:
Identification: Glyphosate TC
Description: Solid, white powder
Lot/Batch #: 20080801
Purity: 964.0 g/kg
Stability of test compound: Stable for two years at ambient temperature
Vehicle and/
or positive control: Negative control: Vehicle (*aqua ad iniectabilia*)
Positive controls:

Assay	Strain	Compound
S9-	TA98	2-Nitrofluorene
S9-	TA100; TA1535	Sodium azide
S9-	TA1537	9-Aminoacridine
S9-	TA102	Methyl methane sulfonate
S9+	TA98; TA102; TA1537	2-Aminoanthracene
S9+	TA100; TA1535	Cyclophosphamide

S9 = metabolic activation

Test organisms/cells:	
Species:	*S. typhimurium*
Strain:	TA98; TA100; TA102; TA1535, TA1537
Source:	Dr. Bruce N. Ames
Metabolic activation system:	
	S9 (microsomal fraction of rat liver induced with Aroclor 1254)
Test concentrations:	
Preliminary cytotoxicity test	0.316, 1.0, 3.16, 10.0, 31.6, 100.0, 316.0, 1000.0, 3160.0, 5000.0 µg/plate
Mutation assay:	31.6, 100.0, 316.0, 1000.0, 3160.0 µg/plate

Preliminary cytotoxicity assay:
A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 0.316 and 5000.0 µg/plate. Toxicity was defined as appearance of scarce background lawn and / or reduction of revertants by more than 50 %.

Mutation study:
Each sample was prepared by mixing 0.1 mL of test item, 0.1 mL of a fresh bacterial suspension, 0.5 mL S9 mix or phosphate buffer (with / without metabolic activation) and 2.0 mL of top agar. Each suspension was incubated on selective agar plates for 48-72 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 31.6 and 3160.0 µg/plate. The protein concentration of the S9 fraction was 26.6 mg/mL.

The mutation study was performed as two independent experiments each with and without metabolic activation. The first experiment was a plate incorporation method where all components were mixed and directly plated. The second experiment was a preincubation method, where test strain, test item and S9 mix were incubated at 37°C for 20 min prior to mixing with agar and plating as described above.

Liability check:
As quality criteria the genotypes, i.e. histidine and biotin requirement (his^-) (bio^-), deep rough character (rfa^-), UV-sensitivity (uvr B^-), Ampicillin resistance (pKM 101) and Ampicillin / Tetracycline resistance (pAQ1) (only strain TA102) of the test strains were regularly confirmed.

Results and discussion
Preliminary cytotoxicity assay: Cytotoxicity was noted at concentrations of 3160.0 and 5000.0 µg/plate. Therefore, the mutation assay was carried out with a maximum concentration of 3160.0 µg/plate.
Mutation assays: No mutagenic effect was observed for glyphosate TC up to the cytotoxic concentration of 3160.0 µg/plate in the two independent experiments with and without metabolic activation.
Liability check: The genotypes of the 5 strains used were confirmed regularly.

Conclusion by the Notifier
The test material glyphosate technical was non-genotoxic.

RMS comments:
The study is considered acceptable. The test substance proved non-gentotoxic. The choice of the highest concentration is sufficiently explained. In addition, some precipitation was observed in the pre-test with TA100 at 5000 µg/plate.

9th new Ames test (Sokolowski, 2010)

Reference:	IIA, 5.4.1/09
Report:	Sokolowski, A. 2010 *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with Solution of Glyphosate TC spiked with Glyphosine Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Data owner: HAG Report No.: 1332300 Date: 2010-04-07 Unpublished, ASB2012-11470
Guidelines:	OECD 471
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: 17/03/2010 – 22/03/2010

Materials and methods

Test material:	
Identification:	Glyphosate TC (5000 mg/L) spiked with glyphosine (32 mg/L)
Description:	An aqueous solution of glyphosate technical grade active ingredient (purity 97.16 % w/w), containing 0.63 % (w/w) glyphosine in the technical grade active ingredient.
Lot/Batch #:	2009051501 (Glyphosate TC)
Purity:	971.6 g/kg
Stability of test compound:	Stable for two years at ambient temperature
Vehicle and/ or positive control:	Negative control: Vehicle (deionised water)

Positive controls:

Assay	Strain	Compound
S9-	TA98, TA1537	4-nitro-o-phenylene-diamine
S9-	TA100; TA1535	Sodium azide
S9-	WP2 uvrA	Methyl methane sulfonate
S9+	TA98; TA100; TA1535; TA1537, WP2 uvrA	2-Aminoanthracene

S9 = metabolic activation

Test organisms/cells:

Species: *S. typhimurium*

Strain: TA98; TA100; TA1535, TA1537

Species: *Escherichia coli*

Strain: WP2 uvrA

Source: Trinova Biochem GmbH (35394 Gießen, Germany)

Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Phenobarbital/β-Naphthoflavone)

Test concentrations:

Preliminary cytotoxicity test: 3; 10; 33; 100; 333; 1000; 2500; 5000 µg/plate

Mutation assay: 3; 10; 33; 100; 333; 1000; 2500; 5000 µg/plate

Preliminary cytotoxicity assay:
A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 0.316 and 5000.0 µg/plate. Toxicity was defined as appearance of scarce background lawn and/or reduction of revertants.

Mutation study:
Each sample was prepared by mixing 1.0 mL of test item, 0.1 mL of a fresh bacterial suspension, 0.5 mL S9 mix or S9 substitution buffer (with / without metabolic activation) and 1.0 mL of top agar. Each suspension was incubated on selective agar plates for at least 48 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 3.0 and 5000.0 µg/plate. The protein concentration of the S9 fraction was 34.3 mg/mL.

The mutation study was performed as two independent experiments each with and without metabolic activation. The first experiment was a plate incorporation method where all components were mixed and directly plated. The second experiment was a preincubation method, where test strain, test item and S9 mix were incubated at 37 °C for 60 min prior to mixing with agar and plating as described above.

Liability check:
The acceptance criteria of the assay were as follows:
Regular background growth in the negative and solvent control.
Spontaneous revertant colonies of the negative control were in the range of historical data.
Positive controls showed mutagenic activity in all tested strains.

Results and discussion
Preliminary cytotoxicity assay: No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation.
Mutation assays: No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Solution of glyphosate TC spiked with Glyphosine at any dose level, neither in the presence nor absence of metabolic activation (S9 mix).
Liability check:The acceptance criteria were met.

Conclusion by the Notifiers
The test material glyphosate technical was non-genotoxic.

RMS comments:
The study is considered acceptable. No evidence of mutagenicity was obtained. The reason for glyphosine spiking of the test material is not clear but it is assumed that this substance (similar to glyphosate) may occur as an impurity in the technical active ingredient. Thus, this test might become particularly important if a certain specification needs to be assessed from a toxicological point of view.

10th new Ames test (Wallner, 2010)

Reference:	IIA, 5.4.1/10
Report:	Wallner, B. 2010 Reverse Mutation Assay using bacteria (*Salmonella typhimurium*) with Glyphosate TC BSL Bioservice Scientific Laboratories GmbH, Planegg, Germany Data owner: Helm AG Report No.: BSL 101268 Date: 2010-04-08 Unpublished, ASB2012-11471
Guidelines:	OECD 471
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: 25/03/2010 – 06/04/2010

Materials and methods

Test material:

Identification:	Glyphosate TC
Description:	Solid.

Lot/Batch #: 200903051

Purity: 982.0 g/kg

Stability of test compound: Stable for two years at ambient temperature

Vehicle and/
or positive control: Negative control: Solvent controls, consisting of solvent or vehicle alone.

Positive controls:

Assay	Strain	Compound
S9-	TA98, TA1537	4-nitro-o-phenylene-diamine
S9-	TA100; TA1535	Sodium azide
S9-	TA102	Methyl methane sulfonate
S9+	TA98; TA100; TA102; TA1535; TA1537	2-Aminoanthracene

S9 = metabolic activation

Test organisms/cells:

Species: *S. typhimurium*

Strain: TA98; TA100; TA102; TA1535, TA1537

Source: MOLTOX, INC, NC 28607, USA

Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Phenobarbital/β-Naphthoflavone)

Test concentrations:

Preliminary cytotoxicity test 31.6, 100, 316, 1000, 2500 and 5000 µg/plate

Mutation assay: 31.6, 100, 316, 1000, 2500 and 5000 µg/plate

Preliminary cytotoxicity assay:
No preliminary cytotoxicity assay was performed.

Mutation study:
Each sample was prepared by mixing 0.1 mL of test item, 0.1 mL of a fresh bacterial suspension, 0.5 mL S9 mix or S9 substitution buffer (with / without metabolic activation) and 2.0 mL of top agar. Each suspension was incubated on selective agar plates for at least 48 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 3.0 and 5000.0 µg/plate. The protein concentration of the S9 fraction was 33.0 mg/mL.

The mutation study was performed as two independent experiments each with and without metabolic activation. The first experiment was a plate incorporation method where all components were mixed and directly plated. The second experiment was a preincubation method, where test strain, test item and S9 mix were incubated at 37 °C for 60 min prior to mixing with agar and plating as described above.

Liability check:
The acceptance criteria of the assay were as follows:
The bacteria demonstrate their typical responses to ampicillin (TA 98, TA 100, TA 102).
Regular background growth in the negative and solvent control.
Spontaneous revertant colonies of the negative control were in the range of historical data.
Positive controls showed mutagenic activity in all tested strains.

Results and discussion
Preliminary cytotoxicity assay: No preliminary cytotoxicity assay was performed.
Mutation assays: In the plate incorporation test toxic effects of the test item were observed in tester strain TA 100 at a dose of 5000 µg/plate (with and without metabolic activation). In tester strain TA 1535 toxic effects of the test item were noted at doses of 2500 µg/plate and higher (with and without metabolic activation). In the preincubation test toxic effects of the test item were noted in tester strains TA 100 and TA 1535 at a dose of 5000 µg/plate (without metabolic activation).
No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with Glyphosate TC at any concentration level with or without metabolic activation.
Liability check:The acceptance criteria were met.

Conclusion by the Notifiers
The test material glyphosate technical was non-genotoxic.

RMS comments:
The study is considered acceptable. Glyphosate proved non-genotoxic. Cytotoxicity was confined to very high concentrations.

11th new Ames test (Thompson, 1996)

Reference:	IIA, 5.4.1/11
Report:	Thompson, P.W. 1996 Technical glyphosate: Reverse mutation assay "Ames test" using *Salmonella typhimurium* and *Escherichia coli* Safepharm Laboratories, Derby, UK Data owner: Nufarm SPL Project No.: 434/014 Date: 1996-02-20 unpublished, ASB2012-11472
Guidelines:	OECD 471 (1983) Commission Directive (EC) 92/69/EEC (1992), Method B14 US EPA (TSCA) guidelines
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: August 19, 1995 to November 13, 1995

Materials and methods

Test material:	
Identification:	Technical Glyphosate
Description:	White powder
Lot/Batch #:	H95D 161 A
Purity:	95.3 %
Stability of test compound:	No data given in the report.
Vehicle/Controls	Vehicle = sterile distilled water
Negative/solvent control:	Vehicle/solvent controls were performed. Without metabolic activation: N-ethyl-N`-nitro-N-nitrosoguanidine (ENNG): - 2 µg/plate for WP2uvrA; - 3 µg/plate for TA100; - 5 µg/plate for TA1535. 9-Aminoacridine (9AA) 80 µg/plate for TA1537. 4-Nitroquinoline-1-oxide (4NQO) 0.2 µg/plate for TA98.
Positive control:	With metabolic activation: 2-Aminoanthracene (2AA) was used in S9 series of plates in the concentrations: - 1 µg/plate for TA100; - 2 µg/plate for TA1535 and TA1537; - 10 µg/plate for WP2uvrA; - 0.5 µg/plate for TA98.
Activation:	S9 was prepared from the livers of male Sprague-Dawley rats. Each received a single i.p. injection of Aroclor 1254 at 500 mg/kg, 5 days before S9 preparation.
Test organisms:	*Salmonella typhimurium* TA1535, TA 1537, TA98, and TA100 *Escherichia coli* WP2uvrA
Culture medium:	Top agar was prepared using 0.6 % Dicfo Bacto agar and 0.5 % sodium chloride with 5 mL of 1.0 mM histidine/1.0 mM biotin and 1.0 mM tryptophan solution added to each 100 mL of top agar. Base agar plates were prepared using 1.2 % Oxoid Agar Technical No 3 with Vogel-Bonner Medium E and 20 mg/mL D-glucose.
Test concentrations:	0, 50, 150, 500, 1500, and 5000 µg/plate with and withput metabolic activation. In triplicate for each bacterial strain and for each concentration of test material with an without S9-mix.

Study conduct:
For each strain and dose level including the controls, three plates were used with and without S9-mix.
The following materials were mixed in a test tube and poured onto the selective agar plates:
0.1 mL of bacterial suspension;
0.1 mL of test solution at each dose level, vehicle/solvent (negative control) or reference mutagen solution (positive control);
2 ml of molten, trace histidine/tryptophan supplemented media;
0.5 ml of buffer (for test without metabolic activation) or S9-mix (for test with metabolic activation).
Known aliquots (0.1 mL) of one of the bacterial suspensions were dispensed into sets of sterile test tubes followed by 2.0 ml of molten trace histidine/tryptophan supplemented top agar at 45 °C, 0.1 mL of the appropriately diluted test material or vehicle control or reference mutagen solution (with and without metabolic activation) and either 0.5 mL of the S9 liver microsome mix or 0.5 ml of pH 7.4 buffer. The contents of each test tube were mixed and equally distributed onto the surface of Vogel-Bonner agar plates (one tube per plate). Plates were incubated for approximately 48 hours at 37 °C.

Results and discussion
The plates incubated with the test item caused no visible reduction in the growth of the bacterial lawn at any dose level up to maximim recommended dose of 5000 µg/plate either with or without metabolic activation, however a decrease in the frequency of revertant colonies was observed with some bacterial strains.
No significant increase in the frequency of revertant colonies was recorded for any of the bacterial strains with any dose of the test material, either with or without metabolic activation.
Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies.

Conclusion by the Notifiers
In conclusion, the test material was found to be non-mutagenic under the conditions of this test.

RMS comments:
There was no evidence of mutagenicity obtained although there was some cytotoxicity. The study is considered ~~supplementary since~~ acceptable although only the plate incorporation method was applied for testing.

12th new Ames test (Callander, 1996)

Reference:	IIA, 5.4.1/12
Report:	Callander, R.D. 1996 Glyphosate Acid: An Evaluation of Mutagenic Potential Using *S. Typhimurium* and *E. Coli*. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK Data owner: Syngenta Report No.: CTL/P/4874 Date: 1996-02-16 not published; ASB2012-11473
Guidelines:	OECD 471 (1997): OPPTS 870.5100 (1998): 2000/32/EEC

B.13/B.14 (2000)

Deviations: None
GLP: Yes
Acceptability: *See RMS comment*

Dates of experimental work: 1995-10-23 to 1996-02-16

Materials and methods

Test Material:	Glyphosate acid
Description:	Technical; white solid
Lot/Batch number:	P24
Purity:	95.6 % a.i
CAS#:	Not reported
Stability of test compound:	Confirmed by Sponsor

Control Materials:	
Negative:	Dimethylsulphoxide – DMSO
Solvent control (final concentration):	Dimethylsulphoxide – DMSO (10 µL/plate)
Positive control:	Nonactivation: Acridine mutagen ICR191 TA1537 2-Aminoanthracene TA1537, WP2 uA Daunomycin hydrochloride TA98 N-Eethyl-N´-nitro-N-nitrosoguanidine WP2P *uvrA* Mitomycin C WP2P Sodium Azide TA1535 and TA100 Activation: 2-Aminoanthracene TA1535, TA1537, TA98, TA100, WP2 uvrA and WP2P

Mammalian metabolic system: S9 derived

X	Induced		Aroclor 1254	X	Rat	X	Liver
	Non-induced	X	Phenobarbitol		Mouse		Lung
			None		Hamster		Other
		X	Other β-naphthoflavone		Other		

The metabolic activation system (S9-mix) used in this study was prepared as a 3:7:20 mixture of S9 fraction, Sucrose-tris-EDTA buffer (250:50:1 mM) and cofactor solution.
The cofactor solution was prepared as a single stock solution Na_2HPO_4 (150 mM), KCl (49.5 mM), glucose-6-phosphate (7.5 mM), NADP (Na salt) (6 mM) and $MgCl_2$ (12 mM), in sterile deionised water and adjusted to a final pH of 7.4.

Test organisms:

***S. typhimurium* strains**									
	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537		TA1538		list any others		
***E. coli* strains**									
X	WP2P (pKM101)	X	WP2P uvrA (pKM101)						
Properly maintained?							X	Yes		No
Checked for appropriate genetic markers (rfa mutation, R factor)?							X	Yes		No

Test compound concentrations used:
Nonactivated conditions: 5000, 2500, 1000, 500, 200 and 100 µg/plate
Activated conditions: 5000, 2500, 1000, 500, 200 and 100 µg/plate
For all strains triplicate plates were used for all test substance and positive control treatments. For solvent controls 5 plates were used.

Test performance
Preliminary Cytotoxicity Assay: Not performed.
Type of Bacterial assay:
X standard plate test (both experiments –S9, initial experiment +S9)
__ pre-incubation (60 minutes) (second experiment +S9)
__ "Prival" modification (i.e. azo-reduction method)
__ spot test
__ other

Protocol:
Bacterial cultures were prepared from frozen stocks by incubating for 10-12 hours at 37 °C.
The following materials were mixed in a test tube and poured onto the selective agar plates:
100 µL Test solution at each dose level, solvent and positive controls;
500 µL S9 mix or phosphate buffer;
100 µL Bacteria suspension;
2 mL Overlay agar containing 50 µM histidine or tryptophan as appropriate.

In this assay 100 µL aliquots of an overnight culture of each bacteria strain were stored in bijou bottles at room temperature until required (1-2 hours). 500 µL S9 mix (or buffer) was then added by dispensing syringe to the number of bijou bottles of one strain required for one dose level, followed by 0.1 mL of the appropriate concentration of the test substance solution added by micropipette. Finally, 2.0 mL top agar was added to each bijou, the force of addition was sufficient to mix the contents. The mixture was then rapidly poured onto a prepared Vogel Banner agar plate. After the agar was set the plates were incubated upside down for 64 - 68 hours at 37 °C in the dark. For each strain and dose level including the controls, three plates were used.

Following the total incubation period the plates were examined for the lack of microbial contamination and evidence that the test was valid: i.e. there should be a background lawn on the negative control plates and on the plates for (at least) the lower doses of test substance, and that the positive controlshad responded as expected.

The plates were counted using an automated colony counter (AMS 40-10) with the discrimination adjusted appropriately to permit the optimal counting of mutant colonies.

Statistical analysis: None – see Evaluation Criteria below.

Evaluation criteria: A positive response in a (valid) individual experiment is achieved when one or both of the following criteria are met:
a significant, dose-related increase in the mean number of revertants is observed;
a two-fold or greater increase in the mean number of revertant colonies (over that observed for the concurrent solvent control plates) is observed at one or more concentrations
A negative result in a (valid) individual experiment is achieved when:
there is no significant dose-related increase in the mean number of revertant colonies per plate observed for the test substance; and
in the absence of any such dose response, no increase in colony numbers is observed (at any test concentration) which exceeds 2x the concurrent solvent control.
For a positive response in an individual experiment to be considered indicative of an unequivocal positive, i.e. mutagenic, result for that strain/S9 combination, then the observed effect(s) must be consistently reproducible.

Results and discussion
Mutagenicity assay: In two separate experiments, glyphosate acid did not induce any significant increases in the observed numbers of revertant colonies in the four *Salmonella typhimurium* strains (TA1535,TA1538, TA98, TA100) and the two *Escherichia coli* strains (WP2P and WP2 *uvrA*) in either the presence or absence of an auxiliary metabolising system (S9).
The positive controls for each experiment induced the expected responses indicating the strains were working satisfactorily in each case.

Conclusion by the Notifiers
Under the conditions of this assay, glyphosate acid gave a negative, ie non-mutagenic, response in *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 and *E. coli* strains WP2P and WP2 uvrA in both the presence and absence of S9-mix, when tested to a limit dose of 5000 µg/plate.

RMS comments:
The study is considered acceptable. No evidence of mutagenicity was revealed. It should be clarified that the first experiment was performed by means of the plate incorporation method with and without metabolic activation. In the second trial, the same method was used in the absence of S9 mix. A pre-incubation assay was run with S9 mix.

13th new Ames test (Sokolowski, 2009)

Reference:	IIA, 5.4.1/13
Report:	Sokolowski 2009 Salmonella Typhimurium and Escherichia Coli Reverse Mutation Assay Harlan Cytotest Cell Research GmbH, In den Leppsteinswiesen 19, 64380 Rossdorf Germany Data owner: Syngenta Report No.: 1264500

	Date: 2009-12-18 not published; ASB2012-11474
Guidelines:	OECD 471 (1997): OPPTS 870.5100 (1998): 2008/440/EC B.13/B.14 (2008)
Deviations:	None
GLP:	Yes
Acceptability:	See RMS comment

Dates of experimental work: 2009-09-15 to 2009-12-18

This study was performed to investigate the potential of Glyphosate technical (produced via the Nantong Jiangshan (glycine-route)) to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100, and the *Escherichia coli* strains WP2 uvrA pKM 101 and WP2 pKM 101 over the range 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate (Experiment I), and 33; 100; 333; 1000; 2500; and 5000 µg/plate (Experiment II).

Materials and methods

Test Material:	Glyphosate
Description:	white solid
Lot/Batch number:	569753
Purity	96.3 % of glyphosate acid
Stability of test compound:	Not indicated by the sponsor
Control Materials:	
Negative:	Concurrent untreated and solvent controls were performed
Solvent control (final concentration):	100 µL/plate
Positive control:	Nonactivation: Sodium azide 10 µg/plate TA100, TA1535 4-nitro-o-phenylene-diamine, 50 µg/plate TA 1537, 10 µg/plate TA98 methyl methane sulfonate 3 µL/plate WP2 (pKM101), WP2 *uvrA* (pKM101) Activation: 2-Aminoanthracene 2.5 µg/plate TA 1535, TA 1537, TA100, TA98 10 µg/plate WP2 (pKM101), WP2 *uvrA* (pKM101)

Mammalian metabolic system: S9 derived

X	Induced		Aroclor 1254	X	Rat	X	Liver
	Non-induced	X	Phenobarbitol		Mouse		Lung
			None		Hamster		Other
		X	Other β-naphthoflavone		Other		

Before the experiment an appropriate quantity of S9 supernatant was thawed and mixed with S9 co-factor solution. The amount of S9 supernatant was 10 % v/v in the S9 mix. Cofactors are added to the S9 mix to reach the following concentrations in the S9 mix:

8 mM $MgCl_2$
33 mM KCl
5 mM Glucose-6-phosphate
5 mM NADP

in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.
During the experiment the S9 mix was stored in an ice bath.

Test organisms:

***S. typhimurium* strains**									
	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537		TA1538		list any others		
***E. coli* strains**									
X	WP2 (pKM101)	X	WP2 uvrA (pKM101)						
Properly maintained?						X	Yes		No
Checked for appropriate genetic markers (rfa mutation, R factor)?						X	Yes		No

Test compound concentrations used
The test item was tested at the following concentrations:
Pre-Experiment/Experiment I: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
Experiment II 33; 100; 333; 1000; 2500; and 5000 µg/plate

Test performance
Preliminary Cytotoxicity Assay
Not performed.

Type of Bacterial assay
X standard plate test (pre-experiment/experiment I; –S9, +S9)
X pre-incubation (60 minutes) (second experiment ; –S9, +S9)
__ "Prival" modification (i.e. azo-reduction method)
__ spot test
__ other

Protocol:
For each strain and dose level including the controls, three plates were used.
The following materials were mixed in a test tube and poured onto the selective agar plates:
100 µL Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
500 µL S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
100 µL Bacteria suspension (cf. test system, pre-culture of the strains),
2000 µL Overlay agar

In the pre-incubation assay 100 µL test solution, 500 µL S9 mix / S9 mix substitution buffer and 100 µL bacterial suspensions were mixed in a test tube and shaken at 37° C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45 °C) was added to each tube. The mixture was poured on selective agar plates.

After solidification the plates were incubated upside down for at least 48 hours at 37° C in the dark.

* Substitution buffer: 8.5 parts of the 100 mM sodium-ortho-phosphate-buffer pH 7.4 with 1.5 parts of KCl solution 0.15 M

Statistical analysis:
None - see Evaluation Criteria below.

Evaluation criteria:
A test item is considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice the colony count of the corresponding solvent control is observed.

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration.

An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A dose dependent increase in the number of revertant colonies below the threshold is regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remain within the historical range of negative and solvent controls such an increase is not considered biologically relevant.

Results and discussion
Preliminary Cytotoxicity Assay : Not performed.
Mutation assays: Glyphosate technical was assessed for its potential to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100, and the *Escherichia coli* strains WP2 uvrA pKM 101 and WP2 pKM 101.
The assay was performed with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test item was tested at the following concentrations:

Pre-Experiment /Experiment I:	3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
Experiment II:	33; 100; 333; 1000; 2500; and 5000 µg/plate

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments.

No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation.
No precipitation of the test item occurred up to the highest investigated dose.

No substantial increase in revertant colony numbers of any of the six tester strains was observed following treatment with glyphosate technical at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.
Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies.

Conclusion by the Notifiers
In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, glyphosate technical did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

RMS comments:
The study is considered acceptable. It could be shown that also technical glyphosate was not mutagenic when manufactured via the Nantong Jiangshan (glycine) route.

Two more tests were provided in 2014 when the revision of the original (2013) RAR was made. Despite this very late submission, they were reviewed by the RMS but described here only in brief.

Schreib (2012, ASB2014-9133) reported an Ames test that was performed by BSL Bioservice Scientific Laboratories (BSL Study no. 126159) in Planegg (Germany) on behalf of Industria Afrasa S.A. (Paterna, Valencia, Spain). The GLP-compliant study was carried out by means of both the pre-incubation and the plate-incorporation methods on *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102 with and without metabolic activation (S9 mix). Glyphosate (Batch no 20110107-2, purity 97 %) concentrations ranged from 10 to 5000 µg/plate. The solvent was DMSO. No precipitation was observed but cytotoxic effects occurred at dose levels of 2500 µg/plate and above, depending on activation and tester strains. No evidence of genotoxicity of the test substance was obtained. The positive control mutagens gave the expected increases. The study is considered acceptable by the RMS. Glyphosate proved negative under the conditions of this assay.

On behalf of Albaugh Europe Sàrl (Lausanne, Switzerland), Thompson (2014, ASB2014-9148) performed an Ames test with glyphosate (Batch no. 04062014, purity 85.79 %) on *S. typhimurium* strains TA98, TA100, TA1535, and TA1537, and *E.coli* strain WP2*uvrA* with and without metabolic activation (S9 mix). The GLP-compliant study (Harlan study no. 41401854) was run in the Harlan Laboratories in Shardlow (Derbyshire, U.K.). The test item was dissolved in DMSO and was applied at concentrations ranging from 1.5 µg/plate (plate incorporation) or 5 µg/plate (pre-incubation method) up to 5000 µg/plate. No precipitation was seen but the maximum concentration caused some toxicity to the bacteria. No evidence of genotoxicity was obtained whereas the positive control substances induced marked increases in the frequencies of revertant colonies. The study is considered acceptable by the RMS. Glyphosate proved negative under the conditions of this assay.

B.6.4.2 *In vitro* genotoxicity testing – Tests for gene mutation in mammalian cells

In contrast to the numerous Ames tests in bacteria, only few gene mutation assays with glyphosate have been performed in mammalian cells. An overview on the available information is given in Table B.6.4-5

Table B.6.4-5: Summary of *in vitro* genotoxicty tests with glyphosate acid in mammalian cells

Reference, study identification, owner		Type of study	Test organism / test system	Dose levels, batch no., purity, metabolic activation	Results
In vitro tests for gene mutation in mammalian cells					
Studies from the 2001 evaluation	Jensen, 1991; TOX9552372; Cheminova	Mouse lymphoma test	Mouse lymphoma cells (L5178Y)	- S9: 0.61 – 5.0 mg/mL, + S9: 0.52 – 4.2 mg/mL; 206-JaK-25-1; 98.6%	Negative
	Li, 1983*; TOX9552369; Monsanto	HGPRT assay	Chinese hamster ovary (CHO) cells	- S9: 2 – 22.5 mg/mL + S9: 5 – 22.5 (25 ??) mg/mL; Lot XHJ-64; 98.7%	Negative
New study	Clay, 1996, TOX2000-1994; Syngenta	Mouse lymphoma test	Mouse lymphoma cells (L5178Y $TK^{+/-}$)	+/- S9: 296 – 1000 µg/mL; P24; 95.6%	Negative

* Results were also published by Li & Long, 1988 (TOX9500253).

The previously known studies by Jensen (1991, TOX9552372) is reported in detail in the old DAR (1998, ASB2010-10302) and was confirmed to be acceptable upon re-evaluation. In this assay, there was neither evidence of gene mutation (TK locus) nor of chromosome aberrations. A similar study (Clay, 1996, TOX2000-1994) confirming the absence of gene mutation anc chromosome aberration in mouse lymphoma cells *in vitro* is decribed in the following in detail because it had not been submitted for previous EU evaluation. A negative HGPRT test (Li, 1983, TOX9552369) metioned in the old DAR is also considered still acceptable although it is not entirely clear from the original study report which dose level was actually the highest under activation conditions.

<u>Mouse lymphoma assay (Clay, 1996)</u>

Reference: IIA, 5.4.3/01

Report: Clay, P. 1998 Glyphosate Acid: L5178Y $TK^{+/-}$ Mouse Lymphoma Gene Mutation Assay
Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK
Data owner: Syngenta
Report No.: CTL/P/4991
Date: 1996-05-24
not published, TOX2000-1994

Guidelines: OECD 476 (1997): OPPTS 870.5300 (1998): 2000/32/EEC B.17 (2000)

Deviations: The stability, homogeneity and achieved concentration of the test or control substances in the vehicle used were not determined by

analysis and the certified purity and stability of the control substances are not available.
These deviations from the current regulatory guideline are considered not to compromise the scientific validity of the study..

GLP: Yes
Acceptability: *See RMS comment*

Dates of experimental work: 1996-01-29 to 1996-05-24

Materials and methods

Test Material: Glyphosate acid
Description: Technical, white solid
Lot/Batch number: P24
Purity: 95.6 % w/w a.i
CAS#: Not reported
Stability of test compound: Confirmed by the Sponsor

Control Materials:
Negative: DMSO
Solvent control (final concentration): 1 %
Positive control: Absence of S9 mix: Ethylmethanesulphonate (EMS), 750 µg/mL
Presence of S9 mix N-nitrosodimethylamine (NDMA), 600 µg/mL

Mammalian metabolic system: S9 derived

X	Induced		Aroclor 1254	X	Rat	X	Liver
	Non-induced	X	Phenobarbitol		Mouse		Lung
			None		Hamster		Other
		X	Other β-naphthoflavone		Other		
X indicates those that apply							

The co-factor solution was prepared as a stock solution of 75 mM NADP (disodium salt) and 1200 mM glucose-6-phosphate (monosodium salt) in RPMI 1640 culture medium with a final pH adjusted to 7.5. S9 fraction was added at 5 % (1 mL S9 added to the 20 mL cell culture) and co-factors at 1 % (200 µL to the 20 mL cell culture).

Test cells: Mammalian cells in culture

X	Mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)		
	Chinese hamster ovary (CHO) cells		List any others		
Media: RPMI 1640					
Properly maintained?		X	Yes		No
Periodically checked for Mycoplasma contamination?		X	Yes		No
Periodically checked for karyotype stability?			Yes		No
Periodically “cleansed” against high spontaneous background?			Yes		No
X indicates those that apply					

Locus Examined:		**Thymidine kinase (TK)**		**Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)**		**Na+/K+ ATPase**
Selection agent:		Bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		ouabain
		Fluorodeoxyuridine (FdU)		6-thioguanine (6-TG)		
	X	Trifluorothymidine (TFT)				
X indicates those that apply						

Test compound concentrations used:	
Absence of S9 mix	296, 444, 667, 1000 µg/mL
Presence of S9 mix	296, 444, 667, 1000 µg/mL

Cells were exposed to test compound, negative/solvent or positive controls for 4 hours in both the presence and absence of S9 mix.

After washing, cells were cultured for 2 days (expression period) before cell selection.

After expression, 10^4 cells/mL were dispensed, at 200 µL/well, into 96 well plates. The cells were cultured for 10-13 days in selection medium to determine numbers of mutants. Dilutions of the cultures to approximately 8 cells/mL were cultured for 10-13 days without selective agent to determine cloning efficiency.

Cell growth in individual microwell plates was assessed after 10-13 days using a dissecting microscope. The survival plates and viability plates were scored for the number of wells containing no cell growth (negative wells). The mutation plates were scored so that each well contained either a small colony (considered to be associated with clastogenic effects), a large colony (considered to be associated with gene mutation effects), or no colony.

Statistical methods: None required.

Evaluation criteria: Each well of the mutation plates (those containing TFT) was scored as containing either, a small colony, a large colony or no colony according to the following criteria:

Small Colony - a small colony was one whose average diameter was less than 25 % of the diameter of the well and was usually around 15 % of the diameter of the well. A small colony should also have shown a dense clonal morphology.

Large Colony - a large colony was one whose average diameter was greater than 25 % of the diameter of the well. A large colony should also have shown less densely packed cells, especially around the edges of the colony.

Any well which contained more than one small colony was scored as a small colony. Any well which contained more than one large colony was scored as a large colony. Any well which contained a combination of large and small colonies was scored as a large colony.

An empty well was one which contained no cell growth.

Results and discussion

Preliminary toxicity assay: The maximum concentration of glyphosate acid considered appropriate for testing in the mutation experiments was estimated to be 1000 µg/mL as concentrations of 1500 µg/mL and 2000 µg/mL in the presence and absence of S9-mix were found to produce an excessive reduction in the pH of the treatment medium.Very little toxicity was seen at the concentrations tested.

Mutation assay: No significant increases in mutant frequency, compared to the solvent control cultures, were observed in cultures treated with glyphosate acid at any concentration tested in either the presence or absence of S9-mix.

The positive controls, EMS and NDMA, induced substantial increases in mutant frequency in all mutation experiments, demonstrating the activity of the S9-mix and that the assay was performing satisfactorily in being capable of detecting known mutagens.

Conclusion by the Notifiers

Glyphosate acid was not mutagenic to L5178Y $TK^{+/-}$ cells in the presence or absence of S9-mix.

RMS comments:

The study is considered acceptable and the conclusion is agreed with. The selection of the highest concentration of 1000 µg/mL because of pH reduction at dose levels above is reasonable. It is widely accepted that pH changes (as well as increases in osmolality) may alter the mutant frequency. However, it was noted that the resulting top dose level was much lower than in the study by Jensen (1991, TOX9552372) who did not report a decline in pH at concentrations above 1 mg/mL. This obvious difference might suggest some variability in the acidic properties of the test materials although it seems not entirely clear from the study report if Jensen (1991, TOX9552372) had in fact measured the pH after treatment.

B.6.4.3 Tests for DNA damage and repair in mammalian cells and bacteria

A direct interaction of glyphosate acid with DNA, *i.e.*, DNA damage and repair was investigated in a small number of studies in different test systems *in vitro*. A UDS assay in primary rat hepatocytes by Rossberger (1994, TOX9400697) was reported in detail in the previous DAR (1998, ASB2010-10302) that is still considered acceptable. In contrast, the other studies in which this endpoint was adressed by different test methods and that had been provided for the previous EU evaluation must be considered not acceptable from a todays point of view. Thus, the UDS assay by Williams (1983, TOX9552370), a DNA repair assay with the IPA salt in *E. coli* by Wang (1993, TOX9500380), the SCE assays by Wang *et al.* (1993, TOX9500381, again, IPA salt tested) and by Jenkinson (1990, TOX9500269) and a Rec-assay by Shirasu et al. (1980, TOX9552408) were excluded from current re-evaluation of glyphosate. In contrast, a more recent rec assay in *Bacillus subtilis* has been submitted and is described in detail and evaluated by the RMS below Table B.6.4-6.

Table B.6.4-6: Summary of valid *in vitro* tests with glyphosate acid for DNA damage and repair in mammalian cells and bacteria (*provided by the Notifiers*)

Reference; study identification; owner	Type of study	Test organism / test system	Dose levels; batch/lot no.; purity	Results
In vitro tests for DNA damage and repair in mammalian cells				

Reference; study identification; owner		Type of study	Test organism / test system	Dose levels; batch/lot no.; purity	Results
Previously known	Rossberger, 1994; TOX9400697; ADAMA	UDS assay	Primary rat (Sprague-Dawley) hepatocytes	0.20 – 111.69 mM; F/93/032; >98%	Negative
In vitro tests for DNA damage and repair in bacteria					
New study	Akanuma, 1995b; ASB2012-11477; Arysta	Rec assay	*B. subtilis* strains H17 and M45 (+/- S9)	+/- S9 : 7.5 – 240 µg/disk; Lot 940908-1; 95.68%	Negative (supplementary study)

Rec assay in *B. subtilis* (Akanuma, 1995)

Reference: IIA, 5.4.3/02
Report: Akanuma, M. 1995b HR-001: DNA Repair Test (Rec-Assay).
The Institute of Environmental Toxicology, Tokyo, Japan
Data owner: Arysta LifeScience
Study No.: IET 94-0141
Date: 1995-03-14
not published, ASB2012-11477
Guidelines: U.S. EPA FIFRA Guidelines, Subdivision F
Deviations: None
GLP: Yes
Acceptability: *See RMS comment*

Dates of experimental work: 1995-02-14 to 1995-02-15

Materials and methods

Test material:	Glyphosate technical
Identification:	HR-001
Description:	Solid crystals
Lot/Batch #:	940908-1
Purity:	95.68 %
Stability of test compound:	Not mentioned in the report
Solvent used:	Sterile water
control materials:	
Negative:	Kanamycin (without activation)
Solvent/final concentration:	Water / > 12 mg/mL
Positive: non-activation and activation	mitomycin C (without activation) 3-amino-1,4-dimethyl-5H-pyrido [4,3-b] indole (with activation)

activation:	The enzyme activity measured by mutagenicity was good. S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factor. The component of S9 mix were 10 % (v/v) S9 fraction, 8 mM MgCl2, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADH, 4 mM NADPH and 100 mM sodium phosphate buffer.
test organisms:	Recombination-wild (rec+) strain H17 Recombination-deficient (recE-) strain M45 of *Bacillus subtilis*
test concentrations	6 dose level were tested: 7.5, 15, 30, 60, 120 and 240 µg/diskwith and without S9 metabolic activation

Study conduct
DNA-damaging activity was evaluated by a DNA repair test (Rec-Assay), with *Bacillus substilis* strains of recombination wild (*rec*$^+$) H17 and recombination-deficient (*recE*$^-$) M45, at concentrations of 7.5, 15, 30, 60, 120 and 240 µg/disk with and without S9 metabolic activation. The S9 fraction for the metabolic activation was obtained from liver of male SD strain rats previously treated intraperitoneally with 30 mg/kg phenobarbital (x 1), 60 mg/kg phenobarbital (x 3) and 80 mg/kg 5,6-benzoflavone (x 1). Negative control substance, kanamycin (0.2 µg/disk) without S9, and positive control substance mitomycin C (0.01 µg/disk) without S9, and positive control substance 3-amino-1,4-dimethyl-5H-pyrido [4,3-b] indole (Trp-p-1, 5 µg/disk) with S9 were also tested on both strains. In addition a solvent control, sterile water (20 µL/disk) without S9, and sterile water and co-factor solution (20µl: 20 µL/disk) with S9, was included in the experiment.

Paper discs (8 mm diameter) impregnated with 20 µl of the solution of the test substance were placed on the prepared spore agar plate containing the tester organism for each test, with and without metabolic activation. Duplicate plates were used for each experimental point. Diameter of a growth inhibitory zone of each strain was measured after incubation at 37 °C for 24 hours.

Statistics
Results were judged without statistical analysis.

Evaluation Criteria
Results are judged positive when growth inhibitory zone of M45 is larger than that of H17, and the difference in diameter was 5 mm or more at one or more dose levels that caused growth inhibitory zones, or 4 mm or less in diameter in the H17 (rec$^+$) strain.

Results and discussion
Analytical determinations: None
Mutations assays: HR-001 induced a growth inhibitory zone of 1 mm in diameter at the highest dose of 240 µg/disk in the (*recE*$^-$) strain M45 with S9 system (Table hereafter). The differences of growth inhibitory zones between the strains H17 and M45 were 1 mm or less. On the other hand, HR-001 did not induce any growth inhibitory zone in either the (rec$^+$) strain H17 with S9 system or both the strains M45 and H17 without S9 system.
The assay was considered as valid because:
-in the negative control plates treated with kanamycin, the difference of growth inhibitory zones between M45 and H17 strains was 2-3 mm

-in the positive controls of mitomycin C without S9 and Trp-p-1 with S9, growth inhibitory zone in M45 is larger than that of H17, and the difference in diameter is 19 mm and 11-12 mm, respectively.
-in the solvent control, no growth inhibitory zone was observed in either strain.

Table B.6.4-7: Results of the Rec assay

Compound	Dose (µg/disk)	S9 fraction (-)			S9 fraction (+)		
		Inhibitory zone* (mm)		Difference** (mm)	Inhibitory zone* (mm)		Difference** (mm)
		M45	H17		M45	H17	
Solvent control (H_2O)		0	0	0	0	0	0
		0	0	0	0	0	0
HR-001	7.5	0	0	0	0	0	0
		0	0	0	0	0	0
	15	0	0	0	0	0	0
		0	0	0	0	0	0
	30	0	0	0	0	0	0
		0	0	0	0	0	0
	60	0	0	0	0	0	0
		0	0	0	0	0	0
	120	0	0	0	0	0	0
		0	0	0	0	0	0
	240	1	0	1	0	0	0
		0	0	0	0	0	0
Negative control (Kanamycin)	0.2	8	6	2			
		9	6	3			
Positive control (Mitomycin C)	0.01	20	1	19			
		20	1	19			
Positive control (Trp-p-1)	5				11	0	11
					12	0	12

* Diameter of growth inhibitory zone subtracted the diameter of disk (8 mm)
** Diameter of growth inhibitory zone in M45 strain subtracted that in H17 strain

Conclusion by the Notifiers
Under the conditions used in this experiment, HR-001 did not have DNA-damaging activity in the bacteria.

RMS comments:
The study is considered to provide supplementary information only because the Rec assay is not a standard method for this endpoint (DNA damage and repair). Furthermore, dose selection was not explained. However, the study results are valid and it is agreed that the test compound glyphosate proved negative in this experiment, both with and without metabolic activation. This conclusion can be drawn because the evaluation criteria for a positive response were not met. The difference of growth inhibiting zone between the two strains was 1 mm at the highest concentrations anddifferences were 0 at lower dose levels. Thus, they were below the value obtained for the negative control (kanamycin).
When the study description in the dossier was compared to the original study report, it was noted that the study director was Mie Akanuma. Erroneously, the first name had been mentioned in the dossier instead of the authors surname.

B.6.4.4 *In vitro* genotoxicity testing – Tests for clastogenicity in mammalian cells

As mentioned in the 1998 DAR (ASB2010-10302), a clastogenic potential of glyphosate was mainly investigated *in vivo* and only two *in vitro* studies had been submitted for the previous evaluation of which one (György *et al.*, 1989, TOX9650157) was considered not acceptable upon re-evaluation. In contrast, a study by Van de Waart (1995, TOX9651525) is of sufficient quality and may still be used to adress this endpoint. It is considered now supplementary since the dose levels were rather low if compared to the 3 further studies that were provided for this new evaluation of glyphosate. These studies are reported in detail below Table B.6.4-8 Therefore, methodical details are not given in this table.

Table B.6.4-8: Summary of *in vitro* tests for chromosome aberrations with glyphosate acid

Reference (Owner)		Type of study	Test organism / test system	Dose levels*; batch/lot number; purity	Results
Study from the 2001	Van de Waart, 1995; TOX9651525; Agrichem	Cytogenicity	Peripheral human lymphocytes (-S9: 24, 48 h exposure; +S9: 3 h, harvest after 24 or 48 h)	- S9: 33 – 333 µg/mL + S9: 237 – 562 µg/mL; Lot 22021; 96%	Negative (supplementary study)
Studies not reviewed in the 2001 evaluation	Kyomu, 1995; ASB2012-11475; Arysta	Cytogenicity	Chinese hamster lung (CHL) cells	- S9: 62.5 – 500 µg/mL, + S9: 255 – 1000 µg/mL; Lot 949908-1; 95.68%	Negative
	Wright, 1996; ASB2012-11476; Nufarm	Cytogenicity	CHL cells	+/- S9: 312.5 - 1250 µg/mL; Lot H95D161A; 95.3%	Negative
	Fox, 1998; TOX2000-1995; Syngenta	Cytogenicity	Human lymphocytes	- S9: 100 – 1250 µg/mL + S9: 100 – 1250 µg/mL; P24, 95.6%	Negative

* Mostly, higher concentrations were included but these were the dose levels up to which metaphases were analysed.

<u>1st new clastogenicity study *in vitro* (Kyomu, 1995)</u>

Reference: IIA, 5.4.2/01
Report: Kyomu, M. 1995 HR-001: In vitro cytogenicity test.
The Institute of Environmental Toxicology, Tokyo, Japan
Data owner: Arysta LifeScience
Study No.: IET 94-0143
Date: 1995-05-29
not published, ASB2012-11475
Guidelines: U.S. EPA FIFRA Guidelines, Subdivision F
Deviations: None
GLP: Yes
Acceptability: *See RMS comment*

Dates of experimental work: 1995-03-13 to 1995-05-09

Materials and methods

Test Material Glyphosate technical

Description:	HR-001
Lot/Batch #:	940908-1
Purity:	95.68 %
Stability of test compound:	Not mentioned in the report
Solvent used:	Hank's balances salt solution and culture medium

Control Materials

Test solvent control:	Hank's balance salt solution
Positive solvent control:	Physiological saline (without metabolic activation) Benzo(a)pyrene (with metabolic activation)
Positive control:	Mitomycin (without metabolic activation) DMSO (with metabolic activation)

Activation
The enzyme activity measured by mutagenicity was good.
S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factor. The component of S9 mix were 10 % (v/v) S9 fraction, 8 mM MgCl2, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADH, 4 mM NADPH and 100 mM sodium phosphate buffer.

Test organisms:	CHL cells established from the lung of Chinese hamster
Culture medium:	The growth medium was Eagle's MEM supplemented with 10 % newborn calf serum

Test concentrations:

Preliminary cytotoxicity test:	8 doses: up to 1000 µg/L for the 24-hr treatment 8 doses: up to 2000 µg/L for the 48-hr treatment
Metaphase analysis:	4 doses: 125, 250, 500 and 1000 µg/mL for the 24-hr treatment 4 doses: 62.5, 125, 250 and 500 µg/mL for the 48-hr treatment

Replicates:

Preliminary cytotoxicity test:	Duplicate
Metaphase analysis:	Duplicate

TEST PERFORMANCE

In life dates	1995-03-13 to 1995-05-09

Preliminary cytotoxicity test
CHL cells were seeded at a density of 1.0 x 10^5 cells with 5 mL of medium and incubated for 48 hours. In the direct method, the cultures were treated with HR-001 with the doses mentioned above during 24 and 48hours. After the treatment, relative cell growth value of each culture was measured by comparing with the staining density in the concurrent solvent control.

In the metabolic activation method, the medium was replaced with 3 mL of medium containing S9 mix and then test substance was added to the cultures.
The second growth inhibition test with the activation system was carried out with higher doses.
Duplicate cultures were used for each experimental point and their relative values were averaged.

Metaphase analysis
CHL cells were seeded at a density of 2.0 x 10^5 cells with 10 mL of medium and incubated for 48 hours. In the direct method, the cultures were treated with HR-001 with the doses mentioned above during 24 and 48hours.
In the metabolic activation method, the medium was replaced with 5 mL of medium containing S9 mix and then test substance was added to the cultures.
Duplicate cultures were used for each experimental point and their relative values were averaged.
Diploid metaphase cells which possessed the typical karyotype of CHL cells and polyploid metaphase cells were analysed for a structural chromosome aberration.
The following data were recorded:
Number and frequency of polyploid cells
Number and frequency of each structural chromosome aberration
Number and frequency of metaphase cells with structural chromosome aberration

Only plyploid cell having 3 or more copies of haploid number of chromosomes was scored as a numerical chromosome aberration cell.

Statistics: The number of aberrant metaphases and polyploidy cells at each dose were statistically compared with those of corresponding solvent controls using a chi-square test.

Results and discussion
Preliminary cytotoxicity test: In the 24-hr and 48-hr treatments of the direct method, the doses of HR-001 which showed a reduction of the relative cell growth by 50 % or more were 1000 and 500 µg/mL, respectively. Therefore, the doses of 1000 and 500 µg/mL were chosen as the highest doses in the 24-hr and 48-hr treatments of the cytogenetics test, respectively.
In the metabolic activation method, no cell growth inhibition effect was observed at the dose of 1000 µg/mL or less, but at 2000 µg/mL or more, their cell growth was inhibited over 50 %. Therefore, the dose of 2000 µg/mL was determined to be used as the highest dose in the cytogenetics test with the metabolic activation system. It was noticed that in the both methods the color of the culture medium was turned to yellow at 500 µg/mL or more, indicating a decline of pH.

Table B.6.4-9: Preliminary growth inhibition test

Concentration (µg/ml)	**Relative cell growth (%)**			
	Direct method 24 h	Direct method 48 h	Metabolic activation method 6-18 h (1st exp.)	Metabolic activation 6-18 h (2nd exp.)
Solvent control (Hanks)	100	100	100	100
3.9	100	100	100	-
7.8	97	101	99	-
15.6	100	101	108	-

31.3	96	110	104	-
62.5	100	106	97	-
125	102	99	103	-
250	92	82	106	-
500	74	46	112	-
1000	22	9	106	100
2000	-	-	-	22
3000	-	-	-	38
4000	-	-	-	27
5000	-	-	-	25

Metaphase analysis
It was noticed that in the both methods the color of the culture medium was turned to yellow at 500 µg/ml and above, indicating a decline of pH.

In the 24-hr and 48-hr treatments, the frequencies of the aberrant metaphases (excluding gaps) were 0.5 % and 0.0 % to 1.0 %, respectively, showing no significant increases when compared with the concurrent solvent control. The dose of 1000 µg/mL in the 48-hr treatment gave high cytotoxicity to the cells so that chromosome preparations were not obtained from the cultures treated at this dose. There was no increase in the frequencies of polyploid metaphases at any doses of HR-001 in both 24-hr and 48-hr treatments (Table B.6.4-10).

On the other hand, MMC used as a positive control caused a great increase in a frequency of aberrant metaphases that was consistent with the historical control data.

In the presence of a metabolic activation system, the frequencies of the aberrant metaphases (excluding gaps) were in the range of 0.5 % to 1.0 %, showing no significant increases when compared with the concurrent solvent control and with the solvent control, respectively. The dose of 2000 µg/ml gave high cytotoxicity to the cells so that chromosome preparations were not obtained from the cultures treated at this dose. The frequencies of polyploid cells did not significantly increase (Table B.6.4-11). The same holds true when the test compound was tested under identical conditions in the absence of metabolic activation.

On the other hand, B(a)P used as a positive control caused a remarkable increase in the frequency of aberrant metaphases in the presence of S9 mix that was consistent with the historical control data.

Table B.6.4-10: Cytogenetics test (direct method, i.e., without metabolic ativation, 24-hr and 48-hr treatments)

Treatment	**Time (h)**	**S9 Mix**	**Dose (µg/ml)**	**Number of metaphase**	**Mitotic index (%)**	**Polyploid**		**Number of chromosome aberrations**							**Number of aberrant metaphases**		
							Judge	Gap g	ctb	Cte	csb	cse	Fragmen -tation	Others	+g	-g	Jud-ge
Untreated control	24	-	0	100	6.1	0	-	1.0	0	0	0.5	0	0	0	1.5	0.5	-
	48	-	0	100	2.5	0.5	-	0.5	1.0	0	0	0	0	0	1.5	1.0	-
Sovent control (Hanks)	24	-	10%	100	6.0	0.5		0	0	0	0	0	0	0	0	0	
	48	-	10%	100	3.4	0.5		0	0	0	0	0	0	0	0	0	
HR-001	24	-	125	100	6.7	0.5	-	0	0	0	0	0.5	0	0	0.5	0.5	-
			250	100	5.9	0.5	-	1.0	0.5	0	0	0	0	0	1.5	0.5	-
			500	100	5.6	0	-	1.5	0.5	0	0	0	0	0	2.0	0.5	-
			1000														
	48	-	62.5	100	3.0	0	-	1.5	0	0	0	0	0	0	1.5	0	-
			125	100	2.9	0	-	0.5	0.5	0	0	0	0	0	1.0	0.5	-
			250	100	3.1	1.0	-	1.5	0.5	0	0	0.5	0	0	2.5	1.0	-
			500	100	3.0	0	-	1.5	1.0	0	0	0	0	0	2.5	1.0	-
Positive control (MMC)	24	-	0.1	100	3.3	0.5	-	4.0	21.5	39.5	2.5	1.0	0	0.5	50.5	49.0	+
	48	-	0.1	100	2.6	1.0	-	5.5	43.0	49.5	2.5	3.0	1.5	1.5	72.0	70.5	+

Ctb: chromatide break; cte: chromatid exchange; csb: chromosome break; cse: chromosome exchange; +g: including gaps;
-g: excluding gaps; MMC: mitomycin C

Table B.6.4-11: Cytogenetics test (with metabolic activation, 6-hr treatment, followed by fixation for 18 hours)

Treat-ment	Time (h)	S9 Mix	Dose (µg/ml)	Number of metaphase	Mitotic index (%)	Polyploid		Number of chromosome aberrations							Number of aberrant metaphases		
							Judge	Gap g	Ctb	Cte	csb	cse	Fragmen-tation	Others	+g	-g	Judge
Untreated control	6	+	0	100	4.9	0	-	1.5	0.5	0	0	0	0	0	2.0	0.5	-
		-	0	100	5.3	0	-	0.5	1.0	0.5	0	0	0	0	2.0	1.5	-
Sovent control (Hanks)	6	+	10%	100	6.3	0		1.5	0.5	0	0	0	0	0	1.5	0.5	
		-	10%	100	5.7	1.0		1.0	0	0	0	0	0	0	1.0	0	
HR-001	6	+	250	100	6.7	0	-	0	1.0	0	0	0	0	0	1.0	1.0	-
			500	100	5.6	0	-	1.0	1.0	0	0	0	0	0	2.0	1.0	-
			1000	100	7.2	0.5	-	1.0	0.5	0	0	0	0	0	1.5	0.5	-
			2000														
		-	250	100	5.1	0	-	1.5	1.0	0	0	0	0	0	2.0	1.0	-
			500	100	4.9	0	-	0.5	0.5	0	0	0	0	0	1.0	0.5	-
			1000	100	5.7	0.5	-	0.5	0.5	0	0	0	0	0	1.0	0.5	-
			2000														
Positive control (B(a)P)	6	+	40	100	3.8	0	-	3.0	21.0	30.0	2.0	0.5	0	0	39.5	38.5	+
		-	40	100	4.7	0.5	-	0	0.5	0	0	0	0	0	0.5	0.5	-

Ctb: chromatide break; cte: chromatid exchange; csb: chromosome break; cse: chromosome exchange; +g: including gaps; -g: excluding gaps; B(a)P: benzo (a) pyrene

Conclusion by the Notifiers

In the direct and metabolic activation system, there was not a significant increase in the frequencies of abnormal metaphases with structural chromosome aberrations or polyploid metaphases. Based on the results obtained, it was concluded that, under the conditions of this study, the test substance HR-001 did not induce chromosome aberrations in Chinese hamster CHL cells with or without the metabolic activation system.

RMS comments:

The study is considered acceptable. No evidence of an increase in structural or numerical chromosome aberrations was obtained. With regard to the study description in the dossier, it must be clarified that metaphases were analysed up to a concentration of 500 µg/mL in the first series of experiments without metabolic activation (called above and in the study report "direct method") and 24- or 48-hour treatment periods. In the experiments with and without activation and an exposure period of 6 hours (thereafter, cells were fixed for 18 hours), metaphases could be analysed up to a concentration of 1000 µg/mL. At concentrations above, evaluation was avoided by severe cytotoxicity.

2nd new clastogenicity study *in vitro* (Wright, 1996)

Reference:	IIA, 5.4.2/02
Report:	Wright, N.P. 1996 Technical glyphosate: Chromosome aberration test in CHL cells *in vitro* Safepharm Laboratories, Derby, UK Data owner: Nufarm SPL Project No.: 434/015 Date: 1996-03-13 unpublished, ASB2012-11476
Guidelines:	not specified
Deviations:	not specified
GLP:	yes
Acceptability:	*See RMS comment*

Dates of experimental work: 30 August 1995 and 4 January 1996.

Materials and methods

Test material:

Identification:	TECHNICAL GLYPHOSATE
Description:	white powder
Lot/Batch #:	H95D 161A
Purity:	95.3 % w/w
Stability of test compound:	Not specified
Vehicle/Negative Controls	suspended in minimal essential culture media

Positive control:	Mitomycin C (MMC, Sigma Batch No. 104H2504) 0.05 µg/mL for cultures treated for 24 or 48 hours in the absence of metabolising enzymes. Cyclophosphamide both with and without metabolic activation
Activation:	Lot No. Aro. S9/11/0CT/95 SPL was prepared in-house at Safepharm Laboratories on 11/0CT/95. It was prepared from the livers of male SpragueDawley rats weighing - 200 g. These had received a single ip. injection of Aroclor 1254 at 500 mglkg, up to 5 days be
Test organisms::	Hamster CHL line
Culture medium:	Eagle's Minimal Essential medium with Earle's Salts (MEM), supplemented with 10 % foetal bovine serum and antibiotics, at 37' C with 5 % CO2 in AIR
Test concentrations::	19.5 to 5000 µg/mLg

Study conduct:
A preliminary toxicity test was performed on cell cultures using 24 and 48-hour continuous exposure times without metabolic activation and a 6-hour exposure period both with and without metabolic activation, followed by an 18-hour recovery period in treatment-free media. The dose range used was 19.5 to 5000 µg/mL. Growth inhibition was estimated by counting the number of cells at the end of the culture period on an electronic cell counter (Coulter) and expressing the cell count as a percentage of the concurrent vehicle control value. Slides were also prepared from the cells in order to check for the presence of cells in metaphase.
- Without Metabolic Activation
i) 24 hours continuous exposure to the test material prior to cell harvest. The dose levels selected for assessment were 312.5, 625 and 1250 µg/mL
ii) 48 hours continuous exposure to the test material prior to cell harvest. The dose levels selected for assessment were 312.5, 625 and 1250 µg/mL.
- With Metabolic Activation
i) 6 hours exposure to the test material and 59-mix (0.5 mL per 4.5 ml culture medium of 10 % 59 in standard co-factors). A phosphate buffered saline wash and then a further 18 hours in treatment-free media prior to cell harvest. The dose levels selected for assessment were 312.5, 625 and 1250 µg/mL.
ii) 6 hours exposure to the test material without 59-mix. A phosphate buffered saline wash and then a further 18 hours in treatment-free media prior to cell harvest. This group acts as a 'control' for group i). The dose levels selected for assessment were 312.5, 625 and 1250 µg/mL.
After exposure, cells were harvested and scored for chromosome damage.

Results and discussion
Preliminary Toxicity Test:
In all cases except 6 hours with 59, the test material induced some evidence of cell toxicity. Microscopic assessment of the slides prepared from the treatment cultures showed metaphases present up to 5000 µg/mL in the 6-hour with and without 59-mix treatment cases. The maximum dose with metaphases present was 2500 µg/mL in the 24 and 48-hour continuous exposure treatment case. However, when a pH check was performed on culture media dosed with technical glyphosate it was observed that the pH was reduced in a'dose-related way. At the maximum two dose levels the pH was reduced by ~ 1 unit and this was considered to be

unacceptable because alterations in pH have been shown to cause artefactual responses. Therefore the maximum dose level selected for the main study was 1250 µg/mL.

Chromosome Aberration Test
The test material was acidic at 2500 and 5000 µg/mL. Therefore the toxicity observed in the preliminary toxicity test was not relevant, and 1250 µg/mL was selected as the maximum dose for all treatment groups. The vehicle control cultures gave values of chromosome aberrations within the expected range. All the positive control cultures except cyclophosphamide without S9 gave highly significant increases in the frequency of cells with aberrations indicating that metabolic activation system was satisfactory and that the test method itself was operating as expected. The test material did not induce a statistically significant increase in the frequency of cells with aberrations at any dose level in any treatment group.The test material did not induce a significant increase in the numbers of polyploid cells at any dose level in any of the four treatment cases.

Conclusion by the Notifiers:
Technical glyphosate did not induce any statistically significant, dose-related increases in the frequency of cells with chromosome aberrations either in the presence or absence of a liver enzyme metabolising system or after various exposure times. Technical glyphosate is therefore considered to be non-c1astogenic to CHL cells *in vitro*.

RMS comments:
The study is considered acceptable. There were no indications for more frequent occurrence of chromosome aberrations. Thus, the conclusion is agreed with.

3d new clastogenicity study *in vitro* (Fox, 1998)

Reference:	IIA, 5.4.2/03
Report:	Fox, V. 1998 Glyphosate Acid: *In Vitro* Cytogenetic Assay In Human Lymphocytes Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK Data owner: Syngenta Report No.: CTL/P/6050 Date: 1998-10-29 not published, TOX2000-1995
Guidelines:	OECD 473 (1997): OPPTS 870.5375 (1998): 2000/32/EC B10 (2000)
Deviations:	The stability and achieved concentration of the test substance and control substances in the vehicles used were not determined by analysis. This deviation from the current regulatory guideline is considered not to compromise the scientific validity of the study.
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: 1995-07-25 to 1998-10-29

Materials and methods

Test Material:	Glyphosate acid
Description:	Technical; white solid
Lot/Batch number:	P24
Purity:	95.6 % a.i
CAS#:	Not reported
Stability of test compound:	Confirmed by Sponsor
Control Materials:	
Negative:	Supplemented RPMI-1640 culture medium
Solvent control (final concentration):	800 µL/mL
Positive control:	Absence of S9 mix: Mitomycin C, 0.2 µg/mL Presence of S9 mix: Cyclophosphamide 50 µg/mL

Mammalian metabolic system: S9 derived

X	Induced		Aroclor 1254	X	Rat	X	Liver
	Non-induced	X	Phenobarbitol		Mouse		Lung
			None		Hamster		Other
		X	Other β-naphthoflavone		Other		

The metabolic activation system (S9-mix) used in this study was prepared as required (on each day of culture treatment) as a 1:1 mixture of S9 fraction and cofactor solution.
The cofactor solution was prepared as a single stock solution of Na_2HPO_4 (150 mM), KCl (49.5 mM), glucose-6-phosphate (7.5 mM), NADP (Na salt) (6 mM) and $MgCl_2$ (12 mM) in sterile double deionised water and adjusted to a final pH of 7.4.

Test cells: mammalian cells in culture

	V79 cells (Chinese hamster lung fibroblasts)
X	Human lymphocytes. Obtained on the days of culture initiation from healthy, non-smoking donors. Donor 1 was male and Donor 2 was female. Bothl donors had a previously established low incidence of chromosomal aberrations in their peripheral blood lymphocytes.
	Chinese hamster ovary (CHO) cells

Media: RPMI-1640 (Dutch modification)				
Properly maintained?	X	Yes		No
Periodically checked for *Mycoplasma* contamination?		Yes	n/a	No
Periodically checked for karyotype stability?		Yes	n/a	No

Test compound concentrations used

Donor 1 – 68 hours		**Donor 2 – 68 hours**		**Donor 2 – 92 hours**	
+ S9	-S9	+ S9	-S9	+ S9	-S9
100 µg/mL 750 µg/mL 1250 µg/mL	100 µg/mL 750 µg/mL 1250 µg/mL	100 µg/mL 750 µg/mL 1250 µg/mL	100 µg/mL 750 µg/mL 1250 µg/mL	1250 µg/mL	1250 µg/mL

Test performance
Cytogenetic assay: Duplicate human peripheral blood cultures were exposed to the solvent, test substance or positive control substances at appropriate concentrations in the following experiments:

A cytogenetic test using blood from Donor 1 in the presence and absence of S9-mix with a standard sampling time of 68 hours after culture initiation. Solvent and positive control cultures were included.

A second independent cytogenetic test using blood from Donor 2 in the presence and absence of S9-mix with a standard sampling time of 68 hours after culture initiation and a later sampling time of 92 hours after culture initiation. Solvent control cultures were included at both sampling times whereas the positive control cultures were only included at the 68 hour sampling time.

In both experiments a range of concentrations of glyphosate acid was used in order to define suitable concentrations for chromosomal aberration analysis.

The standard sampling time of 68 hours after culture initiation used in this study was based on a measured mean cell cycle time for cultured human peripheral blood lymphocytes of 13.5 hours in this Laboratory. The later sampling time was selected to be 24 hours after the standard sampling time.

Culture and treatment of blood samples: Cultures (10 mL) were established by the addition of 0.5 mL of whole blood to RPMI-1640 (Dutch modification) tissue culture medium supplemented with approximately 10 % foetal bovine serum (FBS), 1.0 IU/mL heparin, 100 IU/mL penicillin and 100 µg/mL streptomycin. The lymphocytes were stimulated to enter cell division by addition of phytohaemagglutinin (PHA; at 5 % v/v) and the cultures were maintained at approximately 37 °C for 48 hours with gentle daily mixing where possible.

Prior to treatment, the cultures were centrifuged and the culture medium was removed and replaced with fresh supplemented RPMI-1640 culture medium in the solvent and positive control cultures.

Approximately 48 hours after culture establishment, 8 mL aliquots of the test substance preparations were administered to duplicate cultures as appropriate to the experiment design. The positive control cultures were treated at a dosing volume of 50 µL/10 mL culture. In addition, 200 µL of a 1:1 mix of S9 and co-factor solution was added to each culture to be treated in the presence of S9-mix.

Cultures treated in the presence of S9-mix were treated for a period of approximately 3 hours at 37 °C, after which the culture medium was removed following centrifugation and replaced with fresh supplemented RPMI-1640 culture medium. The cultures were re-incubated at approximately 37 °C for the remainder of the 68 hour growth period. Cultures treated in the absence of S9-mix were maintained at approximately 37 °C for the remainder of the 68 hour growth period. All cultures due for sampling at the later 92 hour sampling time received an additional culture medium change approximately 68 hours after culture initiation.

The effect of glyphosate acid on the pH and osmolality of the culture medium was investigated, using single cultures containing medium only, as changes in pH and increases in osmolality are well known to result in the production of chromosomal aberrations.

Culture harvesting: Approximately 2 hours prior to harvesting, the cultures were treated with colcemid at a final concentration of 0.4 µg/mL. Sixty-eight hours or 92 hours after culture establishment the cultures were centrifuged, the supernatant was removed and the cells were re-suspended in approximately 10 mL of 0.075 M KCl at room temperature for approximately 10 minutes.

Details of slide preparation: The cultures were centrifuged, the supernatant was removed and the remaining cells were fixed in freshly prepared methanol/glacial acetic acid fixative (3:1 v/v) added dropwise and made up to a volume of approximately 10 mL. The fixative was removed following centrifugation and replaced with freshly prepared fixative. This fixation process was repeated at least twice prior to slide preparation on clean, moist labelled microscope slides. The slides were air dried, stained in filtered Giemsa stain (10 % Gurr's R66 in buffered [pH 6.8] double deionised water) for 7 minutes, rinsed in water, air-dried and mounted with coverslips in DPX.

Slide analysis: Slides were examined to determine that they were of suitable quality and, where appropriate, the mitotic index was determined by examining 1000 lymphocytes per culture and calculating the percentage of cells in metaphase.

For each donor, both in the presence and absence of S9-mix, duplicate cultures treated with glyphosate acid at three concentrations were selected for chromosomal aberration analysis at the 68 hour sampling time along with the appropriate solvent and positive control cultures. In each case the highest concentration was selected on the basis of reduction in the pH of the culture medium and the suitability of the metaphase preparations for chromosomal aberration analysis. In addition, duplicate cultures from the Donor 2 treated with glyphosate acid at the highest concentration selected at the 68 hour sampling time in the presence and absence of S9-mix were selected for chromosomal aberration analysis at the 92 hour sampling time along with the appropriate solvent control cultures.

The slides were coded prior to analysis and one hundred cells in metaphase, where possible, were analysed from each selected culture for the incidence of structural chromosomal damage.

Evaluation criteria: The percentages of aberrant metaphases and the number of aberrations per cell were calculated for each treatment scored, both including and excluding cells with only gap-type aberrations.

The Fisher Exact Probability Test (one-sided) was used to evaluate statistically the percentage of metaphases showing aberrations (excluding cells with only gap-type aberrations). Data from each treatment group, in the presence and absence of S9-mix, was compared with the respective solvent control group value. The data have been interpreted as follows:

No statistically significant increase in the percentage of aberrant cells (at any concentration) above concurrent solvent control values - NEGATIVE.
A statistically significant increase in the percentage of aberrant cells above concurrent solvent control values, which falls within the laboratory solvent control range - NEGATIVE.

An increase in the percentage of aberrant cells, at least at one concentration, which is substantially greater than the laboratory historical solvent control values - POSITIVE.
A statistically significant increase in the percentage of aberrant cells which is above concurrent solvent values and which is above the historical solvent control frequencies value but below that described in (c) may require further evaluation.
Significantly increased incidence of interchanges, exchange figures or re-arrangements (where none of the above criteria are met) may require further evaluation

Results and discussion
Cytogenetic assay: Small reductions in mean mitotic activity, compared to the solvent control values, were observed in cultures (37 % - Donor 1; 33 % - Donor 2) treated with the highest concentrations of glyphosate acid selected for chromosomal aberration analysis. No reductions in mitotic activity were observed for culture treated in the presence of S9-mix and harvested at the 68 hour sampling time or cultures treated in either the presence or absence of S9-mix and harvested at the 92 hour sampling time. Cultures treated with higher concentrations of glyphosate acid were considered not to be suitable for chromosomal aberration analysis due to excessive reductions in the pH of the culture medium.
Chromosomal aberration analysis: No statistically or biologically significant increases in the percentage of aberrant cells, above the solvent control values, were recorded at the 68 hour sampling time in cultures from either Donor 1 or Donor 2 treated with glyphosate acid in either the presence or absence of S9-mix.
No statistically or biologically significant increases in the percentage of aberrant cells, above the solvent control values, were recorded at the 92 hour sampling time for cultures treated with glyphosate acid in either the presence or absence of S9-mix.
The positive control materials, mitomycin C and cyclophosphamide induced statistically and biologically significant increases in the percentage of aberrant cells, compared to the solvent control cultures.

Conclusion by the Notifiers
Glyphosate acid was not clastogenic to cultured human lymphocytes treated *in vitro* in either the presence or absence of S9-mix.

RMS comments:
The study is considered acceptable and the conclusion is agreed with. The long duration of this study is surprising but was apparently due to the fact that the in-life phase was run early in 1996 but slides were evaluated not before 1998.

B.6.4.5 *In vivo* genotoxicity testing (somatic cells) – Metaphase analysis in rodent bone marrow, or micronucleus test in rodents

A number of bone marrow micronucleus tests, mostly in mice, and two cytogenetic studies in mouse bone marrow have been submitted by the different notifieres to adress this endpoint. An overview on these studies is given in Table B.6.4-12.
Three of the previously evaluated studies in mice ([redacted] 1994, TOX9400323; [redacted] 1993, TOX9551100; [redacted] 1991, TOX9552374) and one in rats ([redacted] TOX9552375) that were already mentioned in the 1998 DAR were still found by the RMS to be of acceptable quality. In contrast, the studies by [redacted] (1990, TOX9500255), [redacted] (1989, TOX9650159) and [redacted] (1986, TOX9551957) must be regarded as not acceptable and were not taken into account for the current re-evaluation of glyphosate.

Instead, several studies of this type, mostly performed in mice and more recently, were submitted for this re-evaluation of glyphosate by the EU. All these new studies are reported in detail below Table B.6.4-12 and each was commented by the RMS. It must be emphasised that the studies by [redacted] (2007, ASB2012-11480) and [redacted] (2008, ASB2012-11481) were considered not acceptable because the dose levels employed were much too low. In addition, the first one was flawed by severe toxicity at low levels and an amendment to the latter one ([redacted] 2010, ASB2014-9284) was partly contradictory. Therefore, they have been deleted from the summary table.

Table B.6.4-12: Summary of valid *in vivo* micronucleus assays or cytogenic studies with glyphosate in rodents

	Reference, study identification, owner	Type of study	Test organism / test system, route/ treatment	Dose levels; batch/lot, purity; sampling	Results
Studies from the 2001 evaluation	[redacted] 1994; TOX9400323; ADAMA	Cytogenicity in bone marrow	Swiss albino mice; daily oral applications for 2 successive days	0, 50, 500, 5000 mg/kg bw/day; batch 046, 96.8%; sampling 24 h after second dose	Negative; mitotic index ↓ at 5000 mg/kg bw
	[redacted] 1993; TOX9551100; ADAMA	Micronucleus test in bone marrow	Swiss albino mice; daily oral applications for 2 successive days	0, 50, 500, 5000 mg/kg bw/day; batch 60, 96.8%; sampling 24 h after second dose	♂: negative ♀: weakly positive at highest dose
	[redacted] 1991; TOX9552374; Cheminova	Micronucleus test in bone marrow	NMRI mice, single oral application	0 – 5000 mg/kg bw; 206-JaK-25-1, 98.6%; sampling after 24, 48, 72 h	Negative
	[redacted] 1983*; TOX9552375; Monsanto	Cytogenicity in bone marrow	Sprague-Dawley rats, single i.p. injection	0 – 1000 mg/kg bw; XHJ-64, 98.7%; sampling after 6, 12, 24 h	Negative
Studies not reviewed in the 2001	[redacted] 2006; ASB2012-11478; Nufarm	Micronucleus test in bone marrow	CD-1 mice ♂; single i.p. dose	0, 150, 300, 600 mg/kg bw; H05H016A, 95.7%; sampling after 24 and 48 h	Stat. sign. ↑ in PCE at 600 mg/kg bw (24 h) but within historical control; overall: negative
	[redacted] 1999; ASB2012-11482; Nufarm	Micronucleus test	Swiss albino mice, ♂ + ♀, two i.p. injections (24 h interval)	0, 187.5, 375, 562.5 mg/kg bw; batch 037-919-113, 95.49 %; sampling 24 h after 2nd application	Negative

Reference, study identification, owner		Type of study	Test organism / test system, route/ treatment	Dose levels; batch/lot, purity; sampling	Results
	[redacted] 1996; TOX2000-1996; Syngenta	Micronucleus test in bone marrow	CD-1 mice, 5 ♂+ 5 ♀/dose / sampling point; single oral dose	0, 5000 mg/kg bw; P24, 95.6%; sampling after 24 and 48 h	Negative
	[redacted] 2008; ASB2012-11483; Syngenta	Micronucleus test in bone marrow	NMRI mice 6 ♂/dose/ sampling point; single oral dose	0, 2000 mg/kg bw, sampling after 24 and 48 h, 500 & 1000 mg/kg bw sampling after 24 h only; Batch 20070545, 99.1%	Negative
	[redacted] 2012; ASB2014-9277; Dow	Micronucleus test in bone marrow	Swiss albino mice, ♂, two oral. injections (24 h interval)	0, 2000 mg/kg bw, sampling after 24 h; Lot 20061109, 98.9 %	Negative
	[redacted] 2012; ASB2014-9333; Syngenta	Micronucleus test in bone marrow	NMRI mice 7 ♂/ sampling point; single oral dose	0, 2000 mg/kg bw, sampling after 24 and 48 h; Batch 56753, 96.3 %	Negative
	[redacted] 2009; ASB2012-11479; Helm	Micronucleus test in bone marrow	CD rat, single oral application	0, 500, 1000, 2000 mg/kg bw/day; batch 20080801, 98.8%; sampling after 24 and 48 h	Negative

* Results were also published by Li & Long, 1988 (TOX9500253).

Mouse

1st new micronucleus test in mice (Durward, 2006)

Reference: IIA, 5.4.4/01
Report: [redacted] 2006, Glyphosate Technical: Micronucleus Test In The Mouse
[redacted]
Data owner: Nufarm
Report No.: 2060/014
Date: 2006-02-08
Unpublished; ASB2012-11478
Guidelines: OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000), USA EPA, JMAFF
Deviations: None
GLP: Yes
Acceptability: *See RMS comment*

Dates of experimental work: 2005-06-07 - 2005-07-20

Materials and methods

Test material:	
Identification:	Glyphosate Technical
Description:	White crystalline solid
Lot/Batch #:	H05H016A
Purity:	95.7 %
Stability of test compound:	At room temperature stable until March 2008.
Vehicle and/ or positive control:	PBS
Test animals:	
Species:	Mouse
Strain:	CD-1
Source:	[redacted]
Age:	Approx. 5 - 8 weeks
Sex:	Males
Weight at dosing:	21 - 29 g
Acclimation period:	At least 7 days
Diet/Food:	Certified Rat and Mouse Diet Code 5LF2, BCM (IPS Ldt., London UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	In groups up to seven in solid-floor polypropylene cages with wood flake bedding.
Environmental conditions:	Temperature: 19 - 25 °C Humidity: 30 - 70 % Air changes: approx. 15/hour 12 hours light/dark cycle

Animal assignment and treatment:
The test was conducted using young male CD-1 mice. Groups of seven mice each were dosed via the intraperitoneal route at 150, 300 and 600 mg/kg bw.
One group from each dose level was killed by cervical dislocation 24 hours following treatment and another group dosed with test material at 600 mg/kg bw after 48 hours. In addition, three further groups of mice were included in the study; two groups (each of seven mice) were dosed via the intraperitoneal route with the vehicle alone (PBS) and a third group (five mice) was dosed orally with the positive substance cyclophosphamide. The vehicle controls were killed 24 or 48 hours following dosing and positive control group animals were killed 24 hours following dosing.
Immediately following termination both femurs were dissected from each animal, aspirated with foetal calf serum and bone marrow smears prepared following centrifugation and re-suspension. The smears were air-dried, fixed in absolute methanol, stained in May-Grünwald/Giemsa, allowed to air-dry and coverslipped using mounting medium.

Results and discussion

Mortality: No mortality occurred.

Clinical observations: Clinical signs were observed in animals dosed with the test material at and above 150 mg/kg bw in both the 24 and 48-hour groups where applicable, these included as follows: hunched posture, ptosis, ataxia and lethargy.

Evaluation of bone marrow slides:

A statistically significant decrease in the percentage PCEs per 1000 erythrocytes was observed in the 24-hour 600 mg/kg bw group when compared to the control. A similar decrease was also observed in the 48-hour 600 mg/kg bw group, but the larger standard deviation resulted in no statistical significance being applied. This finding, accompanied by the presence of clinical signs, was taken to indicate that systemic absorption had occurred and exposure to the bone marrow was confirmed.

There was a small but statistically significant increase in the incidence of micronucleated PCEs in animals dosed at 600 mg/kg bw in the 24-hour group when compared to the control group. However, the response was very modest and within the historical range (see Table B.6.4-13 and Table B.6.4-14). The response seen is considered to be most likely due to a haematopoietic effect induced by the cytotoxic effect of the test material on the bone marrow rather than any genotoxic mechanism. The increased erythropoiesis caused by the test material toxicity might cause some cells to cycle more quickly than in the vehicle control animals and, therefore, there may also be less opportunity to repair spontaneously-occurring DNA damage before the final mitosis and enucleation, resulting in small increases in micronucleated cells. Therefore the response was considered to have no genotoxic significance.

The positive control group showed a marked increase in the incidence of micronucleated PCEs hence confirming the sensitivity of the system to the known clastogenic activity of cyclophosphamide under the conditions of the test.

Table B.6.4-13: Summary of results

Treatment group / sampling time	**Number of PCE with micronuclei/2000 PCE**		**% PCE with micronuclei**		**% PCE / 1000 erythrocytes**	
	Group mean	SD	Group mean	SD	Group mean	SD
Vehicle control (10 mL/kg) / 48h	2.0	2.4	0.10	0.12	36.01	4.39
Vehicle control (10 mL/kg) / 24h	1.3	1.1	0.06	0.06	38.46	4.58
Positive control (50 mg/kg) / 24h	60.6***	9.7	3.03***	0.49	51.46	4.45
Glyphosate (150 mg/kg) / 24 h	1.4	0.8	0.07	0.04	45.23	6.12
Glyphosate (300 mg/kg) / 24 h	1.1	1.1	0.06	0.05	38.57	8.69
Glyphosate (600 mg/kg) / 24 h	3.9*	1.5	0.19*	0.07	27.71**	4.95
Glyphosate (600 mg/kg) / 48 h	1.9	2.1	0.09	0.1	28.16	14.23

PCE = polychromatic erythrocytes
SD = standard deviation
* : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$

Table B.6.4-14: Historical control data for relative frequency categories of micronuclei per 1000 PCE*

24-h sampling			**48-h sampling**		
Frequency	Groups	%	Frequency	Groups	%

categories			categories		
0.0 – 0.4	15	25	0.0 – 0.4	21	35
0.5 – 0.9	25	42	0.5 – 0.9	18	30
1.0 – 1.4	14	23	1.0 – 1.4	14	23
1.5 – 2.0	3	5	1.5 – 2.0	7	12
2.1 – 2.5	3	5	2.1 – 2.5	0	0

* Data from 60 studies

Conclusion by the Notifiers

The test material glyphosate technical was considered to be non-genotoxic under the conditions of the test.

RMS comments:

The study is considered acceptable. The selection of the highest dose level of 600 mg/kg bw is appropriate since the application route was intraperitoneal (for oral dosing, it might be too low). In fact, in a range-finding test, deaths were observed after i.p. application of 800 and 1000 mg/kg bw. The use of only male mice is also justified because males are known to be the more sensitive sex for micronucleus formation. The conclusion is supported. The increase in micronucleated PCE in high dose males after 24 hours (3.9/2000 = 1.95/1000) is covered by the historical controls even though it is close to its upper edge. Furthermore, no evidence of an increase was seen after 48 hours. Thus, there is no concern about clastogenicity because of this study and the conclusion of the notifiers may be agreed with.

2nd new micronucleus test in mice ([redacted], 2007)

Reference: IIA, 5.4.4/03

Report: [redacted] 2007 Mammalian Erythrocyte Micronucleus Test for Glifosato Téchnico Helm
[redacted]
Data owner: HAG
Report No.: 3393/2007-3.0MN-B
Date: 2007-12-13
Unpublished; ASB2012-11480

Guidelines: OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000), USA EPA, JMAFF

Deviations: None

GLP: Yes

Acceptability: *See RMS comment*

Dates of experimental work: 26/11/2007 – 28/11/2007

Materials and methods

Test material:

Identification:	GLIFOSATO TECNICO HELM
Description:	Solid,
Lot/Batch #:	2007091801
Purity:	980.1 g/kg
Stability of test compound:	Stable (CIPAC MT 46, 54 °C, 14 days)

Vehicle and/ or positive control:	Vehicle: deonized water Positive Control: Cyclophosphamide
Test animals:	
Species:	Swiss mice
Source:	[redacted]
Age:	09 – 10 weeks
Sex:	Male
Diet/Food:	Commercial food (Biobase Biotec), *ad libitum*
Water:	*ad libitum*
Housing:	Animals were kept in groups of 6 animals in solid cages bedded with wooden chips.
Environmental conditions:	Temperature: 18 - 21 °C Humidity: ~57 % 12 hours light/dark cycle

Animal assignment and treatment:
The test was conducted using young male mice. Groups of six mice were dosed via oral route at 8.0, 15.0 and 30.0 mg/kg bw. The animals were treated twice at 0 and 24 h. Sampling was performed 24 hours after last treatment. Both femurs from each rat were dissected, aspirated with foetal calf serum and bone marrow smears prepared following centrifugation and re-suspension. The smears were air-dried, fixed and stained, allowed to air-dry and coverslipped using mounting medium. 3000 polychromatic erythrocytes (PCE) per animal were scored for the incidence of micronuclei. The ratio of PCE to normochromatic Erythrocytes (NCE) was determined for each animal by counting a total of 2000 erythrocytes.

Results and discussion
Mortality: No mortality occurred.
Evaluation of bone marrow slides: When animals treated with GLIFOSATO TECHNICO HELM were compared to the concurrent negative control group, no statistically significant increase in the number of micronuclei was observed at dosage of 8 or 15 mg /kg bw (see Table B.6.4-15). At 30 mg /kg bw results obtained were statistically significant when compared to the control group but they did not have biological relevance when compared to historical control data (that is however, partly based on published data and not considered robust by the RMS).
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Table B.6.4-15: Summary of results

Treatment group / sampling time	Number of PCE with micronuclei /18000 PCE	χ^2
Vehicle control (5 mL/kg)	11	n.a.
Positive control (75 mg/kg)	347	315.4 ($p < 0.001$)
Glyphosate TC (8 mg/kg)	19	2.14 ($p = 0.144$)
Glyphosate TC (15 mg/kg)	21	3.12 ($p = 0.077$)
Glyphosate TC (30 mg/kg)	25	5.44 ($p = 0.020$)

PCE = polychromatic erythrocytes

In the third column, the frequency of micronucleated PCE in the individual groups was compared to the negative control group. This calculation was not checked or repeated by the RMS since the study is considered not acceptable.

Conclusion by the Notifiers
The test material glyphosate technical was non-genotoxic.

RMS comments:
The study is considered not acceptable since it was seriously flawed (see below) and because the dose levels were much too low for any meaningful conclusion with regard to micronucleus formation, in particular when application by the oral route is taken into consideration. In the original report, some justification for dose selection is given, based on a range-finding test suggesting effects at rather low dose levels. In fact, two animals that were administered 2000 mg/kg bw, died on day 3 after having shown ataxia and prostration before. The same observations were made in 3 animals which received an oral dose of 320 mg/kg bw. They all died on day 2. Even at a dose level of 50 mg/kg bw, one out of three treated animals died on day 1. No mortality was seen at 30, 20, and 12.5 mg/kg bw. Therefore, 30 mg/kg bw was considered the MTD and was selected as the highest dose for the micronucleus assay. ~~These findings~~ The occurrence of deaths and clinical signs at relatively low dose levels ~~were~~ was obviously in contradiction to ~~more reliable~~ the available acute toxicity tests with glyphosate in the mouse ([redacted] 1995, ASB2012-11382; [redacted] 1991, TOX9551089; [redacted]d, 1991, TOX9552329; [redacted] 1994, TOX9551624). In addition, in five other micronucleus assays or cytogenetic studies in mice with substance administration by the oral route ~~described in this section~~ ([redacted] 1991; [redacted] 1993 and 1994; [redacted], 1996; [redacted] 2008; see Table B.6.4-12) much higher dose levels could be used. A single study cannot contravene or even outweigh all this data coming from a number of (independent) laboratories even though this was suggested by a comment that was provided in the public consultation. It is much more likely that the micronucleus assay by [redacted] (2007) was seriously flawed by severe toxicity that was completely unexpected and cannot be explained if the whole toxicological profile of glyphosate is taken into consideration. Either, strong methodical mistakes have been made when the study was conducted or the test material was not glyphosate even though it was claimed as such. Both possibilities would turn the study completely unreliable and make it unsuitable for any regulatory use. Because of this general assessment, there is no need to discuss the weak "increase" in micronuclei at 30 mg/kg bw that is in complete contradiction to what was seen in the other, much more reliable studies.

3d new micronucleus test in mice (Costa, 2008)

Reference:	IIA, 5.4.4/04
Report:	[redacted] 2008 Evaluation of the mutagenic potential of Glyphosate Technical Micronucleus assay in mice [redacted] Data owner: HAG (original sponsor: Jingma Chemicals, Longyou Zhejian, China) Report No.: RF - 3996.402.395.07 Date: 2008-09-29 Unpublished; ASB2012-11481
Guidelines:	OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000), USA EPA, JMAFF

Deviations: None
GLP: Yes
Acceptability: See RMS comment

Dates of experimental work: 19/05/2008 – 13/08/2008

Materials and methods

Test material:

Identification:	GLYPHOSATE TECHNICAL
Description:	Solid,
Lot/Batch #:	20070606
Purity:	980.0 g/kg
Stability of test compound:	Stable to hydrolysis at pH 3, 6 and 9 (5-35 °C)
Vehicle and/ or positive control:	Vehicle: sterile corn oil Positive Control: Cyclophosphamide

Test animals:

Species:	Swiss mice
Source:	[redacted]
Age:	07 – 12 weeks
Sex:	Male and Female
Weight at dosing:	approx. 30 g
Diet/Food:	Commercial food (Purina Labina, Agribrands Purina do Brsail, Ltda.)
Water:	*ad libitum*
Housing:	Animals were kept in groups of 5 animals by sex in solid cages bedded with sterile sawdust.
Environmental conditions:	Temperature: 20 - 24 °C Humidity: 50 - 70 % Air changes: approx. 10 - 15/hour 12 hours light/dark cycle

Animal assignment and treatment:
The test was conducted using young male and female mice. Groups of five male and five female mice were ~~dosed via oral route (positive reference item was administered via intraperitoneal route)~~ administered technical grade glyphosate by intraperitoneal injection twice after a 24 hours interval at dose levels of 15.62, 31.25 and 62.5 mg/kg bw in a final volume of 15 mL per dose. Sampling was performed after 24 hours following the second injection for all groups ~~and after 48 hours for the vehicle control and the highest dose group~~. Both femurs from each rat were dissected, aspirated with foetal calf serum and bone marrow smears prepared following centrifugation and re-suspension. The smears were air-dried, fixed and stained, allowed to air-dry and coverslipped using mounting medium. 2000 polychromatic erythrocytes (PCE) per animal were scored for the incidence of micronuclei. It is stated in the orginal report that the ratio of PCE to normochromatic erythrocytes (NCE) was

determined for each animal ~~by counting a total of 1000 erythrocytes~~ when the first 2000 PCE had been detected.

Results and discussion

Mortality: No mortality occurred.

Evaluation of bone marrow cells: The statistical analysis of the results pointed out that the test substance did not induce an increase in micronuclei number in polychromatic erythrocytes of the bone marrow when compared to the negative control at any evaluated concentrations. No adverse effect was observed in the ratio of polychromatic erythrocytes to normochromatics in animals treated with the test substance glyphosate technical, at any evaluated concentrations. A significant statistical increase of micronucleated cells in polychromatic erythrocytes and a slight change in the ratio of polychromatic to normochromatic erythrocytes was observed in animals treated with cyclophosphamide, as expected (see Table B.6.4-17).

~~**Table B.6.4-16: Summary of results**~~

~~**Treatment group / sampling time**~~	~~**Male**~~		~~**Female**~~	
	~~Number of PCE with micronuclei /2000 PCE, Group mean~~	~~PCEs/NCEs~~	~~Number of PCE with micronuclei /2000 PCE, Group mean~~	~~PCEs/NCEs~~
~~Vehicle control (15 mL/kg) / 24h~~	~~0.0~~	~~1.78197~~	~~0.0~~	~~1.77195~~
~~Positive control (27 mg/kg) / 24h~~	~~0.0~~	~~1.76831~~	~~0.0~~	~~1.79107~~
~~Glyphosate TC (15.62 mg/kg) / 24 h~~	~~0.0~~	~~1.74353~~	~~0.0~~	~~1.76047~~
~~Glyphosate TC (31.25 mg/kg) / 24 h~~	~~0.0~~	~~1.71071~~	~~0.0~~	~~1.78676~~
~~Glyphosate TC (62.5 mg/kg) / 24 h~~	~~23.0**~~	~~1.54855~~	~~12.2**~~	~~1.72844~~

~~PCE = polychromatic erythrocytes~~
~~SD = standard deviation~~
~~**p<=0.01~~

When this section of the RAR was reviewed, this table was found to be wrong, due to a technical error. The (expected) in increase in the positiv control group had been erroneously allocated to the group receiving 62.5 mg glyphosate/kg bw in the GTF dossier and the RMS reviewers had not noticed this error when the original RAR was prepared. Furthermore, not all figures were precisely those that are given in the original report ([redacted] 2008, ASB2012-11481). Therefore, it was replaced now by a new one giving the appropriate allocation of test results to the individual test groups. The sampling time was always 24 hours after the second i.p. dose.

Table B.6.4-17: Summary of results

Group	**Males**		**Females**	
	Number of PCE with micronuclei /2000 PCE, Group mean	PCE/NCE ratio	Number of PCE with micronuclei /2000 PCE, Group mean	PCE/NCE ratio
Vehicle control	0.0	1.78197	0.0	1.77195

Positive control (27 mg/kg bw)	23.0**	1.54855	12.2**	1.72844
Glyphosate TC (15.62 mg/kg)	0.0	1.76831	0.0	1.79107
Glyphosate TC (31.25 mg/kg)	0.2	1.74353	0.0	1.76047
Glyphosate TC (62.5 mg/kg)	0.6	1.71071	0.0	1.78676

**p<=0.01 (Mann-Whitney test); PCE = polychromatic erythrocytes

Conclusion by the Notifiers
The test material glyphosate technical was non-genotoxic.

RMS comments:
~~*The study is considered not acceptable since the dose levels were much too low for any meaningful conclusion with regard to micronucleus formation.*~~
Some of the information given in the dossier was apparently wrong but, unfortunately, was corrected in the revised RAR only. In the original RAR, the study was considered not acceptable since the dose levels were much too low for any meaningful conclusion with regard to micronucleus formation even though it must be taken into consideration that the exposure was via the intraperitoneal route. (Thus, the study can be hardly compared to most other micronucleus tests with glyphosate in which the test substance was administered via the oral route.) In the original report, dose selection for the"definitive test" was justified with the outcome of a preliminary test. In this range finding experiment, 3 males and 3 females per dose level received i.p. glyphosate doses of 62.5, 125, 250, 500, or 1000 mg/kg bw. The top dose level resulted in 100 % mortality and at the next lower dose level of 500 mg/kg bw, one male and two female mice died. Based on a clear decrease in the PCE/NCE ratios in both sexes, the intermediate dose of 250 mg/kg bw was found to be cytotoxic. It was recommended that 125 mg/kg bw was the most appropriate high dose to be employed in the definitive test but, without further justification, 62.5 mg/kg bw was actually the highest dose used. That was much lower than in other studies in which the i.p. route had been also chosen [redacted], 1999, ASB2012-11482; [redacted] 2006, ASB2012-11478).
In 2014, an amendment to this study was submitted ([redacted] 2010, ASB2014-9284). In this document, some results of testing glyphosate at dose level of 125, 250, and 375 mg/kg bw are reported. Clinical signs but no mortality were seen at all dose levels. It is not clear in which way this data is linked to the preliminary test that was performed as part of the original study since the dose levels were not exactly the same and the number of animals was different (this time 5 per sex and dose). Furthermore, in the amendment, more data on micronucleus incidences and PCE/NCE ratios at the dose levels of 15.62, 31.25, and 62.5 mg/kg bw was given, apparently based on 10 animals per sex and dose. It was confirmed that there was no clastogenic potential of the test substance. However, treatment of these animals was simply not described in the original report and the amendment cannot be considered a full study report. Taking all these deficiencies and uncertainties in the amendment as well as the use of only very low dose levels into account, assessment of the study as "not acceptable" by the RMS is maintained.

4th new micronucleus test in mice ([redacted], 1999)

Reference: IIA, 5.4.4/05
Report: [redacted]. 1999 A micronucleus study in mice for glyphosate técnico Nufarm
[redacted]
Data owner: Nufarm

	Study No.: RF-G12.79/99 Date: 1999-12-27 unpublished, ASB2012-11482
Guidelines:	Not specified. Internal SOP M 069 - Micronucleus Test
Deviations:	Not applicable
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: 28/0ctober/1999

Materials and methods

Test material:	
Identification:	GLIFOSATE TECNICO NUFARM
Description:	White powder
Lot/Batch #:	3578/99
Purity:	95 %
Stability of test compound:	No data available
Vehicle and/ or positive control:	Water
Test animals:	
Species:	Mouse
Strain:	Swiss albino
Source:	[redacted]
Age:	7-12 weeks
Sex:	Male / Female
Weight at dosing:	30.22 gg
Acclimation period:	Not specified
Diet/Food:	commercial pelleted diet (Labina, Purina)
Water:	Tap water, ad libitum
Housing:	on wood shavings, in propylene rodent cages (five of the same sex per cage) with stainless mesh lids.
Environmental conditions:	Temperature: 20-24 °C Humidity: 50-60 % Air changes: not specified 12-hour light/dark cycle

In life dates: 28/0ctober/1999

Animal assignment and treatment:
Three levels of the GLIFOSATE TECNICO NUFARM were tested: 187.5, 375 and 562.5 mg/kg bw. These dose levels were claimed to correspond~~ing~~ to 25 %, 50 % and 75 % of the (intraperioneal) LD50 for mice as determined before, with ten animals (five male and five female) per level. The animals were dosed twice with intraperitoneal injections in volumes that were adusted to be 0.45 mL per 30 g bw~~/animal~~ within a 24 hours interval, and sacrificed 24 hours after the second injection. Negative control with water and positive control with cyclophosphamide (1.66 mg/mL in physiological solution, corresponding to 25 mg/kg), were

also applied with the two injections protocol. Mice were killed by cervical dislocation 24 hours after the econd dosing. From the freshly killed animal both femora were removed in total. The bones were then freed from muscle, the distal epiphyseal portion was tom off by gentle traction and the proximal end of the emur was shortened with scissors until a small opening ofthe marrow was visible. The bone marrow cells were gently flushed out with fetal calf serum. After centrifugation at 1,000 rev./min. for 5 min., the bone marrow cells were resuspended in fetal calf serum and smeared on glass slides which were air dried overnight. The following day, the smears were fixed in ethanol 70 % for 10 min. air dried and stained for 20 min. with Eosin-Methylene Blue solution. The slides were coded and observed with a 1,000X magnification objective in a Olympus microscope. The technicians were not allowed to know the corresponding coding in the slides. For each animal 1,000 polychromatic erythrocytes (PCEs) and 1,000 normochromatic erythrocytes (NCEs) were examined for the presence of micronuclei (MN). The relation PCEslNCEs were determined in the first 1,000 PCEs or NCEs enumerated. Differences in the incidence per animal of MNPCEs and MNNCEs per 1000 cells and the relation PCEslNCEs were compared using the Kruskal Wallis test for independent samples. All the tests were compared to the negative control. The criteria for a positive response was the detection of a reproducible and statistically significant (p :0; 0.05) positive response for at least one dose level and the increase in the number of micronuclei to be at least twice the vehicle control. The test is considered valid only if the number of micronuclei in the vehicle control stays within the historic value of the laboratory.

Results and discussion

Mortality: There were no mortalities during the study.
Clinical observations: No systemic or local signs of toxicity were observed during the study period.
Body weight: No significant changes observed.
Necropsy: No macroscopic changes of significance were noted.
Bone marrow evaluations

Table B.6.4-18: Summary of results

	Micronuclei in 1000		**Number of**		
Group	PCE	NCE.	PCE	NCE	PCE/NEC ratio
Vehicle	0.6	0	879	997.7	0.9151
187.5 mg/Kg	0.3	0.1	779.2	978.1	0.81341
375 mglKg	0.6	0.3	871.7	948.4	0.9348
562.5 mg/Kg	0.5	0.3	832.8	987.8	0.8513
Cyclophospha-mide	4.8*	2.0*	648.9	1029.5	0.6296

* p= 0.05, Kruskal Wallis test

Conclusion by the Notifiers

Presence of micronuclei in PCEs and NCEs were similar to control animals. Animals treated with the positive control cyclophosphamide showed a significant increase in micronuclei. Therefore, under the test conditions, the GLIFOSATE TECNlCO NUFARM did not have mutagenic activity in mice.
Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate is not to be classified for this endpoint.

RMS comments:

The study is considered acceptable and the conclusion is agreed with. The real dates for the experimental phase were from 28 October to 10 December 1999.

5th new micronucleus test in mice [redacted], 1996)

Reference:	IIA, 5.4.4/06
Report:	[redacted] 1996 Glyphosate Acid: Mouse Bone Marrow Micronucleus Test [redacted] Data owner: Syngenta Report No.: [redacted]/P/4954 Date: 1996-03-21 not published, TOX2000-1996
Guidelines:	OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000)
Deviations:	The stability, homogeneity and achieved concentration of the test and control substances in the vehicles used were not determined by analysis. The certified purity and stability of the control substances are not available. The above deviations from the current regulatory guideline are considered not to compromise the scientific validity of the study
GLP:	yes
Acceptability:	*See RMS comment*

Dates of experimental work: 1995-10-04 to 1996-03-21

Materials and methods

Test Material:	Glyphosate acid		
Description:	Technical, white solid		
Lot/Batch number:	P24		
Purity:	95.6 % w/w a.i		
CAS#:	Not reported		
Stability of test compound:	Confirmed for the duration of the study.		
Control Materials:			
Negative control (if not vehicle) :	N/A	Final Volume: N/A	Route: N/A
Vehicle:	Physiological saline	Final Volume: 20mL/kg	Route: oral
Positive control :	Cyclo-phosphamide	Final Doses: 65 mg/kg	Route: oral
Test Animals:			
Species	Mouse		
Strain	CD-1		
Age/weight at dosing	6-7 weeks / 22.8-37.6 g		
Source	[redacted]		
Housing	Up to 5/cage		

Test Animals:	
Acclimatisation period	At least 5 days
Diet	CT1 (supplied by Special Diets Services, Stepfield, Witham, Essex, UK *ad libitum*
Water	Mains water *ad libitum*
Environmental conditions	Temperature: 19-23 °C Humidity: 40-70 % Air changes: 15/hour Photoperiod: 12hours dark/12 hours light

Test compound administration:

	Dose Levels	Final Volume	Route
Preliminary: Main Study:	5000 mg/kg 5000 mg/kg	20 mL/kg 20 mL/kg	oral oral

Preliminary Toxicity Assay: A maximum tolerated dose (MTD) was determined, based on patterns of lethalities or severe toxicity observed over a four-day observation period following a single oral dose of 5000 mg/kg.

Micronucleus Test: Male and female mice were weighed and given a single oral dose and sacrificed 24 or 48 hours after dosing as shown in the table below:

Table B.6.4-19: Experimental Design

Treatment	**Dose**	**Number of Animals /Time of kill**	
		24 hours	48 hours
Glyphosate acid	5000 mg/kg	5 male and 5 female	5 male and 5 female
Vehicle control	10 mL/kg	5 male and 5 female	5 male and 5 female
Positive control (cyclophoshamide)	65 mg/kg	5 male and 5 female	

Slide Preparation: All animals were killed by over-exposure to halothane followed by cervical dislocation.

Femurs were removed and stripped clean of muscle. The iliac end of the femur was removed and a fine paint brush was rinsed in saline, wiped to remove the excess and wetted with a solution of albumin (6 % w/v in physiological saline). This was then dipped into the marrow canal and two smears were painted on an appropriately labelled clean, dry microscope slide. This procedure was repeated to give four smears of marrow per slide.

The slides were allowed to air dry and were stained with polychrome methylene blue and eosin using an automatic staining machine.

Slide Analyses: Slides were coded and scored blind. Two thousand immature polychromatic erythrocytes were examined for the presence of micronuclei for each animal. The slides were also examined for evidence of cytotoxicity, which may be manifest by alterations in the ratio of different cell types in the bone marrow. This was assessed by counting the ratio of immature to mature erythrocytes in a sample of 1000 erythrocytes.

Statistics: The incidence of micronucleated polychromatic erythrocytes and percentage polychromatic erythrocytes in the erythrocyte sample, were considered by analysis of variance at 24 and 48 hours, separately for males and females.
Analyses were carried out using the GLM procedure in *SAS (1989)*. Each treatment group mean was compared with the control group mean at the corresponding sampling time using a one-sided Student's t-test, based on the error mean square in the analysis.

Results and discussion
Preliminary toxicity assay: As no clinical signs or lethalities were observed over a four day observation period, at the limit dose level of 5000 mg/kg, this was selected to represent the maximum tolerated dose for both males and females.
Micronucleus test: No adverse reactions to treatment were observed for either males or females dosed with glyphosate acid at the limit dose of 5000 mg/kg.
No statistically or biologically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control values, were observed in either males or females at either sampling time investigated.
No statistically significant differences in the percentage of polychromatic erythrocytes, between the vehicle control and glyphosate acid treated animals, were observed in either males or females at either sampling time investigated.
The test system positive control, cyclophosphamide, induced statistically and biologically significant increases in the frequency of micronucleated polychromatic erythrocytes in both male and female mice at the 24 hour sampling time.

Conclusion by the Notifiers
Glyphosate acid, under the conditions of test, was not clastogenic in the mouse micronucleus test.

RMS comments:
The study is considered acceptable and the conclusion is agreed with.

6th new micronucleus test in mice ([redacted] 2008)

Reference:	IIA, 5.4.4/07
Report:	[redacted] 2008 Glyphosate Technical – Micronucleus Assay in Bone Marrow Cells of the Mouse [redacted] Data owner: Syngenta Report No.: 1158500 Date: 2008-06-09 not published, ASB2012-11483
Guidelines:	OECD 474 (1997): EPA OPPTS 870.5395 (1998): 2000/32/EEC B.12 (2000)
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: 2008-02-11 to 2008-06-09

Materials and methods

Test Material: Glyphosate technical
Description: solid, white
Lot/Batch number: 20070545
Purity: 99.1 % w/w glyphosate (estimated error ± 0.3 %)
CAS#: 1071-83-6
Stability of test compound: not available

Control Materials:			
Negative control (if not vehicle) :	N/A	Final Volume: N/A	Route: N/A
Vehicle:	0.5 % CMC	Final Volume: 20 mL/kg	Route: oral
Positive control :	Cyclophosphamide	Final Doses: 40 mg/kg	Route: oral

Test Animals:
Species mouse
Strain NMRI
Age/weight at dosing 7-8 weeks
Source [redacted]
Housing 1/cage
Acclimatisation period At least 5 days
Diet *ad libitum*
Water tap water *ad libitum*
Environmental conditions Temperature: 19-25 °C
Humidity: 30-70 %
Air changes: 15/hour
Photoperiod: 12hours dark/12 hours light

Test compound administration:			
	Dose Levels	Final Volume	Route
Preliminary: Main Study:	2000 mg/kg b.w. 500, 1000, 2000 mg/kg b.w.	20 mL/kg b.w. 20 mL/kg b.w.	oral oral

Preliminary Toxicity Assay: A maximum tolerated dose (MTD) was determined, based on patterns of lethalities or severe toxicity observed over a two-day observation period following a single oral dose.

Micronucleus Test:

Table B.6.4-20: Experimental Design

Treatment	**Dose**	**Number of Animals /Time of kill**	
		24 hours	48 hours
Vehicle control	10 ml/kg	6*	6*
Positive control	40 mg/kg	6*	
Test substance	2000 mg/kg	6*	6*
Test substance	1000 mg/kg	6*	
Test substance	500 mg/kg	6*	

*: the 6th animal was used as a reserve.

Slide Preparation: All animals designated for bone marrow smears were killed by over-exposure to CO_2 followed by bleeding.
The animals were sacrificed using CO_2 followed by bleeding. The femora were removed, the epiphyses were cut off and the marrow was flushed out with foetal calf serum using a syringe. The cell suspension was centrifuged at 1500 rpm (390 x g) for 10 minutes and the supernatant was discarded. A small drop of the re-suspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald (Merck, D-64293 Darmstadt)/Giemsa (Merck, D-64293 Darmstadt). Cover slips were mounted with EUKITT (Kindler, D-79110 Freiburg). At least one slide was made from each bone marrow sample.

Slide Analysis: Slides were coded and scored blind. Two thousand immature erythrocytes were examined for the presence of micronuclei for each animal. The slides were also examined for evidence of cytotoxicity, which may be manifest by alterations in the ratio of different cell types in the bone marrow. This was assessed by counting the ratio of immature to mature erythrocytes and expressed in immature erythrocytes per 2000 erythrocytes.

Results and discussion
Preliminary toxicity assay: In a pre-experiment 4 animals (2 males, 2 females) received orally a single dose of 2000 mg/kg b.w. glyphosate technical formulated in 0.5 % CMC. The volume administered was 20 mL/kg b.w..
The animals treated with 2000 mg/kg b.w. did not express any toxic reactions.
Micronucleus test: In the main experiment for the highest dose group 12 males received orally a single dose of 2000 mg/kg b.w. glyphosate technical formulated in 0.5 % CMC. For the mid and low doses 6 males per group received orally a single dose of 1000 or 500 mg/kg b.w. Glyphosate Technical formulated in 0.5 % CMC. The volume administered was 20 mL/kg b.w.
Neither the test item treated animals nor those treated with the vehicle control (0.5 % CMC) expressed any toxic reactions.
The mean number of polychromatic erythrocytes was not decreased after treatment with the test item as compared to the mean value of PCEs of the vehicle control, indicating that glyphosate technical did not have any cytotoxic properties in the bone marrow.
In comparison to the corresponding vehicle controls there was no biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item. The mean values of micronuclei observed after treatment with Glyphosate Technical were near to the value of the vehicle control group and within the historical vehicle control range.

Conclusion by the Notifiers
In conclusion, it can be stated that during the study described and under the experimental conditions reported, the test item did not induce micronuclei as determined by the micronucleus test in the bone marrow cells of the mouse.

RMS comments:
The study is considered acceptable and the conclusion is agreed with. It seems that technical material from a different (Chinese) source and of rather high purity was tested.

The following two studies have been provided to the RMS in 2014 and were not part of the original dossier of the GTF. Here, they are described in brief:

7th new micronucleus test in mice ([redacted] 2012)

An *in vivo* bone marrow micronucleus assay was conducted on behalf of DowAgroSciences (DAS study no. 120709) in a laboratory of the [redacted] The study ([redacted] 2012; ASB2014-9277; [redacted] study no. 485-1-06-4696) was performed according to OECD Guideline 474, was GLP-compliant (certified in 2011 by the Dutch Food and Consumer Product Safety Authority) and audited by an internal QAU. The study may be considered acceptable.
Male Swiss albino mice (6 per group) were administered either glyphosate (Lot no. 20061109 of Chinese production, purity 98.9 %) at a dose level of 2000 mg/kg bw/day or the vehicle (vegetable oil) by oral gavage (dose volume 10 mL/kg bw) on two consecutive days. A third (positive control) group of same size received a single intraperitoneal injection of 1 mg mitomycin C/kg bw on day 2 of the study. All animals were sacrificed and slides from femur bone marrow prepared 24 hours following the last treatment. In each animal, at least 2000 polychromatic erythrocytes were scored for the presence of micronuclei.
The mice tolerated the treatment without mortality or exhibiting clinical signs. The ratio of polychromatic to total erythrocytes was nearly the same in the vehicle control and glyphosate-treated groups but depressed in the animals that had received the positive control substance. Treatment with glyphosate did not induce micronuclei in any of the animals (0 % group mean). In the vehicle control group, a mean percentage of 0.033 % of micronucleated polychromatic erythrocytes was noted whereas injection of mitomycin C had caused the expected and statistically significant increase in such cells (2.492 %).
Thus, glyphosate proved negative for clastogenicity under the conditions of this assay.

8th new micronucleus test in mice ([redacted] 2012)

An *in vivo* bone marrow micronucleus assay was conducted on behalf of Syngenta by [redacted]. The GLP-compliant study ([redacted] 2012; ASB2014-9333; Report no. 1479200) was performed according to OECD Guideline and audited by an internal QAU. The study may be considered acceptable.
Male NMRI mice (7 per sampling time in the test and 5 in the negative control groups) were obtained from [redacted] The animals were administered by oral gavage a single dose (dosing volume 20 mL/kg bw) of technical grade glyphosate (batch no. 56753, purity 96.3 %) at a dose level of 2000 mg/kg bw/day or of the vehicle (1 % CMC). A third (positive control) group of 5 mice received a single oral dose of 40 mg cyclophosphamide/kg bw in a dosing volume of 10 mL/kg bw. The animals from the test and negative control groups were sacrificed and slides from femur bone marrow prepared 24 hours or 48 hours following treatment. In the positive control group, sampling was performed only 24 hours after dosing. In each animal, 2000 polychromatic erythrocytes were scored for the presence of micronuclei.
After glyphosate treatment, the animals did not exhibit any clinical signs. The ratio of polychromatic to total erythrocytes was nearly the same in all groups. Treatment with glyphosate did not induce an increase in micronucleus incidence in the polychromatic erythrocytes (0.114 % group mean after 24 h and 0.057 % after 48 h). In the vehicle control group, similar mean percentages of 0.160 % (24 h) and 0.070 % (48 h) were observed. In contrast, 24 h after administration of cyclophosphamide, a mean incidence of 2.010 % was noted proving the validity of the test.
Glyphosate proved negative for clastogenicity under the conditions of this assay.

Rat

1st new micronucleus test in rats ([redacted] 2009)

Reference:	IIA, 5.4.4/02
Report:	[redacted] 2009 Micronucleus test of Glyphosate TC in Bone Marrow Cells of the CD Rat by oral administration [redacted] Data owner: HAG Report No.: LPT 23917 Date: 2009-05-18 Unpublished; ASB2012-11479
Guidelines:	OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000), USA EPA, JMAFF
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: 04/02/2009 – 06/03/2009

Materials and methods

Test material:	
Identification:	Glyphosate TC
Description:	Solid, white powder
Lot/Batch #:	20080801
Purity:	988.0 g/kg
Stability of test compound:	Stable for two years at ambient temperature
Vehicle and/ or positive control:	Vehicle: 0.8 % hydroxypropylmethylcellulose Positive Control: Cyclophosphamide
Test animals:	
Species:	Rat
Strain:	CD
Source:	[redacted]
Age:	Males: 32 – 33 days Females: 33 – 34 days
Sex:	Male and Female
Weight at dosing:	Males: 106 – 132 g Females: 88 – 111 g
Diet/Food:	Commercial ssniff® R/M-H V1534, feeding was discontinued approx. 16 hours befor administration
Water:	Tap water, *ad libitum*
Housing:	Animals were kept in groups of 2 – 3 animals by sex in solid cages with wood flake bedding.

Environmental conditions: Temperature: 19 - 25 °C
Humidity: 30 - 70 %
Air changes: approx. 15/hour
12 hours light/dark cycle

Animal assignment and treatment:
The test was conducted using young male and female CD rats. Groups of five male and five female rats were dosed via oral rout (positive reference item was administered via intraperitoneal route) at 500, 1000 and 2000 mg/kg bw. Sampling was performed after 24 hours for all groups and after 48 hours for the vehicle control and the highest dose group. Both femurs from each rat were dissected, aspirated with foetal calf serum and bone marrow smears prepared following centrifugation and re-suspension. The smears were air-dried, fixed in absolute methanol, stained in Mayers Haemaleum and eosin, allowed to air-dry and coverslipped using mounting medium. 2000 polychromatic erythrocytes (PCE) per animal were scored for the incidence of micronuclei. The ratio of PCE to normochromatic Erythrocytes (NCE) was determined for each animal by counting a total of 1000 erythrocytes.

Results and discussion
Mortality: No mortality occurred.
Clinical observations: No signs of systemic toxicity were noted after administration of glyphosate TC up to the highest reasonable dose level of 2000 mg glyphosate TC/kg b.w. until 48 hours after administration (the last sampling time point).
Evaluation of bone marrow slides:
No test item-related increase of micronucleated polychromatic erythrocytes was observed in the treated groups as compared to the corresponding vehicle control group (see Table B.6.4-21) at the two sampling times. The positive reference item group which received cyclophosphamide (27 mg/kg b.w., i.p.) exhibited a significant increase in the number of micronucleated polychromatic erythrocytes. Historical control data is shown in Table B.6.4-22.

Table B.6.4-21: Summary of results

Treatment group / sampling time	**Number of PCE with micronuclei/2000 PCE (male animals)**		**Number of PCE with micronuclei/2000 PCE (female animals)**	
	Group mean	SD	Group mean	SD
Vehicle control (20 mL/kg) / 48h	2.0	1.9	2.2	1.3
Vehicle control (20 mL/kg) / 24h	1.6	1.1	1.8	0.4
Positive control (27 mg/kg) / 24h	30.2	10.5	24.0	4.9
Glyphosate TC (500 mg/kg) / 24 h	1.0	1.2	1.2	1.3
Glyphosate TC (1000 mg/kg) / 24 h	0.8	0.4	1.6	0.9
Glyphosate TC (2000 mg/kg) / 24 h	1.2	0.8	0.8	0.8
Glyphosate TC (2000 mg/kg) / 48 h	1.6	0.9	0.8	0.8

PCE = polychromatic erythrocytes
SD = standard deviation

Table B.6.4-22: Historical control data

Sex		Group mean ratio PCE/-NCE[#1]	Group mean frequency of micronucleated PCE (per 1000) [#1]	Animals (%) with 0, 1 or more micronucleated PCE (per 1000) [#2]						
				0	1	2	3	4	5	>6
Males	Mean Range	0.87 0.26 – 2.94	1.97 0.4 – 5.7	11.3	34.7	30.0	10.7	6.7	4.0	2.7
Females	Mean Range	0.76 0.32 – 1.47	1.86 0.4 – 4.7	14.0	30.0	21.3	18.7	7.3	5.3	3.3

#1 Average of group means from the most recent background data. Data from 24, 48 and 72 hour samplings are combined.
#2 Individual animal profile based on the above experiments; data from 300 animals.
m male
f female
PCE polychromatic erythrocytes
NCE normochromatic erythrocytes

Conclusion by the Notifiers
The test material glyphosate technical was non-genotoxic.

RMS comments:
The study is considered acceptable and the conclusion is agreed with. Thus, absence of clastogenicity in vivo was also confirmed in the rat.

B.6.4.6 *In vivo* studies in germ cells

For the previous EU glyphosate evaluation, dominant lethal assays in rats and mice had been submitted in which genotoxic effects on germ cells had been examined in. The studies by [redacted] (1992, TOX9551102) in rats and by [redacted] (1980, TOX9552377) in mice may be still considered acceptable. No genotoxcic effect of glyphosate on germinal tissues was found up to the highest dose levels. No new data became available since then.

Table B.6.4-23: *In vivo* germ cell genotoxicty testing of glyphosate acid in rats (*provided by the Notifiers*)

Reference; study identificaion; owner		Type of study	Test organism / test system	Dose levels; batch, purity	Results
Studies from the 2001 evaluation	[redacted] 1992; TOX9551102; (ADM)	DLT	Wistar rats, single oral dose, 10 successive one-week mating periods (1:1 sex ratio)	0, 200, 1000, 5000 mg/kg bw/day; batch 60, 96.8 %	Negative
	[redacted], 1980; TOX9552377; Monsanto	DLT	CD-1 mice, single oral dose; each treated male mated with a total of 16 females over a period of 8 weeks	0, 200, 800, 2000 mg/kg bw/day; XHJ-64, 98.7 %	Negative

DLT = dominant lethal test

B.6.4.7 Genotoxicity of formulations (taking into account published data that were released before 2000)

In contrast to studies with the active ingredient that were, with one possible exception ([redacted] 1993, TOX9551100), negative, findings obtained with formulations were contradictory. This concern has been addressed by the RMS during previous EU evaluation yet (Addendum of 02 August 2000 to the DAR on glyphosate; ASB2013-2748). Because this information is considered relevant also for current re-evaluation, the descriptions and evaluations of studies and published information on mutagenicity of formulations from this addendum have been transferred into this RAR. Afterwards (see B.6.4.8), scientific publications are addressed that have been published since 2000, *i.e.*, after the addendum had been prepared.

Amended copy from the 2000 addendum (ASB2013-2748)
(current study identification by the RMS was amended and enumeration of tables was adjusted, two additional studies are reported)

I. Original studies

A total of eight mutagenicity studies using four different glyphosate formulations was made available to the Rapporteur by the companies Monsanto and Cheminova. For each of these formulations, an Ames test and a mouse bone marrow micronucleus test were submitted. The studies are reliable since they were performed at least to a large extent in compliance with current OECD guidelines (Guideline 471 for bacterial reverse mutation tests and Guideline 474 for mammalian erythrocyte micronucleus tests) under GLP-like conditions. They are all scientifically valid and may be used for risk assessment although the studies with the formulation *Glifos* are considered of limited value for this purpose only. Both test systems are widely accepted for mutagenicity testing of chemicals and respective data for glyphosate active ingredient are available allowing a direct comparison between the active substance and some of its formulations. Unfortunately, these data do not refer to those formulations for which acute toxicity studies have been submitted for purposes of EU re-evaluation of glyphosate.

The studies on *Rodeo®* were submitted as part of the joint dossier of Monsanto and Cheminova since this formulation is considered representative for the glyphosate IPA salt without any further chemicals contained. The six other study reports were kindly provided by Monsanto on request for purposes of this addendum and were not part of the original EU submission.
Following a short characterization of the products investigated, test conditions and results are summarized in Table B.6.4-24 (*in vitro* testing) and Table B.6.4-25 (*in vivo* studies). The individual studies are briefly listed below.

Brief description of formulations tested:
Rodeo® is a formulation containing 54 % glyphosate IPA salt and water but no surfactants. According to information obtained by Monsanto, it is especially intended for aquatic use. The studies have been performed and data submitted to facilitate the assessment of genotoxicity of the IPA salt since in most mutagenicity studies the test material was glyphosate acid.

The *Roundup®* formulation tested by Monsanto *(MON 2139)* is made of 31 % glyphosate (acid equivalents), [redacted] (*MON 0818*, i.e., a surfactant), and water.

The third tested Monsanto product *Direct® (MON 14445)* contains 72% glyphosate acid equivalents formulated as ammonium salt with also a [redacted] (Ethomeen T25, C20-C25 [redacted] surfactant. According to the Rapporteurs database, it is the only glyphosate ammonium salt tested for mutagenicity.

The product called *Glifos* in Brazil (in Europe *Glyphos*) is a formulation of glyphosate manufactured by Cheminova. As indicated by the test facility [redacted], it contains the IPA salt at a concentration of 360 g/L. According to the German national registration data files, the product is made of the IPA salt, the by-product Berol 907, and water.

Overview on mutagenicity studies:

Table B.6.4-24: Genotoxicity studies on herbicidal formulations containing glyphosate - *In vitro* testing in bacteria (Ames test)

Study type	Test material	Test system	Dose range/ Test conditions	Result	Reference
Ames test	Rodeo® (containing IPA salt and water only)	*S. typhimurium* strains TA 98, 100, 1535, 1537	50 - 5000 µg/plate; -/+ S9	Negative; no signs of cytotoxicity	Kier et al., 1992 TOX9552373
Ames test	MON 2139 (Roundup® containing IPA salt, a [redacted] surfactant and water)	*S. typhimurium* strains TA 98, 100, 1535, 1537	5 - 500 µg/plate (-S9)/ 15 - 1500 µg/plate (+S9)	Negative; cytotoxic at the maximum dose levels, occasionally also at lower concentrations	Kier et al., 1992 TOX1999-239
Ames test	MON 14445t (Direct®, containing ammonium salt, a [redacted] surfactant and water)	*S. typhimurium* strains TA 98, 100, 1535, 1537	5 - 500 µg/plate (-S9)/ 15 - 1500 µg/plate (+S9)	Negative; cytotoxic at the maximum dose levels, occasionally also at lower concentrations	Kier et al., 1992 TOX1999-320
Ames test	Glifos formulation (IPA salt, Berol 907 and water)	*S. typhimurium* strains TA 97a, 98, 100 and 1535	1, 10, 100, 1000, 5000 µg/plate; -/+ S9	Negative; cytotoxic at the two upper concentrations	Vargas, 1996* TOX1999-884

* study of limited value for risk assessment only
In all trials, the solvent was distilled water.

Kier, L.D.; Stegeman, S.D.; Costello, J.G. and Schermes, S. (1992, TOX9552373): Ames/*Salmonella* mutagenicity assay of Rodeo®. Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; EHL study no. 91184, Sponsor Project no. ML-91-441. Dates of experimental work: 26 November 1991 - 30 December 1991. GLP: yes (self-certification of the laboratory). A respective statement of the Quality Assurance Unit (QAU) is included. The study is considered acceptable.

Kier, L.D.; Stegeman, S.D.; Costello, J.G. and Schermes, S. (1992, TOX1999-239): Ames/*Salmonella* mutagenicity assay of MON 2139 (ROUNDUP® herbicide formulation).

Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; EHL study no. 91183, Project no. ML-91-440, Report no. MSL-11729. Dates of experimental work: 26 November 1991 - 06 January 1992. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.

Kier, L.D.; Stegeman, S.D.; Costello, J.G. and Schermes, S. (1992, TOX1999-320): Ames/*Salmonella* mutagenicity assay of MON 14445 (DIRECT® herbicide formulation). Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; EHL study no. 91185, Project no. ML-91-442, Report no. MSL-11731. Dates of experimental work: 26 November 1991 - 30 December 1991. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.

Vargas, A.A.T. (1996, TOX1999-884): The *Salmonella typhimurium* reverse mutation by GLIFOS. BioAgri (Biotecnologia Agricola Ltda.), Piracicaba, Sao Paulo, Brazil on behalf of Cheminova; BioAgri Report G.1.1 - 050/96. Dates of experimental work: 12 October 1996 - 23 December 1996. GLP: No. However, a QAU statement is included. The study is considered of limited value for risk assessment only since a legal statement on GLP compliance is lacking and since there were some minor reporting deficiencies in particular regarding the negative (absolute and solvent) and positive control values.

Table B.6.4-25: Genotoxicity studies on herbicidal formulations containing glyphosate - *In vivo* experiments (micronucleus test)

Study type	Test material	Test system	Dose range/ Test conditions	Result	Reference
Micro-nucleus test	Rodeo® for-mulation in 0.9% saline	CD-1 mice (m/f), bone marrow, single i.p. administration	0-850-1700-3400 mg/kg bw; sampling after 24, 48 and 72 h	Negative for chromosome aberrations; overt toxicity (clinical signs, bw↓, death) at the upper dosages	[redacted] 1992 TOX9552376
Micro-nucleus test	Roundup® formulation in 0.9% saline	CD-1 mice (m/f), bone marrow, single i.p. administration	0-140-280-555 mg/kg bw; sampling after 24, 48 and 72 h	Negative (no chromosome aberrations); toxic to mice at 555 mg/kg bw with some deaths occurring, cytotoxic to the bone marrow (PCE/NCE ratio↓ at 48-h sampling) at this top dose level	[redacted] 1992 TOX1999-242
Micro-nucleus test	Direct® formulation in 0.9% saline	CD-1 mice (m/f), bone marrow, single i.p. administration	0-91-183-365 mg/kg bw; sampling after 24, 48 and 72 h	Negative for chromosome aberrations; signs of general toxicity at the top and, although less pronounced, mid dose level	[redacted] 1992 TOX1999-322
Micro-nucleus test	Glifos formulation in distilled water	Swiss albino mice (m/f), two i.p. injections with 24-h inverval	0-68-137-206 mg/kg bw; sampling at 24 h after the second dose	Negative. No indications of cytotoxic effects to the bone marrow. No information regarding general toxicity in the main study.	[redacted], 1996* TOX1999-253

m/f male and female mice used
* study of limited value for risk assessment only

[redacted] (1992, TOX9552376): Mouse micronucleus study of RODEO® herbicide formulation. Monsanto [redacted] on behalf of Monsanto; EHL study nos. 91201 (toxicity range-finding study,

not tabulated above) and 91205 (micronucleus test), Sponsor study nos. ML-91-435/ML-91-438. Dates of experimental work: 13 November 1991 - 26 December 1991. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.

[redacted] (1992, TOX1999-242): Mouse micronucleus study of ROUNDUP® herbicide formulation. [redacted] on behalf of Monsanto; EHL study nos. 91200 (toxicity range-finding study, not tabulated above) and 91204 (micronucleus test), Sponsor study nos. ML-91-434/ML-91-437. Dates of experimental work: 13 November 1991 - 26 December 1991. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.

[redacted] (1992, TOX1999-322): Mouse micronucleus study of DIRECT® herbicide formulation. [redacted] on behalf of Monsanto; EHL study nos. 91202 (two toxicity range-finding experiments, not tabulated above) and 91206 (micronucleus test), Sponsor study nos. ML-91-436/ML-91-439. Dates of experimental work: 13 November 1991 - 26 December 1991. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.

[redacted] (1996, TOX1999-253): A micronucleus study in mice for the product GLIFOS. BioAgri (Biotecnologia Agricola Ltda.), Piracicaba, Sao Paulo, Brazil on behalf of Cheminova; BioAgri Report G.1.2 - 060/96. Dates of experimental work: 08 October 1996 - 19 November 1996. Dose levels were chosen on the basis of a preliminary toxicity test (LD_{50} determination) described in the study report. GLP: No. However, a QAU statement is included. The study is considered of limited value for risk assessment only since a legal statement on GLP compliance is lacking and since there was no information regarding general health effects of treatment to the animals. Therefore, it is not clear whether the highest possible dose was actually reached.

Assessment:
Four glyphosate formulations were tested for mutagenicity in the reverse mutation assay in bacteria as well as *in vivo* by means of the mouse bone marrow micronucleus test. Unequivocally, all these products proved negative in both test systems. Thus, it can be concluded that the formulations Rodeo®, Roundup® (MON 2139), Direct® and Glifos® containing either the IPA or the ammonium salt of glyphosate, alone or in combination with different surfactants, do not cause point (gene) mutations in various *Salmonella typhimurium* strains and are devoid of a clastogenic potential *in vivo*.

However, when the studies of the same type (Ames test and Micronucleus test) for the active substance and the formulations are compared, it becomes obvious that the highest concentrations or dosages to be tested were generally lower with the formulations except Rodeo®. This is apparently due to a higher degree of cytotoxicity as well as of general mammalian toxicity related to the formulations containing other ingredients than glyphosate salt and water.

To facilitate direct comparison of the Ames tests, the respective table from the monograph is reproduced here once more. *(Remark, 2013: Deleted by the RMS since a few of these studies*

that were used for previous EU evaluation are now considered not acceptable. The interested reader is kindly referred to Table B.6.4-1*)*

It is clearly to be seen that much higher concentrations of the active substance could be tested without causing significant cytotoxicity. According to the literature (Chan and Mahler, 1992, TOX9551954), even concentrations up to 10,000 µg/plate have been reached. With the formulations described above, only Rodeo® which is made of glyphosate IPA salt and water could be succesfully tested at such high concentrations. In contrast, strong cytotoxicity avoided meaningful evaluation of mutagenicity of the three other formulations at least at the highest of the selected concentrations and was still to be seen at much lower dose levels. Therefore, it can be assumed that cytotoxicity is due to the surfactants contained but not to glyphosate or its salts. The effects appeared more pronounced with Roundup® and Direct® than with Glifos® suggesting a particularly high cytotoxic activity of tallowamine surfactants.

Regarding the micronucleus tests, a similar pattern becomes apparent. A number of micronucleus studies with glyphosate a.i. in mice is available. However, all these experiments were performed using the oral route. General and cytotoxicity (i.e. bone marrow effects) were confined to very high doses of 4000 or 5000 mg/kg bw corresponding to the known low acute oral toxicity of this compound. The only miconucleus test using the i.p. route (as with the formulations) was performed in rats. The highest dose of 1000 mg glyphosate a.i./kg bw did not cause clastogenicity ([redacted] 1983; TOX9552369, also published by Li and Long, 1988, TOX9500253). As shown by [redacted] (1992, TOX9552373), the IPA salt when dissolved in water (Rodeo® formulation) can be given intraperitoneally to mice at a similar dose level (850 mg/kg bw) without causing neither toxicity nor clastogenic effects. Toxicity was confined to higher dosages (1700 and 3400 mg/kg bw) but genotoxicity was not observed. In contrast, toxicity of the other formulations containing surfactants was much higher although, again, no evidence of a clastogenic effect was obtained [redacted] 1992, TOX1999-242 and TOX1999-322; [redacted] 1996, TOX1999-253).

II. Published literature (before 2000)

During the past few years, a number of studies was published dealing with possible mutagenic effects of glyphosate formulations in different test systems. Scientific assessment of these data is very difficult for at least two reasons.

One main deficiency is the lack of precise description of the test material. Usually, source, composition and/or purity neither of the formulations nor, if tested, of the active ingredient are not stated at all or, at least, not sufficiently reported in the publications. It should be also taken into consideration that different formulations may be marketed in different countries under the same trademark, e.g. Roundup®. Further confusion comes from the fact that sometimes by-products in formulations (e.g. surfactants) were replaced by others but the name of the product was not changed. On request, data on the ingredients were submitted by the manufacturer Monsanto but even this information was not sufficient to clarify all uncertainties about the test substances. However, on the basis of the information available so far, it can be be stated that the Roundup products used in the different published studies were not identical. Thus, it is questionable whether results obtained with one product will apply to others containing different non-active ingredients in different concentrations.

A second point of concern is the frequent use of less validated test systems with no proven relevance of the findings for human health risk assessment even if such systems may be well accepted to predict special environmental hazards. With regard to health effects, there are no current guidelines for these test methods and there is no actual experience how to assess positive findings in such test systems. For other test methods used, OECD guidelines do exist but the experiments were not carried out in compliance with these recommendations.

To facilitate presentation of data, it was decided to start with those experiments for which, in principle, widely agreed guidelines are available. Because of the large background database, the SCE assays were also included here. In the subsequent part of this section, investigations in test systems less frequently used for examination of plant protection products and with no guidelines existing are reported. As a result of this approach, one and the same publication may be referred to repeatedly on different sites.

It should be mentioned that in some publications also experiments are reported which were carried out with glyphosate active substance (i.e., the acid or one of its salts) being the test material. These data were not included in the monograph since the respective publications, for different reasons, were considered unacceptable for evaluation purposes (for justification, see description of experimental conditions below) in particular when the current OECD criteria for assessment of published data were applied. However, the findings are reported in this addendum since a direct comparison between active ingredient and formulation data may be of particular interest.
Although various test systems measuring different endpoints were used, it was tried to summarize the available studies in Table B.6.4-26 (see next pages) to facilitate general overview before the individual publications were discussed in greater detail below. For practical reasons, in particular to facilitate direct comparison, the studies were divided into sections according to the test systems and methods and the experiments separately tabulated.

Table B.6.4-26: Overview on published studies on mutagenicity of glyphosate, its salts and formulations

Test method/ test system	Test material	Dose levels/ Dose range	Results	Remarks	Reference
Ames test in S.typhimurium strains TA98 and TA100 (+/- S9 mix)	Roundup (48% glyphosate IPA; polyoxyethylene [redacted] surfactant)	0 - 1440 µg/plate (calculated as glyphosate IPA salt)	Equivocal. Occasional increase in mutation rate but no clear dose response. Marked cytotoxicity from 360 or 720 µg/plate onwards.	Study not acceptable for evaluation purposes due to serious deficiencies. Reliable assessment avoided by cytotoxicity.	Rank et al., 1993 Z82234
Micronucleus test in mouse bone marrow; single i.p. administration; sampling after 24 or 48 h	Glyphosate IPA salt (1:1 mixture) and Roundup (48% glyphosate IPA; [redacted] surfactant)	0, 100, 150, 200 mg/kg bw (glyphosate IPA); 0, 133, 200 mg/kg bw (Roundup, calculated as IPA salt)	Negative. Indication of dose-related bone marrow cytotoxicity with the Roundup formulation but not with glyphosate IPA.	Supplementary study confirming previous results.	Rank et al., 1993 Z82234
Micronucleus test in mouse bone marrow; two i.p. administrations with a 24-h interval between; sampling after 6 and 24 h after the final dose	Glyphosate a.i. (99.9% pure) and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0, 300 mg/kg bw (2X150 mg/kg bw/d) for glyphosate; 0, 450 mg/kg bw (2x225 mg/kg bw/d) for Roundup	Weakly positive for glyposate after 24 h and for Roundup at both sampling times. Some evidence of bone marrow cytotoxicity of Roundup.	Supplementary study (methodical deficiencies) revealing an increase in micronucleus frequency, data in contrast to previous results.	Bolognesi et al., 1997 Z59299
SCE assay in human lymphocytes	Glyphosate a.i. (99.9% pure)and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0 - 6 mg/ml for glyphosate; 0, 0.1, 0.33 mg/ml for Roundup	Positive for glyphosate from 1 mg/ml onwards and for Roundup at both concentrations. With Roundup, complete cytotoxicity at concentrations >0.33 mg/ml.	Insufficient data. In addition, a positive result in this assay is of equivocal biological significance against the background of more appropriate mutagenicity studies.	Bolognesi et al., 1997 Z59299
SCE assay in human lymphocytes	Roundup (not specified)	0, 250, 2500, 25000 µg/ml	Weakly positive at the low and mid dose level (for one of two donors). Cytotoxic at the high dose.	see comment above	Vigfusson and Vyse, 1980 TOX9700576 / ASB2012-12044
Alkaline elution assay for DNA single-strand breaks and formation of alkali labile sites in DNA obtained from liver and kidneys of mice following single i.p.administration	Glyphosate a.i. (99.9% pure)and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0, 300 (glyphosate a.i.), 900 (Roundup) mg/kg bw; sampling after 4 and 24 h	Weakly positive after 4 h in both organs suggesting possible transient DNA damage.	Supplementary study (methodical deficiencies). Biological significance equivocal. Results in contrast to the negative outcome of the UDS assay. Effects might be also due to toxicity.	Bolognesi et al., 1997 Z59299

Test method/ test system	Test material	Dose levels/ Dose range	Results	Remarks	Reference
Investigations for oxida-tive damage in liver and kidney of i.p. treated mice by measuring the number of 8-OHdG (hydroxydesoxyguanosine) adducts	Glyphosate a.i. (99.9% pure)and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0, 300 (glyphosate a.i.), 900 (Roundup) mg/kg bw (single i.p. administra-tion); sampling after 4 and 24 h	Evidence of stimulation of oxidative metabolism in the liver (only glyphosate) or kidney (only Roundup) after 24 h.	Finding not indicative of mutagenicity but could indicate one possible mechanism of toxicity.	Bolognesi et al., 1997 Z59299
Measuring of DNA adducts by means of ^{32}P-postla-beling technique in the liver and kidney of mice following single i.p. administration	Glyphosate IPA salt and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0, 130, 270 mg/kg bw (glyphosate IPA); 0, 400, 500, 600 mg/kg bw (Roundup)	Weak dose-related increase in adducts with Roundup; no adducts seen with the IPA salt alone and in the control group.	Indication of possible DNA damage, however, biolo-gical significance of this finding equivocal. Further characterization of ad-ducts needed. Toxicity not adressed. However, non-mutagenic toxic effects can also cause DNA adducts.	Peluso et al., 1998 TOX1999-318

Table B.6.4-25: Overview on published studies on mutagenicity of glyphosate, its salts and formulations (continued)

Test method/ test system	Test material	Dose levels/ Dose range	Results	Remarks	Reference
Comet assay for single-strand DNA breaks in tadpole erythrocytes	Roundup (41% glyphosate IPA; [redacted] surfactant)	0-1.69-6.75-27-108 mg/l water	Dose-related effect on DNA at 6.75 and 27 mg/l; completely lethal at 108 mg/l.	Impact of this formulation on tadpole DNA under environmental conditions indicated. Effect could be also due to toxicity. No relevance for human health risk evaluation.	Clements et al., 1997 Z101728
Test for lethal mutations in Drosophila melanogaster after treatment of larvae	Roundup (assumed to contain 41% glyphosate IPA and [redacted] surfactant); Pondmaster (probably made from 41% glyphosate IPA; alkyle sulfate surfactant)	Not specified but indicated to be around LC50 concentration.	Positive.	Not predictive for mutagenicity in mammals. Concentrations used were expected to exhibit high toxicity making evaluation of results very difficult.	Kale et al., 1995 Z73986
Anaphase-telophase allium test for chromosome aberrations in onion root cells	Glyphosate IPA salt (1:1 mixture) and Roundup (48% glyphosate IPA; [redacted] surfactant)	0-720-1440-2880 µg/l (for Roundup calculated as IPA salt)	Roundup: increase in chro-mosome aberrations at the two upper levels indicating rather polyploidy than clastogenicity, no clear dose response Glyphosate IPA: negative	Effects in plant cells not predictive for mutagenicity in mammals. Testing a herbicide for genotoxic effects in plants generally doubtful since cytotoxicity may be expected.	Rank et al., 1993 Z82234
Chromosome aberration (CA) and Sister chromatid exchange (SCE) in human lymphocytes	Glyphosate (purity ≥ 98 %)	0-5.0-8.5-17.0-51.0 µM	Increase in CA and SCE frequency	Increase of SCE not dose related in highest dose group	Lioi et al, 1998a ASB2013-9836
CA and SCE in bovine lymphocytes	Glyphosate (purity ≥ 98 %)	0-17-85-170 µM	Increase in CA and SCE frequency	Increase of SCE not dose related in highest dose group	Lioi et al, 1998b ASB2013-9837

a.e. acid equivalents

Studies in test systems for which guidelines exist and/or much experience is available (i.e. Ames test, micronucleus test, SCE assay)

[redacted] (1993, Z82234) studied the mutagenic potential of the herbicide Roundup and of glyphosate isopropylamine salt in different test systems *in vitro* as well as *in vivo*. An Ames test (plate incorporation test) was performed with Roundup only in the *Salmonella typhimurium* strains TA 98 and TA 100 with and without S-9 mix for metabolic activation. Evidence of mutagenicity was confined to the strain TA 98 under non-activation conditions as indicated by a slight but significant increase in the mean number of revertants at a concentration level of 360 µg/plate (calculated as IPA salt) which was also confirmed in the repeat experiment. With activation, however, no increase in mutation rate was seen up to this dose level. From the next higher concentration (720 µg/plate) onwards, cytotoxicity became apparent with and without metabolic activation avoiding meaningful evaluation. The study authors also reported a positive result for TA 100 in the presence of S9 mix at a concentation of 720 µg/plate but already the next lower dose of 360 µg had markedly reduced the number of revertants as compared to the control suggesting a cytotoxic effect. Furthermore, a dose response was lacking. Thus, the marked increase in mutation frequency at 720 µg/plate is not reliable. In the second experiment, this dose level was not included. Without activation, concentrations from 720 µg/plate onwards appeared cytotoxic. At lower concentrations, no increase in mutation rate was noted with strain TA 100.
In a micronucleus test in mouse bone marrow erythrocytes following single i.p. administration, Roundup as well as the IPA salt (i.e., a 1:1 mixture of glyphosate technical and isoproplyamine) proved negative up to the highest dose of 200 mg/kg bw. However, with Roundup but not with the glyphosate IPA salt alone, there was evidence of bone marrow cytotoxicity at this top dose level as indicated by a significantly lower percentage of polychromatic erythrocytes.

Comment: According to the publication and to further information submitted by Monsanto, it is assumed that the Roundup formulation used was made of 48 % IPA salt, [redacted] *surfactant, and water. The study design of the Ames test does not comply with current guideline requirements, e.g. the plate number scored was inconsistent throughout the study. The data obtained are so controversial that a reliable interpretation is not possible. Unfortunately, a complete confirmatory experiment was not performed since repeated testing was confined to the dose of 360 µg/plate and an additional concentration of 180 µg/plate was included. A more extensive study by* [redacted] *(1992, TOX1999-242, see above in section I) using four S. typhimurium strains including also TA 98 and TA 100 failed to elicit any indications of mutagenicity. This latter trial was conducted in compliance with OECD guideline 471 requirements and is of higher reliability, therefore. Of course, the Roundup formulations tested by Rank and her group and by* [redacted] *were not identical but similar since both contained only the active substance formulated as IPA salt,* [redacted] *surfactant, and water. The cytotoxicity of Roundup was described by both groups but the respective concentrations were different.*

The design of the micronucleus test was also not in compliance with guideline requirements. A direct comparison between results obtained with the IPA salt and Roundup is not feasible since not exactly the same dose levels were used and since there was a difference in sampling time (24 and 48 h post dosing for the IPA experiment versus only at 24 h after administration of Roundup). The negative outcome of previous micronucleus studies with the IPA salt (Rodeo® formulation, [redacted] *1992, TOX9552376) and with a similar Roundup formulation in mice (*[redacted] *1992, TOX1999-242) was confirmed. The reported weak*

bone marrow cytotoxicity occurring already after single i.p. administration of 200 mg Roundup/kg bw (amount calculated as the IPA salt to facilitate comparison) may be considered a possible formulation-related effect when the observations in other micronucleus studies (see section I) are taken into consideration.

In contrast, [redacted] (1997, Z59299) reported positive results from a micronucleus test in mouse bone marrow erythrocytes. Either glyphosate a.i. (declared as 99.9 % pure) or a Roundup formulation were administered to Swiss mice once daily by the i.p. route on two consecutive days. Cell samples were harvested at 6 and 24 hours following the final dose. A weak positive effect was observed at total dose levels of 300 mg/kg bw (2 x 150 mg/kg bw/day) after 24 hours for glyphosate and of 450 mg/kg bw (2 x 225 mg/kg bw/day) at both sampling times for Roundup.
Further data in this publication indicated for high purity glyphosate a significant and dose-dependent increase in SCE frequency in human lymphocyte cultures obtained from two female donors from a concentration of 1000 µg/mL onwards. For Roundup, this effect became apparent even at lower concentrations of 100 and 330 µg/mL.

<u>Comment:</u> The outcome of the micronucleus test with glyphosate a.i. is at least surprising since much higher doses of this compound had been tested before and did not reveal indications of clastogenicity (see section B.5.4.2.1 in the monograph). A direct comparison is not possible since the only available test using the i.p. route in which the highest dose of 1000 mg/kg bw proved negative ([redacted] 1983, TOX9552369) was performed in rats. The respective study by [redacted] (1993, Z82234, see above) was conducted in mice but the test material was glyphosate IPA salt and the dose administered was probably too low for meaningful evaluation. However, a number of well-performed micronucleus tests with oral administration to mice is available. Even when the low oral absorption rate of glyphosate (about 30%) is taken into account, the dose levels (up to 5000 mg/kg bw nominal) are much higher than those given by Bolognesi and her co-workers but no convincing evidence of a potential to cause chromosome aberrations in vivo was obtained. It should be emphasized that the increase in the incidence of micronucleated polychromatic erythrocytes as reported in this publication was rather weak only. The test was not performed according to the current OECD guideline. In particular, the number of animals used (three male mice per dose group) was too low since a group size of at least five is recommended. A dose response cannot be assessed since only one dose level was included. The basis for statistical comparison is questionable since it is not clear when the six control animals were sacrificed because only one group mean value was indicated. Due to these deficiencies, this isolated positive finding is not considered to provide sufficient evidence to contravene the previously obtained negative results regarding the active substance.
The same methodical shortcomings apply to the experiment with the Roundup fomulation. The formulation tested is reported to contain 30.4 % glyphosate acid equivalents. The a.i. is formulated as the IPA salt. Alkyl sulfate surfactant (MON 8080) is also contained (source of information: Monsanto). The weak positive response is in contrast to the beforementioned GLP-like study by [redacted] (1992, TOX1999-242) in which Roundup® proved negative. However, these two Roundup formulations were not identical since the glyphosate concentrations were nearly the same but the surfactants contained were different making a direct comparison of the study results difficult. Little is known on mutagenicity of alkyle sulfate itself, however, MON 8080 proved negative in the Ames test but was clearly cytotoxic at relatively low concentrations (see section III of this addendum). Some evidence of bone marrow cytotoxicity was obtained with both Roundup products as indicated by a decrease in the ratio between polychromatic and normochromatic erythrocytes. Cytotoxicity could have

also an impact on chromosome aberration frequency. An overall, unequivocal conclusion from the experiment of Bolognesi and her group cannot be drawn, however an actual clastogenic response is not very likely. Even if a positive result could be confirmed, it would not be applicable to products containing other surfactants.
A higher SCE frequency is not considered to provide evidence of mutagenicity against the large number of studies in which glyphosate proved clearly negative. The two other studies of this type which have been submitted for purposes of toxicological evaluation of glyphosate ([redacted] 1990, TOX9500269 and [redacted] 1993, TOX9500381, the latter using the IPA salt) did not reveal an increase in sister chromatid exchange frequency but, unfortunately, did not include the high concentrations as tested by the group of Bolognesi (see section B.5.4.1.3 of the monograph). Apart from general doubts about biological significance of a positive result in an SCE assay, some methodical deficiencies became obviously in this publication. For statistical reasons, the number of only two subjects to be included in the study appears too low for meaningful evaluation. Furthermore, the data from two experiments were pooled for the two donors and individual values were not given. Therefore, a possible influence of interindividual variation could not be sufficiently assessed by the reviewer. As shown below, this variation may well reach a considerable level. Again, the positive result obtained with Roundup at least might be also due to cytotoxicity of the formulation avoiding further testing at dose levels exceeding 330 µg/ml since no mitotic cells were present any more.

[redacted] (1980, TOX9700576) also reported a weak but statistically significant increase in SCE frequency in human lymphocytes obtained from two donors when the cultures were exposed to Roundup (not specified) at concentrations of 250 and 2500 µg/mL. At the next higher concentration of 25000 µg/mL, the test substance was absolutely cytotoxic.

<u>Comment:</u> *The reported increase is doubtful since a dose response was seen in the cultures from one of the two donors only. Furthermore, this increase in SCE frequency over the control was weak only and the statistically increased values in the cultures provided from donor 1 were below the control value from donor 2. Furthermore, possible cytotoxicity was not adressed in this paper. Generally, the SCE assay is not accepted to provide convincing evidence of mutagenicity but is rather a screening test. For clarification, the study authors themselves recommended further mutagenicity tests to be conducted.*

[redacted]. (1998a, ASB2013-9836 and 1998b, ASB2013-9837) reported an increase in CA and SCE frequency in human lymphocytes of 3 donors in concetrations between 5 an 51 µM and in bovine lymphocytes between 17 and 170 µM.

<u>Comment:</u> *The results are questionable because a number of well performed and validated studies in vitro im mammalian cells and in vivo in mammals did not register comparable effects even in dose levels more than 10 times higher than the doses used in the studies described by [redacted] (1998a and 1998b, ASB2013-9836 and 1998b, ASB2013-9837). A replication would be needed to confirm such aberrant results.*

<u>Other test systems (Comet assay in tadpole erythrocytes, tests for DNA adducts in rats and mice, *Drosophila melanogaster*, plant cells)</u>

Clements et al. (1997, *Z101728*) investigated the genotoxicity of selected herbicides in *Rana catesbeiana* (bullfrog) tadpoles using the single-cell gel DNA electrophoresis test (`Comet´ assay). After a previous study had shown a higher amount of DNA damage in bullfrog tadpoles inhabiting small bodies of water in agricultural areas as compared to non-agricultural

regions, the impact of Roundup and some other commonly used herbicides on the DNA of tadpole erythrocytes was investigated in this test system under alkaline conditions. This modification allows the detection of single-stranded DNA breaks which are indicated by an increase in length:width ratio of the DNA mass following electrophoresis. DNA was obtained from tadpole erythrocytes (nucleated cells in amphibians) after the animals had been exposed to different concentrations of Roundup in the surrounding water for 24 hours. Whereas the low dose of 1.69 mg/L did not cause evidence of DNA damage, a clear and dose-dependent effect became apparent at the following concentrations of 6.75 mg/L and 27 mg/L. At 27 mg/L, the effect level caused by the positive control substance methylmethanesulphonate (MMS) was already approached. The intended top dose level of 108 mg/L could not be evaluated since all tadpoles died during the exposure period. According to the study authors, the concentrations tested were well below the recommended application levels suggesting an environmental mutagenic hazard in particular for organisms living in small adjacent bodies of water that are usually the first to be affected by pesticide runoff.

Comment: Generally, information on genotoxic effects of pesticides under natural conditions is scarce and, thus, this test system may provide important information regarding environmental effects.
In this special case, however, it appears equivocal whether the observed impact on the DNA was indicative of a true mutagenic effect or rather caused by cytotoxicity. It is known that a positive response in the Comet assay may be not only the result of direct interaction with cellular DNA but can be also mediated by toxic and other effects causing apoptosis or necrosis. Cytotoxicity is not addressed in the publication because it is not directly measured in this test system. A certain degree of general toxicity can be assumed since the highest dose was completely lethal to the tadpoles. This effect could be well in line with the toxicity of certain glyphosate formulations to aquatic organisms as reported in the monograph. The Roundup product tested by Clements et al. was made of 41 % glyphosate IPA salt and MON 0818, i.e. the [redacted] surfactant which is already known to cause toxic effects in different test systems in vitro as well as in vivo. Of course, although there is some evidence of a cytotoxic mechanism behind the positive result in the Comet assay, a direct impact of the test compound on the DNA cannot be completely excluded.
At this time, it is not clear whether a positive result of this test obtained in tadpole erythrocytes, even if it was actually due to mutagenicity, would be of any relevance to human beings exposed. In particular, this is doubtful when the strong body of evidence that neither glyphosate nor its formulations are mutagenic as coming from many studies in various test systems is taken into consideration. Thus, the outcome of the Comet assay should be rather used for environmental hazard evaluation only. Again, the application of results obtained with one formulation to others must be critically regarded.

A possible impact on the DNA was also investigated by [redacted] (1997, Z59299) in further experiments. A transient but significant effect towards DNA damage in liver and kidney was noted in the alkaline elution assay after glyphosate (300 mg/kg bw) or Roundup (900 mg/kg bw) had been administered once by the i.p. route to mice. This assay may indicate the induction of DNA single-strand breaks and alkali labile sites. A test for DNA oxidative damage suggested glyphosate and the formulation Roundup to stimulate oxidative metabolism in the liver (glyphosate) or in the kidney (Roundup) at 24 hours after application.
In a subsequent study from the same institute ([redacted] 1998, TOX1999-318), a low incidence of DNA adducts was found by means of the very sensitive ^{32}P-postlabeling technique in the liver and kidney of mice following single intraperitoneal administration of Roundup. All tested concentrations (400, 500 and 600 mg Roundup/kg bw, corresponding to

122, 152, and 182 mg glyphosate salt/kg bw) caused DNA adducts in both organs. A dose response was to be seen. In contrast, treatment with the vehicle (i.e., a DMSO/olive oil mixture) and with doses of 130 and 270 mg glyphosate IPA salt/kg bw did not result in DNA adduct formation.

Comment: The data from the tests for DNA damage and stimulation of oxidative metabolism ([redacted]., 1997, Z59299) are hardly to interpret since the results are given in summary figures only which are based on pooled individual data. There are reporting inconsistencies, e.g. it is not clear how many animals were actually used for testing. A positive control substance was not included. Taking into account that glyphosate proved negative in the UDS assay which is generally accepted to indicate a more frequent occurrence of DNA damage and repair (see section B.5.4.1.3 in the monograph), the published findings are not considered to provide convincing evidence of an interaction with the DNA. Positive results in the alkaline elution assay may also occur as a result of toxic but not-mutagenic effects. Stimulation of oxidative metabolism is not a sign of mutagenicity but may elucidate a possible mechanism behind toxic effects.
The results of [redacted] (1998, TOX1999-318) and his group suggest a direct effect on the DNA. It has been shown that the observed effects were related to administration of the formulation only but not to glyphosate IPA salt. Biological significance of the results is equivocal. Generally, it is questionable whether findings after i.p. administration can be applied to more realistic exposure conditions. Of course, the occurrence of such effects also after oral intake would be much more relevant for human health evaluation. Furthermore, some deficiencies of this study make a definitive assessment difficult. It is rather equivocal what a low incidence of DNA adducts per animal as compared to no adducts in the control group actually means since a positive control substance was not included. The degree of variation between the animals is not known because only mean values for the groups comprizing of 3 to 6 mice were reported and individual values are not given but would be helpful for interpretation of the results. Another point of concern is the lacking information on toxicity. At least with Roundup, one could expect marked general toxicity when the observations reported from the micronucleus tests (see section I of this addendum) and from the acute intraperitonal toxicity studies (see section B.5.2.4 in the monograph) were taken into account. It is known that DNA adducts may be formed not only as a result of direct interaction of cellular DNA with chemicals but also occur naturally or can be even related to a treatment-dependent increase in endogenous metabolites. Thus, further characterisation of these adducts and clarification of their nature would be desirable.

[redacted] (1995, Z73986) examined nine agricultural chemicals in the sex-linked recessive lethal test in *Drosphila melanogaster* for their ability to cause genotoxic damage to the germ cells leading to lethal mutations in the subsequent generations. The group of test compounds included two insecticides and seven herbicides among those were the glyphosate formulations Roundup and Pondmaster. Unlike the generally used method of feeding the test substance to adult males only, larvae were treated in this experiment. This modification was expected by the study scientists to improve the sensitivity of the test system. All products tested proved positive.

Comment: This test system is not considered predictive for mutagenicity in mammals. Generally, tests in Drosophila are considered helpful for screening purposes. For glyphosate, however, a large database on the basis of much more reliable test systems does exist. Furthermore, since lethal changes in spermatogonia and spermatocytes were the relevant endpoint, it appears difficult to distinguish between mutagenicity and general toxicity. The

dose level tested was not specified but it is stated in the publication that concentrations around the LC^{50} were used. At such a high dosage, some toxicity must be expected.

An anaphase-telophase allium test in onion root cells was conducted by [redacted] (1993, Z82234) to detect a possible induction of chromosome aberrations. The exposure period was 24 hours. In this plant system, a significant increase in the occurrence of chromosome aberrations was noted at the two upper dose levels when Roundup was tested. However, there was no dose response, since the total incidence of aberrations at 1440 µg/L was twice that seen at 2880 µg/L. The authors attributed this lack of a clear dose response to cytotoxicity, however, mitotic index was not dramatically reduced (24.2 in the mean at 2880 µg/L versus 28.2 at 1440 µg/L). According to the investigators, the type and pattern of aberrations suggest rather spindle disturbances than clastogenicity in particular when compared to the effects caused by the positive control substance MMS. In contrast, the glyphosate IPA salt did not increase the frequency of chromosome aberrations in this experiment.

<u>Comment:</u> The Roundup product tested was made of the IPA [redacted] surfactant, and water (for details see description of the Ames test and the micronucleus test portions of this study above). The more pronounced effect of the formulation as compared to the IPA salt could be explained by an improved uptake by the onion root cells as mediated by the surfactant. However, genotoxic or aneugenic effects in a plant system are generally not accepted to be indicative of mutagenicity in mammals. For glyphosate and its formulations, a number of well-performed studies in mammals is available for this purpose. Generally, it appears questionable whether a herbicide should be tested for mutagenicity in a plant cell system since at least a certain degree of cytotoxicity must be expected.

Assessment

In the whole, the published data are not sufficient to provide convincing evidence of mutagenic effects caused by glyphosate or its formulations. Of cours, the effects observed in different test systems cannot be totally ignored. Looking for an explanation, the data obtained in the mutagenicity studies with formulations (see section I) must be also considered. Taking all the findings together, the effects reported in the literature appear rather due to cytotoxic properties of the formulations than to a genotoxic mode of action. The same conclusion was also reached by the Danish EPA in an assessment (Rasmussen, 1997, ASB2013-9671) which was made available to the Rapporteur. It has been already known before, that cytotoxicity is much more pronounced with glyphosate formulations than with the active substance and, therefore, is probably due to by-products or impurities. In particular, surfactants are the agents to be suspected for causing such effects.

There are even data suggesting the possiblity of a direct interaction of glyphosate formulations with cellular DNA in some test systems. This is evidenced by a higher frequency of DNA adducts in mouse liver and kidneys following i.p. administration (Peluso et al., 1998, TOX1999-318) as well as from the Comet assay in tadpole erythrocytes ([redacted] 1997, Z101728). Since glyphosate active ingredient is apparently devoid of a DNA damaging potential (see monograph), these effects, if occurring, can be certainly assumed to be related to co-formulants. Damage to the DNA is not essentially indicative of mutagenicity but could also result from cytotoxicity. Irrespective of the origin of these effects on DNA level, they appear to be confined to very special exposure situations only and not to represent a health hazard to human beings.

III. Mutagenicity testing of surfactants

On the basis of the extensive mutagenicity database for glyphosate a.i. and formulations, the conclusion can be drawn that neither the active substance nor the formulations so far investigated are genotoxic. A certain potential of some formulations to cause damage to the DNA, however, cannot be excluded and might be related to the marked cytotoxic acitivity of these products. Data suggest that cytotoxicity is rather due to certain by-products used as surfactants than to the active ingredient. Thus, it is of particular interest to look at mutagenicity tests performed with such chemicals which are contained in glyphosate formulations mostly with the intention to improve the uptake of the herbicide glyphosate by the target plants. For three different surfactants, studies on mutagenicity of surfactants have been submitted. The studies are summarised in Table B.6.4-27 and briefly listed below.

Table B.6.4-27: Mutagenicity studies with surfactants contained in glyphosate formulations

Study type	Test material	Test system	Dose range/ Test conditions	Result	Reference
Ames test	MON 8080 dissolved in distilled water	S. typhimurium strains TA 98, 100, 1535, and 1537; plate incor-poration and spot test# performed	0.003 - 3.0 µL/plate (+/-S9) in the plate incorporation test	Negative up to 0.9 µl/ plate with and without activation; cytotoxicity occurring at this dose, complete toxicity at 3 µL/plate avoiding counting of revertants	Flowers, 1981 TOX1999-319
Ames test	MON 0818 dissolved in DMSO	S. typhimurium strains TA 98, 100, 1535, 1537; plate incorpora-tion test with/ without metabolic activation	Lowest concentra-tions: 0.3 or 1 µg/plate, diffe-rent maximum amounts per plate reached for the strains, i.e. TA98:300µg (-S9) 1000µg (+S9); TA100 and TA1535: 100µg (+/-S9); TA1537:100µg(-S9) 300µg (+S9)	Negative. Cytotoxic effects occurring at the maximum dose levels avoiding evaluation and occasionally also at lower concentrations. (Mutagenicity data for TA 1535 (+S9) not given probably due to excessive toxicity.)	Stegeman and Li, 1990 TOX1999-241
Ames test	Dodigen 4022 dissolved in distilled water	S. typhimurium strains TA 98, 100, 1535, 1537, 138; E.coli strain WP2uvrA; plate incorporation test	4 µg/plate - 10000 µg/plate (+/-S9)	Negative for mutagenicity. No cytotoxic effects observed.	Stammberger and Mayer, 1992 TOX1999-324
Cytogenetic study for chromosom e aberrations in vitro	Dodigen 4022 dissolved in cell culture medium	Chinese hamster V79 cells	0-600-3000-6000 µg/mL; (+/-S9); 4 h exposure, sampling at 7, 18 and 28 h after start of treatment	Negative for clastogenicity and polyploidy. Reversible inhibition of cell cycle (mitotic index↓) after 7 h at the highest dose (+/- S9). Cell survival rate↓ at 3000 µg/ml and above (only without activation).	[redacted] 1992,TOX199 9-325

Study type	Test material	Test system	Dose range/ Test conditions	Result	Reference
Micronu-cleus test	MON 0818 dissolved in corn oil	CD 1-mice (m/f), bone marrow erythrocytes	0 and 100 mg/kg bw; single i.p. injection; evaluation at 24 and 48 h after dosing	Negative. Also, no indications neither of general toxicity nor of bone marrow cytotoxicity to be observed.	[redacted] 1998* TOX1999-240

The spot test did not provide indications of a mutagenic response, however, does not allow quantitative assessment. This variation of the Ames test is no longer in use in routine genetic toxicology. Therefore, the data are not shown here.
* supplementary study

Flowers, L.J. (1981, TOX1999-319): Ames/*Salmonella* mutagenicity assay of MON 8080. Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; Project no. ML-80-294/800281; Report no. MSL 1538. Dates of experimental work: 31 Oktober 1980 - 28 November 1980. GLP: No. When the study was performed, GLP was not compulsory. However, a Quality Assurance Audit statement is included. The study is considered acceptable.
Stegeman, S.D. and Li, A.P. (1990, TOX1999-241): Ames/*Salmonella* mutagenicity assay of MON 0818. Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; Project no. EHL 89178/ML-89-461; Report no. MSL-10625. Dates of experimental work: 28 November 1989 - 29 January 1990. GLP: Yes (self-certification of the laboratory). A respective Quality Assurance Audit statement is included. The study is considered acceptable.

Stammberger, I. and Mayer, D. (1992, TOX1999-324): Dodigen 4022: Study of the mutagenic potential in strains of *Salmonella typhimurium* (Ames test) and *Escherichia coli*. Pharma Development Central Toxicology, Hoechst AG, Frankfurt/Main, Germany; Study no. 92.0336, Report no. 92.0467. Dates of experimental work: 03 June 1992 - 19 June 1992. GLP: Yes. The study is considered acceptable.

Stammberger, I. (1992, TOX1999-325): Dodigen 4022: Chromosome aberrations *in vitro* in V79 chinese hamster cells. Pharma Development Central Toxicology, Hoechst AG, Frankfurt/Main, Germany; Study no. 92.0337, Report no. 92.1024. Dates of experimental work: 22 July 1992 - 03 November 1992. GLP: Yes. The study is considered acceptable.

[redacted] (1998, TOX1999-240): Mouse micronucleus screening assay of MON-0818. [redacted] on behalf of Monsanto; Project no. EHL 89182/ML-89-463. Dates of experimental work: 06 November 1980 - 05 February 1990. GLP: Not stated in the report. The study is considered supplementary only since it was not in compliance with OECD recommendations for tests of this type. In particular, the only dose level used was too low for definitive assessment.

Furthermore, the notifier Monsanto submitted to the Rapporteur published data suggesting that also the sorbitol ester surfactants Tween 20 and Tween 80 proved negative in either the mouse lymphoma test or in the Ames test and the mouse micronucleus test, respectively. However, since these co-formulants were not contained in the glyphosate formulations for which mutagenic effects had been reported, the respective data were not reviewed in detail.

Assessment

The available studies clearly show a lack of mutagenicity of the tested surfactants in the limited number of test systems used confirming the negative outcome of respective studies with glyphosate formulations. In contrast, marked cytotoxicity was caused in the Ames test by the [redacted] surfactant MON 0118 as well as by the alkyle sulfate surfactant MON 8080 suggesting that cytotoxicity observed in mutagenicity testing of formulations are mainly due these surfactants. This assumption is supported by the result of an Ames test using the surfactant-free Rodeo® formulation ([redacted] 1992, TOX9552373) with no signs of cytotoxicity occurring. The more recently introduced surfactant Dodigen 4022 proved non-cytotoxic in the Ames test and caused cytotoxic effects in V79 cells at very high concentrations only.

It is widely accepted that cytotoxicity of a compound can result in positive results in mutagenicity assays and it is often difficult clearly to distinguish between true substance-related genotoxic effects and "mutagenicity" mediated by excessive cytotoxicity. A close relation between cytotoxicity and mutagenicity became apparent also in the chromosome aberration test with Dodigen 4022 ([redacted] 1992, TOX1999-325). The markedly reduced mitiotc index at the first sampling time indicating an adverse effect at least of high doses (only the top dose concentration of 6000 µg/mL and the solvent control were assessed after 7 hours) was accompanied by a slight increase in chromosome aberration frequencies including and excluding gaps in the absence as well as in the presence of the metabolically activating S9 mix. However, at the later sampling times (18 and 28 h following substance application), the mitotic index had normalised again and there was no increase in the incidence of chromosome aberrations any more. Therefore, the test substance was considered negative in this test system. This example provides further evidence that suspected mutagenic effects of formulations as reported in section II might be readily due to cytotoxicity.

These results are in line with data suggesting a higher toxicity and irritancy of certain surfactants as compared to the active substance and to formulations as reported in chapter B.5.11 in the monograph. It can be also assumed that specific adverse effects of surfactants might have significantly contributed to the rather unexpected mammalian toxicity of some glyphosate formulations. Despite the low general toxicity of glyphosate technical, a number of poisoning incidents in humans sometimes resulting in death was reported in particular from asian countries (see chapter B.5.9 in the monograph).

Severe intoxication was mainly characterised by a decrease in blood pressure and further cardiovascular symptoms followed by pulmonary dysfunction and renal failure and by signs of irritation in the gastointestinal tract. Pathophyiology of poisoning is assumed to include irritation or corrosion of the intestinal mucosa resulting in electrolyte imbalances, hypovolemic shock and disturbances in the cardiovascular system. The respiratory signs, as well as renal symptoms, are considered secondary to this mechanism beeing caused either by pulmonary edema related to disturbed circulation or by aspiration pneumonia following emesis ([redacted], 1987, Z35531; see also monograph, chapter B.5.9). There is evidence that the first step, i.e. damage to gut mucosa, might be primarily caused by [redacted] surfactants due to their irritating properties.

Of course, the clinical reports on human posionings with glyphosate formulations are often difficult to interpret since most of the severe intoxications were attempts of suicide. In such cases, also the frequent concomitant intake of drugs and alcohol should be considered.

However, the hypothesis of surfactant effects being involved is further supported by mechanistic and pharmacological studies (see section B.5.8.2.3 in the monograph) suggesting

that the acutely toxic effects may be caused by the [redacted] surfactant alone, too, and that toxicity may be even enhanced when complete Roundup formulations were tested.

Furthermore, according to the information available to the Rapporteur, the cases of severe or even fatal intoxication were related to the ingestion of glyphosate products containing [redacted] surfactant. Sawada and Nagai (1987, Z35531) reported two cases of human poisonings with surfactants causing clinical signs resembling very much those observed after ingestion of large amounts of Roundup.

A possible potentiation of toxicity of glyphosate IPA salt and POEA in animals was reported by [redacted] (1991, Z80636) who tested the acute oral toxicity of Roundup formulations in rats. Using the intratracheal route of administration being of clinical relevance in cases of aspiration, the same authors observed a marked toxic effect of Roundup and of POEA alone to the lungs but this was much less pronounced with Polysorbate-80, i.e. another non-ionic surfactant.
Mucosal irritation in the respiratory tract caused by [redacted] surfactant may be also behind the much lower threshold level for adverse effects of a Roundup formulation as compared to glyphosate a.i. upon subacute inhalative exposure (see section B.5.3.3.2 in the monograph, also reported by WHO/IPCS in 1994, TOX9500301).

A statement of the notifier Monsanto was submitted to the Rapporteur in October, 1998. In this paper, it is suggested that the toxic and cytotoxic effects of polyoxyethylenamine (POEA) were responsible for the observed adverse effects on health and environment. Since it is an important objective to use environmentally safe and less toxic products, the polyoxyethylen [redacted] surfactants were replaced at least in some Monsanto products by others. The company stated that this decision was mainly based on the eye irritation potential and the aquatic toxicity related to the formerly used substances. Accordingly, in the formulations for which toxicological data have been submitted as part of the joint dossier of Monsanto and Cheminova, surfactants of this type are not contained any more. Indeed, cytotoxicity of other surfactants, e.g. Dodigen 4022, and their potential to cause acutely toxic or irritating effects are much lower as compared to POEA.

Thus, it can be expected that the replacement of toxic and irritating surfactants like POEA by other and less critical substances may reduce the risk of death or severe health effects following intentional or accidental ingestion of glyphosate products as well as the severity of eye or respiratory tract irritation.

Recently, the notifier Monsanto provided a new assessment explaining that POEA is a group of chemicals not all capable of causing adverse effects. It is suggested that only particular substances belonging to this group might be responsible for the toxic effects described in this addendum. However, since this is clearly relevant for assessment of formulations but not for health evaluation of the active ingredient, this item hould be considered on a Member state level.

B.6.4.8 Published data (released since 2000)

B.6.4.8.1 Introduction

An earlier review of the toxicity of glyphosate and the original Roundup™ formulation concluded that neither glyphosate nor the formulation pose a risk for the production of

heritable/somatic mutations in humans (Williams et al., 2000, ASB2012-12053). This review of subsequent glyphosate genotoxicity publications includes analysis of study methodology and incorporation of all the findings into a weight of evidence for genotoxicity. Two publications provided limited additional support for the conclusion that glyphosate and glyphosate based formulations (GBFs) are not active in the gene mutation assay category. The weight of evidence from *in vitro* and *in vivo* mammalian chromosome effects studies supports the earlier conclusion that glyphosate and GBFs are predominantly negative for this end point category. Exceptions are mostly for unusual test systems but there are also some unexplained discordant positive results in mammalian systems. Several reports of positive results for the SCE and comet DNA damage endpoints have been published for glyphosate and GBFs. The data suggest that these DNA damage effects are likely due to cytotoxic effects rather than DNA reactivity. This weight of evidence review concludes that there is no significant *in vivo* genotoxicity and mutagenicity potential of glyphosate or GBFs that would be expected under normal exposure scenarios.

B.6.4.8.2 General review and analysis considerations

The published studies for review consideration were identified by literature searches for published reports containing references to glyphosate or glyphosate based formulations (GBFs) that also contained searchable terms which indicated that genotoxicity studies were performed. Literature search utilised Chemical Abstracts (provided by Chemical Abstracts Service, a division of the American Chemical Society) and Web of Knowledge (Thompson Reuters), using the following modules: Web of Science^{SM}, BIOSIS Previews®, MEDLINE®, and CAB Abstracts® (CABI) abstracting services. Search criteria were as follows (glyphosate acid and the various salts): glyphosat* OR glifosat* OR glyfosat* OR 1071-83-6 OR 38641-94-0 OR 70901-12-1 OR 39600-42-5 OR 69200-57-3 OR 34494-04-7 OR 114370-14-8 OR 40465-66-5 OR 69254-40-6 OR (aminomethyl w phosphonic*) OR 1066-51-9. Each identified publication was evaluated to verify that it contained original results of one or more genotoxicity studies on glyphosate or GBFs. Emphasis was placed on publications in peer-reviewed journals and abstracts or other sources with incomplete information were not considered. Reviews without original data were not considered for evaluation; however, these reviews were examined to determine if there were any cited publications that had not been detected in the literature searches.

Each relevant publication was examined using several criteria to characterize the scientific quality of the reported genetic toxicology studies. Useful, objective criteria for this purpose were international guidelines for genetic toxicology studies developed by expert groups. These include principles for conducting studies, reporting results and analyzing and interpreting data. Some of the principles of the guidelines are generally applicable to categories of studies or all studies while others are specific for a particular type of test system and end point. Some of the specific types of studies encountered in the review do not yet have international guidelines; however, some of the guideline elements should be generically applicable to these studies. The guidelines for genetic toxicology tests developed for the Organisation for Economic Cooperation and Development (OECD) are a pre-eminent source of internationally agreed and expert guidelines. Other regulatory international and national regulatory genetic toxicology testing guidance are usually concordant with the OECD guidelines. Table B.6.4-28 presents some key OECD guideline criteria that were found to be relevant to analysis of the studies considered in this review.

Comparison of the published studies to the criteria in guidelines used for regulatory purposes does not represent an absolute judgment standard but it does serve to provide one means of

characterization of the various published studies. Some of the criteria are rarely met in scientific publications. For example, data for individual cultures and individual animals are not commonly included in publications in scientific journals. These data are presumably collected but are usually summarised as means with a measure of variance for the treatment and control groups. This is not considered to be a significant omission in a scientific publication. However, other guideline features are more essential in demonstrating scientific quality standards and should be considered as having greater weight in evaluating a study. For example, there are consistent recommendations that assays involving visual scoring (e.g. chromosome aberration, micronucleus and sister chromatid exchange) should use slides that are independently coded so that scoring is performed without knowledge of the treatment or control group being scored. This guidance is good scientific practice and studies that do not include a description of coding or "blind" scoring in the methodology would appear to have a deficiency either in the methodology or the description of the methodology used. Other examples of guideline features that have clear experimental scientific value are the use of concurrent negative and positive controls and concurrent measurement and reporting of toxicity endpoints in main experiments, especially in *in vitro* mammalian cell assays.

Test materials, as described in the publications, were reviewed by industry experts to identify any publicly available and useful information on composition for the reported formulations to assist in interpreting the relevance of findings to glyphosate and/or formulation components. It should be noted that a common problem encountered in the published literature is the use of the terms "glyphosate", "glyphosate salt" or "Roundup" to indicate what may be any GBF that contains additional components such as surfactants. Published results from studies with different formulations have sometimes been incorrectly or inappropriately attributed to the active ingredient. The original Roundup formulation (MON 2139), containing 41 % isopropyl amine glyphosate salt and 15.4 % MON 0818 (a polyethoxylated [redacted] based surfactant blend), is no longer sold in many markets. However, other glyphosate based formulations are sold under the Roundup brand name with varying glyphosate forms, concentrations and surfactant systems. Clear identification of the test material is very important in toxicology studies because toxicity of formulations can be dramatically different than the active ingredient. The fact that test materials identified as Roundup formulations may actually have different compositions should be considered when comparing results of different studies. A major consideration, especially for DNA damage endpoints and for *in vitro* mammalian cell assays, is an assessment of whether observed effects might be due to toxicity or extreme culture conditions rather than indicating DNA-reactive mediated processes. Relevant considerations include control of medium pH and osmolality for *in vitro* mammalian cell studies and whether effects are observed only at cytotoxic doses or in association with severe toxicity to the test system. Other important generic considerations in evaluating experimental results of each published study are evidence of experimental reproducibility and whether a biologically plausible dose response has been demonstrated.

Table B.6.4-28: Genetic Toxicology Test Guideline Criteria

Area	Guidance	Reference
All studies	Test material purity and stability should be reported	OECD 471 (1997) OECD 473 (1997)
	Concurrent negative and positive controls should be included with each assay	
Assays with visual scoring	All slides should be independently coded before analysis (i.e. scored without knowledge of the treatment or control group)	OECD 473 (1997) OECD 479 (1986)
In vitro mammalian cell assays	Assay should be usually be conducted in the presence and absence of an appropriate exogenous metabolic activation system	OECD 473 (1997)
	Cytotoxicity should be determined in the main experiment	

	At least three analyzable concentrations should be used	
	Maximum dose determined by toxicity or 5 µg/ml, 5 mg/ml or 10 mM for soluble non-toxic test materials	
	Individual culture data should be provided	
In vivo mammalian assays	Five analyzable animals per group. Single sex may be used if there are no substantial difference in toxicity between sexes	OECD 475 (1997) OECD 474 (1997)
	Limit dose for non-toxic substances of 2000 mg/kg for treatments up to 14 days and 1000 mg/kg for treatments longer than 14 days	
In vitro chromosome aberration	Treatment for 3-6 hours in one experiment and harvest at 1.5 cell cycles. If negative a second experiment with continuous treatment for 1.5 cell cycles	OECD 473 (1997)
	Scoring of at least 200 metaphases ideally divided between duplicate cultures	
In vitro sister chromatid exchange	Treatment for 1-2 hours up to two cell cycles with harvest after two cell cycles in the presence of bromodeoxyuridine	OECD 479 (1986)
	Scoring of 25 metaphases per culture (50 per treatment group)	
In vitro micronucleus	Most active agents detected by treatment for 3-6 hours with harvest at 1.5-2 cell cycles after treatment. An extended treatment for 1.5-2 cycles in the absence of metabolic activation is also used	OECD 487 (2010)
	Scoring of at least 2000 binucleated cells or cells for micronuclei for each treatment or control group	
In vivo bone marrow chromosome aberration	Single treatment with first harvest at 1.5 cell cycles after treatment and second harvest 24 hour later or single harvest 1.5 cycles after last treatment for multiple daily treatments	OECD 475 (1997)
	Three dose levels usually recommended except when limit dose produces no toxicity	
	Concurrent measures of animal toxicity and toxicity to target cells	
	At least 100 cells analyzed per animal	
	Individual animal data should be reported	
In vivo erythrocyte micronucleus	Three dose levels for first sampling time	OECD 474 (1997)
	Treatment once with at least 2 harvests usually at 24 and 48 h after treatment or one harvest 18-24 h after final treatment if two or more daily treatments are used	
	Scoring of 2000 immature erythrocytes per animal or 2000 mature erythrocytes for treatments of 4 weeks or longer	

Table B.6.4-29 presents a summary of genotoxicity test results for glyphosate and GBFs published subsequent to Williams et al. (2000, ASB2012-12053). Test results are organised by the major genotoxicity assay categories of gene mutation, chromosome effects and DNA damage and other end points. Major features presented for each publication are the assay endpoint, the test system, the test material, the maximum dose tested and comments relevant to the reported conduct and results of the assay. For brevity, earlier reviewed individual publications of genotoxicity study results are referred to by citation of (Williams et al., 2000, ASB2012-12053) rather than the original references reviewed in (Williams et al., 2000, ASB2012-12053).

Table B.6.4-29: Genetic toxicology studies of glyphosate and glyphosate formulations published on or after 2000

End point	Test System	Test Material	Maximum Dose	Result	Comment[a]	Reference
In Vitro Gene Mutation						

End point	Test System	Test Material	Maximum Dose	Result	Comment[a]	Reference
Point mutation	**Ames strains**	**Perzocyd 10 SL formulation**	**2 µg/plate (toxic)**	**Negative**	**TA1535 not used**	**Chrusciels ka et al., 2000, (ASB2013-9830)**
Wing spot test	**Drosophila**	**glyphosate (96%)**	**10 mM in larval stage**	**Negative/ inconclusive [c]**	**Negative or inconclusive in crosses not sensitive to recombination events**	**Kaya et al., 2000, (ASB2013-9832)**
In Vitro Chromosome Effects—Mammalian Systems						
Cytokinesis block micronucleus	**Bovine lymphocytes**	**Glyphosate formulation (62% glyphosate Monsanto source)**	**560 µM 48 h –S9**	**Positive?**	**PH, MA, SC, TO**	**Piesova, 2004 (ASB2012-12001)**
Cytokinesis block micronucleus	**Bovine lymphocytes**	**Glyphosate formulation (62% glyphosate Monsanto source)**	**560 µM 48 h –S9 2 h –S9 2 h +S9**	**Positive? Negative Negative**	**PH, SC, TO**	**Piesova, 2005 (ASB2012-12000)**
Chromosome aberration	**Mouse spleen cells**	**herbazed formulation**	**50 µM?**	**Positive**	**Concentrations used not clear. PH, MA, SC, TO, RE**	**Amer et al., 2006 (ASB2012-11539)**
Chromosome aberration	**Bovine lymphocytes**	**Glyphosate formulation (62% glyphosate) Monsanto source**	**1.12 mM (toxic) (24 h)**	**Negative**	**Chromosome 1 FISH analysis. PH, MA, PC, SC, TO, RE**	**Holeckova, 2006 (ASB2012-11847)**
Chromosome aberration	**Bovine lymphocytes**	**Glyphosate formulation (62% glyphosate) Monsanto source**	**1.12 mM (toxic) (24 h)**	**Negative**	**PH, MA, SC, RE**	**Sivikova and Dianovsky, 2006 (ASB2012-12029)**
Chromosome aberration	**Human lymphocytes**	**Glyphosate (96%)**	**6 mM (not toxic)**	**Negative**	**MA, IC, RE**	**Manas et al., (2009 ASB2012-11892)**
Cytokinesis block micronucleus	**Human lymphocytes**	**Glyphosate (technical, 96%)**	**580 µg/mL (toxic) (est. 3.43 mM)**	**Negative (-S9) Positive (+S9)**	**SC, RE**	**Mladinic et al., 2009 (ASB2012-11906)**
Cytokinesis block micronucleus	**Human lymphocytes**	**Glyphosate (technical, 96%)**	**580 µg/mL (toxic) (est. 3.43 mM)**	**Negative (-S9) Positive (+S9)**	**SC, RE**	**Mladinic et al., 2009 (ASB2012-11907)**
In Vitro Chromosome Effects— Non Mammalian Systems						
Chromosome aberration	**Onion root tip meristem**	**Roundup formulation (Bulgaria)**	**1% active ingredient (estimated**	**Negative**	**TO, IC, RE**	**Dimitrov et al., 2006 (SB2012-**

End point	Test System	Test Material	Maximum Dose	Result	Comment[a]	Reference
			4.4-5.9 mM)			**11607)**
Micronucleus	**Onion root tip meristem**	**Roundup formulation (Bulgaria)**	**1% active ingredient (estimated 4.4-5.9 mM)**	**Negative**	**TO, RE**	**Dimitrov et al., 2006 (SB2012-11607)**
In Vivo Chromosome Effects—Mammalian Systems						
Bone marrow erythrocyte micronucleus	**Mouse**	**Glyphosate**	**300 mg/kg i.p.** **Perzocyd 10 SL formulation**	**Negative** **Negative**	**DL, TO, SC, IM, RE** **DL, TO, SC, IM, RE**	[redacted] **2000, (ASB2013-9830)**
Bone marrow erythrocyte micronucleus	**Mouse**	**Roundup 69 formulation**	**2 x 200 mg/kg i.p.**	**Negative**	**TO, SC, IE, RE**	[redacted] **2000 (ASB2013-11477)**
Bone marrow erythrocyte micronucleus	**Mouse**	**Roundup™ formulation (Monsanto)**	**2 x 200 mg/kg i.p.**	**Negative**	**TO, SC, IE, RE**	[redacted], **2002 (SB2012-11834)**
Bone marrow Chromosome aberration	**Rabbit**	**Roundup™ formulation**	**750 ppm in drinking water**	**Positive?**	**DL, PC, TO, SC, IC**	[redacted] **2005 (ASB2012-11841)**
Bone marrow Chromosome aberration	**Mouse**	**Herbazed formulation (84% glyphosate)**	**50 mg/kg i.p. (1,3, 5 days)** **100 mg/kg oral (1,7, 14, and 21 days)**	**Negative** **Positive**	**TO, SC, RE**	[redacted] **2006 (ASB2012-11539)**
Spermatocyte Chromosome aberration	**Mouse**	**Herbazed formulation (84% glyphosate)**	**50 mg/kg i.p. (1,3, 5 days)** **100 mg/kg oral (1,7, 14, and 21 days)**	**Negative** **Positive**	**TO, SC, RE**	[redacted] **2006 (ASB2012-11539)**
Bone marrow Chromosome aberration	**Mouse**	**Roundup formulation (Bulgaria)**	**1080 mg/kg p.o. (1/2 LD50)**	**Negative**	**DL, TO, IC, RE**	[redacted] **2006 (ASB2012-11607)**
Bone marrow erythrocyte micronucleus	**Mouse**	**Analytical glyphosate (96%)**	**2 x 200 mg/kg i.p.**	**Positive**	**Erythrocytes scored?** **TO, SC, IC, RE**	[redacted] **2009 (ASB2012-11892)**
Bone marrow	**Mouse**	**Roundup™**	**50 mg/kg**	**Positive**	**DL, SC, IC, RE**	[redacted]

End point	Test System	Test Material	Maximum Dose	Result	Comment[a]	Reference
Chromosome aberration		**formulation (Monsanto)**	**i.p.**			**[redacted], 2009 (ASB2012-12005)**
In Vivo Chromosome Effects—Non-Mammalian Systems						
Erythrocyte micronucleus	**Oreochromis niloticus (Tilapia)**	**Roundup 69**	**170 mg/kg i.p. (maximum tolerated)**	**Negative?[c]**	**TO, RE**	**[redacted] 2000 (ASB2013-11477)**
Wing spot test	**Drosophila**	**Glyphosate (96%)**	**10 mM in larval stage**	**Positive/inconclusive[b]**		**[redacted] 2000 (ASB2013-9832)**
Erythrocyte micronucleus	**Tilapia**	**Roundup™ formulation (Monsanto)**	**170 mg/kg (abdominal injection)**	**Positive**	**TO, RE**	**[redacted] 2002 (ASB2012-11834)**
Erythrocyte micronucleus	**Crasseus auratus (goldfish)**	**Roundup formulation**	**15 ppm glyphosate in water (2, 4 and 6 days)**	**Positive**	**TO, IE, RE**	**[redacted] 2007 (ASB2012-11587)**
	Prochilodus lineatus (tropical fish)	**Roundup™ formulation (75% of 96 h LC50)**	**10 mg/l (6, 12 and 24 h) in water**	**Negative**	**DL, TO, SC, RE**	**[redacted] 2008 (ASB2012-11586)**
Erythrocyte micronucleus	**Caiman eggs**	**Roundup® Full II formulation**	**1750 ug/egg**	**Positive**	**RE**	**[redacted] 2009 (ASB2012-12002)**
Erythrocyte micronucleus	**Caiman eggs**	**Roundup® Full II formulation**	**Sprayed 2x with 100 litres of 3%/ha 30 days apart**	**Positive**	**DL, TO, RE**	**[redacted] 2009 (ASB2012-12002)**
Micronucleus (and alkaline SCGE)	**Fish (Guppy)**	**Roundup® Transorb**	**5.65 µg/l**	**Positive**		**[redacted] 2013 (ASB2014-7617)**
In Vitro DNA Damage Mammalian Systems						
Alkaline SCGE	**GM38 human fibroblasts and HT1090 human fibrosarcoma**	**Glyphosate (technical grade)**	**6.5 mM**	**Positive**	**MA, PH, TO, SC, RE**	**Monroy et al., 2005 (ASB2012-11910)**
Sister chromatid exchange	**mouse spleen cells**	**herbazed formulation**	**50 µM?**	**Positive**	**Concentrations used not clear MA, PH, TO, SC, RE**	**Amer et al., 2006 (ASB2012-11539)**
Sister chromatid exchange	**bovine lymphocytes**	**Glyphosate formulation (62%**	**1.12 mM (toxic)**	**Positive**	**PH, SC, RE**	**Sivikova and Dianovsky,**

End point	Test System	Test Material	Maximum Dose	Result	Comment[a]	Reference
		glyphosate, Monsanto)				**2006 (ASB2012-12029)**
Alkaline single cell gel electrophoresis (SCGE, comet)	**Hep-2 cells**	**Glyphosate (analytical, 96%)**	**7.5 mM (limited by toxicity)**	**Positive**	**MA, PH, RE**	**Manas et al., 2009 (ASB2012-11892)**
Alkaline SCGE	**Human lymphocytes**	**Glyphosate (technical, 96%)**	**580 µg/ml (toxic) (est. 3.43 mM)**	**Positive (-S9) Positive (+S9)**		**Mladinic et al., 2009 (ASB2012-11906)**
SCGE	**Human lymphocytes (compared with Tilapia erythrocytes and Tradescantia nuclei)**	**Glyphosate (96%)**	**700 µM**	**Positive (according to authors)**	**Inconsitent and not clear dose dependent**	**Alvarez-Moya et al., 2014 (ASB2014-6902)**
SCGE	**Human buccal epithelial cells**	**Glyphosate (95%) and Roundup Ultra Max**	**200 mg/l**	**Positive**	**Higher activity of formulation than pure a. s.**	**Koller et al., 2012 (ASB2014-7618)**
In Vitro DNA Damage Non-Mammalian Systems						
SOS	**E. coli**	**Roundup BIO formulation**	**2.5 ug/sample**	**Positive**		**Raipulis et al. 2009 (ASB2012-12008)**
Alkaline SCGE	**Tradescantia flowers and nuclei**	**Glyphosate(technical, 96%)**	**700 µM**	**Positive**	**PH, SC**	**Alvarez-Moya et al., 2011 (ASB2012-11538)**
In Vivo DNA Damage Mammalian Systems						
Spermatocytes and bone marrow	**Mouse**	**herbazed formulation (84% glyphosate)**	**200 mg/kg p.o.**	**Positive**	**TO, SC, RE**	**Amer et al., 2006 (ASB2012-11539)**
SCGE blood cells, liver cells,	**Mouse**	**Glyphosate (96%) and AMPA**	**400 mg/kg bw/day Glyphosate or 100 mg/kg bw/day AMPA**	**Glyphosate and AMPA positive**		**Manas et al., 2013 (ASB2014-6909)**
In Vivo DNA Damage Non-Mammalian Systems						
Erythrocyte alkaline SCGE	**Crasseus auratus (goldfish)**	**Roundup formulation**	**15 ppm glyphosate in water (2, 4 and 6 days)**	**Positive**	**TO, RE**	[redacted] **2007 (ASB2012-11587)**
Erythrocyte and gill cell alkaline SCGE	**Prochilodus lineatus (tropical fish)**	**Roundup™ formulation (75% of 96 h LC_{50})**	**10 mg/l (6, 12 and 24 h) in water**	**Positive**	**DL, TO, RE**	[redacted] **2008 (ASB2012-11586)**
Erythrocyte	**Caiman**	**Roundup®**	**1750**	**Positive**	**RE**	[redacted]

End point	Test System	Test Material	Maximum Dose	Result	Comment[a]	Reference
alkaline SCGE	**eggs/hatchlings**	**Full II formulation**	**µg/egg**			**[redacted]., 2009 (ASB2012-12002)**
Erythrocyte alkaline SCGE	**European eel**	**Roundup formulation**	**166 µg/liter**	**Positive**	**DL, SC, RE**	**[redacted] 2010 (ASB2012-11836)**
Erythrocyte alkaline SCGE	**Caiman eggs/hatchlings**	**Roundup® Full II formulation**	**Sprayed 2x with 100 l of 3%/ha 30 days apart**	**Positive**	**DL, RE**	**[redacted] 2009 (ASB2012-12002)**
SCGE blood cells	**European eel**	**Roundup Ultra and Glyphosate and POAE**	**116 µg/l 35.7 µg/l 18.6 µg/l**	**positive**	**No increased effect of glyphosate in combination with POAE**	**Guilherme et al., 2012 (ASB2014-7619)**
SCGE	**Fish (Prochilidus)**	**Roundup Transorb and Glyphosate**	**5 mg/l 2.4 mg/l**	**positive**	**Inconsistent and not clearly dose dependent**	**Moreno et al., 2014 (ASB2014-7522)**

[a] MA, Mammalian metabolic activation system not used and short exposure not used;
PH, no indication of pH or osmolality control;
DL, less than three dose levels used; PC, no concurrent positive control;
TO, no concurrent measurement of toxicity reported or toxicity not observed for highest dose level;
SC, independent coding of slides for scoring not indicated for visually scored slides;
IC, less than 200 cells scored per treatment or less than 100 metaphases scored per animal for chromosome aberrations.;
IE, less than 2000 erythrocytes scored per animal;
RE, results not reported separately for replicate cultures or individual animals;.
[b] Positive for small wing spots only in one cross. Negative or inconclusive for all spot categories for three other crosses.
[c] Statistically significant increase in micronucleated PCE frequency only at mid dose level but overall result judged negative.

A new comprehensive review on genotoxicity studies of glyphosate and glyphosate-based formulations was submitted by Kier and Kirkland (2013, ASB2014-9587). The authors concluded that an overwhelming preponderance of negative results in well-conducted bacterial reversion and *in vivo* mammalian micronucleus and chromosomal aberration assays indicates that glyphosate and typical GBFs are not genotoxic in these core assays. Negative results for *in vitro* gene mutation and a majority of negative results for chromosomal effect assays in mammalian cells add to the weight of evidence that glyphosate is not typically genotoxic for these endpoints in mammalian systems. Mixed results were observed for micronucleus assays of GBFs in non-mammalian systems. Reports of positive results for DNA damage endpoints indicate that glyphosate and GBFs tend to elicit DNA damage effects at high or toxic dose levels, but the data suggest that this is due to cytotoxicity rather than DNA interaction with GBF activity perhaps associated with the surfactants present in many GBFs. Glyphosate and typical GBFs do not appear to present significant genotoxic risk under normal conditions of human or environmental exposures.

B.6.4.8.3 Structure Activity Analysis

Glyphosate was evaluated using Derek for Windows (Llhasa Ltd., Leeds, UK, Version 11.0.0, October 24, 2009). No structural alerts were identified for chromosome damage, genotoxicity, mutagenicity or carcinogenicity. This small molecule consists of the amino acid, glycine, joined with a phosphonomethyl group. These moieties are not known to be genotoxic; therefore, the lack of structure activity alerts for glyphosate is expected.

B.6.4.8.4 Gene Mutation

As reviewed by Williams et al., (2000, ASB2012-12053), most gene mutation studies for glyphosate and GBFs were negative. Gene mutation assays included numerous Ames/*Salmonella* and *E. coli* WP2 bacterial reversion assays, *Drosophila* sex-linked recessive lethal assays and a CHO/HGPRT *in vitro* mammalian cell assay. Of fifteen gene mutation assays reported, there were only two positive observations. A reported positive Ames/*Salmonella* result for Roundup formulation was not replicated in numerous other studies. There was one report of a positive result for a GBF in the *Drosophila* sex-linked recessive lethal assay but this was contradicted by a negative result for the same GBF in this assay reported by another laboratory. Further, the positive study had some features that hampered interpretation, including the lack of concurrent negative controls (Williams et al., 2000).

Subsequent to the Williams et al. (2000, ASB2012-12053) review only two gene mutation studies have been reported (Table B.6.4-29). One negative Ames/*Salmonella* assay result was reported for a GBF of undefined composition, Percozyd 10 SL (Chruscielska et al., 2000, ASB2013-9820). Although this result is consistent with a large number of negative Ames/*Salmonella* results for glyphosate and GBFs, the reported study results have significant limitations. One of the recommended test strains, TA1535, was not used and results were only presented as "-" without presentation of revertant/plate data. A positive result for glyphosate was reported in the *Drosophila* wing spot assay which can indicate both gene mutation and mitotic recombination endpoints (Kaya et al., 2000, ASB2013-9832). Small increases in small wing spot frequencies were observed in one of four crosses of larvae treated with up to 10 mM glyphosate. The lack of a positive response in the balancer-heterozygous cross offspring, which are insensitive to mitotic recombination events, suggests that there is no evidence for effects on gene mutation endpoint events such as intragenic mutations or deletions in this publication.

These gene-mutation publications add very limited data to the weight of evidence conclusion that glyphosate and GBFs do not pose significant risk for gene mutation.

B.6.4.8.5 Chromosome effects

Assays to detect chromosome effects such as structural chromosome aberrations and micronucleus incidence constitute a second major genotoxicity end point category. A large number of publications with chromosome effects endpoints have been reported since the Williams et al. (2000, ASB2012-12053) review. These are described in Table B.6.4-29 and are separated into various test system categories which include *in vitro* cultured mammalian cell assays, *in vitro* tests in non-mammalian systems, *in vivo* mammalian assays and *in vivo* assays in non-mammalian systems. A *Drosophila* wing spot test (discussed previously) is also included in this category because results are relevant to somatic recombination.

B.6.4.8.5.1 *In vitro* chromosome effects

Two human and one bovine *in vitro* peripheral lymphocyte chromosome aberration studies of glyphosate were considered in the earlier review (Williams et al., 2000, ASB2012-12053). One human lymphocyte *in vitro* study had negative results for glyphosate tested up to approximately 2-3 mM (calculated from reported mg/ml) in the absence and presence of an exogenous mammalian activation system. The other two studies with human and bovine lymphocytes and no metabolic activation system reported positive results at concentrations more than two orders of magnitude lower. The earlier review noted several other unusual features about the positive result studies including an unusual exposure protocol and discordant positive results for another chemical found negative in other laboratories.
As indicated in Table B.6.4-29 both positive and negative results have been reported for glyphosate and GBFs in the nine *in vitro* chromosome effects assays published after the Williams et al. (2000, ASB2012-12053) review. It is noteworthy that many of these studies have various deficiencies in conduct or reporting compared to internationally accepted guidelines for conduct of *in vitro* chromosome aberration or micronucleus studies (see Table B.6.4-28). Perhaps the most significant deficiency was that coding and scoring of slides without knowledge of the treatment or control group was not indicated in seven of nine publications. This could be a deficiency in conducting the studies or perhaps a deficiency in describing methodology in the publications. Other common deficiencies included failure to indicate control of exposure medium pH, no use of exogenous metabolic activation and no reporting of concurrent measures of toxicity.

Results for glyphosate active ingredient
Three publications reported testing of technical glyphosate for micronucleus or chromosome aberration endpoints in cultured human lymphocytes (Manas et al., 2009, ASB2012-11892; Mladinic et al., 2009, ASB2012-11906; Mladinic et al., 2009, ASB2012-11907). Negative results for the micronucleus or chromosome aberration end points were observed in the absence of exogenous metabolic activation (S9) in all three publications. The maximum exposure concentration in the absence of S9 was in the range of 3-6 mM in these studies.
Two publications by one author reported cytokinesis block micronucleus results for cultured bovine lymphocytes treated with what was reported as 62 % by weight isopropyl amine salt of glyphosate from a Monsanto Belgium source (Piesova, 2004, ASB2012-12001; Piesova, 2005, ASB2012-12000). This test material appears to be a manufacturing batch of the isopropylamine salt of glyphosate in water without surfactants, which is not sold as a GBF. In one publication no statistically significant increases in binucleated cell micronucleus frequency were observed with 24 hours of treatment (Piesova, 2004, ASB2012-12001). For 48 hours of treatment a statistically significant increase in micronucleus frequency was observed in one donor at 280 µM but not at 560 µM and in a second donor at 560 µM but not 280 µM. The second publication reported negative results for the cytokinesis block micronucleus assay in bovine lymphocytes incubated with glyphosate formulation up to 560 µM for two hours in the absence and presence of a mammalian metabolic activation system (Piesova, 2005, ASB2012-12000). This publication also reported positive results for 48 hours of treatment without S9. Curiously, in this second publication the same inconsistent dose response pattern was observed in which a statistically significant increase in micronucleus frequency was observed in one donor at 280 µM but not at 560 µM and in a second donor at 560 µM but not 280 µM. The lack of a consistent dose response pattern between donors suggests that the results with 48 hours of treatment are questionably positive.
Two other publications found negative results for the chromosome aberration endpoint in cultured bovine lymphocytes treated with what appears to be the same test material of 62 %

by weight isopropylamine salt of glyphosate from a Monsanto Belgium source, (Holeckova, 2006, ASB2012-11847; Sivikova and Dianovsky, 2006, ASB2012-12029). Both the studies used a maximum concentration of 1.12 mM which was reported to cause a decrease in mitotic inhibition of >50 %. These two studies have several limitations including that an exogenous mammalian metabolic activation system was not used for chromosome aberration and scoring was not reported to be on coded slides. In addition, Holeckova (2006, ASB2012-11847) only examined effects detectable by staining of chromosome 1 and did not report positive control results (Holeckova, 2006, ASB2012-11847). Despite these limitations and the variable donor results, the results from these two studies are generally consistent with a lack of chromosome aberration effects of the isopropylamine salt of glyphosate on *in vitro* cultured mammalian cells in several experiments using high, toxic dose levels and exposures of 2-24 hours in the absence of S9.

One laboratory reported increases in cytokinesis-blocked micronucleus frequency in cultured human lymphocytes exposed to glyphosate for 4 hours in the presence of an exogenous human liver metabolic activation system (S9) in two publications (Mladinic et al., 2009a; Mladinic et al., 2009b). In both publications a statistically significant increase in micronuclei was observed with S9 at the highest dose level of glyphosate tested (580 µg/mL, ≈ 3.4 mM). Increased proportions of centromere- and DAPI-positive micronuclei were observed for the high dose with S9 suggesting that the induced micronuclei were derived from chromosomes rather than chromosome fragments. Statistically significant increases in the frequency of nuclear abnormalities (buds and bridges) and DNA strand breakage were also observed at the highest dose tested in both publications. In parallel experiments cytotoxic effects such as early apoptosis, late apoptosis and necrosis were observed and these effects were uniquely or preferentially observed in the presence of S9 and at the highest dose level tested (Mladinic et al., 2009, ASB2012-11906). Also, the negative control level of such end points as necrosis and alkaline SCGE tail moment was significantly increased in the presence of S9 (Mladinic et al., 2009, ASB2012-11906). It should be noted that glyphosate is mostly excreted unmetabolised *in vivo* in mammals with only very small levels of aminomethylphosphonic acid (AMPA) or an AMPA-related structure observed ([redacted], 2009, ASB2012-11542; [redacted] 1991, TOX9551791). These observations suggest that the observations of S9 mediated effects by Mladinic et al. are not likely to be due to *in vivo* relevant metabolites. The preponderance of *in vitro* genotoxicity studies conducted with exogenous mammalian metabolic activation systems has been negative, including a previously reviewed chromosome aberration study in human lymphocytes conducted up to a similar dose level (Williams et al., 2000, ASB2012-12053) and a bovine lymphocyte cytokinesis block micronucleus study (Piesova, 2005, ASB2012-12000). Overall these results suggest the possibility of a weak aneugenic rather than clastogenic (chromosome breaking) effect occurring in the presence of S9 at high dose levels of glyphosate. The pattern of activity as well as the failure to observe activity in several other *in vitro* genotoxicity assays conducted with S9 suggests that the activity observed in the Mladinic et al. studies does not have a significant weight of evidence for *in vitro* genotoxicity and is not likely to be relevant to *in vivo* genotoxicity.

The recently published results for mammalian *in vitro* chromosome aberration and micronucleus assays demonstrate a weight of evidence that technical glyphosate and glyphosate salt concentrates are negative for these end points in cultured mammalian cells in the absence of an exogenous mammalian metabolic activation system. Five publications from four laboratories report negative *in vitro* mammalian cell chromosome or micronucleus results in the absence of exogenous activation while three publications from two laboratories report positive results. These results reinforce the Williams et al. (2000, ASB2012-12053) conclusion that positive chromosome aberration results reported for glyphosate in cultured human lymphocytes in the absence of an exogenous metabolic activation system are aberrant.

Recent reports of positive chromosome aberration and micronucleus results for glyphosate in the presence of an exogenous mammalian activation system in cultured human lymphocytes in one laboratory (Mladinic et al., 2009, ASB2012-11906; Mladinic et al., 2009, ASB2012-11907) have no substantial reproducibility verification from other laboratories in the recent *in vitro* chromosome effects studies considered in this review because most of the studies performed by other laboratories (Table B.6.4-29) did not employ an exogenous mammalian activation system. These results are discordant with one previously reviewed result demonstrating a negative result for glyphosate in cultured human lymphocytes with mammalian metabolic activation using the chromosome aberration endpoint (Williams et al 2000, ASB2012-12053) and a negative result in the presence of S9 for the micronucleus endpoint in bovine lymphocytes (Piesova, 2005, ASB2012-12000). The numerous consistent negative results for glyphosate and GBFs in gene mutation studies which employed exogenous mammalian metabolic activation and careful examination of the data suggests that the positive results indicate a possible threshold aneugenic effect associated with cytotoxicity rather than a DNA-reactive mechanism resulting in chromosome breakage. Thus, the weight evidence for the *in vitro* chromosome effect assays indicates a lack of DNA-reactive clastogenic chromosome effects.

Results for GBFs

Amer et al. (2006, ASB2012-11539) reported positive *in vitro* chromosome aberration effects in mouse spleen cells for a formulation described as herbazed, which was reported to contain 84 % glyphosate and 16 % solvent, an unusually high glyphosate concentration for a formulation. The test material is not further characterised, lacking description of the glyphosate salt form and inert ingredients. The glyphosate concentrations used in the study are not clear because there are different descriptions of the concentration units (M or M glyphosate/ml medium) in the publication. Thus, the maximum concentration might have been 5 x 10^{-5} M (50 µM) or 5 x 10^{-5} M glyphosate/mL medium (50 mM). The former concentration, which was reported as toxic, would indicate effects at concentrations well below those typically found toxic for GBFs in cultured mammalian cells. The latter level of 50 mM would be well in excess of the limit level of 10 mM recommended in OECD guidelines (OECD473, 1997). In addition to a question about the concentration used there are several other limitations to the reported study including no indication that pH of treatment solutions was controlled, no use of a mammalian metabolic activation system, no reported concurrent toxicity measurements and no reported use of coded slides for scoring. Given these limitations, the uncertainty about the concentrations used and the nature of the test material, these results should not be considered to have significant relevance or reliability with respect to glyphosate or GBFs.

In addition to *in vitro* mammalian cell studies there is also a report of negative results for the chromosome aberration and micronucleus endpoints in onion root tips incubated with a Roundup formulation (Dimitrov et al., 2006, ASB2012-11607). The maximum exposure concentration (stated as 1 % active ingredient) is estimated to be on the order of 4-6 mM. This study did not employ an exogenous mammalian metabolic activation system; however, it does provide evidence for a lack of chromosome effects for glyphosate and a GBF in a non-mammalian *in vitro* system. The result agrees with earlier reported negative onion root tip chromosome aberration results for glyphosate but is discordant with earlier reported positive results for a Roundup GBF in this system (Williams et al., 2000, ASB2012-12053).

B.6.4.8.5.2 *In vivo* Chromosome Effects—Mammalian Systems

The Williams et al. (2000, ASB2012-12053) glyphosate toxicity review presented results from *in vivo* mammalian chromosome effect assays. Results from several mouse bone marrow erythrocyte micronucleus studies of glyphosate and GBFs (e.g. Roundup, Rodeo and Direct) were negative for micronucleus induction. These included studies from different laboratories mostly following modern guidelines. The intraperitoneal (i.p.) route was used for most of the negative studies and maximum doses for many of the studies were toxic or appropriately close to LD50 values. In addition to i.p. studies a 13 week mouse feeding study was also negative for the micronucleus endpoint with an estimated maximum daily glyphosate dose of over 11,000 mg/kg/day. There was one published report of a weak positive mouse bone marrow micronucleus response observed for glyphosate and Roundup GBF. This study, which employed a smaller number of animals per group than other negative studies, was clearly aberrant from the numerous other negative studies not only in micronucleated cell frequency finding but also the finding of altered polychromatic erythrocyte to normochromatic erythrocyte (PCE/NCE) ratios. The overall weight of evidence from the earlier reviewed studies was that glyphosate and GBFs were negative in the mouse bone marrow erythrocyte micronucleus assay. The earlier review also noted a negative mouse dominant lethal result for glyphosate administered by gavage at a maximum dose level of 2000 mg/kg.
As indicated in Table B.6.4-29, there are numerous subsequent publications of *in vivo* mammalian chromosome effects assays. With one exception, all of the *in vivo* mammalian studies were conducted in the mouse using either the bone marrow chromosome aberration or micronucleus endpoints. It should be noted that there are some fairly consistent limitations in the reported conduct of these studies compared to OECD guidelines. In most studies concurrent indications of toxicity (other than effects on the bone marrow) are not reported, coding of slides for scoring is not reported, individual animal data are not reported and fewer than recommended cells or metaphases per animal were scored. Other limitations encountered include use of only a single or two dose levels rather than three dose levels.

Results for glyphosate active ingredient
Two publications reported results for glyphosate in the mouse bone marrow erythrocyte micronucleus assay. Negative results were reported in one study which used a dose of 300 mg/kg of glyphosate administered once i.p. with sacrifices at 24, 48 and 74 hours after dosing ([redacted] 2000, ASB2013-9820). This study had some limitations including the use of only one dose level, no reporting of toxicity other than PCE/NCE ratio, no reported coding of slides for scoring and scoring of 1000 PCE's per animal (scoring of 2000 PCE's per animal is recommended by OECD guidelines). A second publication reported positive results for glyphosate administered at 50, 100 and 200 mg/kg via i.p. injections repeated at 24 hours apart with sacrifice 24 hours after the second dose ([redacted]., 2009, ASB2012-11892). A statistically significant increase in micronucleated erythrocytes was observed in the high dose group. This study had limitations comparable to the negative study. A more significant potential difficulty with this second publication is that "erythrocytes" rather than polychromatic erythrocytes were indicated as scored for micronuclei. This does not appear to be a case of using "erythrocytes" to mean polychromatic erythrocytes because the term "polychromatic erythrocytes" is used elsewhere in the publication describing measurements of PCE/NCE ratios. Scoring of total erythrocytes instead of immature polychromatic erythrocytes for micronuclei would be inappropriate in an assay with the stated treatment and harvest times because of the transient nature of micronucleated PCE's in bone marrow (OECD474, 1997).

There is no definitive explanation for the discrepancy between the two publications. Although one study used a single dose with multiple harvest times and the second used two doses and a single harvest time, both are acceptable protocols and would not be expected to lead to such discordant results (OECD474, 1997). The negative result reported for the 13 week feeding study in the earlier review (Williams et al., 2000, ASB2012-12053) confirms that positive results are not simply due to repeat dosing. The reported negative result ([redacted]., 2000, ASB2013-9820) seems to be in accord with a majority of earlier reviewed mouse bone marrow micronucleus studies of glyphosate using similar doses and the i.p. or feeding routes (Williams et al., 2000, ASB2012-12053). Also, the apparent scoring of micronuclei in erythrocytes rather than just polychromatic erythrocytes raises a significant methodological question for the reported positive study.

Results for GBFs

There are several publications reporting *in vivo* mammalian bone marrow chromosome aberration and micronucleus endpoint results for Roundup GBFs. Three publications report negative results for Roundup branded GBF in mouse chromosome aberration or micronucleus assays. Negative results were reported for two different Roundup branded GBFs administered at 2 x 200 mg/kg i.p. in mouse bone marrow erythrocyte micronucleus assays ([redacted] 2000, ASB2013-11477; [redacted] 2002, ASB2012-11834). The second study did not report coding of slides for scoring. Another publication reported negative results in mouse bone marrow studies for both the chromosome aberration and erythrocyte micronucleus endpoints ([redacted] 2006, ASB2012-11607) using a dose of 1080 mg/kg administered orally (p.o.). In contrast, one publication reported positive results for Roundup GBF in mouse bone marrow for the chromosome aberration and erythrocyte micronucleus endpoints using a single maximum dose of 50 mg/kg i.p. ([redacted] 2009, ASB2012-12005). Both the positive results and the magnitude of the increases in the chromosome aberration and micronucleus endpoint reported in this study are remarkably discordant with other reported results for Roundup and other GBFs in mouse bone marrow chromosome aberration and erythrocyte studies in a number of laboratories and publications (Table B.6.4-29 and Williams et al., 2000, ASB2012-12053). The reasons for this discordance are not clear. One unusual feature of the positive study is that the Roundup GBF was administered in dimethylsulfoxide. This is an unusual vehicle to use in *in vivo* genotoxicity studies, particularly for glyphosate which is water soluble and especially so in a formulated product. A published toxicity study found that use of a dimethylsulfoxide/olive oil vehicle by the i.p. route produced dramatically enhanced toxicity of glyphosate formulation or the formulation without glyphosate compared to saline vehicle and that the enhanced toxicity observed with this vehicle was not observed when the oral route was used ([redacted]., 2008, ASB2012-11845). These observations suggest that use of DMSO as a vehicle for administration of formulation components by the i.p. route might produce unusual toxic effects that are not relevant to normally encountered exposures. Regardless of the reasons for the discordant positive results it is clear that a large preponderance of evidence indicates that GBFs are typically negative in mouse bone marrow chromosome aberration and erythrocyte assays.

One publication reported positive results for bone marrow chromosome aberration in rabbits administered Roundup GBF in drinking water at 750 ppm for 60 days ([redacted], 2005, ASB2012-11841). This study is relatively unique in terms of species and route of administration. The results do not report water intake in the test and control groups. Given the potential for water palatability issues with a formulated product, this is a significant shortcoming, as any effects noted may be attributable to dehydration. This study had further limitations including the use of only a single dose level and not coding slides for scoring.

Examination of the chromosome aberration scoring results showed that large increases for the treated group were observed for gaps and "centromeric attenuation" which were included in the summation and evaluation of structural chromosome aberration effects. Ordinarily gaps are scored but are not recommended for inclusion in total aberration frequency and centromeric attenuation is not included in ordinary structural aberrations (OECD475, 1997). These unusual scoring and interpretive features raise significant questions about using this study to make conclusions about clastogenicity of the GBF tested.

Two other publications report *in vivo* mammalian chromosome aberration or micronucleus results for GBFs. An uncharacterisd GBF, Percozyd 10L, was reported to be negative in a mouse bone marrow erythrocyte micronucleus assay ([redacted] 2000, ASB2013-8929 and ASB2013-8931). The maximum dose level tested, 90 mg/kg i.p., was reported to be 70 of the i.p LD_{50} as determined experimentally by the authors. This study had several limitations including use of less than three dose levels and no reported coding of slides for scoring. Positive results were reported for another uncharacterized GBF, herbazed, in mouse bone marrow and spermatocyte chromosome aberration studies ([redacted] 2006, ASB2012-11539). No statistically significant increases in aberrant cells were observed in bone marrow cells for i.p. treatment of 50 mg/kg for 1, 3 or 5 days or in spermatocytes for 1 or 3 days treatment. Statistically significant increases in frequency of spermatocytes with aberrations were reported for 5 days of treatment with 50 mg/kg (i.p.). Oral treatment of 50 mg/kg and 100 mg/kg were reported to produce increases in aberrant cell frequency in bone marrow cells after extended treatments (14 and 21 days) but not after shorter 1 and 7 day treatments. Similarly, significant increases in aberrant cell frequencies of spermatocytes were reported at 14 and 21 days of 50 mg/kg oral treatment (negative for 1 and 7 days treatment) and at 7, 14 and 21 days of 100 mg/kg treatment (negative for 1 day treatment). Although not a genotoxic endpoint per se, it should be noted that statistically significant increases in frequency of sperm with abnormal morphology were also observed in mice treated with 100 and 200 mg/kg p.o. for 5 days. The positive results for the uncharacterized herbazed GBF were only observed after extended oral treatments (bone marrow and spermatocytes) and extended i.p. treatments (spermatocytes). The fact that positive results were not observed in an erythrocyte micronucleus test of mice treated with glyphosate up to 50,000 ppm in feed for 13 weeks (Williams et al., 2000, ASB2012-12053) provides direct evidence that extended glyphosate treatment by the oral route does not induce detectable chromosome effects. This treatment was longer and up to much higher glyphosate exposures than those used for the [redacted]. (2006, ASB2012-11539) studies. Thus, it appears likely that these effects were due to some component(s) of the specific herbazed GBF tested rather than glyphosate.

In vivo mammalian assays for chromosome effects are an important category for characterisng genotoxicity that complements the gene mutation category. While some positive results have been reported the preponderance of evidence and published results are negative for glyphosate and GBFs.

B.6.4.8.5.3 In vivo Chromosome Effects—Non-Mammalian Systems

The Williams et al. (2000, ASB2012-12053) review reported a few *in vivo* plant assays for chromosome effects in non-mammalian systems. These included negative results for glyphosate and positive results for Roundup GBFs for chromosome aberrations in an onion root tip assay and negative results for glyphosate with the micronucleus end point in a *Vicia faba* root tip assay.

Subsequent to the earlier review a number of publications reported results for erythrocyte micronucleus assays conducted on GBFs in several non-mammalian fish and reptile species

with discordant results. One publication reported apparently negative results for the erythrocyte micronucleus test in *Oreochromis niloticus* (Nile tilapia) administered a test material described as Roundup 69 GBF, at an upper dose of 170 mg/kg i.p. ([redacted] 2000, ASB2013-11477). Although there was an increase in micronucleated erythrocyte frequency at the mid-dose level this was not observed at the high dose level and considerable variability in frequencies in different groups was noted. Negative results were also reported in another fish species (*Prochilodus lineatus*) exposed to 10 mg/liter Roundup branded GBF for 6, 24 and 96 hours ([redacted]2008, ASB2012-11586). This concentration was reported to be 96 % of a 96 hour LC_{50}. Positive results were reported for the erythrocyte micronucleus assay conducted in the fish *Tilapia rendalii* exposed to 170 mg/kg i.p. of another Roundup GBF ([redacted] 2002, ASB2012-11834). Examination of the micronucleus frequencies in this publication indicated that the negative control micronucleus frequency was considerably lower than the frequencies for all but one of 21 treatment groups for 7 different test materials. This suggests an unusually low control frequency and at least one treatment group was statistically significantly elevated for each of the 7 test materials, including many instances where the statistically significant increases were not consistent with a biologically plausible dose response. The possibility that the apparently significant increases were due to a low negative control value should be considered for this publication. Another publication reported positive erythrocyte micronucleus results in goldfish (*Carassius auratus*) exposed to 5 to 15 ppm of a Roundup GBF for 2 to 6 days ([redacted], 2007, ASB2012-11587). The reasons for the discordant results are not clear for these fish erythrocyte micronucleus assays of Roundup GBFs. Although different species and GBF's were used in the different studies there were pairs of studies with positive and negative results that used similar treatment conditions (170 mg/kg i.p. or 10-15 mg/litre in water).

Results for an unusual test system of exposed caiman eggs are reported by [redacted] 2009, ASB2012-12002. Eggs were topically exposed in a laboratory setting to Roundup Full II GBF, and erythrocyte micronucleus formation was measured in hatchlings ([redacted]., 2009, ASB2012-12002). The GBF tested was reported to contain the potassium salt of glyphosate and alkoxylated alkylamine derivatives as surfactants. Statistically significant increases in micronucleated erythrocytes were observed in hatchlings from eggs treated with 500-1750 µg/egg. This system is quite unusual in the species tested and even more so in using an egg application with measurement of effects in hatchlings. Although there is some experience with a hen's egg erythrocyte micronucleus assay using *in ovo* exposure the erythrocytes are evaluated in embryos with only a few days between treatment and the erythrocyte micronucleus end point. In the reported caiman egg assay there was presumably a single topical exposure followed by an egg incubation period of about 10 weeks before hatching. Biological plausibility raises questions whether genotoxic events *in ovo* can produce elevated micronucleated erythrocyte frequencies detectable after 10 weeks, given the number of cell divisions occurring in development of a hatchling.

One published study reported a weak positive result in a *Drosophila* wing spot assay (Kaya et al., 2000, ASB2013-9832). Statistically significant positive increases were only in one of four crosses for small twin spots and not for the two other wing spot categories (large wing spots and twin wing spots). As discussed above, only negative or inconclusive results were observed for crosses that were not subject to mitotic recombination effects. If the result was actually treatment related it only would indicate an increase in recombination events and not in somatic mutations.

The above *in vivo* chromosome effect assays in non-mammalian systems give discordant results for reasons that aren't precisely defined. Typically these results would be given lower weight than mammalian systems in being predictive of mammalian effects, especially since

there is little or practically no assay experience with these systems in comparison with *in vivo* mammalian chromosome effects assays, such as the rat or mouse bone marrow chromosome aberration or erythrocyte micronucleus assays.

B.6.4.8.6 DNA damage and other end points

A number of studies of glyphosate and GBFs have been published since 2000 which used various DNA damage end points in a variety of *in vitro* and *in vivo* systems. The DNA damage category includes end points such as sister chromatid exchange and DNA repair response in bacteria, but the most common DNA damage end point encountered was the alkaline single cell gel electrophoresis end point (alkaline SCGE) also commonly referred to as the "comet" assay. The alkaline SCGE end point has been applied to both *in vitro* and *in vivo* test systems.
In addition to DNA damage there are a few reports of other types of studies which can be associated with genotoxic effects even though the end points are not specific indicators of genotoxicity per se. These include sperm morphology and carcinogenicity studies.

***In vitro* DNA Damage Studies**
Some positive results for glyphosate or GBFs in the SCE end point were reported in cultured human and bovine lymphocytes in the earlier review (Williams et al., 2000, ASB2012-12053). These results tended to be weak, inconsistent and with limited evidence for dose response. A number of limitations were observed for the studies such as the failure to control pH and abnormally low control values. Additional *in vitro* DNA damage end point results described in the earlier review included negative results for glyphosate in the *B. subtilis* rec-assay and in the primary hepatocyte rat hepatocyte unscheduled DNA synthesis assay.
There are two subsequent publications using *in vitro* cultured mammalian cells and the SCE endpoint. Positive SCE results were reported for the uncharacterised herbazed GBF in mouse spleen cells (Amer et al., 2006, ASB2012-11539). The dose response pattern for SCE response in this study was similar to the response for chromosome aberrations in this publication. Limitations of this study are in common to those described above for the chromosome aberration end point portion of the study; no indication that pH of treatment solutions was controlled, no use of a mammalian metabolic activation system, no reported concurrent toxicity measurements and no reported use of coded slides for scoring. Positive SCE results were also reported for cultured bovine lymphocytes treated with up to 1.12 mM glyphosate for 24 and 48 hours without exogenous mammalian metabolic activation (Sivikova and Dianovsky, 2006, ASB2012-12029). The highest dose of 1.12 mM significantly delayed cell cycle progression with 48 hour treatment. These same concentrations for 24 h exposures did not induce statistically significant increases in chromosome aberrations which provides a clear example of a differential response of the SCE endpoint (Sivikova and Dianovsky, 2006, ASB2012-12029). This is an important consideration in these publications, as chromosome effects are considered more relevant to genotoxicity than DNA damage.
Positive results for glyphosate are reported for the alkaline SCGE end point in three publications. Positive SCGE results were observed for two mammalian cell lines exposed to glyphosate for 4 hours at concentrations of 4.5-6.5 mM (GM39 cells) and 4.75-6.5 mM (HT1080 cells) (Monroy et al., 2005, ASB2012-11910). These concentrations are close to the upper limit dose of 10 mM generally recommended for *in vitro* mammalian cell assays and control of medium pH is not indicated. Characterisation of nuclear damage was done by visual scoring without coding of slides being indicated. Positive alkaline SCGE results were also reported in Hep-2 cells exposed for 4 hours to 3.5-7.5 mM glyphosate (Manas et al.,

2009, ASB2012-11892). Higher concentrations of glyphosate were reported to result in viability of <80 % as determined by dye exclusion. As noted for the preceding publication, the concentrations employed were reasonably close to a limit dose of 10 mM and control of medium pH was not reported. This publication reported negative results for the chromosome aberration endpoint in cultured human lymphocytes exposed to up to 6 mM glyphosate for 48 hours and it should be noted that in this case an appropriate control of medium pH was reported for this human lymphocyte experiment. Positive alkaline SCGE results have also been reported for cultured human lymphocytes exposed to glyphosate at concentrations up to 580 µg/ml (estimated 3.4 mM) for 4 hours (Mladinic et al., 2009, ASB2012-11906). Effects were observed both in the presence and absence of S9 with statistically significant increases in tail intensity at 3.5, 92.8 and 580 µg/ml without S9 and at 580 µg/ml with S9. A modification of the alkaline SCGE assay employing human 8-hydroxyguanine DNA-glycosylase (hOGG1) to detect oxidative damage only indicated statistically significant effects on tail length for treatment with 580 µg/ml with S9. Increases in nuclear abnormalities (nuclear buds and/or nucleoplasmic bridges) were also observed at 580 µg/mL with and without S9 and in micronucleus frequency at 580 µg/ml with S9. Measurements of total antioxidant capacity and thiobarbituric acid reactive substances showed statistically significant increases at 580 µg/ml in the presence or absence of S9. Interpretation of the significance of metabolic activation effects is complicated by the observation that several of the end points (alkaline SCGE tail intensity and nuclear abnormalities) tended to show increases in the presence of S9 in negative controls or at the very lowest concentrations of glyphosate. A reasonable summation of the results in this publication is that alkaline SCGE effects and other effects such as nuclear abnormalities, early apoptosis, necrosis and oxidative damage were consistently observed at 580 µg/mL.

In addition to mammalian cell studies there are publications reporting positive alkaline SCGE effects for glyphosate in *Tradescantia* flowers and nuclei exposed to up to 700 µM glyphosate (Alvarez-Moya et al., 2011, ASB2012-11538) and in the *E. coli* SOS chromotest for DNA damage conducted on a Roundup BIO GBF (Raipulis et al., 2009, ASB2012-12008). Observations of DNA damage in plants exposed to glyphosate are of questionable significance because of the herbicidal nature of glyphosate and the SOS chromotest provides only indirect evidence of DNA damage in a bacterial system.

Overall there appear to be a number of studies in which glyphosate or GBFs have been reported to produce positive responses in DNA damage endpoints of SCE or alkaline SCGE *in vitro* in mammalian cells. Most of these have occurred with exposures to mM concentrations of glyphosate. Although this dose level range is lower than the limit dose of 10 mM recommended for several *in vitro* mammalian cell culture assays (OECD473, 1997; OECD476, 1997; OECD487, 2010), an even lower limit dose of 1 mM was recently recommended for human pharmaceuticals, particularly because of concerns about relevance of positive *in vitro* findings observed at higher dose levels. In addition, many of the studies have limitations such as not indicating control of medium pH and not coding slides for visual scoring.
Concerns over the possibility of effects induced by toxicity have led to several suggestions for experimental and interpretive criteria to distinguish between genotoxic DNA-reactive mechanisms for induction of alkaline SCGE effects and cytotoxic or apoptotic mechanisms. One recommendation for the *in vitro* alkaline SCGE assay is to limit toxicity to no more than a 30 % reduction in viability compared to controls. Importantly, dye exclusion measurements of cell membrane integrity, such as those reported in some of the above publications may significantly underestimate cytotoxicity that could lead to alkaline SCGE effects. Other

recommendations include conducting experiments to measure DNA double strand breaks to determine if apoptotic process might be responsible for alkaline SCGE effects. Measurement of apoptotic and necrotic incidence were only performed in one publication (Mladinic et al., 2009, ASB2012-11906) and these measurements indicated both apoptotic and necrotic processes occurring in parallel with observations of alkaline SCGE effects. These direct observations as well as the reported dose responses, consistently suggest that biological effects and cytotoxicity accompany the observations of DNA damage *in vitro* in mammalian cells and therefore confirm the likelihood that the observed effects are secondary to cytotoxicity and are thresholded.

In vivo DNA damage studies

In the earlier review positive results for DNA strand breakage were reported for mice treated by the i.p. route with glyphosate and GBFs and for the alkaline SCGE endpoint in tadpoles of the frog *Rana catesbiana* exposed to a GBF (Williams et al., 2000, ASB2012-12053).

[redacted]. (2006, ASB2012-11539) reporten an increase in SCE frequency in bone marrow cells of mice treated with uncharacterised herbazed GBF. Statistically significant positive effects were only observed at the highest dose level tested (200 mg/kg administered p.o.).

Several recent publications report alkaline SCGE results for GBFs in aquatic species. Three publications reported positive alkaline SCGE results in aquatic vertebrates exposed to Roundup GBFs in water. These publications have a common feature that alkaline SCGE results were reported as visually scored damage category incidence rather than instrumental measurements of properties such as the tail length or tail intensity. In one publication increases in nuclei exhibiting alkaline SCGE visual damage effects were observed in erythrocytes and gill cells of the tropical fish *Prochilodus lineatus* exposed to 10 mg/litre of a Roundup GBF in water ([redacted] 2008, ASB2012-11586). Results were variable with cell type and incubation; statistically significant positive responses were observed for erythrocytes at 6 hours and 96 hours, but not 24 hours or for branchial cells from the gills at 6 hours and 24 hours. Measurement of erythrocyte micronucleus frequency and nuclear abnormalities did not show statistically significant increases in these endpoints. The concentration used was reported to be 75 % of the 96 hour LC_{50}, but trypan blue dye measurements apparently indicated >80 % viability of cells used in the alkaline SCGE assays. A second publication reported positive alkaline SCGE results in erythrocytes of the goldfish, *Carasseus auratus*, exposed to 5, 10 and 15 ppm of a Roundup GBF for 2, 4 or 6 days ([redacted] 2007, ASB2012-11587). Similar effects were observed for other end points (micronucleus and nuclear abnormalities). In general, effects increased with concentration and time. This publication did not report toxicity measurements or, more specifically, measurements of cell viability in the population studied. Positive results were also reported in erythrocytes of the European eel, *Anguilla anguilla*, exposed to 58 and 116 µg/liter of a Roundup GBF in water for 1 or 3 days [redacted]., 2010, ASB2012-11836). Increases in nuclear abnormalities were also observed in erythrocytes from animals exposed for 3 days. Measurement of toxicity was not reported for the animals or erythrocytes; however, several endpoints relevant to antioxidant responses and oxidant effects were made in whole blood samples. No statistically significant effects were observed for catalase, glutathione transferase, glutathione peroxidase, glutathione reductase or reduced glutathione content. A large statistically significant increase for thiobarbituric acid reactive substances (TBARS, a measure of lipid peroxidation) was observed for the 115 µg/litre concentration group at 1 day. Statistically significant TBARS increases were not observed at 3 days, but, the 3-day negative control value appeared to be several fold higher than the 1-day value.

Significance of DNA damage end point results

DNA damage end points such as SCE or alkaline SCGE are generally regarded as supplementary to the gene mutation and chromosome effects end point categories. DNA damage endpoints do not directly measure effects on heritable mutations or events closely associated with chromosome mutations. *In vitro* DNA damage endpoints such as the SCE or alkaline SCGE can be induced by cytotoxicity and cell death processes rather than from DNA-reactive mechanisms.

The observation of effects of sodium dodecyl sulfate is also interesting because it suggests responses to surfactants which are typically components of GBFs. As a more specific example, polyoxyethylenealkalylmine (POEA), a surfactant component of some GBFs has been shown to elicit cytotoxic effects such as perturbation of the mitochondrial membrane and disruption of mitochondrial membrane potential in cultured mammalian cells ([redacted], 2007, ASB2009-9030). Surfactant effects provide a plausible mechanism for observations of GBFs inducing DNA damage responses. Such responses would be expected to be associated with cytotoxicity-inducing exposures and exhibit a threshold.

B.6.4.8.7 Human and environmental studies

A number of human and environmental studies have been published in or after 2000 where some exposures to GBFs in the studied populations were postulated. These publications are summarised in Table B.6.4-30.

Table B.6.4-30: Studies of Human and Environmental Populations with Reported or Assumed Glyphosate Exposure

Exposed Population	End point	Exposures	Result	Reference
Human Studies				
Open field and fruit farmers	Bulky DNA adducts	glyphosate formulation use reported in only 1 of 29 fruit farmers	No effects attributed to glyphosate formulation exposure	[redacted]., 2007 (ASB2012-11543)
Humans in areas where glyphosate formulation is applied	Lymphocyte cytokinesis block micronucleus (CB MN)	Aerial or manual spraying of glyphosate formulation for illicit crop control and sugar cane maturation	Increase in CB MN but no clear relationship to assumed or reported exposures	[redacted] 2009 (ASB2012-11570)
Floriculturists	Lymphocyte CB MN	Glyphosate formulation use reported in 21/51 workers with average of 106.5 kg applied	Increase in CB MN but not statistically significant	[redacted] 2004 (ASB2012-11572)
Floriculturists	Lymphocyte CB MN	Glyphosate formulation use reported in 57/107 workers. Numerous other pesticides reported as used by a similar number or more of workers	Statistically significant increase in CB MN	[redacted] 2002 (ASB2012-11573)

Exposed Population	**End point**	**Exposures**	**Result**	**Reference**
Agricultural workers	Buccal cell micronucleus	Glyphosate formulation use reported along with numerous other pesticides	Statistically significant increase in MN	[redacted] 2009 (ASB2012-11570)
Fruit growers	Lymphocyte Alkaline SCGE; Ames test on urine	Glyphosate use reported in 2/19 1 day before captan spraying and 1/19 on the day of captan spraying	No effects attributable to glyphosate formulation exposure	[redacted] 2003 (ASB2012-11878)
Agricultural workers	Lymphocyte CB MN; buccal cell micronucleus	Glyphosate formulation use reported in 16% of one of four populations studied (Hungary)	No statistically significant increases in CB MN or buccal cell micronucleus frequencies	[redacted] 2003 (ASB2012-11991)
Individuals on or near glyphosate spraying	Lymphocyte alkaline SCGE	Glyphosate formulation aerially sprayed within 3 km	Statistically significant increases in damaged cells	[redacted] 2007 (ASB2012-11992)
Greenhouse Farmers	Lymphocyte SCE	Glyphosate formulation use reported in 99/102 workers; numerous other pesticides used	Statistically significant increases in SCE	[redacted] 2001 (ASB2012-12025)
Farmers	Lymphocyte CB MN	Glyphosate formulation use reported in 3/11 farmers	Statistically significant increase in micronucleus frequency but not in frequency of binucleated cells with micronuclei	[redacted] 2006 (ASB2012-12045)
Environmental Studies				
Meadow voles living on golf cousres	Blood cell alkaline SCGE; erythrocyte micronucleus	Glyphosate formulation use reported along with numerous other pesticides	Some effects judged possibly related to Daconil® fungicide	[redacted] 2004 (ASB2012-11871)
Fish from dams (various species)	Erythrocyte micronucleus	Glyphosate formulation use reported in adjacent lands along with other pesticides	Higher MN frequencies than normal or expected but no negative concurrent controls used	[redacted] 2011 (ASB2012-12017)

Many of the human studies either found no effects attributable to GBFs or the reported GBF usage by the studied population was too low to be associated with observed population effects ([redacted]., 2007, ASB2012-11543; [redacted]., 2004, ASB2012-11572; [redacted]., 2003, ASB2012-11878; [redacted]., 2003, ASB2012-11991; [redacted]., 2006, ASB2012-12045).

In some studies, incidence of GBF use by the population studied was significant but high incidence of use of other pesticides was also reported ([redacted] 2002, ASB2012-11573; [redacted] 2001, ASB2012-12025). Even though positive effects were observed in these populations, ascribing these effects to any particular environmental exposure is not

scientifically justifiable and such results certainly cannot be considered as definitive evidence for GBF-induced human genotoxic effects.

Two published studies focused on populations believed to be exposed to GBFs by their presence at or near aerial or manual spraying operations. One publication reported induction of alkaline SCGE effects in blood lymphocytes of populations living within 3 km of areas sprayed with glyphosate formulation for illicit crop eradication [redacted] 2007, ASB2012-11992). The populations studied were relatively small (24 exposed individuals and 21 non-exposed individuals). The sprayed material was reported to be Roundup Ultra, a GBF containing 43.9 % glyphosate, polyethoxylated [redacted] surfactant and a proprietary component, Cosmoflux 411F. Specific methods for collection, storage, and transport of blood samples are not described for either the exposed population or control group. The publication also does not indicate that slides were coded for scoring which consisted of visual classification into damage categories and measurement of DNA migration (tail length). There were fairly large differences in ages and sex distribution of the exposed and control populations but these did not appear to be statistically significant. The study reported increases in damaged cell categories and statistically significant increases in DNA migration (tail length) in the presumably exposed population. Interpretation of the results of this study should consider numerous reported signs of toxicity in the exposed population and the reported application rate of 24.3 litres/ha which was stated to be 20 times the maximum recommended application rate. Some of the reported human health effects described by [redacted] (2007, ASB2012-11992) appear to be consistent with severe exposures noted in clinical reports of acute poisoning incidents with GBFs and other pesticide formulations (often self-administered) rather than typical bystander exposures. Given the considerably favorable general toxicology profile of glyphosate as reported by the WHO/FAO Joint Meeting on Pesticide Residues (WHO/FAO, 2004, ASB2008-6266) and in Williams et al. (2000, ASB2012-12053), factors related to either high surfactant exposure, unusual GBF components in this formulation or other undocumented variables appear to be confounding factors in this study. It appears that the reported alkaline SCGE effects could well have been secondary to the ailments reported in this study population.

A second publication reported results for a blood lymphocyte cytokinesis-block micronucleus study of individuals in areas treated with glyphosate formulation by aerial spraying or manual application ([redacted] 2009, ASB2012-11570). Although the title of the publication contains the term “agricultural workers”, most of the populations studied do not appear to be agricultural workers who are involved in application of GBFs. The human lymphocyte culture and scoring methodology employed in the [redacted]. (2009, ASB2012-11570) study appear to be generally consistent with commonly used and recommended practices for this assay. However, there is a significant question as to how long the blood samples used in the study were stored prior to initiating cultures and this may have affected the micronucleus numbers observed in the different sets of samples and populations. Also, the populations in the aerially sprayed regions had a second sampling a few days after the first sampling and this second sampling was not performed in the control populations. The publication reported a small increase in the frequency of binucleated cells with micronuclei and micronuclei per cell in samples collected from people living in three regions after spraying of GBFs compared with control values of samples collected just before spraying. However, the pattern of the increases did not correlate either with the application rate or with self-reported exposure. The largest post-spraying increase in binuclated cell micronucleus frequency was reported for a population with a much lower glyphosate active ingredient application rate and only 1 of 25 people in this region reported contact with sprayed glyphosate formulation. Increases in binucleated cell micronucleus frequency did not have a statistically significant relationship with self-reported exposure for two other populations. Some interpretative statements in

[redacted] (2009, ASB2012-11570) suggest a small transient genotoxic effect of glyphosate formulation spraying on frequencies of binucleated cells with micronuclei, but other statements indicate that causality of the observed effects could not be determined using reasonable criteria and that lack of exposure data precluded conclusions. This study has a combination of uncontrolled or inadequately characterized variables, such as uncharacterisd exposure to ”genotoxic pesticides”, that would appear to preclude using the data to support any conclusion that exposure to GBFs affects binucleated micronucleus frequencies. Actually, the available data, while certainly limited in nature, support a conclusion that the observed effects do not appear to be attritubable to glyphosate formulation exposure. This conclusion is reinforced by [redacted] (2004, ASB2012-11528), where biomonitoring of agricultural workers applying GBFs reports systemic exposures orders of magnitude below *in vivo* model chromosome aberration and micronucleus study doses, the majority of which were negative for glyphosate and GBFs.

There are two publications related to environmental monitoring for genotoxic endpoints. One study using blood cell alkaline SCGE and micronucleus endpoints was conducted on samples from meadow voles living on or near golf courses where pesticides had been applied ([redacted] 2004, ASB2012-11871). Results were significantly inconsistent between two seasons. Although some suggestions of effects were reported, glyphosate was only one of a number of applied pesticides and the effects observed were considered as possibly attributable to exposure to Daconil® fungicide. A second publication reported results for the erythrocyte micronucleus assay applied to fish collected from several dams in Brazil ([redacted], 2011, ASB2012-12017). Glyphosate formulation was one of a number of pesticides reported to be used in the area of the dams. No efforts appear to have been made to measure glyphosate or other pesticide concentrations in any of the ten dams from which fish were sampled. This study reported what were considered to be high levels of micronucleated cell frequency but there were no concurrent negative controls. In the absence of these controls the results cannot be interpretted as indicating any effect of pesticide exposure.

Although there have been a fairly large number of human genotoxicity studies reported where there was some exposure to GBFs, the large majority of these studies do not allow any conclusions about possible effects of glyphosate or GBFs because the exposure incidence was low or because there were reported exposures to a large number of pesticides. One report found an increase in alkaline SCGE effects in humans living in or near areas where a GBF was sprayed but that study had a number of methodology reporting and conduct deficiencies and the reported effects could well have been due to toxicity reported in the study population. A second study found some increases in cytokinesis-block micronucleus frequency in humans possibly exposed to GBFs but the effects were not concordant with application rates or self-reported exposures and thus do not constitute reliable indications of effects for this endpoint in humans exposed to GBFs. Neither of the two environmental monitoring studies in meadow voles or fish provide any reliable evidence of exposures to glyphosate or GBFs or adverse effects resulting from potential exposures to glyphosate or GBFs.

After submission of the first draft of this RAR for public comment the following additional studies have been included.

Koureas et al. (2014, ASB2014-9724) performed a study aimed at estimating the oxidative damage to DNA in different subpopulations in Thessaly region (Greece) and investing its correlation with exposure to pesticides and other potential risk factors. The study produced findings that support the hypothesis that pesticide exposure is involved in the induction of oxidative damage to DNA. A correlation was found in this study between exposure to formulations containing neonicotinoids or glufosinate ammonium and oxidative damage to DNA. However, no significant correlation was reported for glyphosate.

Gentile et al. (2012, ASB2014-9482) submitted results of the micronucleus assay as a biomarker of genotoxicity in the occupational exposure to agrochemicals in rural workers in Argentina. The authors found significant differences in the frequency of micronuclei between occupationally exposed (20 individuals) and unexposed (10 individuals) workers. However, no conclusion on genotoxicity of glyphosate or other specific pesticides is possible on basis of this study.
Da Silva et al. (2014, ASB2014-9358) performed a genotoxic assessment in tobacco farmers at different crop times. The study sought to determine genotoxic effects in farmers occupationally exposed to agrochemicals and nicotine. A significant increase of micronucleated cells in the off-season group was observed. However, no conclusion on genotoxicity of glyphosate or other specific pesticides is possible on basis of this study.
Benedetti et al. (2013, ASB2014-9279) studied genetic damage in soybeans workers exposed to pesticides. The evaluation was performed with the comet and buccal micronucleus assays. The results of both tests revealed DNA damage in soybean workers. No special pesticide can be identified as cause of the observed effects.

B.6.4.8.8 DNA-Reactivity and carcinogenesis

As noted in the earlier review, ^{32}P-postlabelling DNA adduct studies in mice did not indicate formation of adducts from glyphosate and questionable evidence of adducts from Roundup GBF administered as a high 600 mg/kg i.p. dose in an unusual dimethylsulfoxide/olive oil vehicle (Peluso et al., 1998, TOX1999-318; Williams et al., 2000, ASB2012-12053). Another earlier reviewed study reported DNA strand breakage in liver and kidneys of mice injected i.p with glyphosate and Roundup GBF. This study also reported an increase in 8-hydroxydeoxyguanosine (8-OHdG) residues in liver DNA from mice injected with glyphosate but not GBF. Increased 8-OHdG was found in kidney DNA from mice injected with GBF but not glyphosate (Bolognesi et al., 1997, Z59299; Williams et al., 2000, ASB2012-12053). No new direct studies of DNA reactivity of glyphosate or GBFs were encountered in publications since 2000. One publication did report on studies in mice to further investigate toxic effects and 8-OHdG levels associated with the routes, vehicles and dose levels employed in earlier ^{32}P-postlabelling and DNA strand breakage and 8-OHdG studies (Heydens et al., 2008, ASB2012-11845). This publication reported that high i.p. dose levels of GBF induced significant liver and kidney toxicity that were not observed with oral administration. Statistically significant increases in 8-OHdG were not observed in this study under the same conditions as employed by the earlier study. The dimethylsulfoxide/olive oil vehicle dramatically enhanced toxicity of GBF administered by the i.p. route and the toxicity was also observed for formulation components without glyphosate. These results indicated that the effects reported in the earlier studies were associated with high liver and kidney toxicity that was primarily due to the non-glyphosate components of the formulation and which were produced by the i.p. route of exposure to very high dose levels. The enhancement of toxicity by the unusual dimethylsulfoxide/olive oil dosing vehicle further calls into question whether the ^{32}P-postlabelling finding represented effects associated with unusual toxicity rather than being indicative of adducts formed from glyphosate or glyphosate formulation components.

Carcinogenicity is not a direct endpoint for genotoxicity but it is one of the possible consequences of genotoxicity and, conversely, lack of carcinogenicity in well-conducted experimental studies provides some evidence that a significant genotoxic mode of action is not operating *in vivo*. The earlier review of glyphosate concluded that it was not carcinogenic

in mouse or rat chronic studies and notes that glyphosate was not considered carcinogenic by numerous regulatory agencies and scientific organisations (Williams et al., 2000, ASB2012-12053).

B.6.4.8.9 AMPA and POEA

In addition to glyphosate and GBFs, the earlier review included information on the toxicity and genotoxicity of the major environmental breakdown product of glyphosate, aminomethylphosphonic acid (AMPA), and what was at that time a common GBF surfactant mixture of polyethoxylated long chain alkylamines synthesized from animal-derived fatty acids (polyethoxylated tallow amine, [redacted] ethoxylate, POEA). Today a wide variety of surfactant systems are employed by different companies for different regions and end uses.

In the earlier review, summarised genotoxicity results for AMPA included negative results in the Ames/*Salmonella* bacterial reversion assay, an *in vitro* unscheduled DNA synthesis assay in primary hepatocytes and a mouse bone marrow erythrocyte micronucleus assay (Williams et al., 2000, ASB2012-12053). One publication of AMPA genotoxicity results was observed subsequent to 2000. In this publication analytical grade AMPA was reported to have positive effects in several assays including an alkaline SCGE endpoint in cultured mammalian Hep-2 cells, a chromosome aberration endpoint in cultured human lymphocytes and in a mouse bone marrow erythrocyte micronucleus assay (Manas et al., 2009, ASB2012-11891). Experimental limitations in the conduct of the alkaline SCGE assay included no inclusion of mammalian metabolic activation and no reported control of medium pH even though relatively high concentrations of AMPA acid (2.5-10 mM for 4 hours) were used. Although nucleoid images were analyzed with software rather than visual analysis the methodology doesn't indicate that slides were coded and there may have been a visual judgment component in selection of images for analysis. The positive results were statistically significant increases in tail length, % DNA in tail and tail moment at 4.5 to 7.5 mM AMPA. The human lymphocyte chromosome aberration assay also did not employ an exogenous mammalian metabolic activation system but control of medium pH and blind scoring of slides were reported for this assay. A small increase in chromosome aberrations per 100 metaphases was observed in cells exposed to 1.8 but not 0.9 mM AMPA for 48 hours. The increase was marginally significant ($p<0.05$) and no statistically significant increases were observed for any specific chromosome aberration category. Although number of cells with aberrations are commonly used to describe results from *in vitro* chromosome aberration assays (OECD473, 1997) these data were not presented. Given the marginal significance, these omissions are a significant limitation in interpreting the results. Positive results were also reported for a mouse micronucleus bone marrow assay in mice administered 2 x 100 mg/kg or 2 x 200 mg/kg i.p at 24 hour intervals. The methodology description did not indicate that slides were coded for analysis in this assay. Results were reported as a statistically significant increase from a negative control value of 3.8/1000 micronucleated erythrocytes to 10.0 and 10.4/1000 micronucleated erythrocytes in the 2 x 100 and 2 x 200 mg/kg dose groups, respectively. These data do not indicate a reasonable dose response and a third dose level was not employed as recommended for this assay (OECD474, 1997). The publication indicates micronucleus scoring results for "erythrocytes" and not polychromatic or immature erythrocytes as would be appropriate for the acute dose protocol employed. Although this might be an inadvertent error in methodology description the term polychromatic erythrocytes was used in the methods section and PCE was used in the results table to describe scoring of PCE/NCE ratio.

The reported positive effects for AMPA in the *in vitro* studies are not concordant with *in vitro* results for other endpoints or the lack of genotoxic structural alerts in the structurally similar parent molecule moieties from DEREK *in silico* analysis. The alkaline SCGE effect could be due to cytotoxicity, especially considering the relatively high dose levels employed (close to the 10 mM upper limit dose) and the lack of indication of pH control. Although limited cytotoxicity (>80 % viability) was reported using the trypan blue exclusion method this endpoint may grossly underestimate cytotoxic effects observed with other end points.
The *in vitro* chromosome aberration assay positive result was of low magnitude and was of particularly questionable significance, considering the lack of statistical significance for any individual chromosome aberration category and that the results for number or percent of cells with chromosome aberrations were not reported.
There is a clear discordance in results for AMPA in the mouse bone marrow micronucleus assay. In the earlier review negative results were reported for AMPA in a mouse bone marrow micronucleus assay conducted with dose levels up to 1000 mg/kg i.p. (Williams et al., 2000, ASB2012-12053) The maximum dose level was much higher than those used by Manas et al. (2009, ASB2012-11891) Although Manas et al. used a protocol with two doses separated by 24 hours and a single harvest time, this protocol would not be expected to give different results than a single dose with multiple harvest times, particularly when the maximum single dose was much higher (OECD474, 1997). PCE/NCE ratio data from the Manas et al. (2009, ASB2012-11891) study do not indicate that there were detectable bone marrow toxic effects observed under the conditions of their study. It appears possible that Manas et al. may have inappropriately scored erythrocytes for micronuclei instead of polychromatic erythrocytes, but if this is the case lower sensitivity rather than higher sensitivity would be expected. These limitations suggest the possibility that the aberrant result might be that of Manas et al. (2009, ASB2012-11891) but further studies might be necessary to resolve the discordance.
The earlier review reported negative results for POEA in an Ames/*Salmonella* assay (Williams et al., 2000, ASB2012-12053). No other genotoxicity results were reported for POEA individually but numerous genotoxicity results were presented, as described earlier, for GBFs containing POEA. Examination of subsequent literature for this review did not produce any new publications reporting genotoxicity results for POEA as an individual test material (i.e. not as a glyphosate formulation). However, there were some publications confirming that POEA can be a significant contributor to toxicity of GBFs and that it exhibits biological effects consistent with surfactant properties. As noted earlier, experiments with a POEA-containing formulation without glyphosate administered i.p. in DMSO/olive oil vehicle to mice produced the same severe liver and kidney toxicity as a GBF indicating that the toxicity primarily resulted from the formulation components rather than glyphosate (Heydens et al., 2008, ASB2012-11845). Similarly, dose-response curves were superimposed for an *in vitro* system evaluating a GBF and the same formulation without glyphosate present (Levine et al., 2007, ASB2009-9030). Effects on mammalian cells consistent with membrane disruption and consequent cytotoxicity were observed for POEA (Benachour and Seralini, 2009, ASB2012-11561).

B.6.4.8.10 Genotoxicity of glyphosate mixtures and photoactivation

Roustan et al. (2014, ASB2014-8086) assessed the photo-inducible cytogenetic toxicity of glyphosate, aminomethyl phosphoric acid (AMPA), desethyl-atrazine (DEA), and their various mixtures by the *in vitro* micronucleus assay on CHO-K1 cells. Results demonstrated according to the authors that cytogenetic potential of pesticides greatly depends on their

physico-chemical environment. The mixture made with the four pesticides exhibited the most potent cytogenetic toxicity, which was 20-fold higher than those of the most active compound AMPA, and 100-fold increased after light-irradation.

B.6.4.8.11 Genotoxicity Weight of Evidence

The earlier review applied a weight of evidence analysis to the available genotoxicity data. Various weighted components included assay system validation, test system species, relevance of the endpoint to heritable mutation, reproducibility and consistency of effects and dose-response and relationship of effects to toxicity (Williams et al., 2000, ASB2012-12053). The conclusion of this analysis was that glyphosate and Roundup GBFs were not mutagenic or genotoxic as a consequence of direct chemical reaction with DNA. This was supported by a strong preponderance of results indicating no effects in *in vivo* mammalian assays for chromosome effects and consistently negative results in gene mutation assays. Although some DNA damage responses were noted, these were judged likely to be secondary to toxicity rather than DNA reactivity.

Since this earlier review, a large number of genotoxicity studies have been conducted with glyphosate and GBFs. For gene mutation, one of the two primary endpoint categories with direct relevance to heritable mutation, one subsequent publication contains a summary of results from a bacterial gene mutation endpoint assay (Ames/*Salmonella* bacterial reversion assay). Although there were very significant limitations to the information published, the negative result is consistent with the majority of negative results reported for glyphosate and GBFs in Ames/*Salmonella* bacterial reversion assays. Another publication reported results for a *Drosophila* wing spot assay of glyphosate. Results were negative or inconclusive in this assay for crosses that would have detected gene mutation as loss of heterozygosity. The new results provide some support to reinforce the earlier conclusion that glyphosate and GBFs are not active for the gene mutation endpoint category.

The second primary endpoint category with direct relevance to heritable mutation is chromosome effects. The earlier review noted mixed results for two *in vitro* chromosome effects assays in mammalian cells but concluded that the most reliable result was the negative assay. A number of *in vitro* mammalian cell chromosome aberration or micronucleus assay results have been subsequently published using bovine or human lymphocytes. These assays suffer from some technical limitations in conduct or reporting of methodology that frequently included failure to indicate control of medium for pH and failure to indicate coding of slides for visual scoring. Both positive and negative results are reported in these assays. A large preponderance of results in the absence of an exogenous mammalian metabolic activation system were negative up to high (mM) dose levels that were toxic or close to toxic levels observed in parallel experiments. The exceptions were a weak and inconsistent response reported in two publications from the same laboratory and a positive response for the uncharacterized formulation, herbazed. In addition to these findings in mammalian cells negative results were also reported for Roundup GBF in an onion root tip assay conducted without exogenous mammalian metabolic activation. Thus, the preponderance of evidence from assays not employing an exogenous mammalian metabolic activation system indicates that glyphosate and GBFs are not structural chromosome breakage inducers (clastogenic) in *in vitro* mammalian chromosome aberration or micronucleus assays.

Two publications from one laboratory reported an increase in micronucleus frequencies for glyphosate in *in vitro* cultured mammalian cells in the presence of an exogenous S9 metabolic activation system (Mladinic et al., 2009, ASB2012-11906; Mladinic et al., 2009, ASB2012-11907). An enrichment for centomeric-containing micronuclei suggested that the increased

micronuclei observed in these studies were derived from aneugenic processes, probably mediated through toxicity, rather than chromosome breakage. Thus, these two reports of weak micronucleus responses in the presence of exogenous mammalian metabolic activation appear to result from toxicity-associated aneugenic rather than clastogenic mechanisms. A number of other gene mutation and *in vitro* chromosome effect genotoxicity studies are negative with exogenous metabolic activation which supports the conclusion that the weight of evidence does not indicate a DNA-reactive clastogenic activity in *in vitro* assays using mammalian cells.

All except one of a number of *in vivo* mouse bone marrow chromosome aberration or micronucleus assays of glyphosate and GBFs were reported as negative in the earlier review. In the updated review both positive and negative results were reported for glyphosate and GBFs in these types of assays. Many of these studies had limitations or deficiencies compared to international guidelines with the most common and significant being no indication of slide coding for visual scoring. Four publications from three laboratories reported negative results in mouse bone marrow erythrocyte micronucleus assays of glyphosate and GBFs which are consistent with the earlier reviewed studies. These studies used high, peri-lethal dose levels administered by the i.p. or oral routes.

Two publications from two laboratories reported positive results for glyphosate and GBFs in the mouse bone marrow erythrocyte micronucleus assay. One positive result for glyphosate was encountered using dose levels and routes that were similar to those employed in the negative glyphosate studies in the same assay system. The publication reporting this result indicates that erythrocytes rather than polychromatic erythrocytes were scored which would be inappropriate for the treatment protocol but it is possible that this is a misreporting of what types of cells were actually scored. Although there is no definitive explanation for the discordance, the preponderance of mouse bone marrow erythrocyte micronucleus studies of glyphosate are clearly negative. The reported positive result for Roundup GBF is discordant with a number of negative results for Roundup or other GBFs conducted at higher dose levels. The most unique feature of this study was the use of dimethylsulfoxide as a vehicle. The preponderance of mouse bone marrow erythrocyte micronucleus studies for Roundup and other GBF studies is negative.

Positive results were reported in an unusual test system (rabbit) and route (drinking water), but water intake was not reported and effects may therefore be attributable to dehydration. Furthermore, most of the effects were on endpoints not usually considered as indicators of clastogenicity and structural chromosome aberration. One laboratory reported positive results for chromosome aberration effects in bone marrow and spermatocytes after extended dosing. However, the herbazed formulation test material was not characterised.

While more discordant results in the important *in vivo* mammalian chromosome effect assay category have been reported in publications subsequent to the earlier 2000 review the preponderance of evidence continues to indicate that glyphosate and GBFs are not active in this category of end point.

Several *in vivo* erythrocyte micronucleus assay results for GBFs in non-mammalian systems (fish and caiman eggs) have been published since the earlier review. These test systems have relatively little experience and are largely unvalidated in comparison to the mouse bone marrow erythrocyte micronucleus assay. Two publications report negative results and two publications report positive results in different fish species and there is no definitive explanation for the discordance. Both the positive and negative studies employed maximum dose levels that were toxic or close to toxic dose levels. One possible explanation for the discordance is that the positive effects were associated with toxicity that only occurred beyond an exposure threshold and over a fairly narrow dose range. Positive results in hatchlings derived from caiman eggs exposed to Roundup formulation are given relatively

little weight because of extremely limited experience with this assay system and because of significant questions about how DNA damage effects induced in embryos can persist and be evident in cells of hatchlings after several weeks and numerous cell divisions. The reported weak and inconsistent response in one of four crosses for somatic recombination in the *Drosophila* wing spot assay is also accorded relatively low weight. These non-mammalian test systems are generally considered of lower weight for predicting mammalian effects than mammalian test systems. Also, the environmental significance of effects for GBFs should consider the relationship between concentrations or exposures producing effects and likely environmental concentrations or exposures. This is particularly important if the effects are produced by threshold mediated toxic processes.

There have been a significant number of publications since the earlier review of results for assays in the DNA damage category with some SCE and a large number of alkaline SCGE endpoint publications. In general, the DNA damage end point category is considered supplementary to the gene mutation and chromosome effect categories because this endpoint category does not directly measure heritable events or effects closely associated with heritable events. Regulatory genotoxicity testing recommendations and requirements focus on gene mutation and chromosome effect end points for initial core testing, particularly for *in vitro* testing. This consideration is underscored by the observation of some cases of compounds where positive effects are observed in these assays that are not observed for gene mutation or chromosome effect assays. Also, there are numerous examples of responses in these endpoints that do not appear to result from mechanisms of direct or metabolite DNA-reactivity. The unique response consideration is reinforced in this data set by observations of responses in DNA damage endpoints but not in chromosome effect end points.

Many DNA damage endpoint assays of glyphosate or GBFs have produced positive results at high, toxic or peri-toxic dose levels for the SCE and alkaline SCGE endpoints in a variety of test systems including cultured mammalian cells, several aquatic species and caiman eggs. The only new report of positive *in vivo* mammalian DNA damage effects are for an uncharacterised formulation, herbazed. There are several examples of negative results for a chromosome aberration or micronucleus endpoint and positive results for the alkaline SCGE or SCE endpoint in the same publication (Cavalcante et al., 2008, ASB2012-11586; Manas et al., 2009, ASB2012-11892; Mladinic et al., 2009, ASB2012-11906; Sivikova and Dianovsky, 2006, ASB2012-12029). These examples confirm the impression that the DNA damage endpoints are not necessarily predictive of heritable mutation effects and are also consistent with the DNA damage endpoints reflecting toxic effect mechanisms. While the number of reported positive responses in these endpoints does suggest that effects in these endpoints can be induced by glyphosate or GBFs, comparison with results for gene mutation and chromosome effects endpoints, examination of the dose response and association of the effects with toxic endpoints indicates that these effects are likely secondary to toxicity and are threshold mediated. Surfactants in GBFs increase toxicity compared to the active ingredient of glyphosate salts and are shown to induce effects such as membrane damage and oxidant stress which are likely capable of inducing DNA damage effects at cytotoxic doses. These factors as well as other considerations presented in Section 6.3 indicate that these DNA damage effects have negligible significance to prediction of hazard or risk at lower and more relevant exposure levels.

Most of the human studies do not provide interpretable or relevant information regarding whether there are *in vivo* human genotoxic effects of GBFs because the reported incidence of glyphosate formulation exposure in the population was low or because there were reported exposures to a relatively large number of pesticides. Two studies with focus on glyphosate exposure through presence in or near areas of glyphosate formulation spraying found increases in the DNA damage alkaline SCGE end point. In one study clinical signs of toxicity

were reported in the population and spraying concentrations were reported to be many times the recommended application rate. Given the nature of the end point a reasonable interpretation is that effects might well be due to the overt toxicity that was reported in the publication. This would be a threshold mediated, non-DNA reactive mechanism and is consistent with experimental system results showing alkaline SCGE effects in animals exposed to high levels of formulation components. The low weight of evidence for significant genotoxic hazard indicated by this particular endpoint in human monitoring is reinforced by findings that exercise induces alkaline SCGE effects in humans. The other study found increases in binucleated micronucleated cell frequency in population in spraying areas but the increases were not consistent with spraying levels or self-reported exposure. These latter observations are not consistent with the study presenting clear evidence of GBF effects on this endpoint. In sum, the available human data do not provide any clear indications that exposed humans are substantially different in response than mammalian animal models or that exposure to GBFs produces DNA-reactive genotoxicity.

Carcinogenicity is an adverse effect that is a possible consequence of genotoxic and mutagenic activity. Conversely, lack of carcinogenicity in properly conducted animal models is supportive for lack of significant *in vitro* mammalian genotoxicity. The updated review provides one new study of glyphosate formulation which is negative for either initiator or complete carcinogenesis activity which provides additional evidence to reinforce the conclusion from earlier mammalian carcinogenicity assays that glyphosate and GBFs are non-carcinogenic. These findings support the conclusion that glyphosate and GBFs do not have *in vivo* mammalian genotoxicity or mutagenicity.

In addition to considering the results relevant to genotoxicity hazard assessment, an important additional perspective on risk can be provided by comparing levels used in experimental studies with expected human and environmental exposure levels. A study of farmers indicated a maximum estimated systemic glyphosate dose of 0.004 mg/kg for application without protective equipment and a geometric mean of 0.0001 mg/kg (Acquavella et al., 2004, ASB2012-11528). When compared with *in vivo* mammalian test systems that utilize glyphosate exposures on the order of 50-300 mg/kg, the margins of exposure between the test systems and farmers is 12,500-75,000 for the maximum farmer systemic exposure and 0.5-3 million for the geometric mean farmer systemic exposure. These margins are quite substantial, especially considering that many of the *in vivo* genotoxicity studies are negative. Assuming reasonable proportionality between exposure to glyphosate and GBF ingredients, similar large margins of exposure would exist for GBF components. The margins of exposure compared to *in vitro* mammalian cell exposures are estimated to be even larger. Assuming uniform distribution, the systemic concentration of glyphosate from the Aquavella et al. (2004, ASB2012-11528) farmer biomonitoring study would be on the order of 24nM for the maximum and 0.59 nM for the geometric mean exposure. A typical maximum *in vitro* mammalian exposure of 1-5 mM represents a margin of exposure of 42,000-211,000 for the maximum farmer exposure and 1.7-8.4 million for the geometric mean farmer systemic exposures, respectively.

Overall, the weight of evidence of the studies considered in the earlier review as well as the studies considered in this review indicates that glyphosate and GBFs are not genotoxic in the two general endpoint categories most directly relevant to heritable mutagenesis, gene mutation and chromosome effects. This conclusion results from a preponderance of evidence; however, there are reports of positive discordant results in both end point categories. The new studies considered in this review provide some evidence for DNA damage effects induced by high, toxic exposures, particularly for the alkaline SCGE end point and for GBFs containing surfactant. Several considerations, including the lack of response in other endpoint

categories, suggest that these effects result from toxic and not DNA-reactive mechanisms and that they do not indicate *in vivo* genotoxic potential under normal exposure levels.
Regulatory and authoritative reviews of glyphosate supporting registrations and registrations in all regions of the world over the last 40 years have consistently determined that glyphosate is nongenotoxic (Commission, 2002, ASB2009-4191; WHO/FAO, 2004, ASB2008-6266). Scientific publications contrary to these regulatory reviews should be evaluated using a weight of evidence approach with consideration for reliability of the assay used and data quality presented.

Abbreviations
AMPA, aminomethylphosphonic acid ; CB MN, cytokinesis block micronucleus; GBF, glyphosate based formulation; i.p., intraperitoneal ; NCE, normochromatic erythrocyte; OECD, Organization for Economic Cooperation and Development; PCE, polychromatic erythrocyte; POEA, polyethoxylated tallow amine, [redacted] ethoxylate; SCE, sister chromatid exchange; SCGE, single cell gel electrophoresis (comet).

Author(s)	Year	Study title
Alvarez-Moya, C., Silva, M.R., Arambula, A.R.V., Sandoval, A.I., Vasquez, H.C., Gonzales Montes, R.M.	2011	Evaluation of genetic damage induced by glyphosate isopropylamine salt using *Tradescantia* bioassays Genetics and Molecular Biology Volume: 34 Number: 1 Pages: 127-130 ASB2012-11538

Abstract*
Glyphosate is noted for being non-toxic in fishes, birds and mammals (including humans). Nevertheless, the degree of genotoxicity is seriously controversial. In this work, various concentrations of a glyphosate isopropylamine salt were tested using two methods of genotoxicity assaying, *viz*., the pink mutation assay with *Tradescantia* (4430) and the comet assay with nuclei from staminal cells of the same plant. Staminal nuclei were studied in two different forms, namely nuclei from exposed plants, and nuclei exposed directly. Using the pink mutation assay, isopropylamine induced a total or partial loss of color in staminal cells, a fundamental criterion utilised in this test. Consequently, its use is not recommended when studying genotoxicity with agents that produce pallid staminal cells. The comet assay system detected statistically significant ($p < 0.01$) genotoxic activity by isopropylamine, when compared to the negative control in both the nuclei of treated plants and directly treated nuclei, but only the treated nuclei showed a dose-dependent increase. Average migration in the nuclei of treated plants increased, when compared to that in treated nuclei. This was probably due, either to the permanence of isopropylamine in inflorescences, or to the presence of secondary metabolites. In conclusion, isopropylamine possesses strong genotoxic activity, but its detection can vary depending on the test systems used.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Exposure conditions of plants (immersion) not representative for glyphosate. Inappropriate test model

	as herbicides are toxic to plants. Presentation of results not sufficient for assessment. Reporting deficiencies (e.g. positive controls).
Relevance of study:	Not relevant (Due to reliability, and exposure conditions of plants and inappropriate test model).
Klimisch code:	3

Author(s)	Year	Study title
Bolognesi, C. Bonatti, S. Degan, P. Gallerani, E. Peluso, M. Rabboni, R. Roggieri, P. Abbondandolo, A.	1997	Genotoxic activity of glyphosate and its technical formulation roundup Journal of Agricultural and Food Chemistry Volume: 45 Pages: 1957-1962 Z59299

Abstract*
Glyphosate (N-phosphonomethylglycine) is an effective herbicide acting on the synthesis of aromatic amino acids in plants. The genotoxic potential of this herbicide has been studied: the results available in the open literature reveal a weak activity of the technical formulation. In this study, the formulated commercial product, Roundup, and its active agent, glyphosate, were tested in the same battery of assays for the induction of DNA damage and chromosomal effects in vivo and in vitro. Swiss CD1 mice were treated intraperitoneally with test substances, and the DNA damage was evaluated by alkaline elution technique and 8-hydroxydeoxyguanosine (8-OHdG) quantification in liver and kidney. The chromosomal damage of the two pesticide preparations was also evaluated in vivo in bone marrow of mice as micronuclei frequency and in vitro in human lymphocyte culture as SCE frequency. A DNA-damaging activity as DNA single-strand breaks and 8-OHdG and a significant increase in chromosomal alterations were observed with both substances *in vivo* and *in vitro*. A weak increment of the genotoxic activity was evident using the technical formulation.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Basic data given, however, the study is performed with methodological and reporting deficiencies (only data without metabolic activation generated in *in vitro* tests, no positive controls included in *in vitro* SCE and *in vivo* experiments, in some experiments only two test substance concentrations tested).
Relevance of study:	Not relevant (Due to methodological and reporting deficiencies data considered to be supplemental information; i.p. exposure route is not relevant for human exposure)
Klimisch code:	3

Author(s)	Year	Study title
Bolognesi, C., Perrone, E., Landini, E.	2002	Micronucleus monitoring of a floriculturist population from western Liguria, Italy Mutagnesis Volume: 17 Number: 5 Pages: 391-397 ASB2012-11573

Abstract*
A biomonitoring study was carried out to investigate whether exposure to complex pesticide mixtures in ornamental crop production represents a potential genotoxic risk. Exposed and control subjects were selected in western Liguria (Italy). The area was chosen for its intensive use of pesticides. The main crops produced were roses, mimosas, carnations and chrysanthemums, as ornamental non-edible plants, and tomato, lettuce and basil, as edible ones. The levels of micronuclei (MN) were analysed in peripheral blood lymphocytes of 107 floriculturists (92 men and 15 women) and 61 control subjects (42 men and 19 women). A statistically significant increase in binucleated cells with micronuclei (BNMN) was detected in floriculturists with respect to the control population (4.41 +/- 2.14 MN/1000 cells versus 3.04 +/- 2.14, $P < 0.001$). The mean number of BNMN varied as a function of sex and age. Smoking habit had no effect on MN frequency. A positive correlation between years of farming and MN frequency in peripheral blood lymphocytes was observed ($r = 0.30$, $P = 0.02$). The conditions of exposure were also associated with an increase in cytogenetic damage, with a 28 % higher MN frequency in greenhouse workers compared with subjects working only outdoors in fields. Workers not using protective measures during high exposure activities showed an increase in MN frequency. Our findings suggest a potential genotoxic risk due to pesticide exposure.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable for glyphosate
Comment:	MN-test comparable to OECD guidelines, but not equal. Exposures to multiple pesticides with no information on exposure concentrations to individual pesticides make result unreliable for glyphosate.
Relevance of study:	Not relevant (Due to the exposure of multiple pesticides, only general conclusions about pesticide exposure and cytogenicity possible. Not relevant for glyphosate).
Klimisch code:	3

Author(s)	Year	Study title
Bolognesi, C., Landini, E., Perrone, E., Roggieri, P.	2004	Cytogenetic biomonitoring of a floriculturist population in Italy: micronucleus analysis by fluorescence in situ hybridization (FISH) with an all-chromosome centrometric probe Mutation Research Volume: 557 Number: 2 Pages: 109-117 ASB2012-11572

Abstract*
Flower production in greenhouses associated with a heavy use of pesticides is very widespread in the western part of the Ligurian region (Italy). The formation of micronuclei in peripheral blood lymphocytes is a valuable cytogenetic biomarker in human populations occupationally exposed to genotoxic compounds. In the present study we investigated the micronucleus frequency in peripheral blood lymphocytes of 52 floriculturists and 24 control subjects by use of the cytokinesis-block methodology associated with fluorescence in situ hybridization with a pan-centromeric probe that allowed to distinguish centromere-positive (C+) and centromere-negative (C−) micronuclei. The comparison between floriculturists and controls did not reveal any statistically significant difference in micronucleus frequency, although an increase was observed with increasing pesticide use, number of genotoxic pesticides used and duration of exposure. An increase in C+ as well as in C− micronuclei and in the percentage of C+ micronuclei with respect to the total number of micronuclei was detected in floriculturists, suggesting a higher contribution of C+ micronuclei in the total number scored. The percentage C+ micronuclei was not related to the duration of exposure or to the number of genotoxic pesticides used, but a higher percentage (66.52 % versus 63.78 %) was observed in a subgroup of subjects using benzimidazolic compounds, compared with the floriculturist population exposed to a complex pesticide mixture not including benzimidazolics. These results suggest a potential human hazard associated with the exposure to this class of aneuploidy-inducing carcinogens.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable for glyphosate
Comment:	Well-documented study. MN-test comparable to OECD guidelines, but not equal. No information on exposure concentrations to individual pesticides
Relevance of study:	Not relevant (Due to the exposure of multiple pesticides, only general conclusions about pesticide exposure and cytogenic non-statistically significant differences possible. No statistically relevant findings reported for glyphosate alone).
Klimisch code:	2

Author(s)	Year	Study title
Cavas, T., Könen S.	2007	Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (*Carassius auratus*) exposed to a glyphosate formulation using the micronucleus test and the comet assay Mutagenesis 22 263-268 ASB2012-11587

Abstract*
Glyphosate is a widely used broad-spectrum weed control agent. In the present study, an in vivo study on the genotoxic effects of a technical herbicide (Roundup®) containing isopropylamine salt of glyphosate was carried out on freshwater goldfish *Carassius auratus*. The fish were exposed to three doses of glyphosate formulation (5, 10 and 15 ppm). Cyclophosphamide at a single dose of 5 mg/L was used as positive control. Analysis of micronuclei, nuclear abnormalities and DNA damage were performed on peripheral erythrocytes sampled at intervals of 48, 96 and 144 h post treatment. Our results revealed significant dose-dependent increases in the frequencies of micronuclei, nuclear abnormalities as well as DNA strand breaks. Our findings also confirmed that the alkaline comet assay and nuclear deformations in addition to micronucleus test on fish erythrocytes in vivo are useful tools in determining the potential genotoxicity of commercial herbicides.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not Reliable
Comment:	Methodological and reporting deficiencies (e.g. test substance source, no concurrent measurement of toxicity reported, less than 2000 erythrocytes scored per animal and results not reported separately for replicates).
Relevance of study:	Relevant with restrictions (Due to reliability. Discussion confuses glyphosate with glyphosate formulated products.)
Klimisch code:	3

Author(s)	Year	Study title
Guilherme, S. Gaivao, I. Santos, M.A. Pacheco, M.	2010	European eel (*Anguilla Anguilla*) genotoxic and pro-oxidant responses following short-term exposure to Roundup® - a glyphosate-based herbicide. Mutagenesis Volume: 25 Number: 5 Pages: 523-530 ASB2012-11836

Abstract*
The glyphosate-based herbicide, Roundup®, is among the most used pesticides worldwide. Due to its extensive use, it has been widely detected in aquatic ecosystems representing a potential threat to non-target organisms, including fish. Despite the negative impact of this commercial formulation in fish, as described in literature, the scarcity of studies assessing its genotoxicity and underlying mechanisms is evident. Therefore, as a novel approach, this study evaluated the genotoxic potential of Roundup® to blood cells of the European eel (*Anguilla anguilla*) following short-term (1 and 3 days) exposure to environmentally realistic concentrations (58 and 116 mg/L), addressing also the possible association with oxidative stress. Thus, comet and erythrocytic nuclear abnormalities (ENAs) assays were adopted, as genotoxic end points, reflecting different types of genetic damage. The prooxidant state was assessed through enzymatic (catalase, glutathione-S-transferase, glutathione peroxidase and glutathione reductase) and non-enzymatic (total glutathione content) antioxidants, as well as by lipid peroxidation (LPO) measurements. The Roundup® potential to induce DNA strand breaks for both concentrations was demonstrated by the comet assay. The induction of chromosome breakage and/or segregational abnormalities was also demonstrated through the ENA assay, though only after
3-day exposure to both tested concentrations. In addition, the two genotoxic indicators were positively correlated. Antioxidant defences were unresponsive to Roundup®. LPO levels increased only for the high concentration after the first day of exposure, indicating that oxidative stress caused by this agrochemical in blood was not severe. Overall results suggested that both DNA damaging effects induced by Roundup® are not directly related with an increased pro-oxidant state. Moreover, it was demonstrated that environmentally relevant concentrations of Roundup® can pose a health risk for fish populations.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	No positive controls were included, which significantly detracts from the utility of a non-validated, non-standard test method. Less than the standard of a minimum of three dose levels used, independent coding of slides for scoring and results not reported separately for replicates.
Relevance of study:	Not relevant (Non-standard test system, no positive controls to verify test method/study validity.)
Klimisch code:	3

Author(s)	Year	Study title
Kale, P.G. Petty, B.T. Jr. Walker, S. Ford, J.B. Dehkordi, N. Tarasia, S. Tasie, B.O. Kale, R. Sohni, Y.R.	1995	Mutagenicity Testing of Nine Herbicides and Pesticides Currently Used in Agriculture. Environmental and Molecular Mutagenesis Volume: 25 Pages: 148-153 Z73986, ASB2012-11860

Abstract*
Nine herbicides and pesticides were tested for their mutagenicity using the *Drosophila* sex-linked recessive lethal mutation assay. These are Ambush, Treflan, Blazer, Roundup, 2,4-D Amine, Crossbow, Galecron, Pramitol, and Pondmaster. All of these are in wide use at present. Unlike adult feeding and injection assays, the larvae were allowed to grow in medium with the test chemical, thereby providing long and chronic exposure to the sensitive and dividing diploid cells, i.e., mitotically active spermatogonia and sensitive spermatocytes. All chemicals induced significant numbers of mutations in at least one of the cell types tested. Some of these compounds were found to be negative in earlier studies. An explanation for the difference in results is provided. It is probable that different germ cell stages and treatment regimens are suitable for different types of chemicals. Larval treatment may still be valuable and can complement adult treatment in environmental mutagen testing.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Comparable to 1984 OECD guideline, but with several deficiencies (no positive controls reported and thus study validity not verifiable; wild type male treatment age different than recommended, purity of test substances not reported, tested formulation other ingredients such as surfactants not reported.)
Relevance of study:	Not relevant for glyposate (Glyphosate not tested; formulation tested)
Klimisch code:	3

Author(s)	Year	Study title
Manas, F. Peralta, L. Raviolo, J. Garcia Ovando, H. Weyers, A. Ugnia, L. Gonzalez Cid, M. Larripa, I. Gorla, N.	2009	Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests. Ecotoxicology and Environmental Safety Volume: 72 Pages: 834-837 ASB2012-11891

Abstract*
Formulations containing glyphosate are the most widely used herbicides in the world. AMPA is the major environmental breakdown product of glyphosate. The purpose of this study is to evaluate the in vitro genotoxicity of AMPA using the Comet assay in Hep-2 cells after 4h of incubation and the chromosome aberration (CA) test in human lymphocytes after 48 h of exposition. Potential in vivo genotoxicity was evaluated through the micronucleus test in mice. In the Comet assay, the level of DNA damage in exposed cells at 2.5-7.5 mM showed a significant increase compared with the control group. In human lymphocytes we found statistically significant clastogenic effect AMPA at 1.8 mM compared with the control group. In vivo, the micronucleus test rendered significant statistical increases at 200-400 mg/kg. AMPA was genotoxic in the three performed tests. Very scarce data are available about AMPA potential genotoxicity.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Reporting deficiencies (purity of AMPA not specified, several parameters in the MNT not reported, only 2 dose levels used in both CA and MNT). Exposure route used in the MNT is not relevant for human exposure; methodological deficiencies (see guideline deviations).
Relevance of study:	Not relevant (Due to reliability)
Klimisch code:	3

Author(s)	Year	Study title
Manas, F. Peralta, L. Raviolo, J. Garcia Ovando, H. Weyers, A. Ugnia, L. Gonzalez Cid, M. Larripa, I. Gorla, N.	2009	Genotoxicity of glyphosate assessed by the comet assay and cytogenic tests Environmental Toxicology and Pharmacology Volume: 28 Pages: 37-41 ASB2012-11892

Abstract*
It was evaluated the genotoxicity of glyphosate which up to now has heterogeneous results. The comet assay was performed in Hep-2 cells. The level of DNA damage in the control group (5.42±1.83 arbitrary units) for tail moment (TM) measurements has shown a significant increase ($p < 0.01$) with glyphosate at a range concentration from 3.00 to 7.50mM. In the chromosome aberrations (CA) test in human lymphocytes the herbicide (0.20–6.00mM) showed no significant effects in comparison with the control group. In vivo, the micronucleus test (MNT)was evaluated in mice at three doses rendering statistical significant increases at 400 mg/kg (13.0±3.08 micronucleated erythrocytes/1000 cells, $p < 0.01$). In the present study glyphosate was genotoxic in the comet assay in Hep-2 cells and in the MNT test at 400 mg/kg in mice. Thiobarbituric acid reactive substances (TBARs) levels, superoxide dismutase (SOD) and catalase (CAT) activities were quantified in their organs. The results showed an increase in these enzyme activities.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Guideline deviations and reporting deficiencies. Several parameters in the MNT not reported. Blind scoring reported for the CA but not MNT. Exposure route used in the MNT is not relevant for human exposure. (see guideline deviations). No indication of pH or osmolality control for the comet assay. Results not reported separately for replicates.
Relevance of study:	Not relevant (Due to guideline deviations and reporting deficiencies).
Klimisch code:	3

Author(s)	Year	Study title
Mladinic, M. Berend, S. Vrdoljak, A.L. Kopjar, N. Radic, B. Zeljezic, D.	2009	Evaluation of Genome Damage and Its Relation to Oxidative Stress Induced by Glyphosate in Human Lymphocytes *in Vitro* Environmental and Molecular Mutagenesis Volume: 50 Number: 9 Pages: 800-807 ASB2012-11906

Abstract*
In the present study we evaluated the genotoxic and oxidative potential of glyphosate on human lymphocytes at concentrations likely to be encountered in residential and occupational exposure. Testing was done with and without metabolic activation (S9). Ferric-reducing ability of plasma (FRAP), thiobarbituric acid reactive substances (TBARS) and the hOGG1 modified comet assay were used to measure glyphosate's oxidative potential and its impact on DNA. Genotoxicity was evaluated by alkaline comet and analysis of micronuclei and other nuclear instabilities applying centromere probes. The alkaline comet assay showed significantly increased tail length (20.39 µm) and intensity (2.19 %) for 580 µg/mL, and increased tail intensity (1.88 %) at 92.8 µg/mL, compared to control values of 18.15 µm for tail length and 1.14 % for tail intensity. With S9, tail length was significantly increased for all concentrations tested: 3.5, 92.8, and 580 µg/mL. Using the hOGG1 comet assay, a significant increase in tail intensity was observed at 2.91 µg/mL with S9 and 580 µg/mL without S9. Without S9, the frequency of micronuclei, nuclear buds and nucleoplasmic bridges slightly increased at concentrations 3.5 µg/mL and higher. The presence of S9 significantly elevated the frequency of nuclear instabilities only for 580 µg/mL. FRAP values slightly increased only at 580 µg/mL regardless of metabolic activation, while TBARS values increased significantly. Since for any of the assays applied, no clear dose-dependent effect was observed, it indicates that glyphosate in concentrations relevant to human exposure do not pose significant health risk.
* Quoted from article

Klimisch evaluation

Reliability of study:	Reliable with restrictions
Comment:	Non-GLP, non-guideline *in vitro* study, meeting scientific principles
Relevance of study:	Relevant with restrictions (Assessment of Genotoxicity *in vitro* at concentrations relevant to human exposure levels; authors state that no clear dose-dependent effect was observed, and results indicate that glyphosate in concentrations relevant to human exposure do not pose significant health risk.
Klimisch code:	2

Author(s)	Year	Study title
Mladinic, M., Perkovic, P., Zeljezic, D.	2009b	Characterization of chromatin instabilities induced by glyphosate, terbuthylazine and carbofuran using cytome FISH assay Toxicology Letters Volume: 189 Number: 2 Pages: 130-137 ASB2012-11907

Abstract*
Possible clastogenic and aneugenic effects of pesticides on human lymphocytes at concentrations likely to be encountered in residential and occupational exposure were evaluated with and without the use of metabolic activation (S9). To get a better insight into the content of micronuclei (MN) and other chromatin instabilities, lymphocyte preparations were hybridized using pancentromeric DNA probes. Frequency of the MN, nuclear buds (NB) and nucleoplasmic bridges (NPB) in cultures treated with glyphosate slightly increased from 3.5 µg/mL onward. Presence of S9 significantly elevated cytome assay parameters only at 580 µg/mL. No concentration-related increase of centromere (C+) and DAPI signals (DAPI+) was observed for glyphosate treatment. Terbuthylazine treatment showed a dose dependent increase in the number of MN without S9 significant at 0.0008 µg/mL and higher. At concentration lower than 1/16 LD_{50} occurrence of C + MN was significantly elevated regardless of S9, but not dose related, and in the presence of S9 only NBs containing centromere signals were observed. Carbofuran treatment showed concentration dependent increase in the number of MN. The frequency of C + MN was significant from 0.008 µg/mL onward regardless of S9. Results suggest that lower concentrations of glyphosate have no hazardous effects on DNA, while terbuthylazine and carbofuran revealed a predominant aneugenic potential.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-GLP, non-guideline study *in vitro*. Positive and negative control results almost indistinguishable for MN assay without metabolic activation. Negative control NB and NBP results not reported.
Relevance of study:	Not relevant (Proposed mechanism of genotoxicity (*in vitro*) is not relevant to human exposure levels. Authors express confidence that estimated maximum human exposure levels correspond to acceptable safety levels based on evaluated *in vitro* endpoints, and that their findings need to be verified *in vivo*.)
Klimisch code:	3

Author(s)	Year	Study title
Paz-Y-Mino, C. Sanchez, M. E. Arevalo, M. Munoz, M. J. Witte, T. De-La-Carrera, G. O. Leone, P. E.	2007	Evaluation of DNA damage in an Ecuadorian population exposed to glyphosate. Genetics and Molecular Biology Volume: 30 Number: 2 Pages: 456-460 ASB2012-11992

Abstract*
We analyzed the consequences of aerial spraying with glyphosate added to a surfactant solution in the northern part of Ecuador. A total of 24 exposed and 21 unexposed control individuals were investigated using the comet assay. The results showed a higher degree of DNA damage in the exposed group (comet length = 35.5 mu m) compared to the control group (comet length = 25.94 mu m). These results suggest that in the formulation used during aerial spraying glyphosate had a genotoxic effect on the exposed individuals.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Documentation of Comet assay insufficient for assessment.
Relevance of study:	Not relevant (Glyphosate formulation was applied at much higher dose rates than recommended for the intended uses in the EU. In addition, the herbicide was combined with the adjuvant (Cosmoflux 411F) that can increase the biological action of the herbicide. This adjuvant will not be used in the EU.)
Klimisch code:	3

Author(s)	Year	Study title
Peluso, M. Munnia, A. Bolognesi, C. Parodi, S.	1998	^{32}P-postlabeling detection of DNA adducts in mice treated with the herbicide Roundup. Environmental and Molecular Mutagenesis Volume: 31 Number: 4 Pages: 55-59 TOX1999-318

Abstract*
Roundup is a postemergence herbicide acting on the synthesis of amino acids and other important endogenous chemicals in plants. Roundup is commonly used in agriculture, forestry, and nurseries for the control or destruction of most herbaceous plants. The present study shows that Roundup is able to induce a dose-dependent formation of DNA adducts in the kidneys and liver of mice. The levels of Roundup-related DNA adducts observed in mouse kidneys and liver at the highest dose of herbicide tested (600 mg/kg) were 3.0 +/- 0.1 (SE) and 1.7 +/- 0.1 (SE) adducts/10(8) nucleotides, respectively. The Roundup DNA adducts were not related to the active ingredient, the isopropylammonium salt of glyphosate, but to another, unknown component of the herbicide mixture. Additional experiments are needed to identify the chemical specie(s) of Roundup mixture involved in DNA adduct formation. Findings of this study may help to protect agricultural workers from health hazards and provide a basis for risk assessment.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not Reliable
Comment:	A non-guideline study with confounding results based on testing a surfactant containing formulation. Reporting deficiencies (statistical methods). Toxic surfactant effects subsequently verified in Heydens et al. (2008, ASB2012-11845) reporting the same study type with a glyphosate formulated product and an appropriate control; formulation blank without glyphosate.
Relevance of study:	Not relevant (i.p. administration of high doses of a surfactant containing formulation a relevant exposure scanario for human risk assessments. In addition, the DNA adducts observed were not related to the active ingredient (isopropylammonium salt of glyphosate), but to another, unknown component of the herbicide mixture.)
Klimisch code:	3

Author(s)	Year	Study title
Poletta, G.L. Larriera, A. Kleinsorge, E. Mudry, M.D.	2009	Genotoxicity of the herbicide formulation Roundup® (glyphosate) in broad-snouted caiman (*Caiman latirostris*) evidenced by the Comet assay and Micronucleus test Mutation Research Volume: 672 Number: 2 Pages: 95-102 ASB2012-12002

Abstract*
The genotoxicity of pesticides is an issue of worldwide concern. The present study was undertaken to evaluate the genotoxic potential of a widely used herbicide formulation, Roundup® (glyphosate), in erythrocytes of broad-snouted caiman (*Caiman latirostris*) after in ovo exposure. Caiman embryos were exposed at early embryonic stage to different sub-lethal concentrations of Roundup® (50, 100, 200, 300, 400, 500, 750, 1000, 1250 and 1750 µg/egg). At time of hatching, blood samples were obtained from each animal and two short-term tests, the Comet assay and the Micronucleus (MN) test, were performed on erythrocytes to assess DNA damage. A significant increase in DNA damage was observed at a concentration of 500 µg/egg or higher, compared to untreated control animals ($p<0.05$). Results from both the Comet assay and the MN test revealed a concentration-dependent effect. This study demonstrated adverse effects of Roundup® on DNA of *C. latirostris* and confirmed that the Comet assay and the MN test applied on caiman erythrocytes are useful tools in determining potential genotoxicity of pesticides. The identification of sentinel species as well as sensitive biomarkers among the natural biota is imperative to thoroughly evaluate genetic damage, which has significant consequences for short- and long-term survival of the natural species.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-GLP studies in a unique test model. Micronucleus assay followed guideline, Comet assay similar to guideline. Test methods have been modified to be applied caiman species. Methodological deficiencies: housing and feeding conditions of parents not specified; sex not distinguished, stability and homogeneity assessment of test substance preparations not reported. Results not reported separately for replicate individual animals.
Relevance of study:	Not relevant. Highly artificial *in ovo* exposure scenario not relevant to real world environmental exposures. Caiman eggs are covered and not exposed to the surface. Any glyphosate in a potential herbicide overspray would sorb to sediment and organic matter. and not transport to the egg surface.
Klimisch code:	3

Author(s)	Year	Study title
Rodrigues, H.G. Penha-Silva, N. Ferreira Pereira de Araujo, M. Nishijo, H. Aversi-Ferreira, T.A.	2011	Effects of Roundup® Pesticide on the Stability of Human Erythrocyte Membranes and Micronuclei Frequency in Bone Marrow Cells of Swiss Mice The Open Biology Journal, Volume: 4 Pages: 54-59 ASB2012-12010

Abstract*
Pesticides can affect the health of living organisms through different mechanisms such as membrane denaturation. The evaluation of the deleterious effects of chemical agents on biological membranes can be performed through the analysis of the stability of erythrocytes against a concentration gradient of certain chemical agent in physiologic saline solution. This work analyzed the effect of the herbicide Roundup® on the membrane of human erythrocytes in blood samples collected with EDTA or heparin as anticoagulant agent. The results were analyzed through spectrophotometry at 540 nm and light microscopy. There was an agreement between spectrophometric and morphologic analyses. At the concentration limit recommended for agricultural purposes, Roundup® promoted 100 % of hemolysis. The D_{50}Roundup® values obtained for human blood samples collected with EDTA were not significantly different from those obtained for samples collected with heparin. However, the lysis curves presented lower absorbance values at 540 nm in the presence of blood collected with EDTA in relation to that collected with heparin, probably due to haemoglobin precipitation with EDTA. This work also analyzed the effects of three different Roundup® doses (0.148, 0.754 and 1.28 mg/kg) on the micronuclei frequency in bone marrow cells of Swiss mice in relation to a positive control of cyclophosphamide (250 mg/kg). The two highest Roundup® doses showed the same genotoxicity level as the positive control.
* Quoted from article

Klimisch evaluation

1Reliability of study:	Not reliable. Determination of the stability of human erythrocytes: Results are not surprising because surfactants are known to compromise cell membrane integrity. Doses not reflective of physiological concentrations of either glyphosate or surfactant. Micronucleus test *in vivo*: Irrelevant route of exposure for surfactant containing formulated products. Results confounded by presence of surfactant toxicity; refer to Heydens et al. (2008, ASB2012-11845)
Comment:	Non-guideline, non-GLP studies Determination of the stability of human erythrocytes Results attributable to surfactant induced cytotoxicity Micronucleus test *in vivo* Major reporting deficiencies (no information on number of cells evaluated, only graphical documentation of results, no information on absolute MN frequencies).
Relevance of study:	Not relevant (Test material containing surfactant is not appropriately evaluated in either model).
Klimisch code:	3

Author(s)	Year	Study title
Vigfusson, N.V. Vyse, E.R.	1980	The effect of the pesticides Dexon, Captan and Roundup on sister chromatid exchanges in human lymphocytes in vitro. Mutation Research Volume: 79 Pages: 53-57 TOX9700576, ASB2012-12044

Abstract*
Three pesticides at varying concentration were tested for the induction of SCE [sister chromatid exchanges] in human lymphocytes in vitro. The fungicide, Dexon, sodium (4-(dimethylamino)phenyl)diazene sulfonate, caused the greatest increase in SCE frequency and the response was dose related. The herbicide, Roundup, isopropylamide salt of N-(phosphonomethyl)glycine, had the least effect on SCE requiring the use of much higher concentrations to produce an effect. Limited results were obtained with the fungicide Captan, cis-N-((trichloromethyl)thio)-4-cyclo-hexene-1, 2-dicarboximide, because of toxic levels of the fungicide or solvent used.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Test material was a formulated product containing surfactant. Authors acknowledge cytotoxicity was a confounding factor for data interpretation; since the time of this study, around 1980, surfactant effects on *in vitro* test systems have been well documented. Only very minor changes in SCE were reported, with a limited data set of two donors and a lack of dose-response. Statistical analysis was not feasible with this very limited data set.
Relevance of study:	Not relevant (Limited data set, internally consistent findings, no statistics conducted and no dose-response)
Klimisch code:	3

B.6.5 Long-term toxicity and carcinogenicity (Annex IIA 5.5)

Introduction into this chapter by the RMS
The chronic toxicity/carcinogenicity part is mainly based on the extensive descriptions of the available valid studies which were provided by the GTF in its dossier. It was noted that a different approach was taken in the dossier with regard to the studies in rats and those in mice. In the section compiling the rat studies, all of them were reported in detail, including the four long-term studies that had been reviewed during previous EU evaluation. In the section on studies on the mouse, only the new studies are described whereas for those already known reference to the old DAR (DAR, 1998, ASB2010-10302) was made.
For higher efficiency of the review and for the sake of transparency, the descriptions of methods and study results in the GTF dossier were virtually not amended and even the conclusions were kept as provided. However, each study that is described in detail was commented by RMS. These remarks on bottom of each study description are clearly distinguished from the original submission by a caption and are always written in italics. In addition, redundant parts (in particular the so-called "executive summaries") have been deleted and the structure of the original submission was significantly changed to make it more transparent and comprehensible.
With regard to the "old" studies in mice that were not reported in the GTF dossier once more, at least re-evaluation for quality and reliability was performed by the RMS and the NOAELs/LOAELs were checked.
A paragraph on testing of formulations for long-term effects in rats has been included.
The overall assessment of chronic toxicity/carcinogenicity of glyphosate by the RMS is provided in Vol. 1 (2.6.5).
In chapter B.6.5.3 publications on glyphosate and carcinogenicity are presented. These publications include a number of epidemiology studies which are focused on pesticide exposure and associated health outcomes.

B.6.5.1 Long-term toxicity and carcinogenicity in the rat

B.6.5.1.1 New studies in rats

1st study: [redacted] 1996

Reference: IIA, 5.5.1/01
Report: [redacted] (1996)
Glyphosate Acid: One Year Dietary Toxicity Study in Rats
[redacted]
Data owner: Syngenta
Study No.: CTL/P/5143
Date: 1996-10-02
not published TOX2000-1998
Guidelines: OECD 452, US EPA 83-1
Deviations: Several organ weights not determined
GLP: Yes
Acceptability: *See RMS comment*

Dates of experimental work: 1995-04-03 - 1996-06-03

Materials and methods

Test material:	
Identification:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 %
Stability of test compound:	At least 1 year when stored at RT.
Vehicle and/ or positive control:	Diet
Test animals:	
Species:	Rat
Strain:	Wistar (Alpk:AP$_f$SD)
Source:	[redacted]
Age:	22-24 days (on delivery)
Sex:	Males and females
Weight at dosing:	Males: 150.5 – 151.5 g (mean values); females: 126.7 – 133.3 g (mean values)
Acclimation period:	At least 10 days.
Diet/Food:	CT1 diet (Special Diet services Ltd., Essex, UK), *ad libitum*
Water:	Mains drinking water, *ad libitum*
Housing:	Initially in litters, sexes separately, after assignment to experimental groups in group of four rats per sex per cage.
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 55 ± 15 % Air changes: at least 15/hour 12 hours light/dark cycle

In life dates: 1995-04-03 to 1996-06-03

Animal assignment and treatment:
In a chronic toxicity study groups of 24 Wistar-derived rats per sex received daily dietary doses of 0, 2,000, 8,000 and 20,000 ppm glyphosate acid (equivalent to mean achieved dose levels of 0, 141, 560 and 1409 mg/kg bw/day for males and 0, 167, 671 and 1664 mg/kg bw/day for females).
Test diets were prepared in either 30 or 60 kg batches by mixing the appropriate amount of the test substance with the basal diet. The stability and homogeneity of the test substance in the diet was determined in an in-house stability study at 2000 and 20000 ppm.

Observations
Rats were examined for toxic signs, ill-health or behavioural changes and pre-terminal deaths prior to the start of the study and once a day afterwards. Detailed clinical observations were conducted weekly. Ophthalmic examination was done in all animals at the start of the study. The eyes of the control and high dose group were additionally examined one week to termination.

Body weight
Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 14 and every two weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from Week 1 to Week 13, once in Week 16 and every fourth week thereafter.

Haematology and clinical chemistry
Blood was collected from 12 animals per sex and group at Week 14, 27 and at termination (Week 53). The following parameters were measured: Haematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, blood cell morphology, platelet count, total leukocyte count, differential leukocyte count, red blood cell distribution width, prothrombin time, activated partial thromboplastin time, alkaline phosphatase, aspartate amino transferase (AST), alanine aminotransferase (ALT), γ-glutamyl-transferase, creatine kinase, creatinine, urea, total protein, glucose, albumin, total bilirubin, triglycerides, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Urinalysis
Individual urine samples were collected from the same animals as those used for haematology analyses (except for Week 52) at Week 13, 26 and 52. The following parameters were determined: Volume, colour, appearance, specific gravity, pH, glucose, ketones, protein, urobilinogen and blood.

Sacrifice and pathology
Necropsy was conducted on all animals except for Rats 38 and 149-152, which were killed during Week 6/7 due to a sexing error. The following organ weights were determined from all animals surviving to scheduled termination: Adrenals, brain, epididymides, kidneys, liver and testes.
Tissue samples were taken from the following organs: Adrenals, aorta, bone & bone marrow (femur incl. joint), brain (cerebrum, cerebellum, brainstem), caecum, cervix, colon, duodenum, epididymis, eye, gross lesions, Harderian gland, heart, ileum, jejunum, kidneys, liver, lung, lymph nodes (cervical and mesenteric), mammary gland, nasopharyngeal cavity, sciatic nerve, oesophagus, oral cavity, ovary, pancreas, pituitary, prostrate, rectum, salivary glands, seminal vesicles, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus and voluntary muscle.

Statistics
All data were evaluated using analysis of variance and covariance for each specified parameter using the GLM procedure in SAS (1989). Differences from control were tested statistically by comparing each treatment group least- squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis. All statistical tests were two sided.

Results and discussion
Analysis of dose formulations

The mean achieved concentrations of glyphosate acid in each dietary preparation were within 8 % of the nominal concentration and the overall mean concentrations were within 4 % of nominal.
The homogeneity of glyphosate acid in diet at concentrations of 2000 and 20000 ppm was satisfactory; percentage deviations were within 7 % of the overall mean.
The stability tests determined at 2000 and 20000 ppm showed that the test substance is stable for at least 61 days when stored at room temperature.

Mortality
There were no treatment-related death.

Clinical observations
There was a small increase in the number of animals in the 20000 ppm group which had urinary staining (wet or dry). All other clinical observations were of a type and incidence expected for rats of this strain.

Body weight
Body weights of rats receiving 20000 ppm glyphosate acid were lower than those of controls throughout the study. Bodyweights in the intermediate group were slightly reduced throughout the study. The difference from control was not statistically significant in males and was statistically significant in females only from Week 46. As the pattern of the effect was similar to that of the high dose rats for both sexes this minor difference in bodyweight is considered to be related to administration of glyphosate acid.
There was no effect on bodyweight in rats receiving 2000 ppm glyphosate acid.

Food consumption and compound intake
Food consumption was generally lower in rats receiving 20000 ppm than in controls. The difference was most marked at the start of the study. Food consumption was generally slightly lower than controls in rats receiving 8000 ppm glyphosate acid. There was no effect on food consumption in rats receiving 2000 ppm.

The group mean achieved doses are summarised below.

Table B.6.5-1: Group mean achieved dose levels

Dose group	**Dietary concentration (ppm)**	**Mean achieved dose level (mg/kg bw/day)**	
		Males	Females
1 (control)	0		
2 (low)	2000	141	167
3 (mid)	8000	560	671
4 (high)	20000	1409	1664

The results show a higher test material intake for females when compared to males for each dose level. The mean intake for each dose group is 0, 141, 560 and 1409 mg/kg bw/day for males and 0, 167, 671 and 1664 mg/kg bw/day for females for 0, 2000, 6000 and 20000 ppm, respectively.

Ophthalmoscopy
There were no treatment-related effects observed.

Haematology and clinical chemistry
Haematology
A number of statistically significant differences from control were identified but there was no evidence of a relationship to dose and the differences were small and not seen consistently at all the time points and therefore were considered to be unrelated to glyphosate acid administration.

Clinical chemistry
Plasma cholesterol and plasma triglycerides were marginally reduced in males receiving 20000 or 8000 ppm at Weeks 14 and 27.
Moreover, there was a treatment- and dose-related increase in plasma ALP activity throughout the study. For rats receiving 2000 ppm glyphosate acid the increase was marginal and was statistically significant only for females at Week 14. The increase in the activity of plasma ALP in animals at all dose levels was compound-related but as there was no accompanying pathological change in either the liver or bone this is considered not to be of toxicological significance.

All other differences from control were small and/or were not dose-related and are considered to be incidental to administration of glyphosate acid.

Table B.6.5-2: Clinical chemistry findings

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Alkaline Phosphatase (IU/L)								
Week 14	248	161	281	201*	342**	227**	429**	292**
Week 27	221	135	250	171	306**	200**	412**	254**
Week 53	232	87	258	100	291**	114	379**	160**

* $p < 0.05$; ** $p < 0.01$

Urinanalysis
There were no consistent treatment- and dose-related effects seen in the any urinary parameters.

Necropsy
Gross pathology
There were no treatment-related macroscopic effects.

Organ weights
There were no treatment- and dose-related effects on organ weights when corrected for bodyweight.

Histopathology
An increased incidence and severity of focal basophilia of the acinar cells of the parotid salivary gland were seen in both sexes receiving 20000 ppm glyphosate acid. This change was considered to be related to treatment and consequently the salivary glands of the 8000 ppm dose group were examined. The examples of focal parotid basophilia seen at this dose were all of minimal severity and the incidence was comparable to that in the control group.

All other observed differences in the incidence of findings are considered to be unrelated to the treatment with glyphosate acid in view of the spontaneous incidence in this strain. No treatment-related neoplasms were found.

Conclusion by the Notifiers
Based on the study results the NOAEL in rats after chronic exposure to glyphosate acid for 12 month is 8000 ppm (corresponding to 560 mg/kg bw/day in males and 671 mg/kg bw/day in females).

RMS comment:
The study is considered acceptable. In addition to the effects described, food utilization was less efficient at the top dose level. A certain increase in basophilia of acinar cells of the parotid salivary gland was still noted in mid dose females although the incidence in males was indeed comparable to the control group (see additional Table B.6.5-3).

Table B.6.5-3: Incidence of focal basophilia in parotid salivary gland of rats treated with glyphosate acid for one year

	Dose group (ppm)							
	0		*2000*		*8000*		*20000*	
	♂	♀	♂	♀	♂	♀	♂	♀
Focal basophilia								
Minimal	*2*	*2*	*0*	*0*	*3*	*6*	*10*	*8*
Slight	*0*	*0*	*0*	*0*	*0*	*0*	*3*	*5*
Moderate	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*2*

Based on this histopathological alteration, the lower body weight even at the mid dose level towards the end of the study, a lower food consumption and higher AP acitivity at the two upper dose levels, the NOAEL in this chronic study is rather seen at 2000 ppm (equal to a mean daily intake of 141 or 167 mg/kg bw in male or female rats) instead of the next higher dose as suggested by GTF. There was no evidence of carcinogenicity but duration of treatment was too short for a definitive conclusion.

2nd study: [redacted] 1997

Reference: IIA, 5.5.2/02
Report: [redacted] (1997)
HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats
[redacted]
Data owner: Arysta Life Sciences
Study No.: [redacted] 94-0150
Date: 1997-07-01
not published,
ASB2012-11484, ASB2012-11485, ASB2012-11486, ASB2012-11487
Guidelines: OECD 453 (1981), JMAFF 59 NohSan 3850 (1984), US-EPA (1989)
Deviations: None

GLP: yes
Acceptability: *See RMS comment*

Dates of experimental work: 1994-12-19 - 1996-12-25

Materials and methods

Test material:	
Identification:	Glyphosate technical, Code: HR-001
Description:	White crystal
Lot/Batch #:	T-941209; T-950308
Purity:	97.56 %; 94.61 %
Stability of test compound:	No data given the report.
Vehicle and/ or positive control:	Diet
Test animals:	
Species:	Rat
Strain:	Sprague-Dawley (Crj:CD)
Source:	[redacted]
Age:	5 weeks (males), 6 weeks (females)
Sex:	Males and females
Weight at dosing:	65 – 85 g
Acclimation period:	At least one week
Diet/Food:	MF Mash (Oriental Yeast Co., Ltd, Japan), *ad libitum*
Water:	Well water treated with sand and charcoal filter, HCl and UV rays, *ad libitum*
Housing:	In groups of ten animals of the same sex in wire-mesh stainless steel cages during the acclimatisation period. During the study males were housed in groups of 5 per cage until week 72, in groups of ≤3 until week 78 and individually thereafter. Females were housed in groups of five until week 78, and individually thereafter.
Environmental conditions:	Temperature: 24 ± 2 °C Humidity: 55 ± 15 % Air changes: 15/hour 12 hours light/dark cycle

Study design and methods:

In life dates: 1994-12-19 to 1996-12-25

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague-Dawley rats/sex/group received daily dietary doses of 0, 3000, 10000 and 30000 ppm (equivalent to mean achieved dose levels of 0, 104, 354 and 1127 mg/kg bw/day in males and 0, 115, 393 and 1247 mg/kg bw/day in females) HR-001. In addition, 30 rats/sex/group were included for interim sacrifices at 26, 52 and 78 weeks.

Test diets were prepared weekly by mixing a known amount of the test substance with a small amount of basal diet. This pre-mix was then added to a larger amount of basal diet and blended by a blending machine.
The stability of the test substance in food was previously determined in a 4-week dose-range finding study in mice. Homogeneity analyses were performed on samples of each dose level of the first diet preparation. Analyses for achieved concentrations were done for each dose level in monthly intervals.

Observations
Rats of all groups were examined for toxic signs and pre-terminal deaths once a day. In addition a detailed veterinary examination was made at least once per week. Ophthalmic examination was done at the start of the study and at termination.

Body weight
Individual body weights were recorded at weekly intervals until the end of Week 13 and every 4 weeks thereafter and before necropsy, except for dead or moribund satellite animals, which were discarded without body weight determination.

Food consumption and compound intake
Food consumption was measured for a period of three consecutive days weekly from Week 1 to 13 and every four weeks from Week 16 to 104. Mean individual food consumption, group mean food consumption and group compound intake were calculated.

Haematology and clinical chemistry
Blood samples were collected from 10 rats/sex/group of the satellite groups in Week 26, 52, from all surviving animals of the satellite group in Week 78 and from 10 rats/sex/group of the main group in week 104. Before sampling animals were fasted overnight. The following parameters were measured: hematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, platelet count, total leukocyte count, differential leukocyte count, alkaline phosphatise, glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), γ-glutamyl-transpeptidase, creatine phosphokinase, creatinine, blood urea nitrogen, total protein, glucose, albumin, globulin, albumin/globulin ratio, total bilirubin, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Urinalysis
Individual urine samples were collected from 10 rats/sex/group of the satellite groups in Week 26, 52, from all surviving animals of the satellite group in Week 78 and from 10 rats/sex/group of the main group in Week 104. The following measurements were made: density, volume, appearance, pH, protein, glucose, occult blood, ketones, urobilinogen, sediments.

Sacrifice and pathology
Necropsy and histopathological examinations were carried out on all tissues collected at interim and terminal sacrifice. The following organ weights were determined from all animals: adrenals, brain, caecum, kidneys, liver and testis.
Tissue samples were taken from the following organs: adrenals, aorta, bone & bone marrow (sternum and femur incl. joint), brain (cerebrum, cerebellum, pons and medulla oblongata), caecum, colon, duodenum, epididymides, eyes, gross lesions, Harderian glands, heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, lymph nodes (cervical and mesenteric), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands (submaxillary and

sublingual), sciatic nerve, seminal vesicles and coagulating glands, skeletal muscle, skin (females only), spinal cord (cervical, thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus (horns and cervix) and vagina.

Statistics
Statistical significance of the difference between the control group and the treated groups was estimated at 5 % and 1 % levels of probability.
The data of body weight (main group only), food consumption, urine specific gravity, urine volume, haematological parameters, blood biochemical parameters, and organ weights were evaluated by Bartlett's test for equality of variance. When group variances were homogeneous, a parametric analysis of variance of a one-way layout type was conducted to determine if any statistical differences existed among groups. When the analysis of variance was significant, Dunnett's (when sample size of each group was equal) or Scheffé's (when sample size of each group was different) multiple comparison test was applied to evaluate differences between the treated and the control groups. When the group variances were heterogeneous, the data were analyzed by Kruskal-Wallis non-parametric analysis of variance. When significant, Dunnett type (when sample size of each group was equal) or Scheffé type (when sample size of each group was different) mean rank sum test was applied to determine if any significant differences existed between the treated and the control groups.
The data of urinalysis except for specific gravity and urine volume were assessed by Mann-Whitney's U test.
Mortality was analyzed by Life table analysis.
The data of clinical sign (main group only), ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability test.

Results and discussion
Analysis of dose formulations
The coefficient of variation for the homogeneity of the test substance for each dose level was 2.2% and less. Hence, the results indicated a good homogeneity.
Analyses for concentrations showed that the diet preparations achieved 97 - 98% of the target concentration. Thus, the concentrations of the test substance in the test diets were within acceptable limits.

Mortality
In the high dose group neither sex showed an increase in mortality, although mortality in males was lower than the control during the last half of the treatment period with statistical significance in most of the weeks. In all other groups mortality was comparable to control. The final mortality is given in Table B.6.5-4:

Table B.6.5-4: Final mortality at termination of treatment (%)

	Dose group (ppm)*			
Sex	0	3000	10000	30000
Male	32/50 (64)	30/50 (60)	32/50 (64)	21/50 (42)
Female	35/50 (70)	31/50 (62)	34/50 (68)	36/50 (72)

* number of mortalities / total number of rats/group (% mortality)

Clinical observations
In the high dose group, significant increases in incidence of bradypnea, mass, and soiled fur were observed in males when compared to the control. Analysis of location of each mass showed that the ones in the tail were present in 27 males, which was apparently high in

incidence compared to 11 of the control. The incidences of mass in other locations were comparable to the control. With respect to soiled fur, the sign was located at the external genital or perianal region. Males in this group also showed significant decreases in incidence of tactile hair loss, wound, and hair loss. In females, a significant increase in incidence of wetted fur was observed; the sign was mainly seen in the external genital region. Besides the signs mentioned above, loose stool was observed in all cages of this group from Week 24 in males and Week 23 in females until the end of the treatment. Animals showing loose stool could not be identified because of group housing, therefore the sign is only described here in the text but not included in Table B.6.5-5.
In the mid dose group, the incidence of tactile hair loss was significantly decreased in males and significantly increased in females when compared to the respective control.
In the low dose group, significant increases in incidence of decreased spontaneous motor activity, bradypnea, and soiled fur and a significant decrease in incidence of tactile hair loss were observed in males. Analysis of location of the soiled fur demonstrated predominant occurrences of the sign in the external genital region and foreleg. Females in this group showed significant increases in incidence of ptosis and tactile hair loss.

Table B.6.5-5: Statistically significant changes in clinical signs

	Dose group (ppm)							
	0		3000		10000		30000	
Parameters	♂	♀	♂	♀	♂	♀	♂	♀
Decreased spontaneous motor activity	9	23	19*	22	9	20	13	26
Bradypnea	3	7	10*	14	4	6	11*	12
Ptosis	7	4	6	12*	4	6	6	6
Tactile hair loss	5	1	0*	17**	0*	9**	0*	4
Integument								
Wound	7	2	4	2	6	2	0**	1
Mass	22	37	26	36	21	38	37**	43
Hair loss	12	16	7	13	15	21	3*	25
Soiled fur	10	16	20*	17	12	11	21*	18
Wetted fur	9	5	7	5	7	5	16	15*

* $p < 0.05$; ** $p < 0.01$

Body weight
In the high dose group, body weights were lower than the control throughout the treatment period; significant decreases in their body weights were observed during Weeks 1 to 80 in males and at Week 7 and during Weeks 9 to 60 in females. The final group mean body weights of males and females at termination of the treatment period were both 93% of the respective control.
In the mid dose group, males showed a decreased body weight gain during the first few weeks of treatment with a statistically significant difference from the control at Week 6. Their retarded growth persisted throughout the treatment period, and the final group mean body weight at termination of treatment was 95 % of the control. Body weight change in females was comparable to the control throughout the treatment period.
In the low dose group, body weights of both sexes were comparable to the control except for a significant increase in females at Week 16.

Food consumption and compound intake
In the high dose group, consistent with the decreasing body weight or decreasing body weight trends, food consumption showed a decreasing trend in males during the first few weeks.

In the other groups, food consumption in males and females was comparable to the respective control.

The group mean achieved doses are summarised below.

Table B.6.5-6: Group mean achieved dose levels in the main groups

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)	
		Males	Females
1 (control)	0		
2 (low)	3000	104	115
3 (mid)	10000	354	393
4 (high)	30000	1127	1247

The results show a higher test material intake for females when compared to males for each dose level.

Ophthalmologic examinations: No abnormalities were observed.
Haematology and clinical chemistry: Haematological and blood biochemical analyses did not demonstrate apparent toxicity of the test substance in either sex or group.
Statistically significant changes in haematology and blood chemistry are displayed in Table B.6.5-7 and Table B.6.5-8.

Table B.6.5-7: Statistically significant changes in haematology

	Dose group (ppm)					
	3000		10000		30000	
Parameters	♂	♀	♂	♀	♂	♀
Haematocrit	108[a]	99	111	84	131*	96
Platelet count	91	105	88	115	66**	104

[a] Figures represent values in the treated groups when the corresponding control is 100.
* $p < 0.05$; ** $p < 0.01$

Table B.6.5-8: Statistically significant changes in blood chemistry

		Dose group (ppm)					
		3000		10000		30000	
Parameters		♂	♀	♂	♀	♂	♀
Alkaline phosphatase	Week 52	129[a]	127	145	118	136	214**
	Week 78	185*	303	154	106	171	116
Glutamic pyruvic transaminase	Week 52	94	91	148	75	67*	66
Creatinine	Week 26	102	95	99	91*	97	89**
Total protein	Week 52	100	101	100	96*	99	96
Albumin	Week 26	100	92*	100	99	103	95
Globulin	Week 26	98	102	95	95	95	95*
	Week 52	103	101	101	93*	100	99
Glucose	Week 26	101	104	107	99	97	87**
Total bilirubin	Week 26	100	80**	94	96	106	88
Chloride	Week 104	97*	100	97*	100	98	101

[a] Figures represent values in the treated groups when the corresponding control is 100.
* $p < 0.05$; ** $p < 0.01$

Urine analysis

Urinalysis did not demonstrate apparent toxicity of the test substance in either sex or group. Statistically significant changes in urinalysis parameters are displayed in Table B.6.5-9.

Table B.6.5-9: Statistically significant changes in urinalysis

		Dose group (ppm)					
		3000		10000		30000	
Parameters		♂	♀	♂	♀	♂	♀
pH	Week 26			↓*		↓**	↓
	Week 52			↓*		↓**	↓**
	Week 78			↓		↓*	↓
	Week 104			↓		↓**	↓
Protein						↓*	
Volume		↑*					
Appearance			Dark*		Dark*		Dark*

* $p < 0.05$; ** $p < 0.01$

Metabolism of glyphosate after absorption from the intestine is minimal. Thus, most of the glyphosate is excreted via urine as the unchanged parent compound. In the urine glyphosate dissociates into the free acid, which can lead to a reduction of the urinary pH. Therefore, the reduced urinary pH might be of no toxicological significance.

Necropsy
In the high dose group significant increases in incidence of distension of the caecum were observed in both sexes, accompanied by soiled fur in the perianal region in males. Moreover, significant increases in absolute and relative weights of the caecum in both sexes in the high and mid dose group were seen, but not associated with histopathological abnormalities.
The incidences of thickened areas in the skin of the tail, corresponding to the tail mass in the clinical observations, were significantly increased in the mid and high dose group. The lesion was histopathologically diagnosed as follicular hyperkeratosis and/or folliculitis/follicular abscess. An increased incidence of hair loss was also observed in high-dosed females, but it lacked corresponding histopathological changes.

All changes regarding neoplastic lesions were not statistically significant.

From this, it is concluded that the test compound at the doses tested does not cause treatment or dose related gross and histopathological changes and it is not carcinogenic under the testing conditions.

Conclusion by the Notifiers
Based on the slight body weight effects, and necropsy findings without correlating histopathology at the mid-dose the NOAEL in rats after chronic exposure to HR-001 for 24 month is 3000 ppm (corresponding to 104 mg/kg bw/day for males and 115 mg/kg bw/day for females). It is concluded that HR-001 is not carcinogenic in rats.

RMS comment:
The study is acceptable. We agree with the evaluation by the notifier and support the NOAEL of 104 mg/kg bw/day.

3d study: [redacted] 1997

Reference:	IIA, 5.5.2/07
Report:	[redacted] (1997) Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat [redacted] Study No.: 1231 Date: 1997-02-15 not published, ASB2012-11489
Guidelines:	OECD 453 (1981)
Deviations:	Organ weights were not determined for all animals; weights of heart, spleen and (para)thyroids are missing
GLP:	yes
Acceptability:	*See RMS comment*

Dates of experimental work: 1994-06-09 - 1996-06-12

Materials and methods

Test material:	
Identification:	Glyphosate technical
Description:	No data given in the report.
Lot/Batch #:	No data given in the report.
Purity:	No data given in the report.
Stability of test compound:	No data given in the report.
Vehicle and/ or positive control:	Diet
Test animals:	
Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Approx. 6 weeks
Sex:	Males and females
Weight at dosing:	Males: 70.0 – 93.2 g, females: 70.0 – 90.6 g
Acclimation period:	One week
Diet/Food:	Powdered rat feed (Lipton India Ltd, India), *ad libitum*
Water:	Filtered pure water, *ad libitum*
Housing:	Initially in groups of five in polypropylene cages, in groups of three from Week 24 to 52 and in groups of two from Week 53 to termination.
Environmental conditions:	Temperature: 22 - 25 °C Humidity: 50 - 70 % Air changes: 10 - 15/hour 12 hours light/dark cycle

In life dates: 1994-06-09 to 1996-06-12

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague Dawley rats per sex received daily dietary doses of 0, 3000, 15000 and 25000 ppm (equivalent to mean achieved dose levels of 0, 0.15, 0.78 and 1.29 g/kg bw/day (males) and 0, 0.21, 1.06 and 1.74 g/kg bw/day (females)) Glyphosate technical for two years. In addition, for the control and each dose group 20 rats per sex were included for interim sacrifice in Week 52 to study non-neoplastic histopathological changes (chronic toxicity study). Selected dose levels were the same except for the highest dose which was 30000 ppm. Here the dietary doses correspond to 0.18, 0.92 and 1.92 g/kg bw/day (males) and 0.24, 1.13 and 2.54 g/kg bw/day (females) for 3000, 15000 and 30000 ppm, respectively
Test diets were prepared weekly by mixing appropriate amounts of the test substance with the basal diet. The stability and homogeneity of the test substance in food was determined in-house stability study at all dose levels before the start of dosing. Analyses for achieved concentrations were performed monthly during the study period.

Clinical observations
Rats were examined for toxic signs once and pre-terminal deaths twice a day. Ophthalmic examination was done at the start of the study, at interim sacrifice and at termination in the control and high dose group

Body weight
Individual body weights were recorded on Day 0, at weekly intervals thereafter until the end of Week 13 and every 4 weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded once weekly for each group from Week 1 to Week 13 and subsequently in Week 25, 38, 51, 65, 78, 92 and 104.

Haematology and clinical chemistry
Haematology
Individual blood samples were collected from 20 rats/sex/group of the main groups at 3, 6, 12, 18 and 24 months and from all surviving animals of the satellite group at 12 months. Before sampling animals were fasted overnight. The following parameters were measured: Haemoglobin, erythrocyte count, PCV, thrombocytes, total leukocyte count and differential leukocyte count.

Blood chemistry
Individual plasma samples were collected from 10 rats/sex/group of the main groups at 6, 12, 18 and 24 months and from all surviving animals of the satellite group at 12 months. Before sampling animals were fasted overnight. The following parameters were measured: Total serum proteins, albumin, ALT, AST, GGTP, SAP, blood urea nitrogen and blood glucose.

Urinalysis
Individual urine samples were collected from 20 rats/sex/group of the main groups at 3, 6, 12, 18 and 24 months and from all surviving animals of the satellite group at 12 months. The following measurements were made: Specific gravity, volume, appearance, pH, protein, glucose, occult blood, ketones, microscopy of sediments.

Sacrifice and pathology
Necropsy was performed on all animals at scheduled termination.

The following organ weights were determined from 10 rats per sex per main group and on all animals of the satellite groups: adrenals, brain, gonads, kidneys and liver.

Histopathological examination was carried out on all tissues collected at interim sacrifice, control and high dose groups; all pre-terminally dead and moribund sacrificed rats of the low and mid dose groups and on all lesions of the terminally sacrificed rats from the low and mid dose groups.
Tissue samples were taken from the following organs of all animals: adrenals, aorta, body cavities, brain, caecum, colon, duodenum, epididymis, eyes (both), femur, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mesenteric and mandibular), mammary gland, oesophagus, ovaries, pancreas, pituitary, preputial gland, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum with bone marrow, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder and uterus.

Statistics
Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier. Animals withdrawn from study during the interval (those taken for moribund sacrifice) are taken into consideration by giving enough weightage.
The incidence of neoplasms was analysed by Life table analysis for fatal tumour incidence and Peto's incidental tumour analysis.
In addition to these tests the Fisher exact test for pairwise comparisons and the Cochran Armitage linear trend test for dose response trends were carried out. All reported P-values for the tumour incidence analysis are one-sided.
The biochemical, haematological and organ weight data was analyzed for significance using Student 't' test or Cochran 't' test.

Results and discussion
Analysis of dose formulations
Analyses for concentrations showed that the diet preparations recovered 86.1 - 98.3 % of the target concentration. Thus, the concentrations of the test substance in the test diets were within acceptable limits.
Analyses for homogeneity recovered 87.5 - 90.0 % for 3000 ppm, 91.7 - 93.0 % for 15000 ppm, 94.3 - 95.1 % for 25000 ppm and 91.8 - 92.6 % for 30000 ppm. Hence, the results indicated a good homogeneity.
Moreover, stability analyses showed that recovery one month after diet preparation ranged between 87.5 and 95.0 %.

Mortality
No treatment-related clinical signs or deaths were observed in the satellite groups, e.g. the chronic toxicity study.
In the carcinogenicity study, e.g. after 104 weeks, male animals of the high dose group exhibited slight but statistically insignificant higher mortalities.
The numbers of pre-terminal deaths in the main group are displayed in Table B.6.5-10.

Table B.6.5-10: Cumulated mortalities after 104-week dietary exposure to Glyphosate technical*

	Dose group (ppm)			
Sex	0	3000	15000	25000
Male	16/50	17/50 (2)	18/50 (4)	23/50 (14)

Female	19/50	20/50 (2)	20/50 (2)	25/50 (12)

* Values in parentheses indicate increases in mortality compared to control in percent.

Clinical observations
No significant toxic signs were observed in treated or control groups.

Body weight
Significantly reduced body weight gain that lasted throughout study until Week 104 was observed in males receiving the highest dose. In all other groups body weight gain was comparable to the control at termination.

Food consumption and compound intake
There were no treatment-related effects on food consumption for either sex or group noted during the study.
The results show a higher test material intake for females when compared to males for each dose level. The mean intake in the chronic toxicity study for each dose group is 0.18, 0.92 and 1.92 g/kg bw/day (males) and 0.24, 1.13 and 2.54 g/kg bw/day (females) for 3000, 15000 and 30000 ppm, respectively.
The mean intake in the carcinogenicity study for each dose group is 0.15, 0.78 and 1.29 g/kg bw/day (males) and 0.21, 1.06 and 1.74 g/kg bw/day (females) for 3000, 15000 and 25000 ppm, respectively.
The group mean achieved doses are summarised below.

Table B.6.5-11: Group mean achieved dose levels

Dose group	**Dietary concentration (ppm)**	**Mean achieved dose level (mg/kg bw/day)***	
		Males	Females
		Chronic toxicity study (52 weeks)	
low	3000	0.18	0.24
mid	15000	0.92	1.13
high	30000	1.92	2.54
		Carcinogenicity study (104 weeks)	
low	3000	0.15	0.21
mid	15000	0.78	1.06
high	25000	1.29	1.74

* Calculations were done with values from Week 13 (chronic) and Week 25 (carcinogenicity)

Ophthalmological examinations
Ophthalmological examinations revealed no abnormalities.

Laboratory investigation
Haematological examination did not reveal any abnormalities attributable to the treatment. Regarding the clinical chemical investigations, a significant increase in the alkaline phosphatase level was only seen in the high dose of the carcinogenicity study at study termination (see Table B.6.5-12).

Other significant changes observed in haematological, and biochemical parameters were within the range of the historical control data and hence appear to be of no biological significance.

Table B.6.5-12: Statistically significant changes in blood chemistry

	Dose group (ppm)							
	0		3000		15000		25000	
Parameters	♂	♀	♂	♀	♂	♀	♂	♀
Alkaline phosphatase								
Month 6	25.58	24.96	24.97	25.25	24.85	25. 2	23.07	25.11
Month 12	25.64	19.04	25.96	25.35*	27.64	28.3*	22.88	22.88*
Month 18	27.7	24.47	25.94	28.42	28.73	27.71	26.68	25.28
Month 24	26.04	24.87	26.75	26.95*	28.42*	25.75	47.71*	53.86*

* $p < 0.05$

Urinanalysis
Urinalysis did not reveal any abnormalities attributable to the treatment.

Necropsy
Gross pathology
There were no treatment-related macroscopic findings observed during the study period.

Organ weights
Significant and dose-dependent effects in the chronic toxicity study were found in both sexes of the high-dose group. In males, weights of kidneys, brain and testes were increased. In females, in addition to kidneys and brain, the liver weight was increased as well.
In the carcinogenicity study which lasted 52 weeks longer, significant and dose-dependent effects in males consisted of increased weight of brain and testes in the mid and high dose group. Effects on the kidneys were not observed, perhaps due to the lower dose level in the highest group compared to the chronic toxicity study, e.g. 25000 ppm to 30000 ppm, respectively.
In females, significant and dose-dependent effects after 24 months occurred only in kidneys. Like for male animals, this increase could be due to the different high dose levels.

Histopathology
Histopathological changes were found at all dose levels including control, hence it is concluded that these are no treatment-related effects.

Neoplastic changes
There were no treatment-related neoplasms observed.

Conclusion by the Notifiers
Based on the mild toxic effects on body weight gain and the increased organ weights without histopathological changes the NOAEL in rats after chronic exposure to Glyphosate technical for 24 month is 25,000 ppm (corresponding to 1290 mg/kg bw/day for males and 1740 mg/kg bw/day for females). It is concluded that Glyphosate technical is not carcinogenic in rats.

RMS comment:
This study is not acceptable because no core information on the test substance such as batch number or purity was given and, thus, it is not clear what was in fact tested. Even if this data would be available, the study might be considered at best supplementary because of its many additional deficiencies. No storage conditions or expiry date for the test substance was given. Mean daily dietary intake of the test substance was not given but apparently calculated by GTF afterwards. In addition, there were some deviations from OECD guideline 453, in

particular with regard to frequency of observations. Even more important, the reported total incidence of neoplasia was surprisingly low and especially the very low frequency of malignant tumours (one female control animal affected by an mammary gland adenocarcinoma among 400 animals on study) might produce doubts about suitability (i.e., sufficient vulnerability) of the rat strain used. Neither the NOAEL nor an LOAEL were proposed in the study report. In contrast to GTF opinion, the NOAEL is seen by the RMS at 3000 ppm (ca 150 mg/kg bw/day). This might be considered very conservative because it is based mainly on changes in clinical chemistry parameters without concomitant liver pathology. However, it must be taken into account that the number of animals that were subject to histopathological examination and of which organs were weighed was lower than usually required. Provided that is can be shown that glyphosate of a certain batch and precisely determined purity was tested, it would be of interest to get historical control data for tumour incidences in this rat strain and laboratory to have a better chance to assess reliability of the results with regard to carcinogenicity. In addition, the study owner should be mentioned.

4th study: [redacted] 2001

Reference:	IIA, 5.5.2/03
Report:	[redacted] (2001) Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats [redacted] Data owner: Syngenta Study No.: CTL/PR1111 Date: 2001-03-15 not published, ASB2012-11488
Guidelines:	OECD 453 (1981), EEC B.33 (1988), MITI (1992), US OPTTS 870.4300 (1998)
Deviations:	None
GLP:	yes
Acceptability:	*See RMS comment*

Dates of experimental work: 1998-04-07 - 2000-10-16

Materials and methods

Test material:	
Identification:	Glyphosate acid (technical material)
Description:	White solid
Lot/Batch #:	P30
Purity:	97.6 % w/w
Stability of test compound:	At least 2 years when stored at -20 °C.
Vehicle and/ or positive control:	Diet
Test animals:	

Species:	Rat
Strain:	Wistar (Alpk:AP_fSD)
Source:	[redacted]
Age:	3 weeks (on delivery)
Sex:	Males and females
Weight at dosing:	Males: 155.0 – 156.6 g (mean values); females: 136.0 – 138.4 g (mean values)
Acclimation period:	At least 10 days.
Diet/Food:	CT1 diet (Special Diet services Ltd., Essex, UK), *ad libitum*
Water:	Mains drinking water, *ad libitum*
Housing:	Initially in litters, sexes separately, after assignment to experimental groups in group of four rats per sex per cage.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 – 70 % Air changes: at least 15/hour 12 hours light/dark cycle

In life dates: 1998-04-07 to 2000-05-07

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 52 Wistar-derived rats per sex received daily dietary doses of 0, 2,000, 6,000 and 20000 ppm glyphosate acid (equivalent to mean achieved dose levels of 0, 121, 361 and 1214 mg/kg bw/day for males and 0, 145, 437 and 1498 mg/kg bw/day for females).
A further twelve animals per sex were added to each group and were designated for interim kill after one year to study chronic toxicity and non-neoplastic histopathological changes.
Test diets were prepared in 60 kg batches by mixing a known amount of the test substance with 1 kg of basal diet. This pre-mix was then added to the remainder of the 60 kg batch of basal diet and mixed thoroughly. The stability and homogeneity of the test substance in the diet was determined in an in-house stability study at 2000 and 20000 ppm.

Clinical observations
Rats were examined for toxic signs, ill-health or behavioural changes and pre-terminal deaths prior to the start of the study and once a day afterwards. Detailed clinical observations were conducted weekly. Ophthalmic examination was done in all animals at the start of the study, at Week 52 and prior to termination. A functional observational battery including motor activity was conducted in Week 52 in animals allocated to the chronic toxicity assessment of the study.

Body weight
Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 15 and every two weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from Week 1 to Week 14, once in week 16 and every fourth week thereafter.

Haematology and clinical chemistry

Blood was collected from 13 animals per sex and group at Week 14, 27, 53, 79 and at termination. Different animals were used for the tail vein haematology and clinical chemistry samples.
The following parameters were measured: hematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, blood cell morphology, platelet count, total leukocyte count, differential leukocyte count, reticulocyte count, red blood cell distribution width, prothrombin time, activated partial thromboplastin time, alkaline phosphatase, aspartate amino transferase (AST), alanine aminotransferase (ALT), γ-glutamyl-transferase, creatine kinase, creatinine, urea, total protein, glucose, albumin, globulin, albumin/globulin ratio, total bilirubin, triglycerides, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Urinalysis
Individual urine samples were collected from the same animals as those used for haematology analyses at Week 13, 26, 52, 78 and prior to termination. The following parameters were determined: volume, abnormal colour and appearance, specific gravity, pH, glucose, ketones, protein, bilirubin, and blood.

Sacrifice and pathology
Necropsy was conducted on all animals. The following organ weights were determined from all animals surviving to scheduled termination: adrenals, brain, gonads, heart, kidneys, liver and spleen.
Tissue samples were taken from the following organs: adrenals, aorta, bone & bone marrow (femur incl. joint), brain (cerebrum, cerebellum, brainstem), caecum, cervix, colon, duodenum, epididymis, eyes (retina, optic nerve), gross lesions including palpable masses, Harderian gland, heart, ileum, jejunum, kidneys, lachrymal gland, larynx, liver, lung, lymph nodes (cervical and mesenteric), mammary gland, muscle, oesophagus, ovary, pancreas, pharynx, pituitary, prostrate, rectum, salivary glands (submandibular, parotid), seminal vesicles, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder and uterus.

Statistics
All data were evaluated using analysis of variance and/or analysis of covariance for each specified parameter using the MIXED procedure in SAS (1996). Kaplan-Meier survival estimates were calculated separately for each sex and treatment group.
The overall incidence of each tumour type was considered by comparing each treated group and the control group using Fisher's Exact Test. In addition, a test for trend with group number was performed using the Cochran-Armitage Test. Analyses were carried out for all animals, intercurrent deaths and at terminal kill.

Results and discussion
Analysis of dose formulations
The mean achieved concentrations of glyphosate acid in each dietary preparation were within 10 % of the nominal concentration and the overall mean concentrations were within 1 % of nominal.
The homogeneity of glyphosate acid in diet at concentrations of 2000 and 20,000 ppm was satisfactory; percentage deviations were within 2 % of the overall mean for the 20000 ppm group and within 4-9 % of the overall mean for the 2000 ppm group.

The stability tests determined at 2000 and 20000 ppm showed that the test substance stability was satisfactory at room temperature and when stored at -20°C for at least 45 days which covered the period of use in the current study.

Mortality
The male groups were terminated in Week 100 because survival in the control, low and mid dose groups was approaching 25% (criteria for termination of the study). Statistically significantly better survival was observed in males fed 20000 ppm than in the other groups (p = 0.02). A statistically significant overall trend was also observed for males (p = 0.03).
The female groups survived to scheduled termination and there were no significant differences in mortality between the groups.

The survival rates are displayed in Table B.6.5-13.

Table B.6.5-13: Survival rates during up to 104-week dietary exposure to glyphosate technical

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Week 13	0.98	1.00	1.00	1.00	0.98	0.98	1.00	1.00
Week 26	0.95	1.00	1.00	1.00	0.98	0.98	1.00	1.00
Week 39	0.94	1.00	1.00	1.00	0.97	0.98	1.00	1.00
Week 52	0.91	1.00	0.97	1.00	0.97	0.98	0.98	0.98
Week 56	0.89	1.00	0.93	1.00	0.93	0.98	0.98	0.98
Week 60	0.87	1.00	0.92	1.00	0.91	0.97	0.98	0.97
Week 64	0.87	1.00	0.90	0.98	0.91	0.95	0.98	0.97
Week 68	0.87	0.94	0.88	0.96	0.87	0.95	0.98	0.95
Week 72	0.85	0.94	0.84	0.96	0.85	0.93	0.97	0.91
Week 76	0.81	0.94	0.80	0.92	0.82	0.89	0.97	0.91
Week 80	0.73	0.88	0.78	0.87	0.72	0.89	0.89	0.83
Week 84	0.69	0.85	0.67	0.83	0.63	0.89	0.85	0.83
Week 88	0.64	0.81	0.57	0.81	0.59	0.83	0.77	0.81
Week 92	0.56	0.79	0.50	0.81	0.53	0.81	0.71	0.80
Week 96	0.50	0.73	0.46	0.73	0.53	0.77	0.66	0.72
Week 100	0.40	0.69	0.44	0.63	0.42	0.77	0.56	0.66
Week 104	—*	0.62	—*	0.56	—*	0.77	—*	0.57

* Terminated in Week 100 because survival in the control, low and mid dose groups was approaching 25% (criteria for termination of the study).

Clinical observations
At 20000 ppm there was a treatment related increase in the incidence of red-brown staining of tray papers, particularly in males.
There were no other treatment related clinical observations.
There were also no treatment-related effects noted in the functional observational battery.

Body weight
The body weights of the animals fed 20000 ppm glyphosate acid were statistically significantly lower than controls throughout the study. The maximum reduction from control values was approximately 5 % for males and 8 % for females.
There were no treatment related effects in animals fed 2000 or 6000 ppm glyphosate acid.

Food consumption and compound intake
Food consumption was lower throughout the first year of the study in animals fed 20000 ppm glyphosate acid. In females the difference was statistically significant over the first 11 weeks (with a maximum reduction of approximately 5%) and again in weeks 40-56 (with a maximum reduction of 6%). In males, the difference was statistically significant over most of the first 6 months with a maximum reduction of 6%.
The group mean achieved doses are summarised below.

Table B.6.5-14: Group mean achieved dose levels

Dose group	**Dietary concentration (ppm)**	**Mean achieved dose level (mg/kg bw/day)**	
		Males	Females
1 (control)	0		
2 (low)	2000	121	145
3 (mid)	6000	361	437
4 (high)	20000	1214	1498

The results show a higher test material intake for females when compared to males for each dose level. The mean intake for each dose group is 0, 121, 361 and 1214 mg/kg bw/day for males and 0, 145, 437 and 1498 mg/kg bw/day for females for 0, 2000, 6000 and 20000 ppm, respectively.

Ophthalmoscopy
There were no treatment-related effects observed.

Haematology and clinical chemistry
Haematology
Minor variations from control values were obtained for most parameters but showed no consistency and were confined to intermediate time points and/or dose groups and were considered not to be treatment-related. An increased haemoglobin concentration and decreased platelet count was seen in all female treated groups at the interim kill but, in the absence of any apparent dose-response or effects at other time points, these variations from mean control values are considered not to be treatment-related (see Table B.6.5-15).

Table B.6.5-15: Haemoglobin and platelet count

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Haemoglobin (g/dL)								
Week 14	15.9	15.7	16.0	15.5	16.0	15.9	15.8	15.0*
Week 27	15.5	15.7	15.8	15.8	15.8	15.7	15.7	15.6
Interim Kill	14.7	14.4	14.4	15.1**	14.3	14.9*	14.4	15.0*
Week 53	16.1	15.9	15.7*	15.9	15.5**	15.9	15.9	15.8
Week 79	15.9	15.9	15.2	15.8	15.5	16.0	15.4	15.5
Week 105	13.3	14.3	12.9	14.1	13.1	13.8	13.6	14.2
Platelet count ($\times 10^9$/L)								
Week 14	885	911	897	877	892	910	847	948
Week 27	903	909	871	868	917	858	880	830*
Interim Kill	889	821	895	761*	888	740**	860	764*
Week 53	911	842	977	794	911	754	865	814
Week 79	963	854	993	796	950	817	935	855
Week 105	1015	780	980	783	988	750	877	846

* $p < 0.05$; ** $p < 0.01$

Clinical chemistry

In rats fed 20000 ppm glyphosate acid, increases in plasma alkaline phosphatase were present until Week 79 (Table B.6.5-16). Increases in alanine aminotransferase activities were present consistently in males until Week 79 and in females in Weeks 14, 79 and 105. Increased total bilirubin was also present in these males throughout the study and increased plasma aspartate aminotransferase activity was present in males at the interim kill. Plasma triglycerides and cholesterol levels were reduced (from Weeks 14-53 and Weeks 53 onwards, respectively) in males.

In animals fed 6000 ppm, there were small increases in alkaline phosphatase activity over the first year of the study and variable increases in plasma alanine aminotransferase activity at intermediate time points throughout the study.

Plasma creatinine values were lower in all treated female groups at Week 27 and in females receiving 6000 and 20000 ppm at Week 14, but in the absence of any effects later in the study, this is considered to be of no toxicological significance.

Other minor variations from mean control values were confined to intermediate dose groups or time points and/or showed no dose response, and so were considered not to be treatment-related.

Table B.6.5-16: Clinical chemical findings

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Alkaline Phosphatase (IU/L)								
Week 14	234	156	246	177	284**	245**	387**	266**
Week 27	196	121	219	136	239**	166**	327**	203**
Interim Kill	230	82	244	102	269	123*	306**	144**
Week 53	231	92	249	117*	277**	152**	357**	172**
Week 79	208	114	254*	131	244	181**	353**	178**
Week 105	184	144	205	129	218	158	280	173
Alanine Aminotransferase (IU/L)								

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 14	94.9	81.9	103.5	92.5	121.8**	103.9*	143.4**	104.7*
Week 27	91.8	99.5	95.9	113.8	116.8	132.7*	125.9*	101.8
Interim Kill	77.6	83.4	84.0	82.8	97.7	113.2*	123.3**	95.9
Week 53	84.2	90.1	99.8	108.2	103.5	121.5*	133.8*	114.0
Week 79	69.2	90.0	81.2	97.2	102.4**	110.6	105.9**	116.0*
Week 105	64.1	83.5	58.6	78.6	63.9	78.9	82.7	108.2**
Total Bilirubin (µmol/L)								
Week 14	1.23	2.00	1.23	1.92	1.46	2.00	1.85**	2.46*
Week 27	2.08	2.31	2.31	2.08	2.31	2.08	2.62**	2.23
Interim Kill	2.09	2.50	1.91	2.42	2.18	2.58	2.67**	2.64
Week 53	2.62	2.54	2.46	2.31	2.92	2.46	3.46**	3.15**
Week 79	2.46	2.92	2.92	2.31	2.85	2.38	3.15**	3.08
Week 105	1.75	1.19	2.29	1.04	1.67	1.77	2.54	1.40
Aspartate Aminotransferase (IU/L)								
Week 14	107.9	104.5	113.5	112.6	129.2	124.0	148.0*	114.3
Week 27	110.5	156.8	114.8	185.5	138.0	208.4	141.3	148.3
Interim Kill	90.0	117.8	91.5	109.0	110.4	149.3	132.0*	131.5
Week 53	111.8	151.9	124.8	194.4	130.2	219.1*	160.7	214.8*
Week 79	88.2	156.0	102.7	129.2	130.0	177.7	112.2	197.0
Week 105	75.8	130.7	81.4	102.8	78.4	121.8	92.8	168.5
Plasma Triglycerides (mmol/L)								
Week 14	1.33	1.03	1.48	096	1.43	0.96	1.11*	0.94
Week 27	1.40	1.18	1.42	1.22	1.38	0.95*	1.14*	1.09
Interim Kill	1.65	1.00	2.07	1.13	2.09	1.07	1.45	0.99
Week 53	1.53	1.62	1.55	1.75	1.50	1.39	1.15*	1.39
Week 79	1.90	2.15	1.96	2.77	1.67	2.26	1.42	2.31
Week 105	1.83	3.26	1.81	3.58	1.94	3.02	1.67	2.82
Cholesterol (mmol/L)								
Week 14	2.40	2.66	2.51	2.62	2.48	2.80	2.54	2.71
Week 27	2.92	3.19	3.02	3.24	3.18	3.13	2.98	3.15
Interim Kill	4.74	2.69	5.05	2.95	4.83	2.98	3.89*	3.01
Week 53	5.03	3.56	4.57	3.49	5.15	3.45	4.06**	3.66
Week 79	6.87	4.26	6.30	4.64	5.81*	3.92	5.20**	3.96
Week 105	6.76	4.44	7.22	4.54	7.79	4.13	5.72*	4.11
Plasma Creatinine (µmol/L)								
Week 14	58.5	61.4	59.9	59.6	57.2	59.0*	56.8	58.6**
Week 27	60.8	62.7	61.2	60.3*	59.4	60.5*	58.4*	58.2**
Interim Kill	55.8	53.6	58.0	51.8	56.5	52.3	56.6	50.9
Week 53	61.0	58.8	61.5	59.5	62.5	58.1	60.5	58.2
Week 79	80.7	62.7	85.9	59.2	86.2	62.8	66.4	61.8
Week 105	79.1	50.9	80.8	51.4	79.2	53.5	66.2	50.7

* $p < 0.05$; ** $p < 0.01$

Urinanalysis

Urinary pH was lower throughout the study in males fed 20000 ppm glyphosate acid (Table B.6.5-17). Moreover, in the same dose group an increased incidence and severity of blood/red blood cells was present in males and, to a lesser extent, in females.

There were no other treatment related findings in the urinalysis.

Table B.6.5-17: Urinalytical findings

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Urine pH								
Week 13	6.85	6.00	6.77	6.00	6.92	6.08	6.31**	5.85
Week 26	6.77	5.77	6.69	5.85	6.69	6.00	6.15**	5.77
Week 52	6.85	6.15	6.85	6.23	6.85	6.31	6.15**	5.92
Week 78	6.54	6.38	6.28	6.77	6.15	6.46	5.69**	6.00
Week 98	6.08	—	6.00	—	6.00	—	5.85	—
Week 104	—	6.00	—	6.08	—	6.15	—	6.00

** $p < 0.01$; NEG: negative, +: very few (1 or 2); ++: few; +++: many

Necropsy
Gross pathology
Treatment-related macroscopic findings were seen in males fed 20000 ppm and/or 6000 ppm in the kidneys, liver, prostate and testes. These findings consisted of a minor increase in incidence of enlarged kidneys, single masses in the liver, firmness of the prostate and a reduction in the incidence of reduced testes.
Additional findings were not considered to be treatment related.

Organ weights
Significant lower relative adrenal gland weight was noted at the interim kill in females fed 20000 ppm and 6000 ppm glyphosate acid. Furthermore, the liver weight was significantly lower at the interim kill in males fed 20000 ppm glyphosate acid.
There were no other significant and dose-related effects on organ weights.

Histopathology
A minor increase in the incidence but not severity of proliferative cholangitis in the liver was present in males fed 20000 ppm glyphosate acid at interim and terminal kill (see Table B.6.5-18) .
Moreover, in males fed 20000 ppm glyphosate acid an increased incidence of hepatitis and periodontal inflammation was observed. The incidence of prostatitis was higher than the control group in all treated males and there was a decrease in the incidence of tubular degeneration of the testis in males fed 20000 ppm glyphosate acid. The incidence of prostatitis was within historical background levels in all treated groups but, as the control value in this study was low, the relationship to treatment at the high dose level cannot be entirely dismissed.

The main changes in interim and terminal kill males and, to a lesser extent, females fed 20000 ppm glyphosate acid, were observed in the kidney. These changes consisted of slight increased incidence of papillary necrosis with varying degrees of mineralisation of the papilla and/or transitional cell hyperplasia. There was also a very small increased incidence of papillary mineralisation only (males and females fed 20000 ppm glyphosate acid) and transitional cell hyperplasia alone (20000 ppm males only).

All other observed differences in the incidence of findings either fall within the historical background level or are considered to be unrelated to the treatment with glyphosate acid.

Table B.6.5-18: Summary of histopathological findings

	Dietary concentration of glyphosate (ppm)									
	Males (n=64)					Females (n=64)				
Finding	Historical Control	0	2000	6000	20000	Historical Control	0	2000	6000	20000
Liver										
Proliferative cholangitis	-	56	57	55	64	-	55	58	59	61
Hepatitis	4.7 [2 - 8]	8	6	9	13	-	6	7	4	6
Kidney										
Papillary necrosis	0.4 [0 – 2]	0	1	0	14	-	0	1	2	5
Transitional cell hyperplasia	-	2	3	0	5	-	3	1	0	1
Prostate Prostatitis	23.4 [13 – 35]	13	22	23	37	-	-	-	-	-
Testis Unilateral tubular degeneration	-	18	13	18	5	-	-	-	-	-
Periodontal inflammation	-	25	27	23	42	-	18	24	32	28

n = number of animals per group
Historical control (mean and [range])

Neoplastic changes
There was no evidence of carcinogenicity and no differences between the groups in tumour incidence.

Conclusion by the Notifiers
Based on the study results the NOAEL in rats after chronic exposure to glyphosate acid for 24 month is 6000 ppm (corresponding to 361 mg/kg bw/day in males and 437 mg/kg bw/day in females). It is concluded that glyphosate technical is not carcinogenic in rats.

RMS comment:
The study is considered acceptable. We agree with the description of the study and its findings and support the conclusions including the NOAEL. It was surprising that the salivary gland findings reported by Milburn (1996, TOX2000-1998) were not confirmed although the study was run in the same laboratory employing rats of the same strain. No further remarks.

5th study: [redacted]. (2009)

Reference: IIA, 5.5.2/08
Report: [redacted] (2009)
Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat
[redacted]
Study No.: 2060-0012
Date: 2009-04-23, amended 2009-05-08
not published, ASB2012-11490
Guidelines: OECD 453 (1981), JMAFF Guideline 2-1-16 (2005), US OPTTS 870.4300 (1996)
Deviations: None
GLP: yes

Acceptability: *See RMS comment*

Dates of experimental work: 2005-09-01 - 2008-03-19

Materials and methods

Test material:	
Identification:	Glyphosate Technical
Description:	White crystalline solid
Lot/Batch #:	H05H016A
Purity:	95.7 % w/w
Stability of test compound:	No data
Vehicle :	Diet
Test animals:	
Species:	Rat
Strain:	Wistar Han Crl:WI
Source:	[redacted]
Age:	5 – 6 weeks
Sex:	Males and females
Weight at dosing:	Males: 112 – 183 g, females: 98 – 150 g
Acclimation period:	At least ten days
Diet/Food:	Rat and Mouse SQC Ground Diet No.1 (BCM IPS Ltd., London, UK), *ad libitum*
Water:	Mains drinking water, *ad libitum*
Housing:	Initially in groups of three per sex in polypropylene solid-floor cages.
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 55 ± 15 % Air changes: at least 15/hour 12 hours light/dark cycle

In life dates: 2005-09-01 to 2007-08-31

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 51 Wistar rats per sex received daily dietary doses of 0, 1500, 5000 and 15000 ppm (equivalent to mean achieved dose levels of 0, 95.0, 316.9 and 1229.7 mg/kg bw/day) Glyphosate technical. To ensure that a received dose of 1000 mg/kg bw/day overall was achieved, the highest dose level was progressively increased to 24000 ppm.
In addition, three satellite groups with 15 rats per sex each were included for interim sacrifice at the 12th month to study non-neoplastic histopathological changes. The satellite control group with 12 rats per sex served as veterinary control. The animals were to be used for investigations should any health problems have developed with study animals. No such problems occurred and therefore the observations of these animals have not been included in the report.

Test diets were prepared weekly by mixing a known amount of the test substance with a small amount of basal diet for 19 minutes at a constant speed. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes.
The stability and homogeneity of the test substance in the diet was determined in an in-house stability study. The homogeneity and achieved concentrations of the test substance preparations was determined at monthly intervals until Week 26, and in 3-month intervals thereafter.

Clinical observations
Rats were examined for toxic signs, ill-health or behavioural changes once and for pre-terminal deaths twice a day. A routine clinical observation session including veterinary examination was made weekly, including palpation for new or existing masses. Ophthalmic examination was done at the start of the study in all satellite animals and at Week 50 in ten satellite animals per sex of the control and high dose group. Prior to treatment and at weekly intervals thereafter all satellite animals were observed for behavioural toxicity.

Body weight
Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 13 and every four weeks thereafter until termination as well at terminal kill.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from Week 1 to Week 13 and subsequently for one week in each four weeks until termination.

Water consumption
Water intake was observed daily, for each cage group, by visual inspection.

Haematology and clinical chemistry
Haematological examinations were performed on ten animals per sex from the satellite and main groups at 3, 6 and 12 months. Further haematological investigations were performed on 20 animals per sex from the main groups at 18 and 24 months. The following parameters were measured: hematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, platelet count, total leukocyte count, differential leukocyte count, reticulocyte count, prothrombin time, and activated partial thromboplastin time.
Blood chemical investigations were performed on ten animals per sex from the satellite groups at 6 and 12 months and from the main groups at 18 and 24 months. The following parameters were determined: urea, glucose, total protein, albumin, albumin/globulin ratio, sodium, potassium, chloride, calcium, inorganic phosphorus, ASAT, ALAT, alkaline phosphatise, creatinine, total cholesterol, total bilirubin, and cholinesterase.

Urinalysis
Urinalytical investigations were performed on ten animals per sex from satellite groups at 3, 6 and 12 months and from main groups at 18 and 24 months. The following measurements were made: specific gravity, volume, pH, protein, glucose, ketones, blood, urobilinogen, reducing substances and microscopic examination of sediment.

Sacrifice and pathology
Necropsy was conducted for all animals surviving until study termination (main groups: 104 weeks; satellite groups: 52 weeks) as well for all animals found dead or killed *in extremis*.

The following organ weights were determined from 10 rats per sex and main group and from all satellite animals: adrenals, brain, gonads, heart, kidneys, liver, spleen and thymus.

Tissue samples were taken from the following organs: adrenals, aorta (thoraic), bone & bone marrow (sternum and femur incl. joint), brain (cerebrum, cerebellum, pons), caecum, colon, duodenum, epididymides, eyes (with optic nerve), gross lesions including palpable masses, head (pharynx, nasopharynx, paranasal sinuses), heart, Harderian gland, ileum (incl. Peyer's patches), jejunum, kidneys, liver, lungs (with bronchi), lymph nodes (cervical and mesenteric), mammary gland, muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands (submaxillary), sciatic nerve, seminal vesicles, skin (hind limb), spinal cord (cervical, mid-thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus and vagina. A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals. In addition, gross lesions and masses from low and intermediate dose groups at termination were examined microscopically.
Histopathological examination was initially carried out on all tissues collected from control and high dose groups; all pre-terminally dead and moribund sacrificed rats and on all lesions and palpable masses of the terminally sacrificed rats from the low and mid dose groups.
Since there were no indications of treatment-related bone marrow changes, examination was subsequently extended to the remaining treatment groups.

Statistics
Where appropriate quantitative data was analysed by the ProvantisTM Tables and Statistics Module. For each variable, the most suitable transformation of the data was found; the use of possible covariates checked and the homogeneity of means assessed using ANOVA or ANCOVA and Bartletts's test. The transformed data was analysed to find the lowest treatment level that shows a significant effect, using the Williams Test for parametric data or the Shirley Test for non-parametric data. If no dose response is found, but the data shows non-homogeneity of means, the data will be analysed by a stepwise Dunnett (parametric) or Steel (non-parametric) test to determine significant differences from the control group. Finally, if required, pair-wise tests are performed using the Student t-test (parametric) or the Mann-Whitney U test (non-parametric).
Histopathology data were analysed using the following methods to determine significant differences between control and treatment groups for the individual sexes.
1. Chi squared analysis for differences in the incidence of lesions occurring with an overall frequency of I or greater.
2. Kruskal-Wallis one way non-parametric analysis of variance for the comparison of severity grades for the more frequently observed graded conditions.

Results and discussion
Analysis of dose formulations
Stability assessment demonstrated that the test material preparations in the diet were stable for at least six weeks.
Analyses for achieved concentrations showed that the diet preparations were within an acceptable range. On one occasion the achieved concentrations of the low, mid and high-dose group were 79%, 83%, and 87%, respectively. At week 2 the concentration in the mid dose group was 112%. However, these isolated deviations from the nominal range were still considered to be acceptable.

Mortality
No significant treatment-related effects on mortality were observed during the study.
The numbers of pre-terminal deaths in the main group are displayed below:

Table B.6.5-19: Cumulated mortalities after 104-week dietary exposure to glyphosate technical

	Dose group (ppm)			
Sex	0	1500	5000	15000-24000
Male	12	14	13	6
Female	14	17	15	12

Clinical observations
No significant treatment-related clinical observations occurred during the study.
There were no treatment-related effects on behavioural assessments, functional performance tests or sensory reactivity assessments observed.

Body weight
There were no treatment-related effects on male and female overall body weight gain during the conduct of study.

Food consumption and compound intake
There were no treatment-related effects on food consumption or food efficiency for either sex noted during the study.
The group mean achieved doses are summarised below.

Table B.6.5-20: Group mean achieved dose levels

Dose group	**Dietary concentration (ppm)**		**Mean achieved dose level (mg/kg bw/day)**		
			Males	Females	Overall mean
1 (control)	0				
2 (low)	1500		85.5	104.5	95.0
3 (mid)	5000		285.2	348.6	316.9
4 (high)	15000	Week 1-11	1077.4	1381.9	1229.7
	17000	Week 12-15			
	19000	Week 16-26			
	21000	Week 27-39			
	24000	Week 40-104			

The results show a higher test material intake for females when compared to males for each dose level. The mean intake for each dose group is 95.0, 316.9 and 1229.7 mg/kg bw/day for 1500, 5000, and 15000-24000 ppm, respectively. The mean intake values represent the combination of satellite and main group values.

Water consumption
There were no treatment-related effects on water consumption during the study.

Ophthalmoscopy
There were no treatment-related effects observed.

Haematology and clinical chemistry
Haematology
All variations were considered to be incidental and unrelated to treatment because of the lack of either a true dose response, a consistent change throughout the study, a lack of progression of change with time and/or lack of concomitant effect in both sexes.

Clinical chemistry
At the highest dose level there was an increase in alkaline phosphatase activity for satellite group males and females compared with controls at 6 and 12 months. Main group males were also affected at 18 months. Values for all alkaline phosphatase activity values are presented as follows:

Table B.6.5-21: Alkaline phosphatase activity (IU/L)

	Dose level							
	Control		Low		Intermediate		High	
Timepoint	♂	♀	♂	♀	♂	♀	♂	♀
Month 6 (Satellite)	87.8	49.6	94.5	62.9	103.4	62.0	128.5**	91.9**
Month 12 (Satellite)	87.7	46.1	96.5	59.7	116.3*	58.1	140.2**	91.3**
Month 18 (Main)	93.3	65.7	110.5	55.8	110.9	70.9	125.0*	92.7
Month 24 (Main)	107.2	66.0	98.8	58.5	101.0	81.7	111.9	86.8

* $p < 0.05$; ** $p < 0.01$

The magnitude of the effect does not appear to increase with age plus the lack of a consistent effect for females does suggest this to be of limited toxicological importance.

At the 18 month evaluation there was an increase in plasma electrolytes for both sexes. Sodium and chloride values for males and females and potassium values for males only were increased compared with controls. Female calcium levels were lower than controls. These elevations/decrements were also observed at lower dose levels but were not seen in a dose related trend. In addition at the 12 month evaluation for satellite females a lower sodium value was seen for females. Values for all calcium and chloride values are presented as follows:

Table B.6.5-22: Calcium and chloride values (mmol/L)

	Dose level							
	Control		Low		Intermediate		High	
Timepoint	♂	♀	♂	♀	♂	♀	♂	♀
Calcium								
Month 6 (Satellite)	2.587	3.693	2.701	3.752	2.617	3.637	2.508	3.604*
Month 12 (Satellite)	2.530	2.602	2.543	2.587	2.458	2.475	2.514	2.483
Month 18 (Main)	2.231	2.775	2.523	2.645*	2.656	2.554**	2.598	2.468**
Month 24 (Main)	2.431	2.293	2.487	2.396	2.511	2.288	2.297	2.347
Chloride								
Month 6 (Satellite)	107.7	105.8	107.1	106.1	107.0	106.1	108.5	106.7
Month 12 (Satellite)	105.6	103.9	105.1	104.8	104.3	104.7	105.9	104.2
Month 18 (Main)	103.3	101.8	105.8**	104.2**	105.8**	106.4**	107.6**	107.8**
Month 24 (Main)	104.5	103.4	104.4	103.1	104.3	102.2	105.4	102.8

* $p < 0.05$; ** $p < 0.01$

At intermediate level similar findings to the highest dose level were seen for plasma electrolytes at the 18 month evaluation. A slight increase in alkaline phosphatase activity was seen for satellite group males at 12 months. At the low dose level there was a similar effect on

the plasma electrolytes for both sexes at the 18 month evaluation of main group animals. Whilst these observations were seen at the highest dose level, the lack of dose response or the effect being limited to one sex does make the toxicological significance questionable.

All other differences were isolated in their finding and are therefore not toxicologically relevant.

Urinanalysis
There were no treatment-related effects observed.

Necropsy
Gross pathology
There were no treatment-related macroscopic findings observed during the study period.

Organ weights
No effects on organ weight values were observed.

Histopathology
Adipose infiltration of the bone marrow was seen for the majority of animals examined, with both sexes being more or less equally affected in terms of incidence and severity. However, greater effects were seen among male rats dosed at the highest level and this attained statistical significance for terminal kill animals. This data indicates the possibility of myeloid hypoplasia as a consequence of treatment. However, given the normal variability of this condition and the influence of other pathological conditions upon marrow cellularity in ageing rats, the effect was not altogether convincing but cannot be dismissed. A similar effect was not seen among male rats in the remaining treatment groups but among premature deaths for animals of both sexes at the intermediate level and only low-dosed females. However, the variable duration of exposure and significant background pathology for premature death animals further negates this as an effect of treatment upon marrow cellularity for female rats.

Moreover, at the highest dose level there was a significant difference in the site of mineral deposition within the kidneys compared with controls. Pelvic mineralisation was commonly seen in both sexes and was more prevalent among female rats; however corticomedullary mineralisation was seen in female rats only. Nephrocalcinosis in rats is generally considered to be related to diet and hormonal status. There was a lower incidence of pelvic/papillary deposition and an increase in the corticomedullary deposition. At the same time there was a reduction in the incidence of renal pelvic hyperplasia in both sexes; which is considered to be a consequence of the decreased mineral deposition.
The effects on pelvic and corticomedullary mineralisation, and hyperplasia of the pelvic/papillary epithelium were confined to high dose animals with no indication of a similar effect at any other treatment level for either sex.
No other treatment-related changes were observed.

Neoplastic changes
No significant effects associated with tumour development were observed.

Conclusion by the Notifiers
Based on the study results the NOAEL in rats after chronic exposure to glyphosate technical for 24 month is 24000 ppm (corresponding to 1229.7 mg/kg bw/day for combined sexes). It is concluded that Glyphosate technical is not carcinogenic in rats.

RMS comment:
The study is acceptable. In principle, the conclusions are agreed with but the NOAEL is seen rather at the mid dose level of 5000 ppm (equal to 285 mg/kg bw/day in males) because of minor effects that were observed at the highest dose level. These effects comprised a by up to 9 % lower body weight gain in male rats, a transient increase in alkaline phosphatase activity (confirming the same findings in many other studies with glyphosate), fatty infiltration of the bone marrow that might be indicative of hypoplasia and the kidney findings of equivocal relevance. In addition, there were some histological skin changes that resemble those reported by [redacted] (1997, ASB2012-11484, ASB2012-11485, ASB2012-11486, ASB2012-11487). They comprised a slightly higher incidence of areas of necrosis or giant cell reaction to keratin and a non-significant increase in keratoacanthoma in high dose males (occurring in 6/51 animals as compared to 2/51 in the control group, 3/51 in the low and 0/51 in the mid dose groups; incidence in females always 0). However, the assessment of glyphosate as non-carcinogenic in this study is not doubted.

B.6.5.1.2 Previously known long-term studies in rats (already subject to EU evaluation)

1st study: [redacted] 1981

Reference:	IIA, 5.5.2/05
Report:	[redacted] (1981) A Lifetime Feeding Study of Glyphosate (ROUNDUP Technical) in Rats [redacted] Data owner: Monsanto Study/Project No.: 77-2062 Date: 1981-09-18 not published, TOX2000-595
Guidelines:	Not stated; In general accordance with OECD 453 (1981)
Deviations:	None
GLP:	no
Acceptability:	*See RMS comment*

Dates of experimental work: In-life: 1978-07-12 to 1980-09-04

Materials and methods

Test material:	
Identification:	Glyphosate acid (Round-up technical material)
Description:	Fine White powder
Lot/Batch #:	XHJ-64
Purity:	98.7 % w/w
Stability of test compound:	At least 45 days when stored at -20 °C.
Vehicle and/ or positive control:	Diet
Test animals:	

Species:	Rat
Strain:	Sprague-Dawley CD
Source:	[redacted]
Age:	28 days (on delivery), 41 days at initiation of delivery
Sex:	Males and females
Weight at dosing:	Males: 155.0 – 156.6 g (mean values); females: 136.0 – 138.4 g (mean values)
Acclimation period:	12 days.
Diet/Food:	Standard laboratory diet (Purina Lab Chow) *ad libitum.* Freshly prepared weekly
Water:	Mains automated water system (Elizabethtown Water Company), *ad libitum*
Housing:	Individually in elevated stainless steel cages.
Environmental conditions:	Temperature: Monitored but values are not stated Humidity: not stated Air changes: not stated 12 hours light/dark cycle

In life dates: 12-07-1978 to 04-09-1980

Animal assignment and treatment:
In a chronic toxicity and carcinogenic study, groups of 50 male and 50 female Sprague-Dawley rats were administered in the diet glyphosate (Round-up technical) at concentrations of 0, 30, 100 and 300 ppm for the first week and at concentrations of 0 (control) 3.05, 10.30 and 31.49 mg/kg bw/day for the males, and 0 (control), 3.37, 11.22 and 34.02 mg/kg bw/day for the females for 26 months. Males received treatment for 775 - 776 days and females 784 - 785 days before termination.
A 0.5 oz. sample of the test substance was taken at weeks 1, 11, 16, 24, 36, 48, 60, 72, 84 and 96 and submitted to the sponsor for analysis. Samples of control feed and feed for each dose level were collected for analysis.

Observations
Rats were examined for mortality and signs of toxicity twice daily. Detailed physical and clinical examinations were performed weekly and included palpations for tissue masses. Ophthalmic examination was done in all animals at the start of the study, at Week 52 and prior to termination. A functional observational battery including motor activity was conducted in Week 52 in animals allocated to the chronic toxicity assessment of the study.

Body weight
Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 14 and every two weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded pre-test, once weekly for from Week 1 to Week 14 and every second week thereafter until termination.

Water consumption

Water consumption was investigated during the 18 and 24 months of treatment over 2-three day periods in 10 animals/sex/dose group.

Laboratory investigations
Haematology and clinical chemistry investigations were performed on 10 males and 10 females of each dose group during Months 4, 8, 12, 18 and 24 months. Blood was obtained via venipuncture of the orbital sinus (retrobulbar venous plexus) under light ether anaesthesia. Animals were selected randomly; the same animals were used at all intervals when feasible. Rats were fasted overnight prior to blood collections and were not dosed until after samples were collected.

Haematological parameters investigated included haematocrit, haemoglobin, erythrocyte count, platelet count, total leukocyte count and differential leukocyte count. Clinical chemistry parameters were alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactic acid dehydrogenase, blood urea nitrogen, fasting glucose, albumin, globulin, albumin/globulin ratio, total bilirubin and direct bilirubin, triglycerides, cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride. However as a result of a technician error, potassium was not evaluated at months 8 and 12 and inorganic phosphorus was evaluated at months 8 and 12 only.

Urinalysis was performed during the same months as for haematology and clinical chemistry except at 8 months and the parameters reported included gross appearance, specific gravity, pH, glucose, ketones, protein, bilirubin, and blood and microscopic analysis.

Sacrifice and pathology
Necropsy was conducted on all animals which died prematurely or were killed at termination as scheduled. Organ weights were determined for all animals surviving to scheduled termination and included the adrenals, brain, gonads, heart, thyroid, kidneys, liver, pituitary and spleen.
Samples from organs and tissues including the adrenals, aorta, blood smears, bone & bone marrow (costochondral junction), brain (cerebrum, cerebellum, brainstem), caecum, cervix, colon, duodenum, epididymis, eyes (retina, optic nerve), gross lesions including palpable masses, Harderian gland, heart, intestines (Including the caecum, colon, duodenum, ileum and jejunum), kidneys, lachrymal gland, larynx, liver, lung, lymph nodes (cervical and mesenteric), mammary gland, muscle, oesophagus, ovary, pancreas, pharynx, pituitary, prostrate, rectum, salivary glands (submandibular, parotid), seminal vesicles, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus and vagina.

Statistics
Parameters analyzed statistically were bodyweight, food consumption, haematology and clinical chemistry values, terminal organ and body weights, organ/body weight ratios and organ/brain weight ratios.

Statistical evaluation of equality of means was made by the appropriate one way analysis of variance technique, followed by a multiple comparison procedure if needed. First, Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to

determine which means were significantly different from the control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control. A statistical test for trend in the dose levels was also performed. In the parametric case (i.e. equal variance) standard regression techniques with a test for trend and lack of fit were used. In the nonparametric case Jonckheere's test for monotonic trend was used. The test for equal variance (Bartlett's) was conducted at the 1%, two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level.

Results and discussion

Analysis of dose formulations

Results of diet analyses were not reported.

Mortality and clinical signs

There was no significant difference between the control and treated both sexes with regard to the survival rate during the course of this study. Survival was approximately 80-90% through Month 20 of the study for all groups. Thereafter, significant reductions in the number of surviving animals occurred in all groups in roughly an equivalent fashion, culminating in the termination of the study at Month 26. At this time, survival had decreased to 30% in the low dose males and the high dose females, requiring that the study be terminated to insure a sufficient number of animals at the terminal necropsy. At 24 months, survival levels equalled or exceeded 50%, which is comparable to historical control data for rats of this strain.
The survival rates are displayed in Table B.6.5-23.

There were no treatment-related signs of toxicity and physical findings observed including alopecia, excessive lacrimation, nasal discharge and rales were present in all groups without a treatment-related trend.

Table B.6.5-23: Survival rates during up to 26-month dietary exposure to glyphosate technical (%)

	Dose group (mg/kg bw/day)							
	Males				Females			
	0	3.05	10.30	31.49	0	3.37	11.22	34.02
Month 20	74	86	86	96	92	88	88	76
Month 24	44	56	46	66	52	62	64	48
Month 26	30	52	32	52	36	46	60	30

Functional observations

A functional observational battery of tests was not performed. It is not considered to affect the validity of this study.

Body weight

There were no statistically significant differences in mean body weights in males. During part of the growth period, a slight but consistent trend toward reduced body weights in the treated males was evident. The maximum decrease was approximately 6% in high dose males. Thereafter, this difference decreased resulting in little difference in mean body weights between groups at termination. Because this effect was slight and not evident at termination of the study and did not affect survival, it is not considered to be toxicologically significant. In females, no statistically significant difference in mean body weights was observed in treated

animals compared to the controls up to Month 19 of the study. However, for the following 2 months, the treated groups showed statistically significant reductions in mean body weights as compared to the control, although not in a dose-related fashion. The magnitude of the reduction ranged between 10-15% with the greatest difference evident in the low and mid-dose group. Thereafter, the treated females gained weight relative to the control group resulting in nearly identical group mean body weights at termination. The absence of a dose-response relationship in this observation suggests the finding was most likely due to biological variation which is evident from the standard deviation of the mean body weights for all dose groups. The body weight changes are noted to have occurred well after the main growth phase in both sexes and lacked a dose- response relationship in females. The top dose was only statistically significantly reduced compared with controls during weeks 92 and 94 when body weight reduction was approximately 11.5% and 11% respectively; however at the next measurement during Week 96 body weight reduction compared with controls was only 5.7 %.

Food consumption and compound intake
Occasional statistically significant differences were noted in the treated animals of both sexes relative to their respective controls. However, these changes in mean food consumption values were slight and occurred sporadically and showed no treatment-relationship.

The target concentrations for treatment as administered in the first were 0 (control), 30, 100 and 300 ppm which corresponded to approximate compound intakes of 0, 3.05, 10.30 and 31.49 mg/kg bw/day for males and 0, 3.37, 11.22 and 34.02 mg/kg bw/day.

Water consumption
There were no treatment-related effects observed.

Laboratory investigation
Haematology
Haematology data did not indicate any toxicologically significant differences in the findings for both sexes for any of the parameters evaluated. All mean data were within the normal physiological range for the laboratory rat. The few statistically significant differences noted appeared to be due to random variation as no consistent treatment-related pattern was evident. Thus, haematological parameters were unaffected by the treatment of glyphosate.

Clinical chemistry
Clinical chemistry parameters for both males and females were within the normal physiological range and did not deviate significantly in a treatment-related manner from controls. Occasional statistically significant differences were noted, but these appear to be due to random fluctuation, as no treatment-related pattern emerged.

Urinalysis
Urinalysis parameters did not show any significant differences were between treated and control groups. Occasional values outside the normal range were found; however, these values occurred sporadically exhibiting no consistent pattern..

Necropsy
Gross pathology
There were no treatment-related gross pathological findings.

Organ weights
There were no statistically significant differences noted in the terminal organ weights, organ/body weight ratios and organ/brain weight ratios of the treated animals compared with their respective controls.

Histopathology
Neoplastic changes
The most common tumours were found in the pituitary in both sexes of both control and treated animals
In the females, mammary gland tumours were the next most common neoplasm found. In general, the incidence of all neoplasms observed in the treated and control animals were to a similar degree, or occurred at low incidence such that a treatment-related association could not be made (Table B.6.5-24).

Table B.6.5-24: Summary of critical tumour findings in 26-month dietary study with glyphosate technical

	Dose group (mg/kg bw/day)							
	Males				Females			
Dose Groups	0	3.05	10.3	31.49	0	3.37	11.22	34.02
Pituitary tumours								
Adenomas	16/48	19/49	20/48	18/47	34/48	29/48	31/50	26/49
Carcinomas	3/48	2/49	3/48	1/47	8/48	7/48	5/48	12/49
Combined	19/48	21/49	23/48	19/47	42/48	36/48	36/50	38/49

However, the incidence of interstitial cell tumours of the testes in male rats in both the scheduled terminal sacrifice animals as well as for all animals suggested a possible treatment-related finding and was presented along with the most recent historical control data at the time of the study for comparison. It was noted that at 12 months the incidence of interstitial tumours was near zero however in animals aged 24-29 months at necropsy, the incidence increased to approximately 10%. The historical control data for chronic toxicity and carcinogenicity from 5 studies terminated at 24-29 months showed background levels of interstitial cell tumours comparable to that found at the highest dose in the study. The incidence of interstitial cell hyperplasia did not provide evidence of a preneoplastic lesion.

Table B.6.5-25: Summary of the interstitial cell tumour findings in the testes of rats after 26-month dietary exposure to glyphosate technical

	Dose group (mg/kg bw/day)*			
Parameter	0	3.05	10.3	31.49
Interstitial cell tumour				
Terminal sacrifice	0/15 (0%)	2/26 (7.7%)	1/16 (6.3%)	4/26 (15.4%)
All Animals	0/50 (0%)	3/50 (6%)	1/50 (2%)	6/50 (12%)
Interstitial cell hyperplasia				
Terminal sacrifice	1/15 (6.7%)	1/26 (3.8%)	0/16 (0%)	0/26 (0%)
All Animals	1/50 (2%)	1/50 (2%)	1/50 (2%)	0/50 (0%)

*number of animals affected / total number of animals examined
( ): Percentage

Table B.6.5-26: Summary of the concurrent historical control data for interstitial cell tumours in the testes of in chronic toxicity studies

Parameter	Study				
	1	2	3	4	5
Terminal Sacrifice	4/65 (6.2%)	3/11 (27.3%)	3/26 (11.5%)	3/24 (12.5%)	3/40 (7.5%)
All Animals	4/116 (3.4%)	5/75 (6.6%)	4/113 (3.5%)	6/113 (5.3%)	5/118 (4,2%)

*number of animals affected / total number of animals examined
( ): Percentage

The investigators noted that at terminal sacrifice, the incidence in the present study was 15.4% (4/26), while the range in control animals from 5 contemporary studies (historical controls) was 6.2% (4/65) to 27.3% (3/11) with an overall mean value of 9.6% (16/166). When all animals on test are included, the incidence for the Group IV males was 12% (6/50) compared to a range of 3.4% (4/116) to 6.7% (5/75) with a mean of 4.5% (24/535). Therefore, this comparison suggests an incidence of this tumour in the Group I males which is slightly lower (0%), and an incidence in the Group IV males which is slightly higher than recent historical control data. Although an effect on the incidence of this tumour due to the administration of the test substance cannot be ruled out, the data suggests that the incidence in treated rats is within the normal biological variation observed for tumours at this site in this strain of rat.

Conclusion by the Notifier
Based on the study results the NOAEL in rats after chronic exposure to glyphosate acid for 24 month is 31.5 mg/kg bw/day in males and 34.0 mg/kg bw/day in females. It is concluded that glyphosate technical is not carcinogenic in rats. This old study, initiated before the establishment of regulatory testing guidelines, no longer meets current testing guideline criteria due to the low doses employed. Therefore, this study type was repeated by Monsanto with higher doses, in accordance with subsequent regulatory test guidelines.

RMS comment:
It is agreed that this study does not comply to modern standards and should not taken into consideration for evaluation of glyphosate any longer although the NOAEL is agreed with. In particular, the dose levels selected were much too low (in particular to assess carcinogenicity) and there were serious reporting deficiencies.

2nd study: [redacted], 1990

Reference: IIA, 5.5.2/06
Report: [redacted] (1990)
Chronic study of glyphosate administered in feed to Albino rats
Monsanto Agricultural Company, St. Louis, Missouri, USA
Data owner: Monsanto
Project No.: ML-87-148
Date: 1990-09-26
not published, TOX9300244
Guidelines: US-EPA Pesticide Assessment Guidelines Subdivision F, 83-5 (1982); in general accordance with OECD 453

Deviations:	(From OECD 453: only 10 rats/sex for interim sacrifice; overall survival at termination was below 50%)
GLP:	yes
Acceptability:	*See RMS comment*

Dates of experimental work: 1987-08-05 – 1989-08-10

Materials and methods

Test material:	
Identification:	Glyphosate
Description:	White solid
Lot/Batch #:	XLH-264
Purity:	96.5 %
Stability of test compound:	Guaranteed for the study period. Confirmed by analysis.
Vehicle and/ or positive control:	Diet
Test animals:	
Species:	Albino Rat
Strain:	Sprague-Dawley (CD)
Source:	[redacted]
Age:	Approx. 8 weeks (at start of study)
Sex:	Males and females
Weight at dosing:	Males: approx. 284 g; females: approx. 221 g
Acclimation period:	29 days
Diet/Food:	Purina Mills certified Rodent Chow #5002 (Purina Mills), *ad libitum*
Water:	Mains drinking water, *ad libitum*
Housing:	In stainless steel cages with wire mesh bottoms suspended over paper bedding
Environmental conditions:	Animal housing & husbandry were in accordance with the provisions of ´Guide to the Care and Use of Laboratory Animal´; USPHS-NIH Publ. No. 85-23 Temperature: 17.8 – 21.1 °C Humidity: 40 – 70% Air changes: not specified 12 hours light/dark cycle

In life dates: 1987-08-05 to 1989-08-10

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague-Dawley rats per sex received daily dietary doses of 0, 2,000, 8000 and 20000 ppm glyphosate (equivalent to mean achieved dose levels of 0, 89, 362 and 940 mg/kg bw/day for males and 0, 113, 457 and 1183 mg/kg bw/day for females) for 24 months.
A further ten animals per sex were added to each group and were designated for interim kill after 12 month to study chronic toxicity and non-neoplastic histopathological changes.

Test diets were prepared in approximately weekly intervals by mixing a known amount of the test substance with basal diet. The stability of the dietary formulations were determined by analysis of samples of the low- and high-dose levels after storage at room temperature for 7 and 14 days, and frozen after storage for 35 days. The homogeneity of the test substance in the diet was determined for the low- and high-dose level preparations in the first and 88th week of the study. Analyses for achieved concentrations were done for all dose levels for the first six weeks, and for at least one dose level in weekly intervals thereafter. The stability of the neat test substance was verified by analysis before the start of the study, during month 8, 14 and 21, and after termination.

Clinical observations
All rats were examined for mortality and clinical signs of toxicity twice daily. Detailed clinical observations were conducted weekly. An ophthalmic examination was done in all animals before the start of the study, and prior to termination.

Body weight
Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 13 and every four weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded at weekly intervals for the first 13 weeks, and every fourth week thereafter.

Haematology and clinical chemistry
Blood was collected from 10 fasted animals per sex and group at Months 6, 12, 18, and at termination. The following parameters were measured: haematocrit, haemoglobin, total erythrocyte count, MCV, MCH, MCHC, platelet count, total leukocyte count, differential leukocyte count, reticulocyte count, alkaline phosphatase, aspartate amino transferase (AST), alanine aminotransferase (ALT), creatinine, blood urea nitrogen, total protein, glucose, albumin, globulin, total bilirubin, direct bilirubin, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Urinalysis
Individual urine samples were collected from the same animals as those used for haematology analyses at Month 6, 12, 18 and prior to termination. Sampling was done over a period of about 18-hours via metabolism trays. The following parameters were determined: appearance, specific gravity, pH, glucose, ketones, protein, bilirubin, urobilinogen and blood. In case that blood and / or protein in excess of the control urine samples were found, the sediment was examined for the presence of bacteria, epithelial cells, erythrocytes, leukocytes, casts or abnormal crystals.

Sacrifice and pathology
A gross necropsy was conducted on all surviving animals at scheduled sacrifice after 12 and 24 month. The following organ weights were determined: brain, kidneys, liver, and testes with epididymides.
Tissue samples were taken from the following organs and subjected to a histopathological examination: adrenals, aorta, bone & bone marrow, brain, caecum, colon, duodenum, eyes, gross lesions including palpable masses, Harderian gland, heart, ileum, jejunum, kidneys, liver, lung (with main stem bronchi), lymph nodes (mesenteric and submandibular), muscle, nasal turbinates, oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, sciatic nerve,

seminal vesicles, skin (with mammary tissue), spinal cord (cervical, thoracic, lumbar), spleen, stomach, submaxillary salivary gland, testes with epididymis, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus (corpus and cervix).

Statistics
Dunnett's Multiple Comparison Test (two-tailed) was used for body weights, cumulative body weight changes, food consumption, absolute leukocyte counts, reticulocyte counts, urine pH, urine specific gravity and clinical chemistry data obtained at Months 6, 12 and 18. Fisher´s exact test (one-tailed) was used for incidence of selected ocular lesions, as well as in combination with Bonferroni inequality procedure for incidences of non-neoplastic (at $p \leq 0.01$) and neoplastic lesions (at $p \leq 0.01$ and ≤ 0.05). EHL decision tree analysis was used for evaluation of terminal haematology, clinical chemistry, body weight, absolute and relative organ weight data and organ to brain weight ratios. Depending on the results either parametric (Dunnett´s Test and linear regression) or nonparametric (Kruskal-Wallis, Jonckheere´s and / or Mann-Whitney Tests) were applied. Mortality data were analysed by SAS lifetable procedure, and Peto Analysis was used for evaluation of histopathological data.

Results and discussion
Analysis of dose formulations
The stability analyses proved the neat test substance to be stable throughout the study period. The stability and homogeneity of glyphosate in diet at concentrations of 2000 and 20000 ppm was satisfactory. The mean achieved concentrations of glyphosate in each dietary preparation were 95 % of the nominal concentration.

Mortality
There were no statistically significant differences in the group survival rates. The percentage of survival in each of the dose groups are summarised below.

Table B.6.5-27: Percentage survival at termination after 24-month dietary exposure to glyphosate

	Dose group (ppm)			
Sex	0	2000	8000	20000
Male	29	38	34	34
Female	44	44	34	36

Clinical observations
There were no treatment-related clinical sings noted except the ophthalmological findings (see below).

Body weight
There were no effects on body weight noted in males of any dose group. In high-dose females body weights were statistically significant reduced from Week 7 through approximately the 20th month. During this time, absolute body weights gradually decreased to 14 % below the control value. The maximum difference in body weights was observed at 20th month. At this time-point the cumulative body weight gain in high-dose females was 23 % lower as compared to controls
There were no treatment-related effects in females fed 2000 or 8000 ppm glyphosate.

Food consumption and compound intake

There were no statistically significant decreases in food consumption in any group of either sex during the study period. However, significant increased food consumption was noted frequently in high-dose males, and on some occasions in low-dose males. The group mean achieved doses are summarised below.

Table B.6.5-28: Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)	
		Males	Females
1 (control)	0	0	0
2 (low)	2000	89	113
3 (mid)	8000	362	457
4 (high)	20000	940	1183

Ophthalmoscopy

There were no treatment-related ocular effects observed in females of any dose group, as well as of males of the low-, and mid-dose group. In high-dose males a statistically increased incidence ($p \leq 0.05$) of cataractous lens changes were observed at the ophthalmic examination prior to termination. However, the observed incidence of 25% was within the historical control range of 0-33 %. A second independent ophthalmic examination also performed prior to termination confirmed a statistically significant increase ($p \leq 0.05$) in the incidence of cataractous lens changes in high-dose males (1/14 (control) compared to 8/19 (high dose)). The results are summarised in the table below.

Table B.6.5-29: Incidences of cataract and lens fibre degeneration in males observed during ophthalmic examinations

	Dose group (ppm in diet)*			
	0	2000	8000	20000
1st examination	0/15	1/22	3/18	5/20**
2nd examination	0/14	2/22	3/17	8/19**

* number of rats affected / number of rats examined
** statistically significant from control ($p \leq 0.05$)

The histopathological examination confirmed a slightly, but not statistically, increased incidence of degenerative lens changes (i.e. cataract and/or lens fibre degeneration) in high-dose males (see Table B.6.5-30 below).

Table B.6.5-30: Histopathological confirmed incidences of cataract and lens fibre degeneration in males

	Dose group (ppm in diet)*			
	0	2000	8000	20000
Terminal sacrifice	2/14	3/19	3/17	5/17
All animals	4/60	6/60	5/60	8/60

* Number of rats affected / number of rats examined

Due to the small number of rats examined ophthalmologically and affected at termination, the results are difficult to interpret. Nevertheless, the occurrence of degenerative lens changes in high-dose males appears to be exacerbated by treatment.

Haematology and clinical chemistry
Haematology and clinical chemistry evaluations noted various changes in the examined parameters. However, the changes were not consistently noted at more than one time point, were within historical control ranges, small in magnitude, and/or did not occur in a dose-related manner. Therefore, they were considered to be either unrelated to treatment or toxicologically insignificant.
The statistically increased alkaline phosphatase level observed in high-dose females at termination was mostly due to an extremely high value for one animal. However, this finding is in line with observation made in other long-term studies in rats.

Urinanalysis
Urine specific gravity was statistically significant increased at the Month 6 examination. The observed statistically significant decreased urinary pH at 6, 18 and 24 months might be related to the renal excretion of glyphosate, which is an acid.

Necropsy
Gross pathology
There were no treatment-related gross pathological findings observed at necropsy.

Organ weights
At interim kill after 12 months relative liver weights were slightly, but statistically significant increased in high-dose males. At terminal sacrifice absolute liver weights, as well as liver to brain weight ratios were also statistically increased in high-dose males. There were no other significant and dose-related effects on organ weights.

Histopathology
Non-neoplastic lesions
Apart from the eye findings mentioned above histopathological examination showed only one other lesion that reached statistical significance. This was an increased incidence of inflammation of the stomach squamous mucosa in females fed 8,000 ppm glyphosate (see Table B.6.5-31).

Table B.6.5-31: Incidence of inflammation and hyperplasia of the stomach squamous mucosa

		Dose group (ppm in diet)*			
		0	2000	8000	20000
Males	Inflammation	2/58	3/58	5/59	7/59
	Hyperplasia	3/58	3/58	4/59	7/59
Females	Inflammation	0/59	3/60	9/60**	6/59
	Hyperplasia	2/59	3/60	7/60	6/59

* Number of rats affected / number of rats examined
** statistically significant at p≤ 0.01 (Fisher exact test with Bonferroni inequality)

Although the incidence of this lesion in mid-dose females (15 %) was slightly outside the historical control range (0 – 13.3 %) for the laboratory, there was no dose-related trend across all groups of females, and there was also no significance difference in male rats. Therefore, this finding is considered to be incidental and not related to treatment with glyphosate.

Neoplastic lesions
The only statistically significant difference in neoplastic lesions was an increased incidence of pancreatic islet cell adenomas observed in low-dose males (see Table B.6.5-32). The incidence (14%) in low-dose males was outside the historical control range (1.8 – 8.5 %) for this laboratory, but was in the historical control range (≥ 17 %) observed in reports from other laboratories. In addition, there was no dose-related trend for this finding in the male groups, as indicated by the lack of statistical significance in the Peto trend test. Due to the lack of a dose-related proliferative effect (hyperplasia) and or progression (carcinoma) of this lesion, and as such effects were not observed in females, this finding was not considered to be treatment-related.

Table B.6.5-32: Incidence of pancreatic islet cell findings

Finding	**Sex**	**Dose group (ppm in diet)***			
		0	2000	8000	20000
Hyperplasia	Males	2/58	0/57	4/60	2/59
	Females	4/60	1/60	1/60	0/59
Adenoma	Males	1/58	8/57**	5/60	7/59
	Females	5/60	1/60	4/60	0/59
Carcinoma	Males	1/58	0/57	0/60	0/59
	Females	0/60	0/60	0/60	0/59

* Number of rats affected / number of rats examined
** Statistically significant at p≤ 0.01 (Fisher exact test with Bonferroni inequality)

Conclusion by the Notifiers
Based on the study results the NOAEL in rats after chronic exposure to glyphosate for 24 month is 8000 ppm (corresponding to 362 mg/kg bw/day in males and 457 mg/kg bw/day in females). It is concluded that glyphosate is not carcinogenic in rats.

RMS comment:
The study is considered acceptable. However, based on stomach mucosal irritation (although not strictly dose-related) at the two upper dose levesl, a NOAEL of 2000 ppm (89 mg/kg bw/day) was established. Further toxic effects on body weight, liver and eyes (cataracts) were apparently confined to the top dose level.

3d study: [redacted] 1993

Reference: IIA, 5.5.2/04
Report: [redacted]
(1993)
Glyphosate – 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks)
[redacted]
Data owner: Cheminova
Study No.: 438623; Report No.: 7867
Date: 1993-04-07
GLP:
not published, TOX9750499

Guidelines:	US-EPA Pesticide Assessment Guidelines Subdivision F, 83-5 (1982)
Deviations:	None
GLP:	yes
Acceptability:	*See RMS comment.*

Dates of experimental work**:** 1990-02-16 to 1992-03-09

Materials and methods

Test material:	
Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	229-Jak-5-1; 229-Jak-142-6
Purity:	98.9 %; 98.7 %
Stability of test compound:	At least two years at ambient temperature in the dark
Vehicle and/ or positive control:	Diet
Test animals:	
Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Approx. 4 weeks upon arrival at testing facility
Sex:	Males and females
Weight at dosing:	Males: 85 ± 5 g, females: 60 ± 5 g
Acclimation period:	14 days
Diet/Food:	SQC Expanded (Fine Ground) Rat and Mouse Maintenance Diet No. 1 (Special Diet Services Limited, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	In groups of five per sex in suspended polypropylene cages with stainless steel wire grid tops and bottoms
Environmental conditions:	Temperature: 20 ± 2 °C Humidity: 55 ± 10 % Air changes: 15 – 20 / hour 12 hours light/dark cycle

In life dates: 1990-02-16 to 1992-03-09

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague-Dawley rats per sex received daily dietary doses of 0, 10, 100, 300 or 1000 mg/kg bw/day glyphosate technical. An additional five groups with 35 rats per sex receiving daily dietary doses of 0, 10, 100, 300, or 1000 mg/kg bw/day were included for the toxicity study. Fifteen rats per sex and per dose of the toxicity study were scheduled for interim sacrifice after 12 months. The dose levels were selected based on the results of a 13-week dietary toxicity study in rats.

Test diets were prepared once per week for the first 13 weeks and at least once every two weeks thereafter by direct admixture of the test substance to the plain diet and mixing for 20 minutes.
Analyses for achieved concentrations of the test substance in the diet were conducted from formulated diets at approximately fortnight intervals for the first 12 weeks and in intervals of 2 month thereafter.
The stability and homogeneity of the test substance in the diet was determined prior to the start of the study.

Clinical observations
A check for mortality was made twice daily on all animals throughout the study. In addition, all animals were examined for clinical signs during each day. A detailed clinical examination and check for palpable masses were done once each week on every animal. An ophthalmoscopic examination was conducted on 20 rats per sex of each group of the oncogenicity study before the start of the study and on 20 rats per sex of the control and high-dose group of the oncogenicity study at weeks 24 and 50. In addition, an opthalmoscopic examination was conducted on all control and high-dose rats of the oncogenicity and toxicity study at week 102.

Body weight
Individual body weights were recorded for each animal before dosing, at weekly intervals until the end of week 13 and approximately every 4 weeks thereafter until termination.

Food and water consumption and compound intake
Food consumption was recorded once weekly for each cage group starting one week before treatment until Week 13 and subsequently every 4 weeks until termination. Water consumption was monitored by visual inspection throughout the study period.
Achieved dosages were calculated from nominal dietary concentration, taking into account actual food consumption and body weight data.

Haematology and clinical chemistry
Individual blood samples for haematology and clinical chemistry evaluations were collected from the orbital sinus of 10 rats/sex of each study group of the toxicity study after approximately 14, 25, 51, 78 and 102 weeks. Samples were taken where possible, on the same animals at each time point. Individual blood smears for differential blood counts were taken from the tail vein after approximately 52, 78, and 103 weeks of dosing from all surviving animals of the oncogenicity study.

Haematology
The following parameters were measured: Haemoglobin, haematocrit, total erythrocyte count total leukocyte count, differential leukocyte count, platelets, and clotting time. Absolute indices were calculated.
Differential blood counts were evaluated with blood smear samples from all control and high-dose animals of the oncogenicity study at weeks 53 and 79. In addition, differential blood cell counts were evaluated on all surviving animals of the oncogenicity study at week 104.

Blood chemistry
The following parameters were measured: Total proteins, albumin, albumin-globulin ratio, ALT, AST, ALP, blood urea nitrogen, blood glucose, sodium, potassium, chloride,

cholesterol, creatinine, calcium, phosphate, total bilirubin, plasma cholinesterase, creatinie phosphokinase and red blood cell cholinesterase.

Brain cholinesterase activity determination
Brain cholinesterase activity was determined from 10 rats per sex from each dose group at the week 52 and 104 necropsies. Approximately 0.5 g of brain was removed at the week 52 and 104 necropsies and stored at -20°C until analysis.

Urinalysis
Individual urine samples were collected from 10 rats/sex of each study group of the toxicity study after approximately 14, 25, 51, 78 and 102 weeks. Samples were taken where possible, on the same animals at each time point. Samples were collected over a period of 4 hours of food and water deprivation in metabolism cages. The following measurements were made: volume, specific gravity, pH, urobilinogen, bilirubin, blood pigments, protein, glucose, ketones, microscopy of sediments.

Sacrifice and pathology
At interim kill after 52 weeks 15 rats per sex from each toxicity study group were sacrificed and necropsied. All remaining toxicity study and surviving oncogenicity study animals were killed and necropsied after 104 weeks. All pre-terminally dead and moribund sacrificed rats were also necropsied.
The following organs were weighed from all interim kill animals of the toxicity study and from 10 rats per sex per group of the oncogenicicty study: adrenals, brain, heart, kidneys, liver, lungs, ovaries (with fallopian tubes), parotid salivary glands, pituitary, prostrate, sublingual and submaxillary salivary glands (weighed together), spleen, testes including epididymides, thymus and uterus.
The following organs were collected: adrenals, aortic arch, any abnormal tissue, bladder, bone and bone marrow (sternum and rib), brain, ears, eyes, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidneys, liver, lungs, mammary gland, lymph nodes (mesenteric and submandibular), muscle (thigh), nasal cavity (oncogenicity study only), oesophagus, optic nerve, ovaries (with fallopian tubes), pancreas, parotid salivary glands, pituitary, prostrate, sciatic nerve, seminal vesicles, skin, spinal cord (cervical, thoracic and lumbar), spleen, stomach (glandular and non-glandular), sublingual salivary glands, submaxillary salivary glands, testes with epididymes, thymus, thyroid/parathyroid, tongue, trachea, uterus and vagina.
A detailed histopathological examination was performed on all tissues collected from the control and high-dose animals at interim kill, all oncogenicity study animals, and all animals that died or were killed in extremis. In addition, a histopathological examination of the liver, kidneys and lungs was performed on all other toxicity study animals at interim kill and all oncogenicicty study animals. Furthermore, the salivary glands of all low- and mid-dose animals at interim kill and the oncogenicity study were examined.

Statistics
Haematology, clinical chemistry, organ weight and body weight data were analysed for homogeneity of variance using the F-max test. If the group variances appeared homogeneous a parametric ANOVA was used and pair wise comparisons made via Student's t-test using Fisher´s F-protected LSD. If the variances were heterogeneous log or square root, transformations were used. If the variances remained heterogeneous a non-parametric test (e.g., Kruskal-Wallis ANOVA) was used. Organ weights were also analysed conditional on body weight (i.e., ANOVA). Differences in survival between the control and test substance

groups from the oncogenicity study were assessed graphically using Kaplan-Meier plots and tested formally using the Gehan-Wilcoxon test. Because no notable survival differences were evident, histological lesion incidences were analysed using Fisher Exact test.

Results and discussion

Analysis of dose formulations

Analyses for achieved concentrations showed that the diet preparations of all dose groups were within an acceptable degree of accuracy (± 10%).

Mortality

There were 336 pre-terminal deaths throughout the study. There was no evidence to suggest that any of these deaths were treatment related. There were also no significant treatment-related effects on the survival times in the oncogenicity study.

The numbers of pre-terminal deaths are summarised in Table B.6.5-33 below.

Table B.6.5-33: Cumulated mortalities after 104-week dietary exposure to glyphosate technical

	Dose group (mg/kg bw/day)*				
Sex	0	10	100	300	1000
Male	27/85	32/85	25/85	26/85	26/85
Female	42/85	41/85	42/85	40/85	35/85

*number of dead / total number

Clinical observations

The only notable clinical sign was pale faeces, from weeks 16-104, the majority or all the cages of animals (males and females) in the 300 and 1000 mg/kg /day dose groups had pale faeces. However, this clinical sign was not considered to be toxicologically significant. There were no other notable clinical signs considered to be treatment related.

Opthalmoscopy examinations demonstrated no inter-group differences.

Body weight

The high-dose group males and females had statistically significant reductions in body weight throughout the study. Reductions started at week one of dosing and were still apparent at week 104. The high-dose group males displayed the greatest reduction in body weights and body weight gains. The mean body weight gain data are summarised in Table B.6.5-34 below.

Table B.6.5-34: Body weight development (mean values) after 52 and 104-week dietary exposure to glyphosate technical – oncogenicity study

	Dose group (mg/kg bw/day)									
	0		**10**		**100**		**300**		**1000**	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Weight gain (g) (0-52 weeks)	514	265	498	285	523	270	500	274	450	243
% of control	--	--	97	108	102	102	97	103	88	92
Weight gain (g) 0-104 weeks	635	376	609	445	644	391	623	405	549	333
% of control	--	--	96	118	101	104	98	108	86	89

Food and water consumption and compound intake
There were no treatment-related effects on food and water consumption for either sex noted during the study.
The overall group mean achieved doses are summarised in Table B.6.5-35 below.

Table B.6.5-35: Group mean achieved dose levels – oncogenicity study

Dose group	**Nominal dose (mg/kg bw/day)**	**Mean achieved dose level (mg/kg bw/day)**		**Mean achieved dose level (% of nominal)**	
		Males	Females	Males	Females
1 (control)	0	--	--	--	--
2 (low)	10	10	10	100	100
3 (mid I)	100	101	103	101	103
4 (mid II)	300	306	311	102	104
5 (high)	1000	1007	1018	101	102

Over the entire study duration the mean achieved dosages in all groups were close to the nominal.

Haematology and clinical chemistry
Haematology
Haemoglobin, haematocrit and mean corpuscular haemoglobin were occasionally increased in 100 and 1000 mg/kg bw/day dose group males. Haemoglobin was also increased in males from the 300 mg/kg bw/day dose group and females from the 1000 mg/kg bw/day group. Females of the 1000 mg/kg bw/day dose group also had increased levels of mean corpuscular haemoglobin.
The haematological changes were not considered to be treatment related due to the lack of a clear dose–response relationship. In addition, the differences observed were rather small and no consistent trend became obvious throughout the study. In the absence of any histopathological change these small increases are not considered to be of toxicological significance (see Table B.6.5-36).

Clinical chemistry
Clinical chemistry measurements showed significant increased alkaline phosphatase levels in males at 1000 mg/kg bw/day and in females at 100, 300 and 1000 mg/kg bw/day. Although the increases were of small magnitude they were consistent and might be treatment-related. However, in the absence of any histopathological changes these small changes are not considered to be of toxicological significance (see Table B.6.5-37). All other changes in clinical chemistry parameters were not considered to be treatment-related.

Table B.6.5-36: Haematology findings (group mean values)

	Dose group (mg/kg bw/day)									
	0		10		100		300		1000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Haemoglobin (g/dL)										
Week 14/15	15.6	15.5	15.8	15.0*	16.2	15.0*	16.2	15.5	16.2	15.9
Week 25/26	15.3	15.2	15.5	14.9	16.1***	14.9	15.9*	15.4	16.4***	15.6
Week 51/52	15.3	14.7	15.5	14.6	15.9	14.5	15.4	14.7	15.6	15.3*
Week 78/79	15.1	14.1	14.3	13.8	15.7	14.4	14.6	14.4	15.4	15.1
Week 102/103	14.0	12.1	13.1	13.6	14.3	13.1	13.8	13.3	14.6	12.9
Haematocrit (L/L)										
Week 14/15	0.397	0.396	0.405	0.386	0.406	0.387	0.407	0.395	0.411	0.407
Week 25/26	0.388	0.392	0.389	0.389	0.409**	0.384	0.399	0.398	0.409**	0.403
Week 51/52	0.406	0.394	0.415	0.388	0.415	0.386	0.410	0.392	0.414	0.408
Week 78/79	0.405	0.382	0.386	0.375	0.415	0.382	0.392	0.387	0.411	0.406
Week 102/103	0.392	0.343	0.365	0.381	0.394	0.367	0.387	0.369	0.401	0.363
MCH (pg)										
Week 14/15	21.3	22.6	21.1	22.5	21.7	22.4	21.9	22.4	21.8	22.8
Week 25/26	21.2	22.4	21.4	22.4	21.9	22.4	21.9	22.2	22.0	22.8
Week 51/52	20.2	22.1	20.1	22.3	21.1*	22.1	20.8	22.2	20.9*	22.7
Week 78/79	20.1	22.3	19.7	22.4	20.8*	22.4	20.6	23.0	20.9*	23.1**
Week 102/103	20.4	22.3	20.1	22.3	20.1	22.0	20.9	22.6	20.6	22.7
WBC (x 10^{-9}/L)										
Week 14/15	14.0	12.0	14.5	13.3	13.4	12.0	13.7	11.1	14.2	12.0
Week 25/26	13.4	8.8	13.2	10.3	11.8	9.9	12.2	8.9	12.7	10.5
Week 51/52	12.8	7.9	13.7	9.1	11.7	7.7	12.9	7.4	12.4	8.8
Week 78/79	12.4	7.7	13.6	7.3	10.9	8.1	13.6	6.8	10.6	7.0
Week 102/103	10.5	10.1	12.2	7.1*	10.3	6.4**	11.6	7.3*	9.5	8.4
Lymphocytes (x 10^{-9}/L)										
Week 14/15	11.7	10.8	12.6	11.9	12.0	10.9	11.8	9.2	12.2	10.7
Week 25/26	10.7	7.1	10.8	8.2	9.6	8.1	10.1	7.4	10.3	8.6
Week 51/52	10.9	6.5	11.0	7.4	9.7	6.6	10.8	6.0	10.3	7.5
Week 78/79	10.0	5.7	10.3	5.6	8.7	6.4	10.1	4.8	8.5	5.6
Week 102/103	7.6	5.7	8.0	4.8	7.3	4.3**	7.8	4.7*	6.7	5.6

* p < 0.05; ** p < 0.01; *** p < 0.001

Table B.6.5-37: Clinical chemistry findings (group mean values)

	Dose group (mg/kg bw/day)									
	0		10		100		300		1000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
ALP (IU/L)										
Week 14	287	182	329	158	320	213	334	223	461***	244*
Week 25	251	148	272	152	267	201*	306	227**	367**	225**
Week 51	308	144	293	143	310	190*	353	195*	403	221**
Week 78	258	124	286	139	284	172	351*	207**	414***	186*
Week 102	212	190	265	161	287*	193	267	228	365***	286*

* p < 0.05; ** p < 0.01; *** p < 0.001

Urine analysis
Urinary pH was slightly reduced in males at 1000 mg/kg bw/day. This change was consistent with that found in a previously conducted 13-week toxicity study with glyphosate.

Necropsy
Gross pathology
There were no treatment-related macroscopic findings observed at the interim and terminal kill necropsies.

Organ weights
At the interim kill (week 52) absolute liver weights were reduced in males and females at doses of 100 mg/kg bw/day and above. However, for males this finding was not confirmed by the sensitive means of covariance analysis, i.e., with correction for final body weight. Absolute adrenal weights were reduced in males at 300 and 1000 mg/kg bw/day. However, this finding was also not confirmed by the sensitive means of covariance analysis, i.e., with correction for final body weight.
At the terminal kill (week 104) no statistical significant decrease in liver and adrenal weights was noted in any dose group. Absolute kidney weight was reduced in males at 100 and 1000 mg/kg bw/day after 104 weeks, but a clear dose relationship was lacking.

At 52 weeks parotid salivary gland weight was increased in males at 100, 300 and 1000 mg/kg bw/day. Combined sublingual and submaxillary gland weight was increased in high-dose males and females. However, salivary gland weights were not affected at week 104 at any dose level.

Histopathology
The most notable histological finding was seen in the salivary glands where cellular alteration was seen in submaxillary and parotid salivary glands in males and females at 300 and 1000 mg/kg bw/day at week 52, and in both sexes at 100, 300 and 1000 mg/kg bw/day at week 104. These changes followed a dose-related pattern and are considered to be treatment related; however, these cellular alterations are similar to those seen occasionally in other subchronic or long-term dietary studies and are considered an adaptive response due to oral irritation from the ingestion of glyphosate, an organic acid, in the diet and are of no adverse consequence.

Another histopathological finding was a decreased incidence of nephropathy in males at 100, 300 and 1000 mg/kg bw/day at interim kill. This finding was also noted in high-dose males at 104 weeks, but with reduced severity. Nephropathy is a common finding in old rats and as the incidence is decreased this finding is not considered as toxicologically significant.
In addition, the decreased incidence of urothelial hyperplasia in high-dose females at week 52 and 104, as well as in females at 300 mg/kg bw/day at week 104, is also not considered to be of toxicological significance.

Neoplastic changes
Neoplastic lesions were seen in all dose groups, however there was no dose relationship in the incidence of any individual tumour or in the incidence of animals with tumours.

It is concluded that the test compound at dose levels up to and including 1000 mg/kg bw/day produced no carcinogenic effect.

Conclusion by the Notifiers

Based on the study results and the lack of toxicological significance of the salivary gland findings, as well as a slight increase of plasma alkaline phosphatise observed at 300 mg/kg bw/day, the NOAEL in rats after chronic exposure to glyphosate technical for 104 weeks is considered to be 300 mg/kg bw/day. It is concluded that glyphosate technical is not carcinogenic in rats.

RMS comment:

The study is considered acceptable. However, the NOAEL is rather 100 mg/kg bw/day because histological alterations of the salivary glands were clearly more pronounced at the two upper dose levels and were accompanied by a clear increase in parotid gland weight after 1 year. The AP increase at these dose levels were in parallel to a liver weight at least after one year. Therefore, 300 mg/kg bw/day is considered the LOAEL. Effects at the NOAEL were still be seen but minor in nature. This evaluation is in line with the most recent one of WHO/FAO (JMPR, 2004, ASB2008-6266).

4th study: [redacted] 1996

Reference:	IIA, 5.5.2/01
Report:	[redacted] (1996) Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats [redacted] Data owner: ADAMA Agan Ltd Study No.: 886.C.C-R Date: 1996-07-18 GLP: not published, TOX9651587
Guidelines:	OECD 453 (1981)
Deviations:	Individual animals exceed the 20% range in body weight; organ weights were not determined for all animals; weights of heart, spleen and (para)thyroids are missing
GLP:	yes
Acceptability:	*See RMS comment*

Dates of experimental work: 1992-03-04 - 1994-03-04

Materials and methods

Test material:

Identification:	Glyphosate technical
Description:	White odourless crystals
Lot/Batch #:	60; 046
Purity:	96.8 %; 96.8 %
Stability of test compound:	More than two years at ambient temperature
Vehicle:	Diet

Test animals:

Species:	Rat
Strain:	Wistar
Source:	[redacted]
Age:	6 weeks
Sex:	Males and females
Weight at dosing:	Males: 90 – 179 g, females: 80 – 151 g
Acclimation period:	At least one week
Diet/Food:	Standard "Gold Mohur" (M/S Lipton India Ltd, India), *ad libitum*
Water:	Deep bore well water treated with charcoal filter and UV rays, *ad libitum*
Housing:	Initially in groups of five per sex in polypropylene cages and in groups of three from Week 12 onwards.
Environmental conditions:	Temperature: 19 -25 °C Humidity: 40 - 70% Air changes: not reported 12 hours light/dark cycle

In life dates: 1992-03-04 to 1994-03-04

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 50 Wistar rats per sex received daily dietary doses of 0, 100, 1000 and 10000 ppm (equivalent to mean achieved dose levels of 0, 7.4, 73.9 and 740.6 mg/kg bw/day for 24 months respectively) glyphosate technical. In additional one vehicle control with ten rats per sex and one high dose group with 20 rats per sex were included for interim sacrifice at the 12th month to study non-neoplastic histopathological changes.

Test diets were prepared fortnightly by mixing a known amount of the test substance with a small amount of basal diet. This pre-mix was then added to larger amount of basal diet and blended for further 20 minutes.
The stability of the test substance in food was determined in an in-house stability study at 2000 and 20000 ppm.

Observations
Veterinary examination was made before and after grouping and at the end of each month of experimental schedule. Rats were examined for toxic signs and pre-terminal deaths once a day. Ophthalmic examination was done at the start of the study and at termination.

Body weight
Individual body weights were recorded before dosing, at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from Week 1 to Week 13 and subsequently over one week in every 4 weeks until termination.

Haematology and clinical chemistry
Haematology
Individual blood samples were collected from 20 rats/sex/group at 3, 6, 12, 18 and 24 months. The following parameters were measured: Haemoglobin, haematocrit, erythrocyte count, clotting time and total leukocyte count and differential leukocyte count.

Blood chemistry
At the scheduled intervals of 6, 12, 18 and 24 months, blood collected from 10 rats/sex/group was subjected to clinical chemistry analysis. The following parameters were measured: Total proteins, albumin, ALT, AST, GGT, ALP, blood urea nitrogen and blood glucose.

Urinalysis
Individual urine samples were collected from 10 rats/sex/group at 3, 6, 12, 18 and 24 months. The following measurements were made: Volume, appearance, pH, nitrite, urobilinogen, bilirubin, erythrocytes, protein, glucose, ketones, microscopy of sediments.

Sacrifice and pathology
Histopathological examination was carried out on all tissues collected at interim sacrifice, control and high dose groups; all pre-terminally dead and moribund sacrificed rats of the low and mid dose groups and on all lesions of the terminally sacrificed rats from the low and mid dose groups.

The following organ weights were determined from 10 rats per sex per group: adrenals, brain, gonads, kidneys and liver.
Tissue samples were taken from the following organs: adrenals, aorta (main group animals), bone & bone marrow (sternum and femur incl. joint), brain, caecum, colon, duodenum, epididymides (main group animals), eyes (with optic nerve), heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, lymph nodes (mesenteric, mandibular and mediastinal), muscle (femoral), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles and coagulating glands, skin, spinal cord (cervical, thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, tumour/mass, urinary bladder and uterus.
A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed in extremis. In addition, gross lesions and masses from low and intermediate dose groups at termination were examined microscopically.

Statistics
Using specific computer programs, body weight, net body weight gain, food consumption, haematology, clinical chemistry and organ weight data of different groups were compared by Bartlett's test for homogeneity of intra group variances. When the variances proved to be heterogeneous, the data were transformed using appropriate transformation. The data with homogeneous intra group variances were subjected to one-way analysis of variance (ANOVA - Snedecor and Cochran). When 'F' value was significant, Dunnett's pair wise comparison (Scheffe) of means of treated groups with control mean was done individually.
Net food intake (g/kg bw/d) and test compound intake (mg/kg bw/d) was calculated for the whole study period using calculated means and food intake was statistically analysed by the procedure given above. Incidence of gross, histopathological changes of mass(es) and incidence of benign and malignant neoplasia in the treatment groups were statistically compared with control group by Z-test wherever it was applicable/necessary. The incidence of

neoplasms was analysed by Cochran-Armitage linear trend test, Life table analysis for fatal tumour incidence and Peto's incidental tumour analysis.
When a significant difference to the control was observed in any of the treatment groups, the dose correlation co-efficient was estimated and subjected to t' test.

Results and discussion
Analysis of dose formulations
Analyses for achieved concentrations showed that the diet preparations of the control, low, mid- and high dose group were within an acceptable range. The mean achieved concentrations of the test substance of eight batches of the prepared test substance diets were 0.0, 99.1 ± 4.7, 995.3 ± 36.8 and 9993.1 ± 277.5 ppm, for the control, low, mid and high dose group, respectively.

Mortality
There were no treatment-related deaths observed during the study.
The numbers of pre-terminal deaths in the carcinogenicity study groups are displayed in Table B.6.5-38.

Table B.6.5-38: Cumulated mortalities after 104-week dietary exposure to glyphosate technical

	Dose group (ppm)			
Sex	0	100	1000	10000
Male	30	30	32	21
Female	26	24	17	29

Clinical observations:
There were no treatment-related clinical signs of toxicity observed during the study.

Body weight:
There were no treatment-related effects on male and female overall body weight gain during the conduct of study.

Food consumption and compound intake
There were no treatment-related effects on food consumption for either sex noted during the study. The group mean achieved doses are summarised below.

Table B.6.5-39: Group mean achieved dose levels in the main groups

Dose group	**Dietary concentration (ppm)**	**Mean achieved dose level (mg/kg bw/day)**		
		Males	Females	Overall mean
1 (control)	0			
2 (low)	100	6.3	8.6	7.4
3 (mid)	1000	59.4	88.5	73.9
4 (high)	10000	595.2	886.0	740.6

The results show a higher test material intake for females when compared to males for each dose level. The mean intake for each dose group is 7.4, 73.9 and 740.6 mg/kg bw/day for 100, 1000, and 10,000 ppm, respectively.

Haematology and clinical chemistry

The following significant dose related changes of the blood chemistry parameters were seen at the high dose:
decrease in GGT level at 12 months in male rats
decrease in Albumin level at 6 months in female rats
increase in AP (alkaline phosphatase) level at 6 months in female rats

No other dose or treatment related significant changes were observed in haematological, and biochemical parameters. These changes observed were only temporal and were not consistently seen at all sampling periods throughout the study. The dose related changes were also within the range of the historical control data and hence appear to be of no biological significance.

Table B.6.5-40: Statistically significant changes in blood chemistry

Parameters	**Timepoint**	**Dose group (ppm)**							
		0		100		1000		10000	
		♂	♀	♂	♀	♂	♀	♂	♀
Albumin (g/dL)	6 month	4.0	3.7	3.9	3.7	4.0	3.7	3.9	3.5*
Alkaline phosphatase (U/L)	6 month	213	133	251	146	227	153	185	235*
GGT (U/L)	12 month	8.3	5.8	8.3	7.7	8.4	6.3	5.1*	5.3

* $p < 0.05$;

Urinanalysis
There were no treatment-related findings.

Necropsy
Gross pathology
There were no treatment-related macroscopic findings observed during the study period.

Organ weights
There were no treatment-related findings observed in organ weights or relative organ weights.

Histopathology
None of the significant microscopic changes, both increased and decreased incidences (in liver, spleen, lymph nodes, adrenals, thymus, gonads, uterus, mammary gland) observed have shown dose relationship, hence appeared to be incidental and not related to the treatment with the test compound.

Neoplastic changes
The historical data on neoplasm incidence for the test species indicates that the incidences of various tumours observed in the present study are within the range. The types of tumours seen were also comparable to the historical records.
No statistically significant inter group difference between the control and low, mid and high dose treatment groups has been recorded in respect of the number of rats with neoplasms, number of malignant neoplasms and incidence of metastasis either sexwise or for combined sex.

Table B.6.5-41: Summary of neoplastic histopathological findings

	Dose group (ppm)							
	Males				**Females**			
	0	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**

Findings for dead and moribund sacrificed animals								
Cholangiocarcinoma	0/30	2/30	2/32	2/21	1/26	0/23	0/17	0/29
Hepatocellular adenoma	9/30	9/30	6/32	6/21	2/26	8/23	3/17	5/29
Hepatocellular carcinoma	12/30	12/30	9/32	5/21	4/26	4/23	2/17	5/29
Intrahepatic bile duct adenoma	1/30	1/30	0/32	0/21	0/26	0/23	0/17	0/29
Histiocytic sarcoma	2/30	0/30	2/32	1/21	1/26	0/23	0/17	0/29
Fibrosarcoma	0/30	1/30	0/32	0/21	0/26	0/23	0/17	0/29
Findings for animals sacrificed at termination								
Cholangiocarcinoma	1/20	1/20	0/16	1/29	0/24	0/25	0/32	0/21
Hepatocellular adenoma	15/20	13/20	4/20	15/20	16/24	10/25	16/32	8/21
Hepatocellular carcinoma	9/20	16/20	9/16	19/29	6/24	11/25	12/32	4/21
Intrahepatic bile duct adenoma	1/20	0/20	0/16	0/29	6/24	11/25	12/32	4/21
Histiocytic sarcoma	0/20	1/20	1/16	0/29	0/24	1/25	0/32	0/21
Benign mixed intra-hepatic bile duct adenoma	0/20	0/20	1/16	0/29	0/24	0/25	0/32	0/21

Incidentally, the number of benign tumours in the low and mid dose group males and combined sex was lower and higher in the mid dose group females. There was no dose-response relationship and the significances were considered incidental.
The different liver tumours observed in the dead and moribund sacrificed and terminally sacrificed rats included hepatocellular adenoma, intrahepatic bile duct adenomas, cholangiocarcinoma, hepatocellular carcinoma, histiocytic sarcoma, fibrosarcoma and lymphosarcoma. Of these, hepatocellular adenomas and carcinomas occurred more frequently, as often observed in ageing rats. The occurrence of these tumours appeared to be incidental and not compound-related as their frequency of occurrence was not dose dependent. No reasons could be ascribed for the decrease in the number of benign tumours in the low and mid dose group males and for combined sex and for an increase seen in the mid group dose females (see Table B.6.5-41).

From this, it is concluded that the test compound at the doses tested does not cause treatment or dose related gross and histopathological changes and it is not carcinogenic under the testing conditions.

Conclusion by the Notifiers
Based on the study results the NOAEL in rats after chronic exposure to glyphosate technical for 24 month is 595 mg/kg bw/day for males, and 886 mg/kg bw/day for females (740 mg/kg bw/day for combined). It is concluded that glyphosate technical is not carcinogenic in rats**.**

RMS comment:
The study is considered acceptable. However, it should be amended that AP activity was increased in female rats not only at 6-month sampling but also after 12 and 18 months achieving statistical significance for the two upper dose levels (unfortunately not shown in Table B.6.5-6 but in the EU DAR). Because this increase that was observed in other studies with glyphosate, too, was not accompanied by further liver findings, it is not considered adverse. However, there was a slight increase in cataract frequency with 3, 4, 2, and 7 males and 1, 4, 5, and 4 females being affected in the control and the treatment groups at study termination. Since a similar finding was reported by [redacted] (1990, TOX9300244), a treatment-related and adverse effect of glyphosate administration cannot be excluded. Accordingly, the NOAEL was set at 1000 ppm, i.e., ca 60 mg/kg bw/day.

The RMS is not aware of any further long-term studies with the active ingredient, apart from an earlier IBT study ([redacted] 1974, Z35230) on rats in which only low dose levels were employed with some minor liver effects occurring at the highest dose of 300 ppm after 2 years. This study was mentioned by [redacted] (1981, TOX2000-595) but was not submitted for EU evaluation.

B.6.5.2 Carcinogenicity in the mouse

The carcinogenicity studies by [redacted] (1983, TOX9552381) and by [redacted] (1993, TOX9552382) were reported in detail in the original DAR (1998, ASB2010-10302). They have been both re-evaluated by the RMS and were confirmed as acceptable. Core information, NOAELs/LOAELs and the outcome of re-evaluation are mentioned in Vol. 1 (2.6.5).
Two more long-term studies in mice had been submitted for previous EU evaluation ([redacted] 1988, TOX9551831; [redacted], 1982/1992, TOX9650154) but were considered not acceptable for reliable assessment of carcinogenicity. Thus, they were not taken into consideration for this re-evaluation. A brief description may be found in the original DAR (1998, ASB2010-10302).

In the following, descriptions of the new studies (*i.e.*, those not previously used for EU evaluation) are given. Reporting of these studies is based on the GTF dossier. Comments by the RMS and additional information, mainly historical control data from the performing laboratories but also from the literature, may be found below the individual studies. A summary view on carcinogenicity studies in the mouse is presented in Vol. 1 (2.6.5).

1st new long-term study in mice ([redacted] 2001)

Reference:	KIIA 5.5.3
Report:	[redacted] (2001) Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice [redacted] Data owner: ADAMA Agan Ltd Study No.: Toxi: 1559.CARCI-M Date: 2001-10-10 not published, ASB2012-11491
Guidelines:	OECD 451 (1981)
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: 1997-12-18 - 1999-06-29

Materials and methods

Test material:	Glyphosate technical
Identification:	Glyphosate
Description:	Solid white, odourless crystals
Lot/Batch #:	01/06/97
Purity:	> 95 % (w/w)
Stability of test compound:	Expiry: December 1999
Vehicle and/ or positive control:	Diet
Test animals:	
Species:	Mouse
Strain:	Swiss albino, HsdOla: MF1
Source:	[redacted]
Age:	6 weeks
Sex:	Males and females
Weight at dosing:	Males: 25 – 47 g, females: 21 – 26 g
Acclimation period:	5 days
Diet/Food:	Ssniff rat/mouse powder food maintenance meal – low in germs (M/s Ssniff Spezialdiäten, D-59494 Soest, Germany), *ad libitum*
Water:	Well water passed through activated charcoal filter and exposed to UV rays, *ad libitum*
Housing:	In groups of five per sex in polypropylene mouse cages with stainless steel top grill and staem sterilized clean paddy husk bedding.
Environmental conditions:	Temperature: 19 - 25 °C Humidity: 30 - 70 % Air changes: 12 - 15/hour 12 hours light/dark cycle

In life dates: 1997-12-23 to 1999-06-29

Animal assignment and treatment:
In a carcinogenicity feeding study groups of 50 Swiss albino mice per sex received daily dietary doses of 0, 100, 1000 and 10000 ppm (equivalent to mean achieved dose levels of 0, 14.5, 149.7 and 1453 mg/kg bw/day for males, and 0, 15.0, 151.2 and 1466.8 mg/kg bw/day for females) glyphosate technical in diet for 18 month. The dose levels were chosen based on results of a 50-day pre-study in mice. Test diets were prepared prior to start of treatment and then in intervals ranging from 10 to 23 days. Diets were prepared in quantities of 10, 12 or 15 kg. For preparation of 12 kg diet mixtures 1.2 g, 12 g and 120 g for the low-, mid- and high-dose group, respectively, of the test substance was mixed with approximately with 0.5 kg basal diet and blended for 3 minutes. This pre-mix was then mixed manually with approximately 0.5 kg food and then added in portions to the remaining bulk amount of food (approximately 11.0 kg) and blended in a stainless steel ribbon mixer for 20 minutes.
The homogeneity of the test material in diet was determined at beginning of treatment, and at 12 and 18 month. Analyses for achieved concentration were done at three and six month of

the study. The stability of glyphosate technical in the diet was determined prior to start of the study for the 100 and 10000 ppm dose levels.

Clinical observations
A detailed veterinary examination of all mice was done before and after grouping and monthly thereafter. A check for clinical signs of toxicity, appearance, behaviour, and neurological changes and mortality was made once daily on all mice. For mice with observed tumours a separate record was maintained with details of the tumour development.

Ophthalmological examination
Ophthalmological examinations were performed on all mice prior to start of treatment at 6, 12 and 18 month of the study. Mydriasis was induced before examination by adding 1% Tropicamide solution into the eyes. All other grossly visible eye findings were recorded also at the daily observations.

Body weight
Individual body weights were recorded on Day 1 (prior to treatment) and at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from week 1 to week 13 and subsequently at weeks 26, 39, 52, 65 and 68. Food efficiency and compound intake was calculated from the recorded food consumption data.

Haematology
Blood smear samples were collected at 9 month and at termination (18 month) from all surviving animals, and from mice that were killed in extremis. Differential white cell counts were performed on all blood smear samples.

Sacrifice and pathology
All animals that died or were killed in extremis during the conduct of the study, were necropsied immediately or preserved in 10% buffered neutral formalin until necropsy.
All surviving mice were sacrificed at scheduled termination. A gross pathological examination was performed on all mice. Any macroscopic findings were recorded.
The following organ weights were determined from 10 mice per sex per group: adrenals, kidneys, liver and gall bladder, ovaries, and testes.
Tissue samples were taken from each mice from the following organs and preserved in 10% buffered neutral formalin: adrenals, bone & bone marrow (sternum and femur (incl. joint)), brain (incl. cerebrum, cerebellum pons), caecum, colon, duodenum, epididymides, eyes (with optic nerve), heart, jejunum, kidneys, larynx, liver and gall bladder, lungs, lymph nodes (mandibular, mesenteric, and superficial inguinal), muscle (femoral), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles and coagulating glands, skin, spinal cord (cervical, mid-thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus and all lesions and tumours/masses.
A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed in extremis. In addition, tissues of gross lesions and masses from all mice were examined microscopically.

Statistics
Body weight, body weight gain, food consumption and differential leukocyte counts of different groups were compared by Bartlett´s test for homogeneity of intra group variances. Heterogeneous data were transformed using log transformation. Data with homogeneous intra group variances were subjected to one-way analysis of variance using ANOVA. When "F" values were significant, Dunnetts pair wise comparison of means of treated groups with control means was done individually.
Incidence of gross lesions and non-neoplastic histopathological changes and incidences of benign and malignant neoplasms in the test substance groups were statistically compared with control group by Z-test where necessary. The incidence of neoplasms was analysed by Cochran-Armitage linear trend test, Life table analysis for fatal tumour incidence and Peto´s incidental tumour analysis. When a significant difference over the control group was observed in any of the treatment groups, the dose correlation co-efficient was estimated and subjected to t-test. All analyses and comparisons were evaluated at the 5 % level and statistically significant differences ($p \leq 0.05$) were indicated

Results and discussion
Analysis of dose formulations
Stability analyses indicated that the dose preparations were stable for up to 30 days with a loss of 8.37 % at the 100 ppm level and 6.99 % at the 10000 ppm level, when stored at room temperature in PE bags inside stainless steel drums.
Analyses for homogeneity at the start and at 12 and 18 month of treatment indicated that the dose preparations were homogeneous. Analyses for achieved concentration demonstrated that the mean prepared dietary admixture concentrations were within ± 10% of the nominal concentration for all diet samples. The overall mean achieved concentrations were 94.0 ± 1.66, 949.5 ± 15.84 and 950.7 ± 142.28 as compared to the nominal concentrations of 100, 1000 and 10000 ppm, respectively.

Mortality
The cumulated pre-terminal deaths (including moribund sacrifice) are summarised in Table B.6.5-42 below.

Table B.6.5-42: Cumulated mortalities after 78-week dietary exposure to glyphosate technical

	Historical control#		**Dose group (ppm)****			
Sex	Min- max*	Mean ± SD	0	100	1000	10000
Male	11/50 – 27/50	18 ± 5	22 (6)	20 (6)	22 (8)	27 (8)
Female	12/50 – 20/50	16 ± 3	16 (7)	16 (7)	20 (2)	20 (3)
Combined sex	12/100 – 47/100	17 ± 4	38 (13)	36 (13)	42 (10)	47 (11)

Derived from the control groups of 9 studies performed in the timeframe embracing the study summarised here
* Number of dead animals / total number of animals
** Total number of animals per group = 50
() number of animals killed in extremis

The percentage of survival in each of the dose groups are summarised below.

Table B.6.5-43: Percentage survival at termination after 18-month dietary exposure to glyphosate technical

	Dose group (ppm)			
Sex	0	100	1000	10000
Male	56	60	56	46
Female	68	68	60	60
Combined	62	64	58	53

The survival percentage was slightly decreased at the high dose level but the decrease did not attain statistical significance.
As can be seen from the historical control data, the mortality in the high-dose group is still within the historical control range even though at the upper end.

Clinical observations
There were no significant treatment-related clinical signs of toxicity observed.

Body weight
There were no significant treatment-related effects on male and female body weight and overall body weight gain during the conduct of study.
In males, incidences of slightly decreased body weights in week 10 at 100 ppm and in months 7 and 8 at 1000 ppm were considered incidental, since no effects on body weights were observed in the high-dose group.
In females, decreased net body weight gain was observed in month 18 at 100 ppm only. Therefore, this finding was also considered as incidental.

Food consumption and compound intake
There were no treatment-related effects on food consumption for either sex noted during the study.
The observed slightly lower food consumption observed in males in week 1 at 100 ppm and in weeks 1 and 7 at 10000 ppm was considered incidental since the changes were minimal and the effects was not consistent during the remaining parts of the study period.
In females, lower food consumptions were observed in week 2 for all dose levels and in week 26 at 10000 ppm. Higher food consumption occurred in week 11 at 100 ppm and in weeks 3 and 4 at 10000 ppm. These findings were also considered incidental since the changes were minimal and food consumption during the remaining parts of the study was comparable with the control group.
The calculated mean daily test substance intake is summarised in Table B.6.5-44 below.

Table B.6.5-44: Group mean daily compound intake levels

Dose group	**Dietary concentration (ppm)**	**Mean daily test substance intake (mg/kg bw/day)***		
		Males	Females	Combined
1 (control)	0	0.0	0.0	0.0
2 (low)	100	14.5	15.0	14.7
3 (mid)	1000	149.7	151.2	150.5
4 (high)	10000	1453.8	1466.8	1460.3

* based on actual food intake and body weight data

Haematology
Differential leukocyte counts at 9 and 18 month
There were no significance treatment-related changes in the white blood cell counts for either sex at both 9 and 18 month. Slightly higher neutrophile counts and slightly lower lymphocyte counts in high dose males at 9 month were within the historical control ranges. The slightly higher eosinophil counts, higher neutrophil and monocyte counts, as well as slightly lower lymphocyte counts observed in high dose females at 18 month were comparable with historical control values and therefore considered incidental.

Differential leukocyte counts of moribund sacrificed mice
Although the differential leukocyte count data were not statistically analysed, they appeared to be within the range of biological variation.

Ophthalmological examination
There were no treatment-related findings observed at the ophthalmological examinations performed at 6, 12 and 18 month of treatment.

Gross pathology
There were no treatment-related macroscopic findings observed for any mice sacrificed at termination or mice that died or were killed in extremis during the study period.
In animals found dead or sacrificed moribund across control and all dose levels the incidence of enlargement of superficial inguinal lymph nodes and thymus in mid dose females and in the high dose for combined sexes was statistically significantly increased. These enlargements were associated with neoplasms of the hemolymphoreticular system. Other changes included enlargement of various lymph nodes, and thymus, both associated with neoplasms of the hemolymphoreticular system, enlargement of the spleen, associated with neoplasia and amyloidosis and increased extramedullary haematopoiesis. The low incidence of observed liver enlargements was associated with neoplasia and amyloidosis. However, none of these findings were dose-dependent.
In mice sacrificed at termination the following changes were observed: Kidney surface rough/uneven in high dose males, discoloration / enlargement of mesenteric lymph nodes in high dose females and discoloration in high dose combined sex, and enlargement of spleens in both sexes combined at the high dose were significantly higher than in control mice. Since none of these changes showed a dose-dependency, and the corresponding histopathological changes were not significantly higher in these groups, the findings were considered incidental.

Organ weights
There were no treatment-related findings observed in organ weights or relative organ weights.

Histopathology
There were no treatment-related histopathological findings observed in any dose group of either sex.

In mice found dead or sacrificed moribund during the study period the following significant histopathological changes were seen (Table B.6.5-45): Cystic glands of the stomach were significantly increased in high dose males and for both sexes combined. However, the incidence of these findings was similar to historical control data and did not show a dose dependency. Therefore, these finding was considered incidental. Increased haematopoiesis was seen in the bone (femur) of high dose males, mid- and high-dose animals combined sex. Cell debris in tubules of epididymides was increased in mid dose males and the incidence of

sub-capsular cell hyperplasia was increased in adrenals of low dose males. In addition, the incidence of kidney nephropathy in mid-dose females, as well as the incidence of lymphocyte infiltration of epididymides in mid dose males was significant decreased. All these findings were also observed at lower doses and/or were not dose dependent. Thus, these findings were also considered incidental. Furthermore, it is necessary to consider the frequency of this finding in animals surviving to scheduled termination.

In mice sacrificed at termination the following more frequent observed changes were observed (Table B.6.5-45):
Cystic glands of the stomach were significantly increased in low-, mid- and high-dose males but without a dose-response.
Degenerative heart changes were higher in high-dose males and females, and significant higher when sexes were combined. Since the incidences were similar or slightly higher than in the historical controls, and since no dose-dependency was observed, this finding is considered incidental.
In mandibular lymph nodes lymphoid hyperplasia was significantly increase in low and mid-dose males and combined sex, whereas the incidence was significantly lower in high dose females.
In addition, extramedullary haematopoiesis was significantly increased in these lymph nodes at the mid-dose level in combined sex. In spleen extramedullary haematopoiesis was significantly increased in females and combined sex at the low dose level. In the absence of any dose-relation these findings, as well as several not statistically significant changes considered incidental.

Table B.6.5-45: Summary of non-neoplastic histopathological findings for dead and moribund animals

	Dietary concentration of glyphosate (ppm)											
	Males				Females				Combined sex			
Finding	0	100	1000	10000	0	100	1000	10000	0	100	1000	10000
Number examined	22	20	22	27	16	16	20	20	38	36	42	47
Stomach												
Cystic glands (n)	8	8	9	16	1	4	5	6	9	12	14	22+
Kidney												
Nephropathy (n)	9	7	10	12	5	1	1	3	14	8	11	15
Bone (femur) Increased haematopoises (n)	1	1	8+	5	0	1	2	3	1	2	10+	8+
Epididymes Cell debris in tubules (n)	0	1	4	0	--	--	--	--	--	--	--	--
Epididymes Lymphocyte infiltration (n)	4	1	0	3	--	--	--	--	--	--	--	--
Heart Degenerative changes (n)	11	14	13	16	4	2	4	1	15	16	17	17
Adrenals sub-capsular cell hyperplasia (n)	3	8+	7	10	12	11	13	15	15	19	20	25
Mandibular LN extramedullary haematopoiesis (n)	3	2	5	3	1	1	1	2	4	3	6	5

n = number of animals affected; LN = lymph node
+ significantly increased; -- not examined/determined

The number of malignant lymphoma was slightly elevated in the high dose group compared to control. This tumour of the hemolymphoreticular system is one of the most common tumours of mice accounting for the highest percentage of spontaneous tumours in this species. Therefore, the observed tumour incidence is considered incidental and not treatment-related. In addition, the incidences of this tumour varied with sex and fate (i.e. pre-terminal and terminal deaths).

Table B.6.5-46: Summary of non-neoplastic and neoplastic histopathological findings at termination

	Dietary concentration of glyphosate (ppm)											
	Males				Females				Combined sex			
Finding	0	100	1000	10000	0	100	1000	10000	0	100	1000	10000
Stomach(N)	28	30	28	23	34	-	-	30	62	64	58	53
Cystic glands (n)	9	19+	22+	17+	22	-	-	19	31	-	-	36
Kidney(N)	28	6	4	23	34	2	1	30	62	64	58	53
Nephropathy (n)	7	4	3	6	5	2	0	2	12	6	3	8
Bone (femur) (N)	28	-	-	23	34	2	1	30	62	--	--	53
Increased haematopoises (n)	1	-	--	0	1	0	0	2				
Epididymes (N)	28	1	-	23	--	--	--	--	--	--	--	--
Lymphocyte infiltration (n)	0	0	--	1	--	--	--	--	--	--	--	--
Mandibular LN (N)	28	30	28	23	34	33	28	30	62	64	58	53
Extramedullary haematopoiesis (n)	5	7	9	9	3	9	7	4	8	16	16+	13
Heart (N)	28	2	--	23	34	--	-	30	62	--	--	53
Degenerative changes (n)	14	1	--	17	2	--	-	6	16	--	--	23+
Adrenals (N)	28	--	--	23	34	-	-	29	62	--	--	53
sub-capsular cell hyperplasia (n)	15	--	--	13	27	--	--	22	42	--	--	35
Hemolymphoreticular system (N)	28	30	28	23	34	34	30	30	62	64	58	53
malignant lymphoma (n)	1	3	3	6	9	10	6	13	10	13	9	19

N = number examined; n = number of animals affected; LN = lymph node
+ significantly increased; -- not examined/determined

Table B.6.5-47: Incidences of malignant lymphoma and comparison with historical control

			Dietary concentration of glyphosate (ppm)							
			Males				Females			
	♂	♀	0	100	1000	10000	0	100	1000	10000
Dead & moribund										
Number examined	75	77	22	20	22	27	16	16	20	20
Number affected	20	49	9	12	13	13	9	10	13	12
Percentage affected	26.7	63.6	41.0	60.0+	59.0+	48.0	56.0	63.0	65.0	60.0
Mean %	26	61.8	--	--	--	--	--	--	--	--
Range %	0-44	0-100	--	--	--	--	--	--	--	--
			--	--	--	--	--	--	--	--
Terminal sacrifice										
Number examined	175	175	28	3028	23	34	34	30	30	28
Number affected	26	50	1	3	3	6+	9	10	6	13
Percentage affected	14.9	28.9	3.6	10.0	10.7	26.1+	26.5	29.4	20.0	43.3+
Mean %	14.8	28.8	--	--	--	--	--	--	--	--
Range %	8-24	20-43	--	--	--	--	--	--	--	--
			--	--	--	--	--	--	--	--
All fates										
Number examined	250	250	50	50	50	50	50	50	50	50
Number affected	46	99	10	15	16	19+	18	20	19	25
Percentage affected	18.4	39.6	20.0	30.0	32.0	38.0+	36.0	40.0	38.0	50.0+
Mean %	18.4	41.6	--	--	--	--	--	--	--	--
Range %	6-30	14-58	--	--	--	--	--	--	--	--

+ significantly increased; -- not examined/determined

Conclusion by the Notifiers

Based on mortality at the upper limit of the historical control range, the NOAEL in mice after chronic exposure to Glyphosate technical for 18 month is conservatively set at 1000 ppm, corresponding to 149.7 mg/kg bw/day for males, 151.2 mg/kg bw/day for females, and 150.5 mg/kg bw/day for both sexes combined. It is concluded that Glyphosate is not carcinogenic in mice.

RMS comments

The study is considered acceptable and the NOAEL of 150 mg/kg bw/day is agreed but should be based on different considerations. In fact, mortality was slightly higher in males receiving the high dose (54% as compared to 44 % in the control) and in females which were fed the mid and high dose (40% in both vs. 32 % in the control) but these differences were not that convincing. As shown in Table B.6.5-42, mortality was ut the upper edge of the historical control range. Based on additional information that can be obtained from the original study (occurrence of first pre-terminal deaths in the individual groups, slopes for cumulative mortality curves, mean survival for the animals that were found dead pre-terminally or were sacrificed moribund, investigations on causes of deaths, so far performed), it is not likely that this slightly lower survival rate in these few groups was due to glyphosate feeding.

In contrast, two pathological findings should be given more attention.

There was a more frequent occurrence of cystic glands of the stomach in male mice that becomes more clear when the incidences in animals found dead or moribund and those sacrificed at scheduled termination are combined (Table B.6.5-48). Indeed, the difference between the treated groups is not large and, taking into account the large dose spacing, a clear dose response may be doubted but, according to the study author, the incidence was

higher than the historical control data. Unfortunately, this historical control data was not given. In contrast, no increase became apparent in females.

Table B.6.5-48: Stomach cystic glands in the study by Kumar (2001, ASB2012-11491) in Swiss albino mice, total incidence

Sex	***Males***				***Females***			
Dose	*0*	*100*	*1000*	*10000*	*0*	*100*	*1000*	*10000*
No. examined	*50*	*50*	*50*	*50*	*50*	*16*	*20*	*50*
Animals with cystic stomach glands	*17*	*27*	*31*	*33*	*23*	*4*	*5*	*25*

Based on this table and the statistical significance mentioned in Table B.6.5-47 for male animals, there was no NOEL in this study because it cannot be excluded that this finding was due to treatment. The clinical relevance of cystic glands of the stomach is not clear. In any case, there was no increase in severity (always minimal to mild) and, more important, the cysts formation did not progress to any other pathological lesion, even at a dose level that was 100 times higher than the lowest. Thus, this finding should not be taken into account when the NOAEL for this study is set.

As can be seen in Table B.6.5-46 and Table B.5.6-47, an increase in malignant lymphoma was noted in both the male and female groups receiving the highest dose. The incidence was statistically significantly elevated as compared to the actual control groups in this study, was above the mean values of the (relatively small) historical control and, for males, outside the historical control range. Even though malignant lymphoma is a common tumour in mice (accounting for 54.6% of all tumours in this study), it cannot be completely excluded that the higher incidence in the top dose groups were somehow related to treatment. The RMS conclusion is that there was limited evidence of a carcinogenic potential of glyphosate in this mouse strain at the very high dose level of 10000 ppm (about 1460 mg/kg bw/day for sexes combined) in this study, with male animals being more affected. The NOAEL should be set at the mid dose level of ca 150 mg/kg bw/day confirming a previous figure established by [redacted] (1983, TOX9552381) even though effects at higher dose levels were completely different.

The issue of malignant lymphoma was discussed in length on the PRAS 125 expert meeting held in February, 2015, in Parma. On request of this meeting, the RMS collected additional data from the literature to substantiate the claim of a high background incidence of this tumour type in mice in general and in particular in the Swiss mouse. In fact, a few articles could be identified and are briefly reported in the following. Even though some of them were rather old, they clearly demonstrate a frequent, however variable, occurrence of malignant lymphoma. For historical data on lymphoma incidence in CD-1 mice, see the RMS comments below the descriptions of the next two studies.

In an article going back to the 1960ies, Swiss mice were considered to be prone to the induction of malignant lymphoma by leukemogenic agents such as 7,12-dimethylbenz(α)anthracen (Toth et al., 1963, ASB2015-2536). In control animals which apparently survived for up to 70 – 80 weeks, the incidence of malignant lymphoma was 13.6% in males and 10.5% in females but the number of animals per group was low. In another,

similar experiment, the incidence in males was lower (5.5%) but, this time, accounted for 36.3% in females. This latter information may be considered the first published evidence of a remarkable sex difference in the frequency of this tumour type and a higher vulnerability of female mice as it was nearly consistently reported thereafter.
More than 10 years later, Sher (1974, Z22020) published a review on spontaneous tumour incidences in various non-inbred mouse strains, based on scientific articles that had been released between 1960 and 1974. For Swiss random-bred strains, lymphomas and leukemias were mentioned to occur as the most common tumours. However, again, extremely variable incidences ranging from 0 to 21.4% were reported in long term studies for untreated males, depending on strain and source. In female Swiss mice, the incidences varied even between 0 and 36.4%. The maximum incidence had been noted in minimally inbred Carworth CF-1 mice (not related to Swiss mouse strains) with 53% in females.
Roe and Tucker (1974, ASB2015-2534) reported an incidence of 22.5 to 27.5% of (not further specified) lymphoreticular neoplasms in male Swiss mice (n=80) if fed ad libitum but a much lower tumour rate when diet was restricted.
Tucker (1979, Z83266) found 18% of male Swiss albino mice (Alderley Part strain) and 28% of the females with lymphoma, nearly all of them malignant. Her analysis was based on 50 males and 50 females fed ad libitum from weaning for their lifespan with the last, very few surviving animals killed after 3 years.
A large colony of (minimally inbred)"Swiss-derived" Icr:Ha(ICR) mouse had a 15% incidence of lymphoma in total with an approximate 2:1 ratio between females and males (precise percentages not given). In addition, 5% of the mice had developed leukemia (Eaton et al., 1980, ASB2015-2537). Only lung tumours occurred more frequently (23%). With regard to Swiss mice in general, the authors emphasised that "... differences occur between colonies and even within a colony with the passage of time so that contradictory results may be obtained using 'Swiss' stock from different sources. For example, the incidence of spontaneous neoplasia, although seldom reported in detail, varies with source and age."
According to a more recent article (Taddesse-Heath et al., 2000, ASB2015-2535), a much higher incidence of hematopoietic neoplasia of 58% was observed in a colony of CFW Swiss mice in the USA. Lymphoma (mostly of B-cell origin) accounted for 85% of these cases giving a total incidence of nearly 50%. The authors ascribed these tumours mainly to "infectious expression of murine leukemia viruses". It is not known to which extent such a latent infection might have contributed to lymphoma incidences reported earlier or even in the studies described in this RAR. A possible etiologic role of oncogenic viruses had been suspected by Roe and Tucker (1974, ASB2015-2534) yet who complained that many scientists performing long-term studies would often ignore this problem.

2nd new long-term study in mice ([redacted] 2009)

Reference: IIA, 5.5.3/02
Report: [redacted] (2009b), Glyphosate technical: Dietary Carcinogenicity Study in the Mouse
[redacted]
SPL Project No.: 2060-0011
Data owner: Nufarm
Date: 2009-04-22
not published, ASB2012-11492
Guidelines: OECD 451 (1981), JMAFF guideline 2-1-15 (2005), US-EPA

	OPPTS 870.4200 (1996)
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: 2005-10-10 - 2007-11-19

Materials and methods

Test material:	Glyphosate technical
Identification:	Glyphosate
Description:	White crystalline solid
Lot/Batch #:	H05H016A
Purity:	95.7 %
Stability of test compound:	Expiry: 2008-03-25
Vehicle and/ or positive control:	Diet
Test animals:	
Species:	Mouse
Strain:	CD-1, Crl:CD-1 (ICR) BR
Source:	[redacted]
Age:	Approx. 5 – 6 weeks
Sex:	Males and females
Weight at dosing:	Males: 22 – 32 g, females: 18 – 28 g
Acclimation period:	At least ten days
Diet/Food:	Rat and Mouse SQC Ground diet No. 1, Special Diet Services Limited, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Initially in groups of three per sex in polypropylene solid-floor cages.
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 55 ± 15 % Air changes: at least 15/hour 12 hours light/dark cycle

In life dates: 2005-10-10 to 2007-11-19

Animal assignment and treatment:
In a carcinogenicity feeding study groups of 51 CD-1 mice per sex received daily dietary doses of 0, 500, 1500 and 5000 ppm (equivalent to mean achieved dose levels of 0, 84.7, 266.8 and 945.6 mg/kg bw/day) Glyphosate technical in diet. Additional 12 mice per sex, designated for veterinary controls, were housed and maintained alongside treated animals. Ten animals per sex from each group were set aside for an interim kill (toxicity assessment), which was carried out on the survivors after 39 weeks of dosing. The remaining 50 mice per sex and dose-level were dosed for a maximum of 79 weeks (carcinogenicity assessment).

Test diets were prepared prior to start of treatment and then weekly by mixing a known amount of the test substance with a small amount of basal diet and blending for 19 minutes. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes.
The stability and homogeneity of the test material in diet were determined. Samples of each dietary admixture were analysed for achieved concentration monthly for the first six months and then every three months thereafter.

Clinical observations
A check for clinical signs of toxicity, ill health and behavioural changes was made once daily on all mice and recorded weekly. Observations for morbidity, and mortality were made twice daily. Additional unscheduled examinations were performed on animals that showed ill-health.
All surviving animals were palpated weekly for size, position and appearance of new or existing masses.

Body weight
Individual body weights were recorded on Day 1 (prior to treatment) and at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination. Body weights were also determined before sacrifice. Body weight data were reported only until Week 77.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from Week 1 to Week 13 and subsequently over one week in every 4 weeks until termination. Food consumption data were reported only until Week 77. Food efficiency and compound intake was calculated from the recorded food consumption data.

Water consumption
Water intake was observed daily, for each cage group, by visual inspection of the water bottles for any overt changes.

Haematology
Blood smear samples were collected after 12 months and at termination from all animals, and from mice that were killed in extremis. Differential white cell counts were performed on all control and high-dose animals and on the animals killed in extremis.

Sacrifice and pathology
All animals that died or were killed in extremis during the conduct of the study, and all animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined from 10 mice per sex per group: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, spleen, and testes.
Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta (thoracic), bone & bone marrow (sternum and femur (incl. stifle joint)), brain (incl. cerebrum, cerebellum and pons), caecum, colon, duodenum, epididymides, eyes (with optic nerve), gross lesions incl. palpable masses, head (incl. pharynx, nasopharynx and paranasal sinuses), heart, Harderian and lacrimal glands, ileum, jejunum, kidneys, larynx, liver and gall bladder, lungs (with bronchi), mammary gland, lymph nodes (cervical and mesenteric), muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, preputial gland, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin (hind limb), spinal cord

(cervical, mid-thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus and vagina.
A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed in extremis. In addition, tissues of the liver, lungs and kidneys, as well as gross macroscopic lesions and palpable masses from low and intermediate dose groups at termination were examined microscopically.

Statistics
All data were summarised in tabular form and analysed by computerised analysis using ProvantisTM Tables and Statistics Module. For each variable the of variance incorporating Student´s t-test and F-test. For each variable the most suitable transformation of data was found, the use of possible covariates checked and the homogeneity of means assessed using ANOVA or ANOVA and Bartlett´s test. The lowest treatment-related significant effects were determined using the Williams Test for parametric data or the Shirley Test for non-parametric data. If no response is found, but the data showed non-homogeneity of means, data were further analysed by a stepwise Dunnet (parametric) or Steel (non-parametric) test to determine significant differences from control. If required, pair-wise tests are performed using Students t-test (parametric) or the Mann-Whitney U test (non-parametric)
The levels of probability chosen as significant were $p < 0.01$** and $p < 0.05$*.
Histopathology data were analysed using Chi squared analysis (differences in the incidence of lesions occurring with an overall frequency of 1 or greater) and the Kruskal-Wallis one-way non-parametric analysis of variance (comparison of severity grades).
The levels of probability chosen as significant were $p < 0.001$, $p < 0.01$, $p < 0.05$, and $p < 0.1$.

Results and discussion
Analysis of dose formulations
Analyses for homogeneity and stability indicated that the dose preparations were homogeneous and stable for at least six weeks. Analyses for achieved concentration demonstrated that the mean prepared dietary admixture concentrations were within ± 5 % of the nominal concentration for all but 1 sample (500 ppm –level), which was + 10 % of the nominal concentration.
The group mean achieved doses are summarised below.

Table B.6.5-49: Group mean achieved dose levels

Dose group	**Dietary concentration (ppm)**	**Achieved dose level (mg/kg bw/day)***				
		Males		Females		Overall mean
		Mean	Range	Mean	Range	
1 (control)	0					
2 (low)	500	71.4	33 – 104	97.9	55 – 155	84.7
3 (mid)	1500	234.2	101 – 365	299.5	176 – 466	266.8
4 (high)	5000	810	461 - 1143	1081.2	610 - 1728	945.6

* based on actual food intake and body weight data

The results show a higher test material intake for females when compared to males for each dose level. Highest intakes were achieved within the first few treatment weeks, with subsequent decline thereafter. The mean intake for each dose group (sexes combined) is therefore 84.7, 266.8 and 945.6 mg/kg bw/day for 500, 1500, and 5000 ppm, respectively.

Mortality

No treatment-related effects on the deaths occurred during the study, as well as no treatment-related effects on the time of death. From three male mice that were killed in extremis, examination results suggest that the morbidity of these animals was due to fighting between cage mates.

Table B.6.5-50: Cumulated mortalities after 78-week dietary exposure to Glyphosate technical

	Dose group (ppm)			
Sex	0	500	1500	5000
Male	12 (6)	10 (8)	12 (6)	16 (6)
Female	14 (10)	13 (7)	13 (10)	11 (8)

( ): number of animals killed in extremis

The percentage of survival in each of the dose groups are summarised below.

Table B.6.5-51: Percentage survival at termination after 78-week dietary exposure to glyphosate technical

	Dose group (ppm)			
Sex	0	500	1500	5000
Male	76	80	76	69
Female	73	75	75	78

Clinical observations
There were no significant treatment-related clinical signs of toxicity observed.

There were no trends in the proportion of palpable masses observed during the study period. A significant proportion observed showed evidence for regression before the animal reached the point of death or termination. Based on the results (see Table B.6.5-52) no treatment-related effect on the development of palpable masses is seen for either sex. The slight increase in the mean number of masses per animal for high-dose females and mid-dose males was considered a coincidence. The median time to appearance of palpable masses was comparable for all dose groups of either sex.

Table B.6.5-52: Group summary of palpable masses

Dose	**Total number of animals in group**		**Number of animals with palpable masses**		**Total number of masses per group**		**Mean number of masses per animal**		**Median time (weeks) to appearance of masses**	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
0	51	51	28	23	45	38	0.88	0.75	42.00	45.75
500	51	51	32	28	49	49	0.96	0.96	42.00	46.08
1500	51	51	39	23	60	38	1.20	0.75	42.43	44.83
5000	51	51	25	23	49	51	0.96	1.00	41.67	42.50

Body weight
There were no treatment-related effects on male and female overall body weight gain during the conduct of study.

Food consumption and compound intake

There were no treatment-related effects on food consumption for either sex noted during the study.

Water consumption
There were no treatment-related effects on water consumption for either sex noted during the study.

Haematology
There were no significance differences in the proportions of white blood cell counts for either sex at both 12 and 18 month.

Necropsy
Gross pathology
There were no treatment-related macroscopic findings observed for any mice sacrificed at termination or mice that died or were killed in extremis during the study period.

Organ weights
There were no treatment-related findings observed in organ weights or relative organ weights.

Histopathology
There were no treatment-related histopathological findings observed in any dose group of either sex.

Conclusion by the Notifiers
Based on the study results the NOEL and NOAEL in mice after chronic exposure to Glyphosate technical for 18 month is 810 mg/kg bw/day for males, and 1081 mg/kg bw/day for females. It is concluded that Glyphosate technical is not carcinogenic in mice.

RMS comments
The study is considered acceptable and setting of the NOAEL at the highest dose level of 5000 ppm (eqivalent to 810 mg/kg bw/day in males and 1081 mg/kg bw/day in females) is supported. Indeed, there was no evidence for carcinogenicity up to this dose level and the very comprehensive ranges of tissues that were examined histologically does not suggest an increase in any non-neoplastic pathological lesion. In an amendment to the study report ([redacted] 2011, ASB2014-9149) it was clarified that there was also no increase in (bilateral) testicular atrophy between the control and the high dose group, correcting a misleading statement in the original report. As further confirmed again by [redacted] (2011, ASB2014-9150) in a response to a "question" (not mentioned, by whom it was raised) the latter one was an artefact due to incorrect data management. Apparently, there had been no appropriate differentiation between the two testes of the animals when effects were reported.
Survival and growth of the animals were not affected. However, the dose levels choosen, although sufficiently high for a study of this type, were much lower than in other long-term studies with glyphosate in mice.
It was noted that histological examination of salivary glands covered submaxillary, sublingual and parotid glands. However, no lesions similar to those found by [redacted] (1992, TOX9551954, see B.6.3.2) in another mouse strain following administration of glyphosate ober 90 days at higher doses were reported.
~~*There was no increase in malignant lymphoma.*~~

There was a weak increase in malignant lymphoma incidence in male mice at the top dose level. The actual numbers of affected animals were 0, 1, 2, and 5 in the control, low, mid and high dose groups (n=51 in each of them). In females, the respective figures were 11/51, 8/51, 10/51 and, again, 11/51. Thus, no evidence of any change in lymphoma frequency was seen in female mice in this study. Even in males, the difference was not statistically significant but a possible effect might be suspected and should be clarified because of the increase in malignant lymphoma in the study by [redacted] (2001, ASB2012-11491, "1st new study", see above) and because of a weakly higher incidence in the study by [redacted] (1997, ASB2012-11493, "3d new study", see below). On request of the RMS, the GTF submitted historical control data for malignant lymphoma from the performing laboratory ([redacted] 2015; ASB2015-2531) but, unfortunately, only after the PRAS 125 meeting that was held in February, 2015. Therefore, the following data was not subject to peer review by the regulatory agencies of the MS.

Nine long-term studies were included which had been conducted in the same mouse strain between 2000 and 2010. The study duration was 104 weeks and, thus, longer than in the study that was under evaluation here. In total, 768 control mice (sexes not distinguished) had been examined. Malignant lymphoma was found in 63 animals, i.e., in 8.2%. (In the submitted document, 12.63% was mentioned but this must be wrong if the whole number of animals under examination is taken into consideration.) In line with that figure, the mean study incidence for this tumour type was 7.51% with a standard deviation of 6.61 pointing to a large variation. In the individual studies, the lymphoma rates ranged from 0 to 32%. Based on this data, the incidences of malignant lymphoma in all groups in the study with glyphosate by [redacted] (2009, ASB2012-11492) were within the historical control and the incidence of slightly below 10% in top dose males (even if compared to 0% in the concurrent control) was of no concern. However, the quality and regulatory value of the historical control data is very much compromised by the fact that the sexes were not considered separately. Moreover, the data were apparently not all obtained from the same laboratory but, instead, also from other testing facilities of the Harlan group in Europe. At least, this information may be considered as indicative for the high variability in lymphoma incidence in the mouse strain used.

There are more sources to support, based on historical control data, remarkable differences in the occurrence of malignant lymphoma in CD-1 mice. According to information obtained from the "Registry of Industrial Toxicology Animal-data" (RITA) database (Fraunhofer ITEM Institute, Hannover, Germany; http://reni.item.fraunhofer.de/reni,) and made available to the RMS only very recently by the GTF, male CD-1 mice had a mean incidence of 3.4% (of 470 animals in total) in the control groups from nine 18-/19-month long-term studies performed between 1994 and 1998. In the individual studies, incidences ranged from 0 up to 12%. In female mice, the mean control incidence was much higher (16.9% in a total of 350 examined animals). In line with that, actual study incidences in female mice varied between 4 and 32% (Anonym, 2015, ASB2015-2532).
For the Crl:CD1 (ICR) mouse [i.e., the strain that was used by [redacted] (2009, ASB2012-11492), in their glyphosate study], Giknis and Clifford (2010, ASB2015-2529) reported data from a total of 13 (males) or 14 studies (females) with a duration between 78 and 104 weeks that had been performed between 2002 and 2006 by [redacted]. (Also this data was submitted by GTF following PRAS 125 meeting.) In males, malignant lymphoma was more rarely seen than in females since tumours of this type were found in the control groups in 8 out of 13 studies only with a minimum study incidence of 1/75 and a maximum one of 5/49 closely resembling that one at the top dose level of the [redacted] (2009, ASB2012-11492) study with glyphosate. In female CD-1 mice, malignant lymphoma was

observed in all but one of the 14 studies, even though with an extremely variable study incidence ranging from 2/60 up to 22/50.
Based on their retrospective analysis of 20 long-term studies for carcinogenicity (Huntingdon Life Sciences, U.K., 1990-2002) Son and Gopinath (2004, ASB2015-2533) described lymphoma as the most common tumour in young control CD-1 mice. This result was based on an analysis of premature deaths in these studies. In a total of 101 fatalities occurring up to week 50 of treatment in all these studies among male animals, lymphoma was found in 23 cases. In the 190 males which died between weeks 50 and 80 before scheduled termination, 36 were diagnosed with lymphoma. Among females, there were 68 premature deaths up to week 50 of which 19 had lymphoma suggesting a slightly higher rate than in males (28% vs. 23%). Between weeks 50 and 80, there were 211 deaths and, among them, 61 with lymphoma (ca 29% vs. 19% in males). It was noted that lymphoma incidence in the Huntingdon colony was similar in females as in the ICR mouse (Giknes and Clifford, 2010, ASB2015-2529) or in CD-1 mice included in the RITA database (Anonym, 2015, ASB2015-2532) whereas a more frequent occurrence of this tumour type was noted in males. However, this might be due to a different focus of the analysis. In the RITA database and in the review from [redacted] all animals on study were considered. In contrast, Son and Gopinath (2004, ASB2015-2533) looked only at the premature deaths to which malignant lymphoma might have contributed to a rather large extent.

3d new long-term study in mice ([redacted] 1997)

Reference:	IIA, 5.5.3/03
Report:	[redacted] (1997) HR-001: 18-Month Oral Oncogenicity Study in Mice. [redacted] Data owner: Arysta LifeScience Study No.: [redacted] 94-0151 Date: 1997-06-18 Not published, ASB2012-11493
Guidelines:	Japan MAFF Guidelines 59 NohSan No.4200, 1985 U.S. EPA FIFRA Guidelines Subdivision F, 1984 OECD 451 (1981).
Deviations:	None
GLP:	yes
Acceptability:	*See RMS comment*

Dates of experimental work: 1995-02-21 to 1996-09-06

Materials and methods

Test material:	Glyphosate technical	
Identification:	HR-001	
Description:	Solid crystals	
Lot/Batch #:	T-941209	T-950308
Purity:	97.56 %	94.61 %
Stability of test compound:	Not mentioned in the report	

Vehicle and/ or positive control:	Diet
Test animals:	
Species:	Mouse
Strain:	SPF ICR (Crj:CD-1)
Source:	[redacted]
Age:	5 weeks
Sex:	Males and females
Weight at dosing:	Males: 15 – 25 g, females: 14 – 23 g
Acclimation period:	9 days in males; 7 days in females
Diet/Food:	Certified diet MF Mash (Oriental Yeast Co., Ltd.), *ad libitum*
Water:	Filtered and sterilized water, *ad libitum*
Housing:	In groups of four per sex in aluminium cages with wiremesh floors
Environmental conditions:	Temperature: 24 ± 2°C Humidity: 55 ± 15% Air changes: 15/hour 12 hours light/dark cycle

In life dates: 1995-02-21 to 1996-09-06

Animal assignment and treatment:
Groups of 50 males and 50 females Specific –Pathogen-Free (SPF) ICR (Crj : CD-1) mice received the test material by incorporating it into the basal diet at a level of 0, 1 600, 8 000 or 40 000 ppm for a period of 18 months.

Clinical observations
All animals were conducted a cage-side observation daily for clinical signs and their deaths during the study. In addition, a detailed examination including palpation of the body was performed at least once a week. Moribund animals showing marked delibility were euthanized by exsanguinations under deep ether anesthesia and necropsied when an unfavourable prognosis was predicted. Dead animals were taken from the cage as soon as possible after discovery o minimize the loss of tissues by cannibalism and necropsied.
Mortality was expressed as ratios of cumulative number of animals found dead or killed in extremis to effective number of animal group.

Body weight
Individual body weights were recorded weekly from week 1 to 13 and every 4 weeks from week 16 to 76. Body weights were also measured at week 78, at the end of treatment, and used for calculation of relative organ weights. Group mean body weights were calculated at each measurement.

Food consumption and compound intake
Food consumption by each cage was recorded for a period of 3 or 4 consecutive days weekly during the first 13 weeks and every 4 weeks from week 16 to 76. Food efficiency and compound intake was calculated from the recorded food consumption data.

Haematology
Blood smear samples were collected at week 52 and at termination (18 month) from all surviving animals, and from mice that were killed in extremis. Differential white cell counts were performed on all blood smear samples.

Sacrifice and pathology
All animals that died or were killed in extremis during the conduct of the study, were necropsied immediately.
All surviving mice were sacrificed at scheduled termination. A gross pathological examination was performed on all mice. Any macroscopic findings were recorded.
The following organ weights were determined from 10 mice per sex per group: brain, adrenals, kidneys, spleen, liver and gall bladder, ovaries, and testes.
Tissue samples were taken from each mice from the following organs and preserved in 10% buffered neutral formalin: brain, spinal cord, sciatic nerve, pituitary, thymus, thyroids with parathyroids, adrenals, spleen, bone with marrow, tibio-femoral joint, lymph nodes, heart, aorta, salivary glands, esophagus, stomach, liver with gallblader , pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, head, pharynx, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus, vagina, harderian glands, eyes, skeletal muscle, skin, mammary gland, all gross lesions.
A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed in extremis. In addition, tissues of gross lesions and masses from all mice were examined microscopically. The following tissues were examined: brain, spinal cord, sciatic nerve, pituitary, thymus, thyroids with parathyroids, adrenals, spleen, bone with marrow, tibio-femoral joint, lymph nodes, heart, aorta, salivary glands, esophagus, stomach, liver with gallblader , pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus, vagina, harderian glands, eyes, skeletal muscle, skin, mammary gland, all gross lesions

Statistics
Body weight, food consumption and organ weights were evaluated by Bartlett´s test for homogeneity of intra group variances. When group variances were homogenous, a parametric analysis of variance of a one way layout type was conducted to determine if any statistical differences exist among groups. When the analysis of variance was significant, Dunnett's or Scheffe's multiple comparison test was applied. When the group variance were heterogeneous, the data were evaluated by Kruskal-Wallis non-parametric analysis of variance. When significant Dunnett type mean rank test or Scheffe's type mean rank test was applied.
Mortality was assessed by a life table analysis.
Urinalysis were analyzed by Mann-Whitney's U test to compare data between the treatment groups and the controls.
Mann-Whitney's U test was used to analyze difference of the differential leukocyte counts between the high dose groups and the controls. For comparison of the data from all groups, Dunnett's and Scheffe's multiple comparison test was applied. The data from males killed in extremis during the treatment were examined by Mann-Whitney's U test.
Fisher's exact probability test was used to analyze the data of clinical signs and incidences of gross lesions at necropsy and histopathological lesions.

Results and discussion
Analysis of dose formulations

Stability analyses indicated that the dose preparations were stable for up to 30 days with a loss 8.37 % Homogeneity of the test substance in diet was analysed on the samples taken from the top, middle, and bottom portion of the mixer. The coefficient of variation for each test diet was within 5.2 % or less. The results indicated that homogeneity of the test substance in diet was satisfactory in each test diet.
In order to verify concentration of the test substance in test diets, every batch of test diet was analysed during the treatment period. Mean concentration of the test substance in test diet at a nominal level of 1 600, 8 000 or 40 000 ppm was 1 561± 86.7, 7 790±394.4 or 38 783±1 655.0 (mean + standard deviation) ppm, respectively. The values were within 97-98 % of the target concentrations and satisfied the acceptable limit of concentration for test substance.

Mortality
No significant differences were noted for mortality between the treated groups and the respective control of either sex. Cumulative mortality of each group of either sex is shown in the following table:

Table B.6.5-53: Final mortality at termination of treatment (%)

Dose group (ppm)	Male	Female
0	24/50 (48)	18/50 (36)
1 600	16/50 (32)	14/50 (28)
8 000	23/50 (46)	10/50 (20)
40 000	21/50 (42)	15/50 (30)

Clinical observations
Statistically significant changes in clinical signs observed in the treated groups of either sex are shown in the following table:

Table B.6.5-54: Statistically significant changes in clinical signs:

	Male				Female			
	Dose group (ppm)							
	0	1 600	8 000	40 000	0	1 600	8 000	40 000
Number of animals examined	50	50	50	50	50	50	50	50
Perinasal region : tactile hair loss	0	3	3	6*	5	13*	9	8
Anus : mass(es)	0	0	0	8**	0	0	0	0
Integument :								
Wound	22	16	20	6*	3	0	0	0
Erosion/Ulcer	9	5	12	8	16	4**	1**	2**
Swelling	16	6*	13	9	6	2	0	1
Mass(es)	15	13	13	10	13	11	9	4*
Pale-colored skin	2	3	6	10*	4	2	6	6
Hair loss	11	12	21*	12	22	23	18	14
Wetted fur	11	9	7	4*	1	1	1	1

* : p<0.05 ; **, p<0.01 (Fisher's exact probability test).

In the 40 000 ppm group, males showed increased incidences of tactile hair loss, pale-colored skin, and mass(es) of anus and decreases of wound and wetted fur. In females of this group decreased incidences were observed in ulcer/erosion and mass(es) of skin. Although, in addition to these signs, loose stool was observed in the cages of both sexes beginning at week 21 in males and 20 in females, the group housing failed to identify which animal excreted the loose stool.

In the 8 000 ppm group, males showed an increased incidence in hair loss of the skin and females represented decreases in ulcer/erosion and swelling of the skin.
In the 1 600 ppm group, males showed a decrease in swelling of the skin and females represented an increase in tactile hair loss as well as a decrease in ulcer/erosion of the skin.
None of the observed effects seems to be dose-related. Whatever the dose tested, females were more sensitive to erosion/ulcer of the integument than males.

Body weight
In the 40 000 ppm group, males and females showed retarded growth during the treatment manifesting significantly lowered weights at weeks 16 to 36 in males and at weeks 6 and thereafter in females compared to the respective control. At the end of treatment, mean average weights were 93 % and 86 % of the respective control in males and females, respectively.
In the 8 000 ppm group, females showed significantly decreased weights at week 6 and weeks 9 to 24 compared to the control and the final mean average weight was 92% of the control at the end of the treatment, while growth rate in males was comparable to the control.
In the 1 600 ppm group, males and females showed similar growth curves to the controls during the treatment period.
Effects on the body weight were more important in females than in males. These effects were durable in the 40 000 ppm female group whereas they were stopped at week 36 in the male group of the same treatment dose. Sporadic effects were observed in the 8000 ppm female group. No significant effects were seen in the 1600 ppm male and female groups.

Food consumption and compound intake
In the 40 000 ppm group, males showed significant depressions in food consumption at weeks 1 and 68, revealing an overall group mean food consumption at 94% of the control during the treatment period. Females in this group also showed significantly decreased food consumption at weeks 1, 4, 8, 12, 20, 28, 40, 48 and 68, revealing an overall group mean food consumption at 93% of the control during the treatment period.
In the 8 000 ppm group, females showed significantly lowered food consumption at weeks 28, 40, and 68 compared to the control manifesting an overall group mean food consumption at 96 % of the control. Whereas, food consumption in males was comparable to the control during the treatment period.
No statistically significant effects was observed in the 1600 ppm group either in males or females.
The food consumption depressions were more important in female than in males. They were not time-related
Overall average chemical intake in each treated group of either sex was calculated from food consumption and nominal concentration as shown in the following table:

Table B.6.5-55: Calculated test substance intake in mg/kg bw/day:

	Dose level (mg/kg bw/day)	
Dose level (ppm)	Male	Female
1 600	165.0	153.2
8 000	838.1	786.8
40 000	4348	4116

Haematology
Statistically significant changes in differential leucocyte counts observed in the treated group of either sex are shown in the following table.

Table B.6.5-56: Statistically significant changes in haematology parameters:

Parameter	Sex	Fate of animals [a]	Dose group (ppm)		
			1600	8000	40000
Lymphocytes	Males	Ke	ND[b]		↑ 172
	Females	tk			↑ [c]163
Neutrophil (segmented)	Males	ke	ND		↓ 81

Numbers in the above table show values in the treated groups when the corresponding value in the control group is 100.
a: ke, killed in extremis; tk, terminal kill
b: ND, not determined
c: Dunnett's or Scheffe's multiple comparison test
↓↑: Mann-Whitney's U test

In the 40000 ppm group, males killed *in extremis* during the treatment period showed an increase of lymphocytes in differential leukocyte counts and a decrease of neutrophil (segmented form). In females of this group, differential count of lymphocytes was significantly increased at week 78.

There were no significant differences in differential leukocyte counts at other intervals of examination in the 40000 ppm group of both sexes, males killed *in extremis* in the 8000 ppm group, and females at week 78 in the 8000 and 1600 ppm groups compared to the controls. No significant treatment-related effects were conceived in morphology of the leukocytes.

Necropsy
Gross pathology
Statistically significant changes in incidence of macroscopic lesions observed in the treated groups of either sex are shown in the following table.

Table B.6.5-57: Statistically significant changes in macroscopic lesions:

Sex	Male				Female			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
78tk (N=)	(26)	(34)	(27)	(29)	(32)	(36)	(40)	(35)
External appearance: Loss of tactile hair	0	0	1	5*	4	8	8	0*
Soiled fur on external genital region	9	7	2*	6	0	0	0	0
Spleen: Swelling	5	1*	4	2	7	2	3	3
Lung: Mass(es)	4	12	11*	9	8	6	18	8
Cecum: Distention	0	0	0	11**	0	0	0	16**
Kidney: Cyst(s)	4	4	2	0*	2	0	4	1
Uterus: Cyst(s)	-	-	-	-	6	2	2	0*
Skin: Loss of hair	1	4	7*	6	8	11	16	5
Ke/fd (N=)	(24)	(16)	(23)	(21)	(18)	(14)	(10)	(15)
Lymph nodes (mesenteric): Swelling	0	2	0	5*	1	2	1	4
Lymph nodes (others): Swelling	5	2	4	9	0	3	4*	4*
Kidney: Coarse surface	4	2	1	1	6	3	0*	4
Skin: Loss of hair	5	4	7	4	11	5	2*	4
Wound	6	2	3	0*	0	0	0	0
Ulcer/Erosion	6	3	4	6	5	3	0	0
All (N=)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
External appearance: Loss of tactile hair	0	0	1	6*	5	11	9	3

Sex	**Male**				**Female**			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
Lymph nodes (cervical): Swelling	5	3	6	9	12	6	4*	7
Lymph nodes (mesenteric): Swelling	0	2	0	6*	3	2	1	5
Spleen: Swelling	16	4**	12	14	17	8*	8*	10
Lung: Mass(es)	9	14	17	15	10	8	20*	11
Cecum: Distention	0	0	0	14**	0	0	0	18**
Anus: Anal prolapse	0	0	0	5*	0	0	0	0
Kidney: Pale in color	6	2	4	2	7	4	1*	4
Coarse surface	6	2	2	1	7	4	0**	5
Testis: Atrophy	5	2	5	0*	-	-	-	-
Uterus: Cyst(s)	-	-	-	-	6	2	2	0*
Eye: Opacity	1	1	5	2	5	1	0*	0*
Auricle: Partial amputation	6	2	1	0*	4	2	0	1
Skin: Loss of hair	6	8	14*	10	19	16	18	9*
Wound	9	3	3	1*	0	0	0	0
Ulcer/Erosion	7	4	9	6	8	3	1*	0**
Swelling	7	1*	3	1*	3	0	0	0

Tk: Terminal kill
Ke/fd: Killed in extremis or found dead
All: All animals examined
(N=): Number of animals examined
*, p<0.05 (Fisher's exact probability test); **, p<0.01

In the 40000 ppm group, males and females showed significant increases in icidence of distention of the cecum at terminal kill after 78 weeks of treatment. Significant increases in incidence of the lesion were also noted in all animals examined recording 28 % (14/50) in males and 36 % (18/50) in females. Distended cecum was filled with loose stool-like materials. In addition, males showed an increase in loss of tactile hair and a decrease of cyct(s) in the kidney in thosenecropsied at terminal kill, and an increase of swelling in the lymph nodes (mesenteric) and a decrease of wound in the skin in those killed in extremis or foud dead during the treatment period when compared to the controls. Among these, significant differences in incidence were also noted in all animals examined for increases in loss of tactile hair and swelling of the lymph nodes (mesenteric) and a decrease in wound in the skin. Moreover, significant differences in incidence were also noted in all animals examined for an increase in anal prolapse of the anus and decreases in atrophy of the testis, partial amputation of the auricle, and swelling of the skin. Females showed decreases in loss of tactile hair and cyst(s) of the uterus in those necropsied at terminal kill, and an increase in swelling of the lymph nodes (others) and a decrease in ulcer/erosion of the skin in those killed in extremis or found dead during the treatment period. Among these, significant differences in incidence were noted in all animals examined for decreases in cyst(s) of the uterus and ulcer/erosion of the skin. Moreover, significant differences in incidence were also noted in all animals examined for decreases in opacity of the eye and loss of hair of the skin.

In the 8000 ppm group, males showed increases in mass(es) of the lung and loss of hair of skin and a decrease in soiled fur on external genital region in those necropsied at terminal kill when compared to the control. An increased incidence was also noted in all animals examined for loss of hair of the skin. Females killed in extremis or found dead during the treatment period in this group showed an increase in swelling of the lymph nodes (others) and decreases in coarse surface of the kidney and loss of hair of the skin. Moreover, significant differences in incidence were noted in all animals for an increase in mass(es) of the lung and decreases in

swelling of the lymph nodes (cervical) and spleen, pale in color and coarse surface of the kidney, opacity of the eye, and ulcer/erosion of the skin.

In the 1600 ppm group, males showed decreased incidences in swelling of the spleen in those necropsied at terminal kill and in all animals examined and in swelling of the skin in all animals examined, while females disclosed a decreased incidence in swelling of the spleen in all animals examined.

Organ weights
In the 40000 ppm group, males and females showed significant increases in absolute and relative weights of the cecum. The percentages of the values to those of the respective control were 173 % in males and 187% in females for absolute weight, respectively, and 174 % and 212 % for relative weight, respectively. In females, relative weight of the kidney was also increased significantly at a level of 111 % of the control.

Histopathology
Neoplastic lesions
The table below shows neoplastic lesions in the treated groups of aither sex with statistically significant differences in incidence from those of the controls.

Table B.6.5-58: Statistically significant changes in histopathology findings:

Sex	**Male**				**Female**			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
78tk (N=)	*(26)*	*(34)*	*(27)*	*(29)*	*(32)*	*(36)*	*(40)*	*(35)*
Hematopoietic & Lymphatic system: General: Malignant lymphoma	0	0	1	5*	4	8	8	0*

Tk: Terminal kill
(N=): Number of animals examined
*, p<0.05 (Fisher's exact probability test)

As to neoplatic lesions, the incidence of malignant lymphoma was significantly decreased in females of the 1600 ppm group necropsied at terminal kill compared to the control. Neither increases in incidence nor nearly occurrences compared to the controls were noted for neoplatic lesions in the treated groups of both sexes.

Non-neoplatic lesions
Statistically significant changes in incidence of non-neoplastic lesions observed in the treated groups of either sex are shown in the following table.
Statistically significant changes in incidence of non-neoplastic lesions:

Table B.6.5-59: Statistically significant changes in non-neoplastic lesions:

Sex	**Male**				**Female**			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
78tk (N=)	*(26)*	*(34)*	*(27)*	*(29)*	*(32)*	*(36)*	*(40)*	*(35)*
Spleen: Increased extramedullary hematopoiesis	5	2	4	3	6	5	1*	4
Liver: Micro-granuloma	1	5	5	4	15	16	14	7*
Kidney: Cortical cyct(s)	9	6	9	0*	2	1	5	0
Tibio-femoral joint: Proliferation of cartilageous tissue	14	17	11	15	18	14	11*	15
Ke/fd (N=)	(24)	(16)	(23)	(21)	(18)	(14)	(10)	(15)

Sex	**Male**				**Female**			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
78tk (N=)	*(26)*	*(34)*	*(27)*	*(29)*	*(32)*	*(36)*	*(40)*	*(35)*
Bone marrow (femur): Increased hematopoiesis	6	3	7	6	7	1*	1	2
Lymph nodes (cervical): Plasma cell hyperplasia	6	1	5	4	5	3	0	0*
Spleen: Amyloid deposition	2	3	2	0	8	3	0*	1*
Small intestine: Amyloid deposition	1	1	1	0	5	0*	0	2
Liver: Amyloid deposition	3	3	2	0	10	3	0**	1**
Thyroid: Amyloid deposition	2	2	2	0	8	1*	0*	2
Parathyroid: Amyloid deposition	1	1	2	0	7	1	0*	2
Skin: Wound	9	5	9	4	9	5	1*	3
All (N=)	*(50)*	*(50)*	*(50)*	*(50)*	*(50)*	*(50)*	*(50)*	*(50)*
Bone marrow (femur): Increased hematopoiesis	9	3	10	10	9	2*	2*	2*
Bone marrow (sternum): Increased hematopoiesis	9	3	9	10	9	3	2*	2*
Bone marrow (Vertebra): Increased hematopoiesis	9	3	10	10	9	3	2*	2*
Lymph nodes (cervical): Plasma cell hyperplasia	6	2	8	5	8	3	1*	0*
Lymph nodes (mesenteric): Myeloid cell aggregation	5	0*	3	2	4	1	1	1
Spleen: Increased extramedullary hematopoiesis	20	7*	14	14	13	10	5*	9
Amyloid deposition	3	3	4	0	10	3*	0**	1**
Lung: Alveolar epithelial cell hyperplasia	0	5*	1	1	3	4	5	5
Small intestine: Amyloid deposition	2	1	1	0	8	0**	0**	3
Liver: Micro-granuloma	1	6	5	5	16	16	14	7*
Amyloid deposition	5	3	4	0*	12	3*	0**	1**
Kidney: Cortical cyct(s)	10	8	13	2*	5	1	5	0*
Glomerular amyloidosis	1	1	2	0	7	2	0**	2
Uterus: Amyloid deposition	-	-	-	-	6	0*	0*	1
Thyroid: Amyloid deposition	3	2	4	0	11	1**	0**	2**
Parathyroid[c]: Amyloid deposition	2	1	4	0	10	1**	0**	2*
Eye: Cataract	4	5	5	5	5	2	0*	2
Skin: Skin subacutaneous abscess	3	1	2	5	5	1	0*	1

Tk: Terminal kill
Ke/fd: Killed in extremis or found dead
All: All animals examined
(N=): Number of animals examined
*, $p<0.05$; **, $p<0.01$ (Fisher's exact probability test)
c: The number animals examined in the control, 1600, 8000 or 40000 ppm groups were 46, 48, 48 or 46 in males and 48, 48, 50 or 49 in females, repscetively.

In the 40000 ppm group, males showed significant decreases in incidence of amyloid deposition in the liver in all animals examined and cyst(s) in the kidney in those necropsied at terminal kill and in all animals examined, when compared to the control. In these males, erosion/ulcer in the anus was observed in a total of 8 animals including 6 cases killed in extremis or found dead during the treatment period and 2 cases necropsied at terminal kill. There was even a large abscess in one case. Among these, regressive hyperplasia of mucous eithelium of the large intestine was seen in 2 cases with severe lesions in the anus. However, as the histopathological examinations were carried out only on the anus which were observed macroscopic lesions, the incidence of erosion/ulcer in the anus was not assessed by a statistical method. In females of this group, statistical significant decreases in incidence were

noted in all animals examined as follows; increase hematopoiesis in bone marrow (femur, sternum and vertebra), plasma cell hyperplasia in the lymph nodes (cervical), cyst(s) in the kidney, micro-granuloma in the liver, and amyloid deposition in the spleen, liver, thyroid, and parathyroid. Among these, significant decreases in incidence were also noted for micro-granuloma in the liver in those necropsied at terminal kill and plasma cell hyperplasia in the lymph nodes (cervical) and amyloid deposition in the spleen and liver in those killed in extremis or found dead during the treatment period.

In the 8000 ppm group, although males did not show any non-neoplastic lesions with statistically significant differences in incidence from the control, females disclosed significant decreases in incidence of proliferation of cartilageous tissue in the tibio-femoral joint in those necropsied at terminal kill, wound in the skin in those killed in extremis or found dead during the treatment period, and subcutaneous abscess in the skin in all animals examined. In addition, significant decreases in incidence, when compared to the control, were observed in all animals examined as follows; increase hematopoiesis in bone marrow (femur, sternum and vertebra), plasma cell hyperplasia in the lymph nodes (cervical), extramedullary hematopoiesis in the spleen, amyloid deposition in the spleen, small intestine, liver, kidney (glomerular amyloidosis), uterus, thyroid, and parathyroid, and cataract in the eye. Among these, the incidences of extramedullary hematopoiesis in the spleen in those necropsied at terminal kill and amyloid deposition in the spleen, liver, thyroid, and parathyroid in those killed in extremis or found dead during the treatment period were also decreased significantly.

In the 1600 ppm group, males in all animals examined showed a significant increase in incidence of alveolar epithelial cell hyperplasia in the lung and decreases in incidence of myeloid cell aggregation in the lymph nodes (mesentery) and extramedullary hematopoiesis in the spleen. In females of this group, the incidences in all animals examined were decreased significantly in increased hematopoiesis in bone marrow (femur) and amyloid deposition in the spleen, small intestine, liver, uterus, thyroid, and parathyroid. Among these, significantly decreased incidences were also noted for increased hematopoiesis in bone marrow (femur) and amyloid deposition in the small intestine and thyroid in those killed in extremis or found dead during the treatment period

Conclusion by the Notifiers

Based on the results, no oncogenic potential was observed in glyphosate after treated to mice at a dietary level of as high as 40 000 ppm for a period of 18 months (78 weeks).

RMS comments

The study is considered acceptable. Based on the effects in female mice on food consumption and body weight gain at the mid dose level of 8000 ppm, the lowest dose of 1600 ppm (ca. 153 mg/kg bw/day) is considered the NOAEL in this study. In contrast, the masses in lung mentioned in the dossier were not dose-related and there was no convincing evidence of lymph node swelling.

Male mice appeared less vulnerable.

There was an apparent error with regard to carcinogenicity:

If compared to the original study report (Text table 6, p. 48), the figures given by GTF in Table B.6.5-60 (6.5-58 in the dossier) are wrong. The precise figures are as follows:

Table B.6.5-60: Incidence of malignant lymphoma at terminal sacrifice in the study by [redacted] (1997, ASB2012-11493), revised

Sex	***Male***				***Female***			
Dose group (ppm)	*0*	*1600*	*8000*	*40000*	*0*	*1600*	*8000*	*40000*
78 Tk (N=)	*(26)*	*(34)*	*(27)*	*(29)*	*(32)*	*(36)*	*(40)*	*(35)*
Hematopoietic & Lymphatic system:	~~*0*~~	~~*0*~~	~~*1*~~	~~*5**~~	~~*4*~~	~~*8*~~	~~*8*~~	~~*0**~~
General: Malignant lymphoma	*0*	*0*	*0*	*2*	*4*	*0**	*5*	*3*

Tk: Terminal kill
(N=): Number of animals examined
** p<0.05 (Fisher's exact probability test)*

If these figures are used, the paragraph that is written below in the original text becomes clear.

Total incidence of malignant lymphoma (including animals that were prematurely found dead or had to be killed in extremis) is given in the following Table B.6.5-61 that was introduced by the RMS.

Table B.6.5-61: Total incidence of malignant lymphoma in the study by [redacted] (1997)

Sex	***Male***				***Female***			
Dose group (ppm)	*0*	*1600*	*8000*	*40000*	*0*	*1600*	*8000*	*40000*
No. examined	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
Hematopoietic & Lymphatic system:								
General: Malignant lymphoma	*2*	*2*	*0*	*6*	*6*	*4*	*8*	*7*

The slight increase in high dose males was not statistically significant. ~~Unfortunately, no historical control data for malignant lymphoma from the performing laboratory was provided.~~ On request, the GTF submitted historical control data for malignant lymphoma from the performing laboratory (Kitazawa, 2013; ASB2014-9146). A total of 9 long-term studies (no information on actual duration provided) in the same mouse strain was covered that had been performed or at least terminated (perhaps commenced before) between 1993 and 1998, i.e., exactly the time in which the study under review was conducted. In male mice, the total incidence of malignant lymphoma in control groups varied considerably, ranging from ca 4 (actually 3.58) to ca 19 % (19.23). In fact, 8 of 9 studies had a control incidence below 12 % (6 % or lower) as observed now at the top dose level but, in principle, this incidence fell into the historical control range. Thus, the conclusion is that the higher incidence at the exaggerated dose level of 40,000 ppm as compared to the control group is a chance findings and cannot be used to support the assumption of a carcinogenic effects of glyphosate in mice that is based on the results of the study by [redacted] (2001, ASB2012-11491). In female control groups, malignant lymphoma incidence was between 8 and 27 % and, thus, the actual incidences in the control and treated groups were well covered.

Furthermore, it was noted that the study director was actually Mika Kinoshita. The report writer (Kayoko Sugimoto) was as a pathologist involved in histopathological examination.

B.6.5.3 Published data on carcinogenicity (released since 2000)

Epidemiology studies

A number of epidemiology studies over the last decade have focused on pesticide exposure and associated health outcomes. Publications vary in the specificity of their conclusions regarding pesticides in general, classes of pesticides and in some cases individual insecticides, herbicides or fungicides. While some of these publications specifically mention glyphosate, few draw tenable associations with any specific cancer outcome. Publications suggesting glyphosate is associated with any cancer outcome are discussed below.

An essential consideration in both, risk assessment and interpreting the relevance of toxicology data is exposure assessment. An inherent low level of confidence exists for epidemiological studies where tenuous links to exposure exist. Suggested associations between health outcomes and any possible causative agent are merely speculation if exposures are not identifiable. Pivotal to the understanding of glyphosate exposure are data published by Acquavella et al. (2004, ASB2012-11528; 2005, ASB2012-11530), which quantified human systemic glyphosate exposure levels in farmer applicators and their families. The geometric mean systemic dose for farmers applying glyphosate, some of whom applied glyphosate to areas up to 400 acres, was 0.0001 mg/kg/day, approximately 0.03 % of the EU glyphosate acceptable operator exosure Level (AOEL) according to EU Review Report 6511/VI/99-final (21 January 2008, ASB2009-4191). The highest systemic dose, skewed well above the geometric mean, was 0.004 mg/kg/day, which is 1.95 % EU glyphosate AOEL according to EU Review Report 6511/VI/99-final (21 January 2008, ASB2009-4191) and 1.3 % of the current EU glyphosate attapcable daily intake (ADI) according to EU Review Report 6511/VI/99-final (21 January 2008, ASB2009-4191). Even lower systemic doses were determined for spouses and children, 0.00004 mg/kg and 0.0008 mg/kg, respectively. Multiple carcinogenicity studies have since been conducted by numerous glyphosate registrants demonstrating NOAELs of at least ten-fold higher than the highest dose tested in the study driving the current EU ADI calculation.

The largest epidemiological study of pesticide exposure and health outcomes in the United States is the Agricultural Health Study (AHS), which included glyphosate. Dozens of publications have resulted from data generated in this study of approximately 57,000 enrolled farmer applicators. Blair et al. (2009, ASB2012-11566) provided an overview of cancer endpoints associated with different agricultural chemicals reported in earlier AHS publications. Glyphosate was not reported to be associated with leukemia, melanoma, or cancers of the prostate, lung, breast, colon or rectum. De Roos et al. (2005, ASB2012-11605) reported AHS data evaluating glyphosate use and multiple cancer endpoints; no association was noted for glyphosate with all cancers, including cancer of the lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate, melanoma, all lymphohematopoietic cancers, non-Hodgkin's lymphoma (NHL) and leukemia. In an earlier publication based on another data set, however, De Roos et al., (2003, ASB2012-11606) reported an association between NHL and glyphosate use. McDuffie et al. (2001, ASB2011-364) reported a non-significant positive association between self-reported glyphosate exposure and NHL in a Canadian study. Blair et al. (2009, ASB2012-11566) did not report an association between glyphosate use and NHL in the AHS data, but a "possible association" between glyphosate use and multiple myeloma was mentioned. The AHS publication reporting this refers to a "suggested association" between glyphosate use and multiple myeloma (De Roos et al., 2005, ASB2012-11605), yet it did not demonstrate significant increase in relative risk for multiple myeloma. Both De Roos papers will be discussed in more detail below. Interestingly, a subsequent AHS review paper for the President's Cancer Panel (Freeman, 2009, ASB2012-11623) specifically references De Roos (2005 ASB2012-11605) as providing no observed incidents of cancers of any type being associated with glyphosate.

Lee et al. (2005, ASB2012-11882) reported a glyphosate association with gliomas, with the odds ratio differing between self-respondents (OR = 0.4) and proxy respondents (OR = 3.1). The authors expressed concern that higher positive associations observed for proxy respondents with glyphosate and several other pesticides, and suggested perhaps more accurate reporting of proxies for cases, and underreporting by proxies for controls; proxy respondents were spouses in 62 % of cases versus 45 % of controls, lending to lower reported incidents in the control group.

Monge et al (2007, ASB2012-11909) investigated associations between parental pesticide exposures and childhood Leukaemia in Costa Rica. Results are not interpretable for glyphosate as exposure was estimated with "other pesticides", including paraquat, chlorothalanil and "others". No association was noted for paternal exposures, but elevated leukaemias were associated with maternal exposures to "other pesticides" during pregnancy. Similarly, glyphosate is captured under "other pesticides" being associated with NHL by Fritschi et al. (2005, ASB2012-11624) and therefore should not be interpreted as an association with glyphosate.

Some further epidemiologic studies are focused on an association between pesticide exposure and Non-Hodgkin`s Lymphoma (NHL). Hardell and Eriksson (1999, ASB2012-11838) investigated in a case-control study the incidence of NHL in relation to pesticide exposure in Sweden. 404 cases and 741 controls have been included. The authors discussed an increased risk for NHL especially for phenoxyacetic acids. Glyphosate was included in the uni-variate and multi-variate analyses. However, only 7 of 1145 subjects in the study gave exposure histories to this agent. The authors reported a moderately elevated odds ratio (OR) of 2.3 for Glyphosate. This OR was not statistically significant oand was based on only 4 "exposed" cases and 3 "exposed" controls. The major limitations of this study were: the reliance on reported pesticide use (not documented exposure) information, the small number of subjects who reported use of specific pesticides, the possibility of recall bias, the reliance on secondary sources (next-of-kin interviews) for approximately 43 % of the pesticide use information, and the dificulty in the controlling for potential confounding factors given the small number of exposed subjects.

A further study was submitted by Hardell et al. (2002, ASB2012-11839). This study pools data from the above mentioned publication by Hardell and Eriksson (1999, ASB2012-11838) with data from a previously submitted publication from Nordström, Hardell at al. (1998, TOX1999-687).

The authors found increased risks in an uni-variate analysis for subjects exposed to herbicides, insecticides, fungicides and impregnating agents. Among herbicides, significant associations were found for glyphosate and MCPA. However, in multi-variate analyses the only significantly increased risk was for a heterogenous category of other herbicides than above, not for glyphosate. No information is given about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use of prescribed drugs etc.). In all, the above mentioned limitations of the publication from Hardell and Eriksson (1999, ASB2012-11838) are also the limitions of the publication from Hardell et al. (2002, ASB2012-11839).

Fritschi et al. (2005, ASB2012-11624) submitted a case-control study with 694 cases of NHL and 694 controls in Australia. Substantial exposure to any pesticide was associated with an increase of NHL. However, no association between NHL and glyphosate can be made on basis of this study. No information was given about exposure duration, used glyphosate products, exposure duration and application rates. Therefore, the documentation is considered to be insufficient for assessment.

Eriksson et al. (2008, ASB2012-11614) reported a case-control study which included 910 cases of NHL and 1016 controls living in Sweden. The highest risk was calculated for MCPA. Glyphosate exposure was reported by 29 cases and 18 controls, and the corresponding odds ratio (OR) was 2.02. Results and reliability of the study are discussed below.
Alavanja et al. (2013, ASB2014-9174) reviewed studies on cancer burden among pesticide applicators and others due to pesticide exposure. In this article the epidemiological, molecular biology, and toxicological evidence emerging from recent literature assessing the link between specific pesticides and several cancers including prostate cancer, non-Hodgkin lymphoma, leukemia, multiple myeloma, an breast cancer were integrated. Glyphosate was reported to be the most commonly used in conventional pesticide active ingedient worldwide. The only association between the use of glyphosate and cancer burden described in this review was the result of Eriksson et al. (2008, ASB2012-11614) which was described above.

The following epidemiology publications report a lack of association between glyphosate and specific cancer types.

- Alavanja et al. (2003, ASB2012-11535) reported on prostate cancer associations with specific pesticide exposures in the AHS; glyphosate did not demonstrate a significant exposure-response association with prostate cancer.
- Multigner et al, (2008, ASB2012-11917) also reported a lack of association between glyphosate use and prostate cancer. This data appears to have also been reported by Ndong et al. (2009, ASB2012-11922).
- The lack of association between glyphosate use and prostate cancer was also supported recently in an epidemiology study of Farmers in British Columbia, Canada by Band et al. (2011, ASB2012-11555).
- Lee et al. (2004, ASB2012-11883) reported a lack of association between glyphosate use and stomach and esophageal adenocarcinomas.
- Carreon et al. (2005, ASB2012-11585) reported epidemiological data on gliomas and farm pesticide exposure in women; glyphosate had no association with gliomas.
- Engel et al. (2005, ASB2012-11613) reported AHS data on breast cancer incidence among farmers' wives, with no association between breast cancer and glyphosate.
- Flower et al (2004, ASB2012-11620) reported AHS data on parental use of specific pesticides*a* and subsequent childhood cancer risk among 17,280 children, with no association between childhood cancer and glyphosate.
- Andreotti et al. (2009, ASB2012-11544) reported AHS data where glyphosate was not associated with pancreatic cancer.
- Landgren et al. (2009, ASB2012-11875) reported AHS data on monoclonal gammopathy of undetermined significance (MGUS), showing no association with glyphosate use.
- Karunanayake et al. (2011, ASB2012-11865) reported a lack of association between glyphosate and Hodgkin's lymphoma.
- Pahwa et al. (2011, ASB2012-11987) reported a lack of association between glyphosate and multiple myeloma.
- Schinasi and Leon (2014, ASB2014-4819) published the results of epidemiologic research on the relationship between non-Hodgkin lymphoma (NHL) and occupational exposure to pesticides. Phenoxy herbicides, carbamate insecticides, organophosphorus insecticides and lindane were positively associated with NHL. However, no association between NHL an glyphosate was reported.

- Kachuri et al. (2013, ASB2014-8030) investigated the association between lifetime use of multiple pesticides and multiple myeloma in Canadian men. Excess risks of multiple myeloma were observed among men reported using at least one carbamate pesticide, one phenoxy herbicide and ≥ organochlorines. However, no excess risk was observed for glyphosate.
- Cocco et al. (2014, ASB2014-7523) investigated the role of occupational exposure to agrochemicals in the aetiology of lymphoma overall, B cell lymphoma and its most prevalent subtypes. No increased CLL risk in relation to glyphosate was evidenced.
- Alavanja and Bonner (2012, ASB2014-9173) reviewed studies on occupational pesticide exposure and cancer risk. Twenty one pesticides identified subsequent to the last IARC review showed significant exposure-response associations in studies of specific cancers. No significant association was observed for glyphosate.
- El-Zaemy and Heyworth (2012, ASB2014-9473) reported a case control study on the association between pesticide spray drift from agricultural pesticide application areas and breast cancer in Western Australia. The findings support the hypothesis that woman who ever noticed spray drift or who first noticed spray drift at a younger age had increased risk of breast cancer. However, it was not possible to examine whether the observed associations are the result of a particular class of pesticides.
- Pahwa et al. (2011, ASB2014-9625) investigated the putative associaton of specific pesticides with soft-tissue sarcoma (STS). A Canadian population-based case-control study conducted in six provinces was used on this analysis. The incidence of STS was associated with insecticides aldrin and diazinon after adjustment for other independent predictors. However, no statistically significant association between STS and exposure to glyphosate or other herbicides was observed.
- Koutros et al. (2011, ASB2014-9594) studied associations between pesticide and prostate cancer. No statistically significant positive association between pesticides and prostate cancer were observed. There was suggestive evidence on an increased risk (OR>1.0) with an increasing number of days of use of petroleum oil/petroleum distillate used as herbicide, terbufos, fonofos, phorate and methyl bromide. However, no increased risk (OR>1.0) was observed for glyphosate.

In summarizing AHS publications, Weichenthal et al. (2010, ASB2012-12048) noted that increased rates in the following cancers were not associated with glyphosate use; overall cancer incidence, lung cancer, pancreatic cancer, colon or rectal cancer, lymphohematopoietic cancers, leukemia, NHL, multiple myeloma, bladder cancer, prostate cancer, melanoma, kidney cancer, childhood cancer, oral cavity cancers, stomach cancer, esophagus cancer and thyroid cancer.

Mink et al. (2012, ASB2014-9617) submitted a comprehensive review of epidemiologic studies of glyphosate and cancer. To examine potential cancer risks in humans they reviewed the epidemiologic literature to evaluate whether exposure to glyphosate is associated causally with cancer risk in humans. They also reviewed relevant methodological and biomonitoring studies of glyphosate. The review found non consistent pattern of positive assciations indicating a causal relationship between total cancer (in adults or in children) or any site-specific cancer and exposure to glyphosate.

Animal studies

Just recently (*i.e.*, after submission of the GTF dossier), a two-year study in rats was published (Séralini *et al.*, 2012, ASB2012-15514). Its main objective was to show a possible impact of long-term feeding of genetically modified (and glyphosate treated) maize to rats but three of the test groups were administered a commercially available formulation (Roundup

GT Plus, apparently authorised at least in Belgium) containing 450 g glyphosate/L at different concentrations ranging from 0.1 ppb (50 ng glpyphosate/L) to 0.5 % (2.25 g glyphosate/L) in drinking water. In these groups, the authors reported alterations in some clinical chemistry (blood and urine) parameters and hormone levels and histopathological lesions concerning the liver and the gastrointestinal tract but also a higher incidence of mammary tumours in females resulting in a shorter lifespan. This study was heavily discussed in the scientific community as well as in the general public where it gained remarkable attention due to massive promotion although it was clearly flawed by many serious deficiencies. A major point of concern was the small group size of only 10 males and 10 females per dose, *i.e.*, the test design was that one of a subchronic study. Such a small number of animals is not appropriate for a long-term study because age-related changes cannot be adequaetely taken into account. Following the receipt of contributions from many MS authorities, a comprehensive critical assessment was published by EFSA (2012, ASB2012-15513, EFSA Journal, 2012, 10 (11), 2986). The conclusion was that ”the currently available evidence does not impact on the ongoing re-evaluation of glyphosate…”. This opinion on the Séralini study is agreed with and supported by the RMS.
In reaction to this publication a large number of letters was send to the editor: Barale-Thomas (2012, ASB2013-10998), Berry (2012, ASB2013-10988), Grunewald (2012, ASB2013-11001), Hammond et al. (2012, ASB2013-10995), Heinemann (2012, ASB2013-10987), Langridge (2012, ASB2013-10986), Ollivier (2012, ASB2013-11000), Panchin (2013, ASB2013-10937), Pilu (2012, ASB2013-10992), Schorsch (2013, ASB2013-10996), Tester (2012, ASB2013-10994), Tien & Huy (2012, ASB2013-10984), Trewavas (2012, ASB2013-10989), Tribe (2012, ASB2013-10997), Wager (2012, ASB2013-10993), de Souza (2012, ASB2013-10999).

Chruszielska et al. (2000, ASB2013-9829) published a combined long term toxicity and carcinogenicity study in rats. The active substance glyphosate was used in the study and the study was performed on basis of OECD guideline 453. The number of animals per dose group and sex (85 animals) was even higher than required in guideline 453. Therefore, the study is considered to be relevant. No carcinogenic effects have been registered in the study.

George *et al.*, (2010, ASB2012-11829) used a 2-stage cancer model in mice to evaluate a glyphosate formulation for tumor promotion. A known tumor promoter, 12-o-tetradecanoyl-phorbol-13-acetate (TPA) was used as a positive control and for comparison with glyphosate effects after exposure to a tumor initiator, 7, 12-dimethylbenz[a]anthracene. Proteomics were later applied to extrapolate a basis for glyphosate formulation tumor promotion. The results are considered by the authors to indicate a tumor promoting potential of glyphosate. However, the formulation Roundup was used in the study and not the active substance glyphosate. Furthermore, the up- and down-regulation of protein expression is not sufficient to prove a carcinogenic effect.

Mechanistic studies

Andreotti et al. (2012, ASB2014-9198) investigated the interaction between pesticide use and genetic variants involved in lipid metabolism on prostate cancer risk. The authors examined the interactions between 39 pesticides and 220 single nucleotide polymorphisms (SNPs) in 59 genes. They found 17 interactions that displayed a significant monotonic increase in prostate cancer risk with pesticides exposure in one genotype and no significant assciation in the other genotype. The most noteworthy association was for ALOXE3 rs 3027208 and terbufos. A higher risk was also reported with this method for glyphosate and other pesticides. However,

the authors emphasize that glyphosate was not associated with prostate cancer risk in the main effect studies (Agricultural Health Study AHS).
Barry et al. (2011, ASB2014-9247) evaluated interactions between 39 pesticides and 394 tag single-nucleotide polymorphisms (SNPs) for 31 BER genes among 776 prostate cancer cases and 1444 male controls in a nested case-control study of Agricultural Healt Study (AHS) pesticide applicators. The authors used likelihood ratio tests from logistic regression models to determine p-values for interactions between three-level pesticide variables and SNP (assuming a dominant model) and the false discovery rate multiple comparison adjustment approach. The authors observed notable interactions between several pesticides and BER gene variants with respect to prostate cancer. However, only fonofos x NEIL3 rs 1983132 showed an interaction fitting an expected biological pattern that remained significant after adjustment for multiple comparisons. No significant association was observed for glyphosate.

The following studies are described more detailed:

Author(s)	**Year**	**Study title**
Hardell, L. Eriksson, M.	1999	A Case-Control Study of Non-Hodgkin Lymphoma and Exposure to Pesticides. Cancer, Volume: 85, Number: 6, Pages: 1353-1360 ASB2012-11838

Abstract*
Background. The incidence of non-Hodgkin lymphoma (NHL) has increased in most Western countries during the last few decades. Immunodefective conditions are established risk factors. In 1981, the authors reported an increased risk for NHL following exposure to certain pesticides. The current study was designed to further elucidate the importance of phenoxyacetic acids and other pesticides in the etiology of NHL.
Methods. A population-based case–control study in northern and middle Sweden encompassing 442 cases and twice as many controls was performed. Exposure data were ascertained by comprehensive questionnaires, and the questionnaires were supplemented by telephone interviews. In total, 404 cases and 741 controls answered the questionnaire. Uni-variate and multi-variate analyses were performed with the SAS statistical data program.
Results. Increased risk for NHL was found for subjects exposed to herbicides (odds ratio [OR], 1.6; 95% confidence interval [CI], 1.0 –2.5) and fungicides (OR, 3.7; 95% CI, 1.1–13.0). Among herbicides, the phenoxyacetic acids dominated (OR, 1.5; 95% CI, 0.9 –2.4); and, when subclassified, one of these, 4-chloro-2-methyl phenoxyacetic acid (MCPA), turned out to be significantly associated with NHL (OR, 2.7; 95% CI, 1.0–6.9). For several categories of herbicides, it was noted that only exposure during the most recent decades before diagnosis of NHL was associated with an increased risk of NHL. Exposure to impregnating agents and insecticides was, at most, only weakly related to NHL.
ConclusionS. Exposure to herbicides in total, including phenoxyacetic acids, during the decades before NHL diagnosis resulted in increased risk for NHL. Thus, the risk following exposure was related to the latency period. Fungicides also increased the risk for NHL when combined, but this group consisted of several different agents, and few subjects were exposed to each type of fungicide.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Study prone to selection and recall bias. No evidence of relevant glyphosate exposures. Medical history was assessed, but not reported.
Relevance of study:	Not relevant (Exposure to multiple chemicals and though glyphosate exposure data were convincing (7/1145 subjects) and statistically non-significant positive associations reported.)
Klimisch code:	3

Additional comments:
Hardell and Eriksson (1999, ASB2012-11838) conducted a case control study to look for associations between reported pesticide use and non-Hodgkin's lymphoma (NHL). The study included 404 NHL cases and 741 controls. The measure of association in this study was the odds ratio (OR), a statistic that estimates of the ratio of disease rates (in this case NHL rates) for exposed and unexposed populations.
The authors reported statistically significant associations for NHL with: reported use of any herbicide (OR = 1.6), reported use of any fungicide (OR = 3.7), and reported use of 4-chloro-2-methylphenoxyacetic acid (OR = 2.7). The major limitations of this study were: the reliance on reported pesticide use (not documented exposure) information, the small number of subjects who reported use of specific pesticides, the possibility of recall bias, the reliance on secondary sources (next-of-kin interviews) for approximately 43 % of the pesticide use information, and the difficulty in controlling for potential confounding factors, given the small number of exposed subjects.
The authors also reported a moderately elevated OR of 2.3 for glyphosate. This OR was not statistically significant and was based on only four "exposed" cases and three "exposed" controls.
This study has several important limitations: no exposure assessment, dependence on next-of-kin's recollections of study subjects' pesticide use for approximately 43 % of study subjects, potential recall bias, and the very small number of subjects who reported using specific herbicides. The latter leads to findings that are statistically imprecise. Due to the potential for bias and the statistical imprecision, the results of this study are not convincing.

Author(s)	Year	Study title
Hardell, L. Eriksson, M. Nordstrom, M.	2002	Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: Pooled analysis of two Swedish case-control studies. Leukemia & Lymphoma Volume: 43 Number: 5 Pages: 1043-1049 ASB2012-11839

Abstract*

Increased risk for non-Hodgkin's lymphoma (NHL) following exposure to certain pesticides has previously been reported. To further elucidate the importance of phenoxyacetic acids and other pesticides in the etiology of NHL a pooled analysis was performed on two case-control studies, one on NHL and another on hairy cell leukemia (HCL), a rare subtype of NHL. The studies were population based with cases identified from cancer registry and controls from population registry. Data assessment was ascertained by questionnaires supplemented over the telephone by specially trained interviewers. The pooled analysis of NHL and HCL was based on 515 cases and 1141 controls. Increased risks in uni-variate analysis were found for subjects exposed to herbicides (OR 1.75, CI 95% 1.26-2.42), insecticides (OR 1.43, CI 95% 1.08-1.87), fungicides (OR 3.11, CI 95% 1.56-6.27) and impregnating agents (OR 1.48, CI 95% 1.11-1.96). Among herbicides, significant associations were found for glyphosate (OR 3.04, CI 95% 1.08-8.52) and 4-chloro-2-methyl phenoxyacetic acid (MCPA) (OR 2.62, CI 95% 1.40-4.88). For several categories of pesticides the highest risk was found for exposure during the latest decades before diagnosis. However, in multi-variate analyses the only significantly increased risk was for a heterogeneous category of other herbicides than above.

- Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	This publication combines the results of two previous studies by the authors on HNL (Hardell and Eriksson, 1999, ASB2012-11838) and HCL (Nordström,et al., 1998, TOX1999-687). No information about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use of prescribed drugs etc). Study documentation is insufficient for assessment.
Relevance of study:	Not relevant (Due to reliability of data set drawn from Hardell and Eriksson, 1999, ASB2012-11838)
Klimisch code:	3

Additional comments:

This study pools data from the previously reviewed publication by Hardell and Eriksson (1999, ASB2012-11838) with data from Nordström et al. (1998, TOX1999-687). Therefore the discussion of limitations of Hardell and Eriksson (1999, ASB2012-11838) also applies to Hardell et al. (2002, ASB2012-11839) (see above).

Author(s)	Year	Study title
Fritschi, L. Benke, G. Hughes, A. M. Kricker, A. Turner, J. Vajdic, C. M. Grulich, A. Milliken, S. Kaldor, J. Armstrong, B. K.	2005	Occupational exposure to pesticides and risk of non-Hodgkin's lymphoma American Journal of Epidemiology Volume: 162, Pages: 849-857 ASB2012-11624

Abstract*
Pesticide exposure may be a risk factor for non-Hodgkin's lymphoma, but it is not certain which types of pesticides are involved. A population-based case-control study was undertaken in 2000-2001 using detailed methods of assessing occupational pesticide exposure. Cases with incident non-Hodgkin's lymphoma in two Australian states (n = 694) and controls (n = 694) were chosen from Australian electoral rolls. Logistic regression was used to estimate the risks of non-Hodgkin's lymphoma associated with exposure to subgroups of pesticides after adjustment for age, sex, ethnic origin, and residence. Approximately 10 % of cases and controls had incurred pesticide exposure. Substantial exposure to any pesticide was associated with a trebling of the risk of non-Hodgkin's lymphoma (odds ratio = 3.09, 95 % confidence interval: 1.42, 6.70). Subjects with substantial exposure to organochlorines, organophosphates, and "other pesticides" (all other pesticides excluding herbicides) and herbicides other than phenoxy herbicides had similarly increased risks, although the increase was statistically significant only for "other pesticides." None of the exposure metrics (probability, level, frequency, duration, or years of exposure) were associated with non-Hodgkin's lymphoma. Analyses of the major World Health Organization subtypes of non-Hodgkin's lymphoma suggested a stronger effect for follicular lymphoma. These increases in risk of non-Hodgkin's lymphoma with substantial occupational pesticide exposure are consistent with previous work.
Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	No information about exposure duration, used glyphosate products, exposure duration and application rates. Documentation is insufficient for assessment.
Relevance of study:	Not relevant (Multiple pesticide exposures. No definitive association between NHL and glyphosate can be made.)
Klimisch code:	3

Additional comments:
No information about exposure duration, used glyphosate products, exposure duration and application rates. Only multiple pesticide exposures are reported. No association between NHL and glyphosate can be made on basis of this study.

Author(s)	Year	Study title
De Roos, A. J. Zahm, S. H. Cantor, K. P. Weisenburger, D. D. Holmes, F. F. Burmeister, L. F. Blair, A.	2003	Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. Occupational and Environmental Medicine Volume: 60, Number: 9, Pages: -E11 ASB2012-11606

Abstract*
Background: An increased rate of non-Hodgkin's lymphoma (NHL) has been repeatedly observed among farmers, but identification of specific exposures that explain this observation has proven difficult.
Methods: During the 1980s, the National Cancer Institute conducted three case-control studies of NHL in the midwestern United States. These pooled data were used to examine pesticide exposures in farming as risk factors for NHL in men. The large sample size (n = 3417) allowed analysis of 47 pesticides simultaneously, controlling for potential confounding by other pesticides in the model, and adjusting the estimates based on a prespecified variance to make them more stable.
Results: Reported use of several individual pesticides was associated with increased NHL incidence, including organophosphate insecticides coumaphos, diazinon, and fonofos, insecticides chlordane, dieldrin, and copper acetoarsenite, and herbicides atrazine, glyphosate, and sodium chlorate. A subanalysis of these "potentially carcinogenic" pesticides suggested a positive trend of risk with exposure to increasing numbers.
Conclusion: Consideration of multiple exposures is important in accurately estimating specific effects and in evaluating realistic exposure scenarios.
Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	No useful information about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use of prescribed drugs etc were reported. Specific lymphomas are not identified (NHL captures all types of lymphoma other than Hodgkin's lymphoma). Documentation is insufficient to associate exposures with specific NHL diseases.
Relevance of study:	Not relevant (No report of identifying various types of lymphoma under the NHL umbrella; no definite association between specific NHL diseases and glyphosate can be made)
Klimisch code:	3

Additional comments:
No useful information about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use of prescribed drugs etc) were reported. Specific lymphomas are not identified. The reported hierarchical regression did not find a statistically significant odds ratio for ever use of glyphosate and NHL.

Author(s)	Year	Study title
De Roos, A.J. Blair, A. Rusiecki, J.A. Hoppin, J.A. Svec, M. Dosemeci, M. Sandler, D.P. Alavanja, M.C.	2005	Cancer Incidence among Glyphosate-Exposed Pesticide Applicators in the Agricultural Health Study Environmental Health Perspectives Volume: 113, Number: 1, Pages: 49-54 ASB2012-11605

Abstract*
Glyphosate is a broad-spectrum herbicide that is one of the most frequently applied pesticides in the world. Although there has been little consistent evidence of genotoxicity or carcinogenicity from *in vitro* and animal studies, a few epidemiologic reports have indicated potential health effects of glyphosate. We evaluated associations between glyphosate exposure and cancer incidence in the Agricultural Health Study (AHS), a prospective cohort study of 57,311 licensed pesticide applicators in Iowa and North Carolina. Detailed information on pesticide use and other factors was obtained from a self-administered questionnaire completed at time of enrolment (1993–1997). Among private and commercial applicators, 75.5% reported having ever used glyphosate, of which > 97% were men. In this analysis, glyphosate exposure was defined as *a*) ever personally mixed or applied products containing glyphosate; *b*) cumulative lifetime days of use, or "cumulative exposure days" (years of use × days/year); and *c*) intensity-weighted cumulative exposure days (years of use × days/year × estimated intensity level). Poisson regression was used to estimate exposure–response relations between glyphosate and incidence of all cancers combined and 12 relatively common cancer subtypes. Glyphosate exposure was not associated with cancer incidence overall or with most of the cancer subtypes we studied. There was a suggested association with multiple myeloma incidence that should be followed up as more cases occur in the AHS. Given the widespread use of glyphosate, future analyses of the AHS will allow further examination of long-term health effects, including less common cancers.
* Quoted from article

Klimisch evaluation

Reliability of study:	Reliable without restrictions
Comment:	Well documented publication. Study included glyphosate exposure, as well as demographic and lifestyle factors. However, adjusted relative risk calculations eliminated a significant proportion of the data set without justification.
Relevance of study:	Relevant (Evaluation focussed on glyphosate, although other pesticides were also considered in the data evaluation)
Klimisch code:	2

Additional comments:
Study included glyphosate exposure, as well as demographic and lifestyle factors. However, adjusted relative risk calculations eliminated a significant proportion of the data set without justification.

Response 1 – summary from Letter to the Editor by Farmer et al. (2005, ASB2012-11616)

Authors provided an incomplete genotoxicity review which was inconsistent with opinions of regulatory agencies and experts around the world, that glyphosate is not genotoxic. An extensive toxicology review of glyphosate was cited by the authors, mentioning a lack of carcinogenicity with glyphosate exposures, yet neglected to cite the extensive genotoxicity review in the same publication by Williams et al. (2000, ASB2012-12053)

Biological plausibility of a cancer effect should be considered in the light of exposure. Acquavella et al (2004, ASB2012-11528) reported the maximum systemic dose to resulting from application of glyphosate to areas as large as 400 acres was 0.004 mg/kg, and the geometric mean systemic dose was 0.0001 mg/kg in farmers. If these glyphosate applications and exposures continued daily over the course of a lifetime, the systemic dose would be at least 250,000-fold lower than the cancer no-effect level in rodents.

The authors were requested to further evaluate their models for confounding and selection bias in the multiple myeloma analysis.

Response 2 – summary from Lash (2007, ASB2012-11877)

Table 2 of De Roos et al. (2005, ASB2012-11605) noted 32 cases of multiple myeloma associated with "ever-use" of glyphosate and when compared with "never-use" (adjusted for age only) yielded a rate ratio of 1.1 (95 % CI 0.5-2.4). However, when the data set was adjusted for age, demographic and lifestyle factors and other pesticide use, the rate ratio increased to 2.6 (95 % CI 0.7-9.4).

The adjusted estimate merits careful inspection and can only be undertaken with access to the primary data, not made available by the authors.

Bias analysis was conducted, accounting for confounding and exposure misclassification.

Adjustment for confounders in De Roos et al. (2005, ASB2012-11605), which resulted in limiting the data set by 25 % because of missing data on the adjustment variables, likely introduced selection bias and produced the a rate ratio of 2.6 that was substantially biased.

Author(s)	Year	Study title
Eriksson, M. Hardell, L. Carlberg, M. Akerman, M.	2008	Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis International Journal of Cancer Volume: 123, Pages: 1657-1663 ASB2012-11614

Abstract*

We report a population based case-control study of exposure to pesticides as risk factor for non-Hodgkin lymphoma (NHL). Male and female subjects aged 18-74 years living in Sweden were included during December 1, 1999, to April 30, 2002. Controls were selected from the national population registry. Exposure to different agents was assessed by questionnaire. In total 910 (91 %) cases and 1016 (92%) controls participated. Exposure to herbicides gave odds ratio (OR) 1.72, 95% confidence interval (CI) 1.18-2.51. Regarding phenoxyacetic acids highest risk was calculated for MCPA; OR 2.81, 95% CI 1.27-6.22, all these cases had a latency period >10 years. Exposure to glyphosate gave OR 2.02, 95% CI 1.10-3.71 and with >10 years latency period OR 2.26, 95% CI 1.16-4.40. Insecticides overall gave OR 1.28, 95% CI 0.96-1.72 and impregnating agents OR 1.57, 95% CI 1.07-2.30. Results are also presented for different entities of NHL. In conclusion our study confirmed an association between

exposure to phenoxyacetic acids and NHL and the association with glyphosate was considerably strengthened.
Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Multiple avenues for bias were introduced in study design, execution and data processing. No information about exposure duration, used glyphosate products and application rates. Other factors (i.e. smoking habits, medication etc.) were assessed but not included in the evaluation.
Relevance of study:	Relevant with reservation
Klimisch code:	3

Additional comments:
The authors (Eriksson et al. 2008, ASB2012-11614) conducted a population-based case-control study of exposure to a variety of pesticides and non-Hodgkin lymphoma (NHL), including separate analyses of histopathological categories of NHL. Study subjects were males and females, ages 18-74, living in Sweden between December 1, 1999 and April 30, 2002. The final study group included 910 cases and 1016 controls. Exposure, ascertained via an interviewer-administered questionnaire, focused on pesticide and other chemical agents, and included a total work history (although a job-exposure matrix was not used). For pesticide exposure, information on number of years, number of days per year, and approximate length of exposure per day was also obtained. A minimum of one full day of exposure was required for categorization as "exposed."

The authors reported a statistically significant positive association between "herbicide exposure" and NHL (OR = 1.72; 95% CI: 1.18-2.51). Glyphosate exposure was reported by 29 cases and 18 controls, and the corresponding odds ratio (OR) was 2.02 (95% CI: 1.10-3.71). The ORs for glyphosate exposure of <10 days and >10 days were 1.69 (95% CI: 0.70-4.07) and 2.36 (1.04-5.37), respectively. The ORs for glyphosate were 1.11 (95% CI: 0.24-5.08) and 2.26 (95% CI: 1.16-4.40) for "latency" periods of 1-10 years and >10 years, respectively. In analyses of glyphosate and type of NHL, statistically significant positive associations were observed for small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL) (OR = 3.35; 95% CI: 1.42-7.89) and for "unspecified NHL" (OR = 5.63; 95% CI: 1.44-22.0). Odds ratios for the other types (total B-cell lymphomas, grade I-III follicular lymphoma, diffuse large B-cell lymphoma, other specified B-cell lymphoma, unspecified B-cell lymphoma, and T-cell lymphomas) were above 1.0, but were not statistically significant (i.e., the 95% confidence intervals were relatively wide and included the null value of 1.0).

The authors concluded, "Glyphosate was associated with a statistically significant increased OR for lymphoma in our study, and the result was strengthened by a tendency to dose-response effect…" (p. 1662). The authors suggested that their findings are consistent with results of a previous case-control study (Hardell and Eriksson 1999, ASB2012-11838) and pooled analysis (Hardell et al. 2002, ASB2012-11839) that they conducted. In the case-control study, an OR of 2.3 (95% CI: 0.4-13.0), based on 4 exposed cases and 3 exposed controls, was reported for glyphosate and NHL. In the pooled analysis of two case-control studies, which included data from Hardell and Eriksson (1999, ASB2012-11838), an OR of

3.04 (95% CI: 1.08- 8.52) was reported, based on 8 exposed cases and 8 exposed controls. The authors also cited three studies (De Roos et al. 2003, ASB2012-11606; McDuffie et al. 2001; ASB2011-364, De Roos et al. 2005, ASB2012-11605) by other groups as being consistent with their results in that they "also associate glyphosate with different B-cell malignancies such as lymphomas and myelomas." It should be noted, however, that the relative risk (RR) reported by De Roos et al. (2005, ASB2012-11605) for the highest versus lowest category of cumulative exposure days of glyphosate and NHL in the prospective Agricultural Health Study was 0.9.

Interpretation Issues
Identification of Cases and Potential Referral Bias. It is noteworthy that the cases in the current analysis were identified from some of the same hospitals as the authors' prior publication; thus, referral bias may have been an issue. In particular, the researchers approached the patients after diagnosis if the physicians deemed it appropriate. Therefore, if the physicians were concerned that their patient's NHL was associated with agricultural exposures, they may have suggested participation in the study.

Participation Rates and Potential Selection Bias. The authors report a participation rate of 91% and 92% for cases and controls, respectively; however, these figures are based on completed questionnaires out of those who had previously said they would participate in the study. The number of eligible patients (i.e., prior to physician approval to "approach") was not reported, so the computation of an exact participation rate is difficult. Based on information provided in the paper, participation among cases is estimated to be about 80%. Nonparticipation is a concern for several reasons. First, in a case-control study, an odds ratio will be an accurate representation of the exposure-disease association when the cases are representative of all cases and the controls are representative of the exposure experience of the population that gave rise to the cases. If the final study sample is not representative of this "target population" then measures of effect (e.g., the odds ratio) may not be valid. In addition, one must be concerned about selection bias. Selection bias occurs in a case-control study when the exposure distribution for cases and controls differ for those who participate in the study compared to those who are eligible but do not participate in the study. It is not possible to determine whether there is selection bias without information about nonparticipants.

Strengths and Limitations of Using Living Cases Only versus All Cases (Living + Dead).
The authors noted that 88 potential cases died before they could be interviewed and were therefore excluded from the study. It is also stated in the Discussion that restricting the study to living cases and controls was an "advantage" of the study, as interviewing cases and controls directly compared to interviewing next-of-kin was preferable. While it is generally true that this would be an advantage, the following statement by the authors, therefore, is not accurate, "The study covered all new cases of NHL during a specified time" (p. 1660). The study did not include all new cases; it included only those cases who survived until the time of the interview. Thus, while there may have been an advantage to restricting the study to living cases, there was a trade-off in that the study population did not represent all cases, specifically those cases with more aggressive disease. This disadvantage was not discussed by the authors, nor was the potential bias that could have resulted from excluding many eligible cases.

Exposure Measurement and Information Bias. Exposure was ascertained via a questionnaire oriented towards pesticide and other chemical agents. In addition, interviewers collected information by telephone if "important" data were lacking, incomplete, or unclear. It is

unknown what is meant by "important," and the proportion of cases and controls who received phone calls was not reported. Thus, information bias may be a concern. Even though interviewers were blinded to case and/or control status, they may have been able to determine this information during the course of the interview. Furthermore, recall bias may be an issue because exposure information was based on participant response and cases and controls may recall and/or report past pesticide exposures differently. No exposure validation techniques were implemented, nor did an industrial hygienist (or any other type of personnel trained in assessing occupational exposures) independently validate/estimate the frequency and/or intensity of exposure. The authors assumed that "some misclassification regarding quantity of exposure has probably occurred, but such misclassification would most probably be nondependent of case/control status, and therefore only weaken any true risks" (p. 1660). They do not provide any explanation as to why they believe that exposure misclassification would be "most probably" nondifferential. If NHL cases believe that pesticides may be related to their disease, then it is certainly possible that they may recall and/or report pesticide exposure differently than NHL-free controls, which could result in odds ratios that are inflated as a result of bias.

Interpretation of "dose-response" analyses. The referent group in the statistical analyses consisted of participants who were unexposed to all pesticides. The dose-response analyses were based on a dichotomy of the median number of days exposed to a particular agent. It is difficult to analyze "dose-response" when only two exposure categories are considered. Furthermore, the dose-response analyses were based on median values of exposure but heterogeneity of cut-points is evident across agents. For example, glyphosate was analyzed as < 10 days and > 10 days, whereas, "other" herbicides were analyzed as < 32 days and > 32 days. Although analytical cut-points were data driven, interpretation across the wide variety of exposures is complicated by the variability in exposure cut-points. In addition, even though the OR for the higher category of exposure days was greater than the OR for the lower category, the two 95% confidence intervals were wide and overlapped considerably (0.70-4.07 and 1.04-5.37).
Thus, it is not clear whether the two point estimates reported (1.69 and 2.36) are significantly different from each other. Finally, this result cited in the "dose-response" analyses may have been confounded by exposure to other herbicides. In Table II (Eriksson et al. 2008, ASB2012-11614), the authors observed elevated associations for other herbicides, including MCPA, 2,4,5-T and/or 2,4-D. The correlation between exposure to glyphosate and other herbicides was not provided nor were analyses of glyphosate-exposed individuals after accounting for the collinear relation between this agent and other agents. The odds ratio for "ever" exposure to glyphosate was attenuated after additional adjustment for other pesticides (Table VII, Eriksson et al. 2008, ASB2012-11614), but multi-variate -adjusted estimates for the "dose-response" odds ratios were not reported.
Unusual Pattern of Positive Associations. The authors conducted multiple comparisons, and one would expect a certain proportion of their findings to be statistically significant (whether in the positive or inverse direction) simply as a result of chance. It is somewhat surprising, therefore, that the vast majority of the ORs presented in this manuscript are greater than 1.0, regardless of the statistical significance. The authors do note that for some of the analyses (e.g., latency), only chemicals for which ORs were greater than 1.5 and for which there were at least 10 exposed cases, or for which there was a statistically significant OR were evaluated. On the other hand, dose-response was evaluated based on the number of exposed subjects and not on the strength or significance of the findings. The authors do not address this directly, but do state in their Discussion, "…several pesticides are chemically related and may exert their effects on humans through a similar mechanism of action, which may explain the wide range

of pesticides that have been related to NHL over time in different countries and with different exposure conditions" (p. 1661). On the other hand, this pattern of positive findings could be a result of bias, including recall bias (or other information bias), selection bias, uncontrolled confounding, or a combination of these and other factors.

Interpretation of Eriksson et al. (2008, ASB2012-11614) in Context of Other Studies. Despite the statement by the authors that, "Recent findings from other groups also associate glyphosate with different B-cell malignancies such as lymphomas and myeloma" (p. 1662), most multi-variate analyses of glyphosate and NHL do not report statistically significant associations (De Roos et al. 2005, ASB2012-11605; De Roos et al. 2003; ASB2012-11606, Hardell and Eriksson 1999, ASB2012-11838; Hardell et al. 2002; ASB2012-11839, Lee et al. 2004; ASB2012-11883, McDuffie et al. 2001; ASB2011-364, Nordström et al. 1998, TOX1999-687) (Tables B.6.5-62 and B.6.5-63). It is notable that Hardell et al. (2002, ASB2012-11839) reported a significant positive association between glyphosate association and NHL, but the multi-variate -adjusted odds ratio was attenuated and not statistically significant. Similar findings were reported by Eriksson et al. (2008, ASB2012-11614). Specifically, the association reported by the authors in the abstract (OR = 2.02; 95% CI: 1.10-3.71) was adjusted for age, sex and year of diagnosis or enrollment. When other pesticides were added to that model (i.e., agents with statistically significant increased odds ratios, or with an odds ratio greater than 1.5 and with at least 10 exposed subjects), the adjusted odds ratio was 1.51 (95% CI: 0.77-2.94). Thus, the authors' final statement, "Furthermore, our earlier indication of an association between glyphosate and NHL has been considerably strengthened" is questionable. Their previous findings showed a non-significant association after multi-variate adjustment (OR = 1.85; 95% CI: 0.55-6.20). The 2008 study similarly reported a statistically non-significant association between glyphosate and NHL after multi-variate adjustment (OR = 1.51; 95% CI: 0.77-2.94). The results reported for analyses of duration of exposure and latency of exposure did not adjust for other pesticides, and one would expect that those ORs would also be attenuated.

Summary of Findings: Cohort and Case-Control Studies of Exposure to Glyphosate and Non-Hodgkin Lymphoma

Table B.6.5-62: Cohort Studies

Author Year	Description	No. of Exposed Cases	Type of Relative Risk Estimate	Relative Risk Estimate	95% Confidence Limits	Variables Included in Statistical Model
De Roos et al. 2005 (ASB2012 -11605)	57-2,678 vs. 1-20 Cumulative Exposure Days[a]	17	RR	0.9	0.5-1.6	Age at enrollment, education, pack-years of cigarette smoking, alcohol consumption in the past year, family history of cancer in first-degree relatives, and state of residence
	337.2-18,241 vs. 0.1-79.5 Intensity-Weighted Exposure Days[b]	22	RR	0.8	0.5-1.4	Also adjusted for other pesticides

[a] Years of use x days per year; categorized by tertiles
[b] Years of use x days/year x estimated intensity level; categorized by tertiles

Table B.6.5-63: Case Control Studies

Author Year	Exposure Evaluated	Subgroup Description	No. of Exposed Cases	No. of Exposed Controls	OR	95% CI	Variables Included in Statistical Model
De Roos et al. 2003 (ASB2012-11606)	Ever exposure to specific pesticide; men only (all 47 pesticides were regressed simultaneously)	Glyphosate (Logistic Regression)	36	61	2.1	1.1-4.0	Age, study site and other pesticides
		Glyphosate (Hierarchical Regression)	36	61	1.6	0.9-2.8	Second-level model incorporated what was known about each true effect parameter prior to seeing the study data
Hardell and Eriksson 1999 (ASB2012-11838)	Exposure to specific pesticides (ever/never exposed to the specific pesticide vs. no exposure to any pesticide)	Glyphosate (conditional logistic regression; uni-variate analysis)	4	3	2.3	0.4-13	Age and country (matching factors)
		Glyphosate (conditional logistic regression; multi-variate analysis)	4	3	5.8	0.6-54	Multi-variate variables not listed by authors
Hardell et al. 2002 (ASB2012-11839)	Exposure to specific pesticides (ever/never exposed to the specific pesticide vs. no exposure to any pesticide)	Glyphosate (conditional logistic regression; uni-variate analysis)	8	8	3.04	1.08-8.52	Age and county (matching factors); study, study area (county), and vital status
		Glyphosate (conditional logistic regression; multi-variate analysis)	8	8	1.85	0.55-6.20	Multi-variate variables not listed by authors
Lee et al. 2004 (ASB2012-11883)	Exposure to individual pesticides	Glyphosate use, Non-asthmatics	53	91	1.4	0.98-2.1	Age, state, vital status
		Glyphosate use, Asthmatics	6	12	1.2	0.4-3.3	
McDuffie et al. 2001 (ASB2011-364)	Exposure to individual active chemicals	Glyphosate (Round-Up)	51	133	1.26	0.87-1.80	Strata for age and province of residence
		Glyphosate (Round-Up)	NR	NR	1.20	0.83-1.74	Plus statistically significant medical variables

Author Year	**Exposure Evaluated**	**Subgroup Description**	**No. of Exposed Cases**	**No. of Exposed Controls**	**OR**	**95% CI**	**Variables Included in Statistical Model**
Nordström et al. 1998 (TOX1999-687)	Exposure to specific herbicides, insecticides, and fungicides	Glyphosate	4	5	3.1	0.8-12	Age and country (matching factors)
Eriksson et al. 2008 (ASB2012-11614)	Exposure to specific herbicides regardless if they also had been exposed to phenoxyacetic acids or not	Glyphosate	29	18	2.02	1.10-3.71	Age, sex, and year of diagnosis or enrollment
			29	18	1.51	0.77-2.94	Age, sex, and year of diagnosis or enrollment and pesticides with statistically significant increased odds ratios, or with an odds ratio greater than 1.5 and with at least 10 exposed subject
	Exposure to herbicide stratified by median number of days among exposed controls	Glyphosate ≤ 10 days	12	9	1.69	0.70-4.07	Age, sex, and year of diagnosis or enrollment
		Glyphosate >10 days	19	9	2.36	1.04-5.37	
	Exposure to specific herbicides according to different lymphoma entities	Glyphosate: B-Cell lymphomas	NR	NR	1.87	0.998-3.51	Age, sex, and year of diagnosis or enrollment
		Lymphocytic lymphoma/B-CLL	NR	NR	3.35	1.42-7.89	
		Follicular grade I-III	NR	NR	1.89	0.62-5.79	
		Diffuse large B-cell Lymphoma	NR	NR	1.22	0.44-3.35	
		Other specified B-cell lymphoma	NR	NR	1.63	0.53-4.96	
		Unspecified B-cell Lymphoma	NR	NR	1.47	0.33-6.61	
		T-cell lymphomas	NR	NR	2.29	0.51-10.4	
		Unspecified NHL	NR	NR	5.63	1.44-22.0	

Author(s)	Year	Study title
George, J. Prasad, S. Mahmood, Z. Shukla, Y.	2010	Studies on glyphosate-induced carcinogenicity in mouse skin: A proteomic approach Journal of Proteomics Volume: 73, Pages: 951-964 ASB2012-11829

Abstract*
Glyphosate is a widely used broad spectrum herbicide, reported to induce various toxic effects in non-target species, but its carcinogenic potential is still unknown. Here we showed the carcinogenic effects of glyphosate using 2-stage mouse skin carcinogenesis model and proteomic analysis. Carcinogenicity study revealed that glyphosate has tumor promoting activity. Proteomic analysis using 2-dimensional gel electrophoresis and mass spectrometry showed that 22 spots were differentially expressed (>2 fold) on glyphosate, 7, 12-dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoyl-phorbol-13-acetate (TPA) application over untreated control. Among them, 9 proteins (translation elongation factor eEF-1 alpha chain, carbonic anhydrase III, annexin II, calcyclin, fab fragment anti-VEGF antibody, peroxiredoxin-2, superoxide dismutase [Cu–Zn], stefin A3, and calgranulin-B) were common and showed similar expression pattern in glyphosate and TPA-treated mouse skin. These proteins are known to be involved in several key processes like apoptosis and growth-inhibition, anti-oxidant responses, etc. The up-regulation of calcyclin, calgranulin-B and down-regulation of superoxide dismutase [Cu–Zn] was further confirmed by immunoblotting, indicating that these proteins can be good candidate biomarkers for skin carcinogenesis induced by glyphosate. Altogether, these results suggested that glyphosate has tumor promoting potential in skin carcinogenesis and its mechanism seems to be similar to TPA.
* Quoted from article

Klimisch evaluation

Reliability of study:	Reliable with restrictions
Comment:	Non-guideline mechanistic study. Scientifically acceptable study with deficiencies (controls with glyphosate alone, and co-formulants were not included)
Relevance of study:	Relevant with restrictions (Glyphosate formulation not glyphosate alone was tested.)
Klimisch code:	2

Additional comments:
The authors use glyphosate as a synonym for what is really a glyphosate based formulated product. Doses in this study are not representative of human exposures to glyphosate or glyphosate based formulations. Mice in the tumor promoting group VIII received topical applications of concentrated glyphosate formulated product three times per week for over thirty weeks without washing after an initial treatment with the potent tumor initiator DMBA. Glyphosate had been shown to have very low dermal absorption, even in formulated products, and since is non-volatile, would likely accumulate on mouse skin. Surfactants are typically irritating and non-volatile. Given the irritation potential of the unwashed exposed mouse skin over the course of thirty or more weeks, tumor promotion may be a physical response to substantial localized dermal irritation. Epidemiological studies reported above note no association with glyphosate and either skin or lip cancers.

Label directions outline appropriate personal protective equipment such as gloves and long sleeves. Furthermore, any dermal exposure of concentrated product to human skin would prove irritating and prompt handlers to wash off soon after dermal exposure.
Human *in vitro* dermal absorption studies reported for a range of glyphosate based formulations containing different surfactant systems all demonstrate extremely low dermal absorption of glyphosate active ingredient for concentrated products, of less than 0.2 %. Test material recovery in each of the four reported dermal absorption studies was very good, close to 100 %. Most of the glyphosate was removed during skin surface washing at either eight or twenty four hours of in vitro human skin exposure. This also suggests significant potential for accumulation of glyphosate on the surface of the mice skin in George et al. (2010, ASB2012-11829).
The up-regulation / down-regulation of protein expression reported after a single dermal dose of a glyphosate formulated product (proteomics experiment, group II), while interesting, does not demonstrate any toxicological endpoint. Rather, perturbations may well represent normal homeostatic fluctuations and be a natural response to insult.

Author(s)	**Year**	**Study title**
Seralini, G.-E. Clair, E. Mesnage, R. Gress, S. Defarge, N. Malatesta, M. Hennequin, D. Spiroux de Vendomois, J.	2012	Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Food and Chemical Toxicology 50, 4221-4231 ASB2012-15514

Abstract*
The health effects of a Roundup-tolerant genetically modified maize (from 11% in the diet), cultivated with or without Roundup, and Roundup alone (from 0.1 ppb in water), were studied 2 years in rats. In females, all treated groups died 2–3 times more than controls, and more rapidly. This difference was visible in 3 male groups fed GMOs. All results were hormone and sex dependent, and the pathological profiles were comparable. Females developed large mammary tumors almost always more often than and before controls, the pituitary was the second most disabled organ; the sex hormonal balance was modified by GMO and Roundup treatments. In treated males, liver congestions and necrosis were 2.5–5.5 times higher. This pathology was confirmed by optic and transmission electron microscopy. Marked and severe kidney nephropathies were also generally 1.3–2.3 greater. Males presented 4 times more large palpable tumors than controls which occurred up to 600 days earlier. Biochemistry data confirmed very significant kidney chronic deficiencies; for all treatments and both sexes, 76% of the altered parameters were kidney related. These results can be explained by the non linear endocrine-disrupting effects of Roundup, but also by the overexpression of the transgene in the GMO and its metabolic consequences.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	The study was performed to investigate the long term toxicity and carcinogenicity. However the study design does not agree with the OECD guidelines on long term toxicity and carcinogenicity.
Relevance of study:	Relevant with restrictions (Glyphosate formulation not glyphosate alone was tested.)
Klimisch code:	3

Comments:
Seralini et al. (2012, ASB2012-15514) submitted a report of long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. The health effects have been studied 2 years in rats. Six groups of rats were fed with 11, 22 and 22 % of genetically modified NK603 maize either treated or not with Roundup. Three further groups of rats were fed with control diet and had access to water supplemented with 50 ng/L, 400 mg/L and 2.25 g/L of the commercial product Roundup (GT Plus, 450 g/L of glyphosate). The pure active substance glyphosate was not tested in this study.
The study is not considered reliable because of several important limitations. According to the authors the studies have been performed to investigate the long term toxicity and carcinogenicity. However, the number of animals per dose and sex was only 10 and also the further study design does not agree with the OECD guidelines on long term toxicity and carcinogenicity. The spontaneous incidence of mammary tumors in the used Sprague Dawley rats is much higher than in most other rat strains. Therefore, a higher number of animals would be necessary for the differentiation between treatment related carcinogenicity and accidental aberrations. Also for the assessment of mortality and further described toxic effects a higher number of animals would be needed.
The presented results in the publication are incomplete and therefore, an evaluation of the presented results was complicated.
The study was extensively discussed and criticized in the public. In an additional paper Seralini et al. (2013, ASB2013-10985) gave some answers to the critics. The authors admit that the study "should not be considered as a final point in knowing the toxicological effects of NK603 and R (oundup)" and that the study has limits.
Jany (2012, ASB2014-9580) submitted a critical review of the study by Seralini et al. (2012). The authors conclude that the scientific value of this publication would be limited and non conclusions are possible concerning maize NK603 with and without Roundup treatment.
Ollivier (2012, ASB2013-11000) proposes to use the Chi-square test to compare mortality rates in the study of Seralini et al. (2012). In result of this test there would be no statistical significance.
In a further paper Seralini et al. (2014, ASB2014-9632) discuss criticisms which have been published in reaction on the study by Seralini et al. (2012, ASB2012-15514).
John (2014, ASB2014-9584) reacts in a letter on the decision of the publisher to retract the article of Seralini et al. (2012). John concludes that there would be no grounds for retraction.
Wallace-Hayes (2014, ASB2014-9559), the editor-in-chief of Food and Chemical Toxicology, gives answers on questions on the retraction of the paper of Seralini et al. (2012). He concludes once more that "a careful and time-consuming analysis found that the data were inconclusive, and therefore the conclusion described in the article were unreliable. Accordingly, the article was retracted."

Folta (2014, ASB2014-9478) writes in a letter to the editor that he would see this work of Seralini (2012) as a manipulation of the scientific process to achieve activist gains. He stands behind the journal`s decision to retract the work.
Rosanoff (2014, ASB2014-9397) proposes in a letter concerning the Seralini (2012) study that the raw data should be published.
Roberfroid (2014, ASB2014-9393) writes in a letter concerning the Seralini (2012) study that he is ashamed about the decision to retract this paper.
In a further letter Roberfroid (2014, ASB2014-9392) writes that in his understanding the study of Seralini (2012) remains an important scientific (not a regularory) observation that can not be ignored.
Pilu (2012, ASB2014-9387) writes in a letter to the editor on the Seralini (2012) study that mycotoxins in maize could have influenced the results of the study. Therefore, he asks for further information on the mycotoxin content in the maize used in the Seralini study.

Author(s)	Year	Study title
Chruscielska, K. Brzezinski, J. Kita, K. Kalhorn, D. Kita, I. Graffstein, B. Korzeniowski, P.	2000	Glyphosate Evaluation of chronic activity and possible far-reaching effects. Part 1. Studies on chronic toxicity Pestycydy 2000, (3-4), 11-20 ASB2013-9829

Abstract*:
The combined test of chronic toxicity and carcinogenicity of glyphosate was performed on Wistar-RIZ rats. The herbicide was administered in water at concentrations: 0, 300, 900, 2700 m/L. The examination of the peripheral blood parameters and the smears of bone marrow did not reveal harmful effect of the herbicide on haematopoietic system of rats. The biochemical parameters determined on blood and urine only in some cases showed significant deviations in comparison with the control group, but in any examined indices dose-effect-time occurred what could manifest the toxic influence of glyphosate. In pathomorphological studies on the organs no correlation was stated between the number of observed tumours and the concentrations of the herbicide. It indicates lack of pathogenic influence of glyphosate on neoplastic pathogenesis.
* Quoted from article

Klimisch evaluation

Reliability of study:	Reliable with restrictions
Comment:	The published details of the study are limited. However, according to the authors the study was performed on basis of OECD guideline No. 453
Relevance of study:	Relevant
Klimisch code:	2

Comments:
The active substance glyphosate was used in the study and the study was performed on basis of OECD guideline 453. The number of animals per dose group and sex (85 animals) was

even higher than required in guideline 453. Therefore, the study is considered to be relevant. No carcinogenic effects have been registered in the study.

B.6.6 Reproductive toxicity (Annex IIA 5.6)

Introduction into this chapter by the RMS
For higher efficiency of the review and for the sake of transparency, the descriptions of methods and study results in the GTF dossier were virtually not amended and even the conclusions were kept as provided. However, each study that is described in detail was commented by RMS. These remarks on bottom of each study description are clearly distinguished from the original submission by a caption and are always written in italics. In addition, redundant parts (in particular the so-called "executive summaries") have been deleted and the structure of the original submission was significantly changed to make it more transparent and comprehensible.

The overall assessment of reproductive toxicity of glyphosate by the RMS is provided in Volume 1 (2.6.6) of the present RAR.

Comments by the GTF on the first draft of the RAR (July 1013) have been partly included in the present report. Responses by RMS to GTF are written in italcs and given below. This approach was taken to avoid doubling of comments/responses at a later timepoint.

B.6.6.1 Two generation reproductive toxicity in the rat

The reproductive toxicity of glyphosate was tested in a variety of multi-generation studies in rats. For the previous EU evaluation, a total of 8 studies in rats had been submitted of which four were still considered acceptable or, in case of a single one-generation study, at least supplementary upon re-evaluation. The studies by [redacted] (1981, TOX9552385), by [redacted] (1985, TOX9650161) and by [redacted] (both 1988, TOX9551832 and TOX9551965), however, were deleted from current evaluation due to major deficiencies and/or because the dose levels were much too low and therefore one could not expect the occurrence of any toxic effects.
Three new studies were provided in the GTF dossier and were submitted either for the first time for this evaluation or had been subject to JMPR evaluation (JMPR, 2004, ASB2008-6266) yet.

Reference:	IIA, 5.6.1/01
Report:	[redacted] (2007) Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat [redacted] Data owner: Nufarm SPL project no.: 2060/0013 Date:2007-10-31 (amended 2008-04-08 and 2008-08-08) not published ASB2012-11494
Guidelines:	OECD 416 (2001), JMAFF 2-1-17 (2001), US-EPA OPPTS

	870.3800 (1998)
Deviations:	None
GLP:	yes
Acceptability:	*See RMS comment*

Dates of experimental work: 2005-11-18 to 2006-11-06

Materials and methods

Test material:	
Identification:	Glyphosate technical
Description:	White crystalline solid
Lot/Batch #:	H05H016A
Purity:	95.7 % (w/w)
Stability of test compound:	Not reported
Vehicle and/ or positive control:	Plain diet
Test animals:	
Species:	Rat
Strain:	Sprague-Dawley Crl:CD (SD) IGS BR
Source:	[redacted]
Age:	Approximately 8 weeks
Sex:	Males and females
Weight at dosing:	Males: 138 – 257 g; females: 140 – 195 g
Acclimation period:	At least 14 days
Diet/Food:	Rodent PMI 5002 (certified) diet (BCM IPS Limited, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Initially in groups of up to four in polypropylene cages with stainless steel grid floors and tops, suspended over polypropylene trays lined with absorbent paper. During mating animals were house one male : one female. Mated females were housed individually during gestation and lactation in polypropylene cages with solid floors and stainless steel lids, furnished with softwood flakes.
Environmental conditions:	Temperature: 21 ± 2°C Humidity: 55 ± 15% Air changes: at least 15/hour 12 hours light/dark cycle

Study design and methods:
In life dates: 2005-11-18 to 2006-11-06

Animal assignment and treatment:
In a two-generation reproduction study groups of 28 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 0, 1500, 5000 and 15000 ppm (equivalent to mean achieved dose levels of 0, 104, 351 and 1063 mg/kg bw/day for males, and 0, 162, 530 and 1634 mg/kg bw/day for females) glyphosate technical in diet. The dose levels were chosen based on results of a previously conducted study. After 10 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis, to produce the F1 litters. At weaning of offspring from the F0 mating phase, groups of twenty-four male and twenty-four female offspring from each dose group were selected to form the F1 generation. The remaining surviving F0 females and unselected offspring were terminated at Day 21 *post partum*, followed by the termination of all F0 male dose groups. The offspring selected for the F1 generation were dosed for at least 10 weeks and then paired within each dose group to produce the F2 litters. At weaning of the F2 litters all surviving adults and their offspring were killed, followed by the termination of all F1 male dose groups.

Diet preparation and analyses
For preparation of diet mixtures a known amount of the test substance was mixed with a small amount of basal diet at a constant speed for 19 minutes in a Hobart QE200 mixer. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes in a Hobart H800 mixer.
The stability and homogeneity of the test material in diet were determined. Dietary admixtures were analysed for achieved concentration weekly for the first four weeks of the study and monthly thereafter.

Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily.

Body weight
Individual body weights were recorded for F0 males on Day 1 (prior to treatment) and at weekly intervals for F0 and F1 males until termination. F0 and F1 females were weighed daily until mating was evident. Bodyweights for females showing evidence of mating were recorded on Days 0, 7, 14 and 21 *post coitum*. Females with live litters were weighed on Days 1, 4, 7, 14 and 21 *post partum*.

Food consumption and compound intake
During the maturation period, weekly food consumption was recorded for each cage of adults. For females showing evidence of mating, food consumption was recorded for the periods covering Days 0 - 7, 7 - 14 and 14 – 21 *post coitum*. For females with live litters, food consumption was recorded for the period covering Days 1 - 4, 4 - 7, 7 - 14, 14 - 21 *post partum*.
Food conversion efficiency (the ratio of bodyweight change / dietary intake) was calculated retrospectively for males for both the pre-mating and post-mating phases of the study. For females, food conversion efficiency was only calculated for the pre-mating phases of the study. Due to offspring growth, milk production and weaning, food efficiency could not be accurately calculated for the gestation and lactation phases of the study.

Water consumption
Water intake was observed daily by visual inspection of water bottles for any overt change.

Reproduction parameters
Oestrus cycle
Prior to pairing of females for the F0 and F1 mating phases, a vaginal smear was taken daily for twenty-one days and examined microscopically to determine the stage of oestrous.

Pregnancy and parturition
Pregnant females were observed at approximately 0830, 1230 and 1630 hours daily, and at approximately 0830 and 1230 hours on weekends and public holidays. In addition, the females were observed around the period of expected parturition. The date of mating, date and time of start and end of parturition and duration of gestation was recorded.

Litter data
The following litter data were recorded:
The number of offspring born, the number of offspring alive recorded daily and reported on Day 1, 4, 7, 14, 21 post partum. On Days 1, 4 and 21, the sex of individual offspring was recorded. The clinical condition of offspring during lactation, as well as individual offspring and total litter weights were recorded after birth on Day 1, 4, 7, 14.

Physical and sexual development
All live offspring were observed for the detachment and unfolding of pinna, incisor eruption and eyelid separation and assessed for reflexological response to stimuli by assessing surface righting reflex on Day 1 *post partum* and air righting reflex on Day 17 *post partum*. Pupillary reflex and auditory startle response were performed on Day 21 *post partum*.

All selected F1 offspring were observed for sexual development and the bodyweight for each individual animal at the time of sexual maturation was recorded. In addition, the ano-genital distance was recorded for all F2 generation offspring on day 1 post partum

Sacrifice and pathology
All surviving adult females and surviving offspring, except offspring selected to form the F1 generation, as well as surviving males were sacrificed on Day 21 post partum.
All adult animals and offspring, including those dying during the study, were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded. For females the uterine implantation sites were counted. In addition, the corpora lutea of all ovaries from pregnant females were counted at necropsy.

The following organs of F0 males and females from each dose group that were sacrificed at the end of the study sampled, weighed and preserved, except for the thyroids, which were weight after fixation:
adrenals, brain, left cauda epididymis, epididymides, kidneys, liver, ovaries, prostrate, pituitary, seminal vesicles (with coagulating gland and fluids), spleen, testes, thymus, thyroid glands, and uterus (with cervix and oviducts).
The following organs from one male and one female offspring from the F0 and F1 pairings were weighed: brain, spleen, thymus, and uterus.

The following tissues were preserved from all F0 males and females from each dose group in 10 % buffered formalin, except for the right epididymis, right testis, which were fixed in Bouins fluid and 70% IMS: adrenals, coagulating gland, right epididymis, ovaries, right testis, pituitary, prostrate, seminal vesicles, Uterus (with oviducts) and cervix, vagina and all gross lesions.

A detailed histopathological examination was performed on all sampled tissues from all F0 and F1 control and high-dose animals, and on animals that died or were killed in extremis.
During the histopathological examination there were indications of treatment-related changes in the adrenal glands for the F1 animals. Thus, the microscopic examination was subsequently extended to include similarly prepared sections of adrenals from the F1 animals from the 5000 and 1500 ppm dose groups.

Semen assessment
At necropsy of adult F0 and F1 males at least 200 individual sperms were evaluated for motility, motility characteristics, and morphology. In addition, samples of the testis and cauda epididymis of the control and high dose animals were homogenised and examined for homogenisation resistant spermatids.

Evaluation of the oocyte number
From ten control and ten high dose females of the F1 generation slides of the ovaries were prepared and analysed for visible oocytes. The identified oocytes were classified as small, medium or large follicles.

Statistics
Organ weight (absolute and relative to terminal bodyweight), weekly bodyweight gain, litter weights and offspring bodyweights were assessed for dose response relationships by linear regression analysis, followed by one way analysis of variance (ANOVA) incorporating Levene's test for homogeneity of variance. Where variances were shown to be homogenous, pair wise comparisons were conducted using Dennett's test. Where Levene's test showed unequal variances the data were analysed using non-parametric methods: Kruskal-Wallis ANOVA and Mann-Whitney 'U' test.
The non-parametric methods were also used to analyse implantation loss, offspring sex ratio and developmental landmarks and reflexological responses.
Probability values (p) are presented as follows:
$p < 0.001$ ***
$p < 0.01$ **
$p < 0.05$ *
$p \geq 0.05$ (not significant)

Histopathology data were analysed using the following methods to determine significant differences between control and treatment groups for the individual sexes:
1. Chi-squared analysis for differences in the incidence of lesions occurring with an overall frequency of one or greater.
2. Kruskal-Wallis one-way non-parametric analysis of variance for the comparison of severity grades for the more frequently observed graded conditions.
Probability values (p) were calculated as follows:
$p < 0.001$ +++ --- ***
$p < 0.01$ ++ -- **
$p < 0.05$ + - *
$p < 0.1$ (+) (-) (*)
$p \geq 0.1$ N.S. (not significant)
(+)-signs indicate positive differences from the control group, and (-)-signs indicate negative differences. * refer to overall differences between group variation which is non-directional.

Results and discussion

Analysis of dose formulations

Stability analyses indicated that the dose preparations at nominal concentrations of 1500, 5000 and 15000 ppm were stable for at least six weeks at ambient temperature.

Analyses for homogeneity at the start of treatment indicated that the dose preparations were homogeneous.

Analyses for achieved concentration performed on ten separate occasions demonstrated that the prepared dietary admixture concentrations given to the animals were in the range of 83 to 102% of the nominal concentration.

Test compound intake

The group mean achieved dosages are summarised in Table B.6.6-1 below.

Table B.6.6-1: Group mean achieved dose levels

Group	**Dietary concentration**	**Estimated dose level**	**Mean achieved dose level**			
	(ppm)	(mg/kg bw/day)	(mg/kg bw/day)			
			Males	Females		
				Maturation	Gestation	Lactation
Control	0	0	0	0	0	0
Low	1500	75	104	126	108	252
Intermediate	5000	250	351	423	358	808
High	15000	750	1063	1273	1109	2520

Mortality

There were no test substance related mortalities.

Four unscheduled deaths occurred during the study. In the F0 generation one male of the low dose group and one female of the mid dose group was killed on humane reasons on Days 87 and 103, respectively. The male exhibited a mass of about 3 x 4 cm on the lower jar. The female was in extremis following a suspected prolonged parturition. One high dose female was found dead on Day 97 possibly due to complications during parturition.

In the F1 generation one control female was killed on Day 99 following severe clinical signs (pallor of the extremities, lethargy, pilo-erection, hunched posture and staining around the ano-genital region); however the aetiology of the signs was not established.

Clinical observations

No treatment-related clinical signs of toxicity were noted. Clinical signs observed in control and treated animals of the F0 and F1 generation are summarised in Table B.6.6-2 and Table B.6.6-3 below. These signs were considered unrelated to the test substance, since they were either commonly seen in laboratory rats, or caused by physical injury, or occurred in control and treated rats.

Table B.6.6-2: Observed clinical signs in F0 generation

Clinical sign	**Number of rats affected in dose group***							
	Control (0 ppm)		Low (1500 ppm)		Mid (5000ppm)		High (15000 ppm)	
	♂	♀	♂	♀	♂	♀	♂	♀
Abrasion to dorsal region	2/28	0/28	0/28	0/28	0/28	0/28	1/28	0/28
Generalised fur loss	5/28	5/28	3/28	5/28	2/28	6/28	2/28	3/28
Red/brown staining around snout	4/28	0/28	4/28	0/28	1/28	3/28	5/28	0/28
Red/brown staining of fur	1/28	0/28	1/28	0/28	2/28	2/28	1/28	0/28
Red/brown staining around eyes	1/28	0/28	1/28	1/28	0/28	0/28	3/28	0/28
Swollen face (due to overgrowth tooth)	1/28	0/28	0/28	0/28	0/28	0/28	0/28	0/28
Cranial abrasion	0/28	0/28	0/28	0/28	0/28	0/28	2/28	0/28
Red stained urine	0/28	0/28	0/28	0/28	0/28	0/28	1/28	0/28
Facial scab formation	1/28	0/28	0/28	0/28	1/28	0/28	0/28	0/28
Scab formation	1/28	0/28	1/28	0/28	1/28	0/28	0/28	0/28
Large mass under lower jar	0/28	0/28	1/28	0/28	0/28	0/28	0/28	0/28
Mass on dorsal region	0/28	0/28	0/28	0/28	1/28	0/28	0/28	0/28
Scab formation around right eye	0/28	0/28	0/28	0/28	1/28	0/28	0/28	0/28
Physical injury to tail apex	0/28	0/28	0/28	1/28	0/28	0/28	0/28	0/28
Stained fur on head	0/28	0/28	0/28	0/28	0/28	0/28	0/28	1/28
Red swollen ears	0/28	/28	0/28	0/28	0/28	1/28	0/28	1/28
Blood seen without evidence of offspring born	0/28	1/28	0/28	0/28	028	0/28	0/28	0/28
Blood around vagina (suspected prolonged parturition, killed in extremis)	0/28	0/28	0/28	0/28	0/28	1/28	0/28	0/28
Pilo-erection	0/28	0/28	0/28	0/28	0/28	0/28	0/28	1/28
Exophthalmia	0/28	/28	0/28	0/28	0/28	0/28	0/28	1/28

* x/y: number affected / total number of animals in group

Table B.6.6-3: Observed clinical signs in F1 generation

Clinical sign	**Number of rats affected in dose group***							
	Control (0 ppm)		Low (1500 ppm)		Mid (5000ppm)		High (15000 ppm)	
	♂	♀	♂	♀	♂	♀	♂	♀
Generalised fur loss	3/28	4/28	0/28	2/28	0/28	6/28	0/28	4/28
Red/brown staining around eyes	2/28	1/28	0/28	0/28	1/28	0/28	0/28	0/28
Red/brown staining of fur	0/28	1/28	2/28	0/28	0/28	2/28	1/28	1/28
Red/brown staining around snout	1/28	7/28	1/28	0/28	4/28	7/28	1/28	4/28
Scabbing and fur loss around eye	0/28	/28	0/28	/28	0/28	/28	1/28	/28
Protruding sternum	0/28	2/28	0/28	3/28	0/28	3/28	0/28	0/28
Lethargy	0/28	1/28	0/28	0/28	0/28	0/28	0/28	0/28
Hunched posture	0/28	1/28	0/28	0/28	0/28	0/28	0/28	0/28
Staining around ano-genital region	1/28	0/28	0/28	0/28	0/28	0/28	0/28	0/28
Pallor of extremities	1/28	0/28	0/28	0/28	0/28	0/28	0/28	0/28

* x/y: number affected / total number of animals in group

Body weight

No adverse effect of body weight change was evident for treated animals in comparison to controls throughout the treatment period for both the F0 and F1 generations except for *post-partum* females treated with 15000 ppm (see Table B.6.6-4). During the final week of

lactation, both the F0 and F1 generations showed statistically significant less body weight loss in comparison to controls ($p < 0.001$ and $p < 0.01$ respectively).

Table B.6.6-4: Body weight changes during lactation (Group mean values)

Dietary concentration (ppm)	No. of animals		Body weight Change (g) at Day			
			4	7	14	21
			F0 Generation			
0 (Control)	26	mean	15	22	0	-23
		sd	14	9	15	10
1500	27	mean	16	16	3	-26
		sd	9	9	13	13
5000	26	mean	16	18	1	-23
		sd	14	13	11	11
15000	26	mean	18	18	1	-8***
		sd	11	12	14	14
			F1 Generation			
0 (Control)	26	mean	14	9	9	-16
		sd	11	13	14	13
1500	27	mean	14	16	3	-21
		sd	7	11	9	17
5000	26	mean	17	10	5	-17
		sd	12	10	13	13
15000	26	mean	16	11	10	-4**
		sd	9	9	12	13

sd - standard deviation
** - significantly different from control group $p < 0.01$
*** - significantly different from control group $p < 0.001$

Water consumption
Daily visual inspection of water bottles showed no overt intergroup differences in water intake for treated males and females from the F0 or F1 generations, when compared to their concurrent controls.

Reproductive parameters
Oestrus cycle
There were no toxicologically-significant effects on female oestrous cycles.

Mating Performance, Fertility and Gestation
There were no treatment-related effects on mating performance, fertility and gestation length for both F0 and F1 generation animals.

Litter data
Size and Viability
No overt differences in litter size and viability were detected. The mean numbers of corpora lutea and subsequent number of implantations did not indicate any adverse effect of dietary exposure and pre and post implantation loss for treated animals were essentially similar to controls. There were no toxicologically significant differences in sex ratio for both F0 - F1 and F1 - F2 litters.

Growth and Development
No adverse effects on mean offspring bodyweights, bodyweight change or development were detected for male and female offspring in comparison to their controls.

Clinical signs
No clinically observable signs of toxicity were observed for offspring from treated animals.

Pathology
Necropsy
There were no toxicologically significant macroscopic abnormalities detected in the F0 and F1 animals, nor in the offspring.

Organ weights
F0 females treated with 15000 ppm displayed statistically significant increases in liver weights, both absolute and relative to terminal body weight ($p < 0.001$). An increase in liver weights was also noted for F1 females treated with 15000 ppm (absolute: $p < 0.05$, relative: $p < 0.01$). In the absence of any histopathological changes in the liver, and as increased liver weights without histopathological changes were also noted in another repeated dose toxicity study this finding is considered as an adaptive response rather than an adverse effect. Furthermore, F0 females treated with 15000 ppm displayed an increase in kidney weights, both absolute ($p < 0.001$) and relative to terminal body weight ($p < 0.01$) (see Table B.6.6-5). No such observations were detected for males treated with 15000 ppm from either generation.

Table B.6.6-5: Liver and kidney weights (relative and absolute) of females (Group mean values)

Dietary concentration (ppm)	**No. of animals**		**Organ weight (g)**			
			Liver		Kidney	
			Absolute	Relative	Absolute	Relative
			F0 Generation			
0 (Control)	26	mean	15.0328	4.3103	2.4315	0.6977
		sd	1.0493	0.2864	0.1706	0.0548
1500	27	mean	15.1465	4.3027	2.5395	0.7233
		sd	1.4948	0.3435	0.1602	0.0560
5000	27	mean	15.8791	4.3570	2.5654*	0.7062
		sd	1.7649	0.2810	0.2361	0.0592
15000	26	mean	16.9704***	4.6806***	2.7096***	0.7490**
		sd	1.7620	0.2977	0.2203	0.0521
			F1 Generation			
0 (Control)	22	mean	16.4887	4.5970	2.6792	0.7483
		sd	2.0275	0.4038	0.4137	0.1070
1500	23	mean	16.3848	4.6047	2.5777	0.7257
		sd	1.7744	0.2858	0.2776	0.0647
5000	24	mean	17.2591	4.6543	2.8124	0.7585
		sd	2.0969	0.3628	0.5326	0.1229
15000	23	mean	18.0724*	4.9591**	2.7660	0.7578
		sd	1.2434	0.3130	0.2616	0.0517

sd - standard deviation
* - significantly different from control group $p < 0.05$
** - significantly different from control group $p < 0.01$
*** - significantly different from control group $p < 0.001$

There were no toxicologically significant intergroup differences detected for the brain, spleen or thymus for offspring of either sex from either generation. Furthermore, there were no differences in uterus weights for treated females from either generation when compared to controls.

Sperm assessment
There were no toxicologically significant effects on the concentration, motility or morphology of samples of sperm from treated F0 and F1 generation males when compared to their controls. Furthermore, no abnormal sperm were detected in the control and treated males from either generation.

Oocyte assessment
There were no toxicologically significant differences in follicle numbers for F1 females treated with 15000 ppm when compared to controls.

Histopathology
No treatment-related changes were detected in the F0 generation animals.

In the F1 generation cortical vacuolation of the adrenal glands was observed with a lower incidence and with generally lower grades of severity among males treated with 15000 ppm ($p < 0.05$), 5000 ppm ($p < 0.05$ - 0.01), and 1500 ppm ($p < 0.1$ - 0.05) when compared to controls. The group distribution of incidence and of severity grades may also suggest a consequence of treatment. However, the absence of a dose-related response, may suggest that a higher than normal background incidence of the condition among control male rats may have contributed to the effect on this occasion.

Table B.6.6-6: Incidence of adrenal cortical vacuolation in males at terminal kill

	Historical control data	**Dietary concentration (ppm)**							
		0		1500		5000		15000	
Generation	--	F0	F1	F0	F1	F0	F1	F0	F1
Animals examined	234	28	24	27	24	28	24	28	24
adrenal cortical vacuolation									
Absent	153	20	7	--	14	--	16	16	14
Present	81	8	17	--	10*	--	8**	12	10***
Minimal	57	6	10	--	6	--	6	8	7
Slight	23	2	7	--	4	--	2	4	2
moderate	1	0	0	--	0	--	0	0	1
% present	34.6	28.6	71%	--	24	--	33	42.9	42

* - significantly different from control group $p < 0.1 – p < 0.05$
** - significantly different from control group $p < 0.01 – p < 0.05$
*** - significantly different from control group $p < 0.05$

All remaining morphological changes were those commonly observed in laboratory maintained rats of the age and strain employed and, since there were no differences in incidence or severity between control and treatment groups, all were considered to be without toxicological significance.

Conclusion by the Notifiers

The oral administration of glyphosate technical to rats by dietary admixture at a maximum dose level of 15000 ppm for two successive generations resulted in possible treatment-related changes at 15000 ppm. The effects however were considered not to represent an adverse health effect, therefore the NOAEL was considered to be 15000 ppm (equivalent to 1063 and 1634 mg/kg bw/day, for males and females, respectively) for adult toxicity for both the F0 and F1 generations.

The NOAEL for reproductive and developmental toxicity, for both generations and offspring was considered to be 15000 ppm.

Comment by RMS:

The study is considered acceptable. The NOAEL for parental, reproductive and offspring toxicity is considered to be 5000 ppm (approx. equivalent to 351 mg/kg bw/d) instead the proposal by the Notifiers being 15000 ppm (> 1000 mg/kg bw/d): At highest dose level of 15000 ppm increased organ weights in liver (F0 & F1 females) and kidneys (F0 females) were observed. The Notifiers stated that there is no toxicological concern regarding the significant increased liver weights due to the absence of any histopathological changes in the liver. In fact, in the present study no evidence for histopathological examination of the liver was given.

*At this high dose level a significant decrease in homogenisation resistant spermatids (HRS, cauda epididymis) was counted in F0 males (Control: 399.9 million/gram; 15000 ppm: 309.0 million/gram**). No remarkable effects were seen at lower dose levels.*

Furthermore, in F1 male offsprings sexual maturation (preputial separation) was delayed at 15000 ppm without any additional developmental retardation (e.g. body weight, please see Table B.6.6-7 *below*). *The authors of the study considered this finding in F1 males (45.9 d versus control 43.0 d) to be unrelated to treatment, because no effects on sexual maturation were evident for females and there were no differences in mating performance. Sperm changes and histopathological examinations did not reveal any changes in the testis or epididymes. Although, the later onset of preputial separation in male offsprings at 15000 ppm had obviously no impact on reproductive performance in week 29, a treatment related effect on sexual maturation at parental toxic dose cannot be excluded.*

All in all, the NOAEL of 5000 ppm (ca. 351 mg/kg bw/d) is considered to be more appropriate regarding parental, reproductive and offspring toxicity.

Table B.6.6-7: ***Sexual Maturation (balano preputial separation) of F1-males (provided by RMS)***

Diet concentration (ppm)	*Number of animals*		*Age (Days) at completion*	*Body weight (g) at attainment*
0 (Control)	*24*	*mean*	*43.0*	*210*
		sd	*2.3*	*23*
1500	*24*	*mean*	*43.3*	*216*
		sd	*1.6*	*22*
5000	*24*	*mean*	*43.5*	*219*
		sd	*2.3*	*22*
15000	*24*	*mean*	*45.9***	*230***
			3.1	*28*

*** p<0.01*

Reference:	IIA, 5.6.1/02
Report:	[redacted] (2000) Glyphosate acid: Multigeneration reproduction toxicity study in rats [redacted] Data owner: Syngenta, Monsanto Report No.: [redacted]/P/6332 Date:2000-06-16 not published TOX2000-2000
Guidelines:	OECD 416, Annex V 67/548/EEC, 9.ATP 87/302/EEC OJEC, L133, 47-50 (1988), US-EPA OPPTS 870.3800 (1998)
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment.*

Dates of experimental work: 1998-09-01 to 1999-12-10

Materials and methods

Test material:	
Identification:	Glyphosate acid, technical
Description:	White solid
Lot/Batch #:	Y04707/082
Purity:	97.6% (w/w)
Stability of test compound:	At least 10 years at ambient temperature
Vehicle and/ or positive control:	Plain diet
Test animals:	
Species:	Rat
Strain:	Alpk:AP$_f$SD (Wistar-derived)
Source:	[redacted]
Age:	At least 5 weeks old
Sex:	Males and females
Weight at dosing:	Males: approx. 160 g; females: approx. 140 g
Acclimation period:	At least 14 days
Diet/Food:	CT1 diet (Special Diet Services Ltd., Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Rats were house in pairs (same sex) in multiple rat racks (with rats of the same group in adjacent cages). During mating animals were house one male : one female. Mated females were housed individually during gestation and lactation and provided with bedding material. After day 29 females separated from their litter were house in pairs until termination. Males were housed up to four per cage after being used for mating.

Environmental conditions:	Temperature:	22 ± 3 °C
	Humidity:	50 ± 20 %
	Air changes:	at least 15/hour
	12 hours light/dark cycle	

Study design and methods:
In life dates: not reported

Animal assignment and treatment:
In a two-generation reproduction study groups of 26 Alpk:AP_fSD rats per sex of the F0 generation received daily dietary doses of 0, 1000, 3000 and 10000 ppm glyphosate acid in diet. The dose levels were chosen based on results of a previously conducted chronic toxicity study.
After 10 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis, to produce the F1 litters. On Day 29 *post partum*, groups of twenty-six male and twenty-six female offspring from each dose group of the F0 generation were selected to form the F1 generation. F0 males were terminated after the completion of littering and females were terminated on or soon after Day 29 of lactation. Unselected offspring were terminated at Day 29 *post partum*. The offspring selected for the F1 generation were dosed for at least 10 weeks and then paired within each dose group to produce the F2 litters. F2 litters were weaned off on Day 29 *post partum* and terminated thereafter.

Diet preparation and analyses
For preparation of diet mixtures (60 kg) a known amount of the test substance was mixed with a small amount of basal diet in a mortar using a pestle. Further milled diet was added to give a pre-mix of 1000 g. Each pre-mix was grounded at a constant speed for 15 min with an automatic pestle and mortar. This pre-mix was then added to a larger amount of basal diet and blended for further 6 minutes in a Pharma Matrix Blender Model PMA 150S (TK Fielder). Control diet was treated in the same way but without addition of the test substance. The stability and homogeneity of the test material in diet were determined in the lowest and the highest dose. Dietary admixtures were analysed for achieved concentration at a 2 month interval.

Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily.

Body weight
Individual body weights were recorded for F0 adults immediately prior to treatment and weekly thereafter throughout the pre-mating period. F0 males were weighed weekly thereafter until termination. Successfully mated F0 females were weighed on Day 1, 5, 8, 15 and 22 of gestation and on Day 1, 5, 8, 15, 22 and 29 *post partum*. Initial body weights for the F1 adults were recorded at selection on Day 29 *post partum* and weekly thereafter throughout the pre-mating period. F1 males were weighed weekly thereafter until termination. Successfully mated F1 females were weighed on Day 1, 5, 8, 15 and 22 of gestation and on Day 1, 5, 8, 15, 22 and 29 *post partum*. All rats were weighed at termination.

Food consumption and compound intake
Food consumption for each cage was recorded throughout the pre-mating period and calculated on a weekly basis. Food utilisation was calculated as the bodyweight gained by the

rats in the cage per 100 g of food eaten. Food consumption was also recorded for females during gestation and lactation and calculated on a weekly basis.

Reproduction parameters
Oestrus cycle
Prior to pairing of females for the F0 and F1 mating phases, a vaginal smear was taken daily for twenty-one days and examined microscopically to determine the stage of oestrous. A vaginal smear was also taken and examined from all F0 and F1 females at termination.

Reproductive performance
The success of mating (production of viable litter) was established. Length of gestation was measured in days from the date of the positive smear to the date of birth. Pre-coital interval was measured as the number of days from the date of pairing to the date of the positive smear.

Litter data
The following litter data were recorded:
The number of offspring born and the number of offspring alive were counted within 24 h after parturition and thereafter on Day 5, 8, 15, 22 and 29 *post partum*. The sex and the litter weight was also recorded at these times. Any clinical findings were recorded. Litters were examined for dead or moribund pups at least once daily.

Physical and sexual development
All selected F1 offspring were observed for sexual development and the bodyweight for each individual animal at the time of sexual maturation was recorded.

Sacrifice and pathology
All surviving adult females and surviving offspring, except offspring selected to form the F1 generation, were sacrificed on Day 29 post partum. Males were sacrificed at completion of the littering. All adult animals and offspring, including those dying during the study, were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded. For F0 and F1 females the uterine implantation sites were counted.

The following organs of F0 males and females from each dose group that were sacrificed at the end of the study were sampled, weighed and preserved:
adrenal gland, brain, left and right epididymides and caudae, kidneys, liver, ovaries, prostrate, pituitary, seminal vesicles (with coagulating gland and fluids), spleen, testes, uterus (with cervix and oviducts).
The following organs from one male and one female offspring from the F1 pairings were weighed: brain, spleen and thymus.
The following tissues were preserved from all F0 males and females from each dose group in 10% buffered formalin, except for the left epididymis, left testis, which were fixed in Bouins fixative: adrenals, brain, coagulating gland, left epididymis, ovary, left testis, pituitary, prostrate, seminal vesicle, uterus (with oviducts) and cervix, vagina and all gross lesions.

Beside all pups killed in extremis (age 18-29 days) 3 male and 3 female per F2-litter were given a macroscopic examination at termination on Day 29 *post partum*. One of the 3 pups/sex/litter was used for organ weight determination as described above. Following tissues were stored from these pups: brain, spleen, thymus, salivary gland. Abnormal tissue from all these pups were taken and fixed as described earlier.

The reproductive organs from animals suspected of reduced fertility were processed for histopathological examination.

Semen assessment
At necropsy of adult F0 and F1 males sperm were taken from the right distal cauda epididymis. At least 200 individual sperms were evaluated for motility, motility characteristics, and morphology. In addition, samples of the right testis of the control and high dose animals were homogenised and examined for homogenisation resistant spermatids.

Evaluation of the oocyte number
Primordial and small growing follicles were quantified in the left ovary of all F1 females from the control and high dose groups. Quantification was done using five 5 µm thick sections cut from the central third of each ovary and taken at least 100 µm apart and as evenly spaced as possible.

Statistics
One or a combination of the following statistical methods were applied for the evaluation of the measured parameters: analyses of variance (ANOVA), analyses of covariance, ANOVA followed by analyses of covariance, as well as ANOVA following the double arcsine transformation of Freeman and Tukey (1950), or ANOVA following a square root formation, or Fisher's Exact Test.
All analyses were carried out in SAS (1996). For Fisher‘s Exact Tests the proportion in each treated group was compared to the control group proportion. Analyses of variance and covariance, with the exception of pup organ weights, allowed for the replicate structure of the study design.
Least-squares means for each group were calculated using the LSMEAN Option in SAS PROC MIXED. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a Student‘s t-test, based on the error mean square in the analysis.
All statistical tests were two sided.

Results and discussion
Analysis of dose formulations
The chemical stability of glyphosate acid in the diet at nominal concentrations of 1,000 and 10,000 ppm was consistent for at least 6 weeks (at room temperature). Homogeneity of the test substance in the dietary mixture was satisfactory, percentage deviations from the overall mean were within 4 %. The mean achieved concentrations of glyphosate acid in the preparations were within 9 % of the nominal concentrations and the overall mean concentrations were within 3 % of the nominal concentrations.

Test compound intake
The group mean achieved dosages are summarised in Table B.6.6-8 below.

Table B.6.6-8: Group mean achieved dose levels F0 and F1-generation

Group	**Dietary concentration**	**Mean achieved dose level**	
	(ppm)	(mg/kg bw/day)	
		Males*	Females

			Maturation	Gestation	Lactation
Control	0	0	0	0	0
Low	1000	108.0 *[99.4 / F0]* *[116.5 / F1]*	113.9	90.4	227.9
Intermediate	3000	322.2 *[292.6 / F0]* *[351.8 / F1]*	346.8	277.9	752.4
High	10000	1072.9 *[984.7 / F0]* *[1161.0 / F1]*	1136.2	910.9	2424.8

** Values of achieved dose levels, separated in F0 and F1 were inserted by the RMS*

Mortality
There were no test substance related mortalities.
Seven unscheduled deaths occurred during the study. In the F0 generation one control male was killed for humane reasons during week 9 because it was found to have a ruptured eyeball. In the low level dose group one female was killed for humane reasons during week 14 having failed to litter on time, dead foetuses were present in the uterus. In the intermediate level dose group one female was killed in week 14 on gestation day 23 due to difficulties with parturition. In the high-level dose group one female with an imperforate vagina and one male having a subcutaneous mass were killed in week 15 and 18, respectively.
In the F1 generation two control animals were killed in extremis. One male due to an accidental injury in week 2 and one female in week 15 due to difficulties with parturition (one dead foetus present in uterus).

Clinical observations
No treatment-related clinical signs of toxicity were noted.

During the pre-mating period, annular constrictions were visible on the tails of the F0 and F1 male and female rats. Almost all males and approximately half of the females, in all groups, were affected. Scaly tail was also observed in some of the animals. These findings were considered incidental to the administration of glyphosate acid in the diet. Other recorded changes in clinical condition were either isolated occurrences or of an incidence comparable with that of the control group
These signs were considered unrelated to the test substance, since they were either commonly seen in laboratory rats, or caused by physical injury, or occurred in control and treated rats.

Body weight
There was no effect of glyphosate acid on body weight adjusted for initial weight for the F0 rats, males and females, during the pre-mating period. For the F1 males given 10000 ppm, body weight was slightly lower at week 1, in comparison with the control group. Thereafter, body weights adjusted for initial weight remained lower than the controls for the duration of the pre-mating period and were statistically significant different from week 2 through to week 8 (see Table B.6.6-9). There was no effect of 10000 ppm on the body weight of the Fl females and no effect of 3000 or 1000 ppm on the body weight of the F1 males or the F1 females (see Table B.6.6-9). There was no effect of glyphosate acid on body weight adjusted for initial weight for either the F0 or F1 rats during gestation or lactation.

Table B.6.6-9: Body weight during the pre-mating period-F1 generation (Group mean values)

F1 generation	**body weight (g)**							
	Control (0 ppm)		Low (1,000 ppm)		Mid (3,000ppm)		High (10,000 ppm)	
Week	♂ (n=25)	♀ (n=26)	♂ (n=26)	♀ (n=26)	♂ (n=26)	♀ (n=26)	♂ (n=26)	♀ (n=26)
1	80.2	74	81.1	75.2	78.1	74.2	75.3	73.4
2	130.1	115.4	132.3	115.7	128.6	114.7	127.6*	115.2
3	188.5	152.6	190.7	154.7	186.5	151.2	183.3*	152.3
4	246.2	178.3	247.6	180.2	242.8	176.5	237.3**	179.4
5	300.3	201	304.1	202.7	296.5	199.7	289.5**	202.1
6	345	219.8	347.5	224.1	334.5	217.2	328.7**	218.4
7	377.2	231.7	382.4	237.1	369	228.3	360.5**	234.4
8	403.6	241.9	410.1	245.1	395.3	237.2	387.0*	245.6
9	425	250.3	433.3	253.6	416.3	245.1	411.8	252.5
10	443.4	259.7	453.1	263.8	435.1	251.7	431.6	258.1
11	461.7	265.7	471.3	271.2	455.5	258.8	449.7	266.9

* - significantly different from control group $p < 0.05$
** - significantly different from control group $p < 0.01$

Food consumption
There was no effect of glyphosate acid on food consumption for the F0 generation, all F1 females and F1 males of the low and intermediate level dose group during the pre-mating period. Only F1 males of the high-level dose group showed significantly lower food consumption throughout the pre-mating period. There was no effect of glyphosate acid on food utilisation for the F0 generation, all F1 females and F1 males of the low and intermediate dose group during the pre-mating period. Food utilisation was slightly higher for F1 males given 10000 ppm glyphosate acid, the difference from control being statistically significant for weeks 5-8 only. There was no effect of glyphosate acid on food consumption for either the F0 or F1 rats during gestation or lactation.

Reproductive parameters
Oestrus cycle
There were no consistent toxicologically-significant effects on female oestrous cycles.

Mating Performance, Fertility and Gestation
There were no treatment-related effects on pre-coital interval, mating performance, and gestation length for both F0 and F1 generation animals.

Litter data
Size and Viability
No overt effects of glyphosate acid on pup survival or on litter size during lactation were detected.

In both generations the incidence of whole litter losses was low and similar across all groups. Glyphosate acid treatment did not affect the percentage of post-implantation loss. The proportion of F1A and F2A pups born live was slightly higher in the glyphosate acid groupsthan in the control group. There was no effect of glyphosate acid on litter size at birth or during the time of lactation for either the F1A or F2A pups. The proportion of litters with all pups surviving and the proportion of pups surviving during lactation were also unaffected by the treatment. An increased proportion of litters with all pups surviving noted for the F1A

litters in the 10000 ppm group in comparison with the control group were not present for the F2A litters since the F2A controls showed an improvement over the F1A controls. Sex distribution within the litters was not altered by the administration of glyphosate acid.

Growth and Development
There was no effect of glyphosate acid on pup weight at birth for the F1A or F2A pups. Thereafter, the bodyweights of the F1A pups in the 10000 ppm group were lower in comparison with the control group The differences from control were statistically significant for males from day 8 through to day 29 and for females, from day 5 through to day 29. A similar effect was neither observed for the F2A pups in the 10000 ppm group nor for the F1A pups of the low and intermediate dose level groups. There was no effect of glyphosate acid on total litter weight of either generation. Also the day of age when preputial separation or vaginal opening occurred in the F1 parents was unaffected by treatment.

Clinical signs
No clinically observable signs of toxicity were noted for offspring from treated animals.

Pathology
Necropsy
No macroscopic findings that could be attributed to the treatment with glyphosate acid were observed in any animal of the F0 and F1 generation.

The incidence of unilateral pelvic dilatation was slightly higher (9/69) in F2A females in the 10,000 ppm group compared with the other groups. Unilateral pelvic dilatation is a very common spontaneous change in the Alpk:APfSD strain of rat. There was no increase in incidence in the F0 or F1 adults or in the F1A pups and, as an isolated observation, it is considered incidental to treatment with glyphosate acid.

Organ weights
The treatment of rats with glyphosate acid did not affect the weight of the adrenal glands, brain, right cauda epididymis, epididymides, kidney, liver, ovary, pituitary gland, prostate gland, spleen, seminal vesicles, testes or uterus. For the F0 males given 10000 ppm glyphosate acid, liver and kidney weights adjusted for bodyweight were statistically significantly greater than in the control group. Similar changes were not observed in the F1 males given 10000 ppm glyphosate acid. Absolute and relative values were comparable with the control group (see Table B.6.6-10). The weight changes seen in the liver and kidney of the F0 males were therefore considered not to be treatment related. For the F0 males given 3000 or 10000 ppm glyphosate acid, brain weight adjusted for bodyweight was statistically significantly greater than in the control group. Absolute values were comparable with the control group (see Table B.6.6-10). Similar changes were not observed in the F1 animals. The weight changes seen in the brain of the F0 males were therefore considered to be incidental to treatment.

Table B.6.6-10: Liver, kidney and brain weights (relative and absolute) of males (Group mean values)

Dietary concentration (ppm)	No. of animals		Organ weight (g)					
			Liver		Kidney		Brain	
			Absolute	Relative	Absolute	Relative	Absolute	Relative
			F0 Generation					
0 (Control)	25	mean	19.3	3.4	3.20	0.57	2.11	0.38
		sd	2.6	0.2	0.38	0.04	0.09	0.03
1000	26	mean	19.1	3.5	3.17	0.58	2.12	0.39
		sd	2.3	0.2	0.36	0.04	0.08	0.03
3000	26	mean	18.7	3.5	3.11	0.58	2.12	0.40
		sd	1.9	0.2	0.27	0.03	0.07	0.03
10000	25	mean	19.7	3.6	3.23	0.59	2.13	0.40
		sd	2.7	0.2	0.38	0.04	0.07	0.05
			F1 Generation					
0 (Control)	25	mean	21.4	3.7	3.42	0.6	2.11	0.37
		sd	2	0.3	0.31	0.05	0.07	0.02
1000	26	mean	21.4	3.7	3.45	0.59	2.12	0.37
		sd	3.3	0.4	0.37	0.04	0.07	0.02
3000	26	mean	20.1	3.6	3.32	0.6	2.1	0.38
		sd	2.6	0.3	0.31	0.04	0.07	0.03
10000	26	mean	19.7*	3.6	3.36	0.62	2.1	0.39
		sd	2.3	0.3	0.28	0.04	0.07	0.03

sd - standard deviation
* - significantly different from control group $p < 0.05$

There was no effect of glyphosate acid on brain, spleen or thymus weight.

For the F1A female pups in the 10000 ppm group absolute thymus weight was statistically significantly lower than in the control group. There was no effect of glyphosate acid on the thymus weight of the F2A pups. The observation in the F1A females is therefore considered incidental to treatment with glyphosate acid.

Sperm assessment
In F0 and F1 males no effect of glyphosate acid on the number of sperm, sperm motility parameters or sperm morphology was observed.

Oocyte assessment
There was no effect of 10000 ppm glyphosate acid on the number of primordial and small growing follicles in the left ovary of the F1 parent animals.

Histopathology
No treatment-related changes were detected in the F0 and F1 generations.

Conclusion by the Notifiers
The oral administration of glyphosate acid to rats by dietary admixture at a maximum dose level of 10,000 ppm for two successive generations of the Alpk:APfSD rat resulted in possible treatment-related changes at 10,000 ppm, where a reduction in the bodyweight of the F1A pups in the 10000 ppm group with a subsequent reduction in bodyweight of the selected F1 parent males for the duration of the pre-mating period was observed. Therefore the 'No Observed Adverse Effect Level' (NOAEL) was considered to be 3000 ppm (equivalent to 322

and 459 mg/kg bw/day for males and females, respectively) for maternal and offspring for both the F0 and F1 generations.

Comment by RMS:
The study is considered acceptable and the evaluation is confirmed. Addtionally, up to 10000 ppm over to successive generations no effect on the sexuality and fertility was observed in males and females.
Furthermore, the NOAEL for parental and offspring toxicity was considered to be 3000 ppm, which has to be converted into 293 mg/kg bw/d (mean daily intake of glyphosate during pre-mating phase in F0 males.

Comment by GTF on the first draft of the RAR (July 2013):
The GTF suggested that the appropriate NOAEL value should be based on 3000 ppm in F1 males, 352 mg/kg/day, and not on the achieved dose in the parental males of 293 mg/kg/day as proposed by the RMS.

RMS comment (August 2013):
The NOAEL 3000 ppm (293 mg/kg bw/d) was set for both offspring and parents (F1) on the basis of a reduction in body weight of F1 pups and a subsequent reduction in body weight of in F1 males. The dose of 352 mg/kg bw/d was only achieved in F1 males during pre-mating period (please refer to Table B.6.6-8).

Reference:	IIA, 5.6.1/03
Report:	[redacted] (1997) HR-001: A two-generation reproduction study in rats [redacted] Data owner: Arysta Life Sciences Study No.: IET 96-0031 Date: 1997-06-19 not published ASB2012-11495
Guidelines:	OECD 416 (1981), US-EPA FIFREA Guidelines Subdivision F (1984), Japan MAFF Guideline 59 NohSan No. 4200 (1985)
Deviations:	None
GLP:	yes
Acceptability:	*See RMS comment*

Dates of experimental work: 1996-04-16 to 1997-03-31

Materials and methods

Test material:	
Identification:	Glyphosate technical, Code: HR-001
Description:	White crystal
Lot/Batch #:	T-950308
Purity:	94.61 % (w/w)
Stability of test compound:	Not reported
Vehicle and/ or positive control:	Plain diet
Test animals:	
Species:	Rat
Strain:	Sprague-Dawley; Crj:CD (SD)
Source:	[redacted]
Age:	5 weeks
Sex:	Males and females
Weight at dosing:	Males: 132 - 148 g; females: 112 - 126 g
Acclimation period:	7 days
Diet/Food:	Certified pulverized feed (MF Mash, Oriental Yeast Co., Ltd), *ad libitum*
Water:	Filtered, sterilized well water, *ad libitum*
Housing:	During acclimatisation in groups of five per sex in suspended wire-mesh stainless steel cages). During pre-mating, and mating periods animals were housed in groups o 3/sex/cage. During mating one male and one female were housed in aluminium cages with wire-mesh floors and fronts. Mated females were housed individually during gestation and lactation and provided with bedding material. After day 29 females separated from their litter were house in pairs until termination. Males were housed up to four per cage after being used for mating.
Environmental conditions:	Temperature: 22 ± 2 °C Humidity: 55 ± 10 % Air changes: 15/hour 12 hours light/dark cycle

Study design and methods:
In life dates: 1996-04-16 - 1997-03-31

Animal assignment and treatment:
In a two-generation reproduction study groups of 24 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 0, 1200, 6000 and 30000 ppm HR-001 in diet. The dose levels were chosen based on results of a preliminary reproductive study in Crj:CD (SD) rats..
After 10 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis, to produce the F1 litters. The day of proved copulation was

designated Day 0 of gestation. Copulated females were placed individually into breeding boxes with nestle material. The day of completed parturition was designated Day 0 of lactation. On Day 4 *post partum*, litter sizes were reduced to a maximum of 8 pups, preferable to 4 males and 4 females, and the remaining pups were culled. Weaning was done on Day 21 of lactation and all F0 parental animals were sacrificed. Groups of 24 male and 24 female offspring from each dose group of the F0 generation were selected to form the F1 parents. Unselected offspring were sacrificed and subjected to a gross necropsy.

The offspring selected for the F1 generation were dosed for 10 weeks and then paired within each dose group to produce the F2 litters. F2 litters were weaned on Day 21 of lactation and terminated together with F1 parental animals. F1 parental rats which failed to produce F2 offspring (10 males and 10 females with normal external genitalia and oestrus cycle) were mated with untreated rats of the same strain and sacrificed thereafter for fertility assessment (reproductive performance).

Diet preparation and analyses
Diets were prepared monthly during the pre-mating period, and biweekly during the breeding period. For each dose level a specified amount of the test substance was mixed with a small amount of basal diet in a mortar. This pre-mix was stirred into the remaining part of the diet. The diets were stored at about 4 °C in the dark. Analyses for homogeneity were done for each dose level of the first diet preparation. Analyses for achieved concentration were done for all prepared diets.

Clinical observations
A check for clinical signs of toxicity and mortality was made once daily on all F0 and F1 parental animals. A detailed physical examination was performed on males prior to treatment, and weekly during pre-mating and breeding periods and at necropsy. Females were examined prior to treatment, weekly during pre-mating periods and on gestation days 0, 7, 14 and 20, and on days 0, 7, 14 and 21 of lactation, and at necropsy.

Body weight
Individual body weights F0 and F1 males adults were determined prior to treatment, and weekly during pre-mating and breeding periods and at necropsy. F0 and F1 females were weighed prior to treatment, weekly during pre-mating periods and on gestation days 0, 7, 14 and 20, and on days 0, 7, 14 and 21 of lactation, and at necropsy.

Food consumption and compound intake
Food consumption for each cage was recorded and daily food consumption was calculated. Determination of food consumption was made on a weekly basis during the pre-mating period for males and females and during the breeding period for males. In addition, for females total food consumption was determined at the following intervals: Day 0-7, 7-14, 14-20 of gestation and of days 0-7, 7-14 and 14-21 of lactation.
Compound intakes in parental animals were calculated during the pre-mating periods for each sex on a weekly basis.

Reproduction parameters
Oestrus cycle
The oestrus cycle was checked daily by microscopically examination of vaginal smears. Examinations were done for each female for one week prior to mating until copulation was confirmed.

Reproductive performance
Mating indices for males and females were calculated separately after copulation was confirmed. In addition, fertility and gestation indices, the length of gestation, as well as the number of implantation sites were determined.

Sperm assessment
An assessment of motility and morphology of epididymal sperm was done at necropsy for 10 males per group, which were selected for the organ weight measurement, as well as for males that failed to impregnate females.

Litter data
Total number of live and dead pups, and the number of males and females per litter were determined on Day 0 of lactation. The sex ratio was calculated for each group. Viability indices, were determined for each litter on lactation days 0, 4 and 21. Body weights were determined on lactation days 0, 4, 7, 14 and 21.
A check for clinical signs of toxicity and mortality was made once daily during the lactation period on all F1 and F2 pups. A detailed physical examination was done on lactation days 0, 4, 7, 14 and 21.

Sacrifice and pathology
All surviving parental F0 and F1 males and females were sacrificed on Day 21 post partum and subjected to a gross pathological examination. Animals of all generations that died, were found dead or were killed moribund during the study period were necropsied as soon as possible. The following organs and tissues were preserved: adrenals, aorta, brain, caecum, colon, duodenum, epididymis, eyes, gross lesions, head (incl. nasal cavity, paranasal sinuses, buccal mucosa and ears), heart, ileum, jejunum, kidneys, larynx, liver, lung, mammary gland, oesophagus, ovaries, pancreas, pharynx, pituitary, prostrate, rectum, seminal vesicles, spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus (cornua and cervix) and vagina.

F1 and F2 pups that were not selected on Day 4 of lactation were also killed and necropsied on that day. In addition, F1 weanlings that were not selected for parental animals of the F1 generation and all F2 weanlings were necropsied at 22-26 and 21-26 days of their age, respectively. The same organs, as described above, were preserved from one animal per sex per litter of the F1 and F2 weanlings necropsied.

The following organs weights of 10 F0 and F1 males and females from each dose group that were sacrificed at the end of the study, as well as from pairs of parental animals that failed to mate: adrenal gland, brain, epididymides, kidneys, liver, ovaries, prostrate, pituitary, seminal vesicles (with coagulating gland and fluids), testes, uterus.
A histopathological examination was performed on the reproductive organs and pituitary of the control and high dose F0 and F1 parental animals that survived until scheduled termination. A histopathological examination of the reproductive organs and pituitary in the

low and mid-dose group was only performed on pairs of animals that had failed to produce offspring.
In addition, a histopathological examination was performed on organs with significant weight change, and on all organs with gross pathological changes.

Statistics
One or a combination of the following statistical methods were applied for the evaluation of the measured parameters: Bartlett´s test for equality of variance (p=0.05) followed by parametric analyses of variance in one-way classification (p=0.05) or Dunnett´s t-test or Scheffé ´s multiple comparison test (p=0.05, 0.01 or 0.001); or Bartlett´s test followed by Kruskal-Wallis test (P=0.05) and Dunnett-type mean rank test or Scheffé-type mean rank test ((p=0.05, 0.01 or 0.001). Fisher´s exact probability test ((p=0.05, 0.01 or 0.001) and Mann-Whitney´s U-test (p=0.05 or 0.01) were also used.

Results and discussion
Analysis of dose formulations
Based on the results of the dose-finding study the chemical stability of the test substance in the was given for 5 weeks (at room temperature) in sealed plastic bags in the dark, and for at least 2 weeks after being released from the plastic bags.
Homogeneity of the test substance in the dietary mixtures was satisfactory, percentage deviations from the overall mean were within 4%. The mean achieved concentrations of HR-001 in the diet preparations were in the range of 90 – 105 % of the nominal and therefore acceptable.

Mortality
F0 and F1 males
Seven unscheduled deaths occurred during the study. In the F0 generation one control male was killed for humane reasons during week 9 because it was found to have a ruptured eyeball. In the low level dose group one female was killed for humane reasons during week 14 having failed to litter on time, dead foetuses were present in the uterus. In the intermediate level dose group one female was killed in week 14 on gestation day 23 due to difficulties with parturition. In the high-level dose group one female with an imperforate vagina and one male having a subcutaneous mass were killed in week 15 and 18, respectively.
In the F1 generation two control animals were killed in extremis. One male due to an accidental injury in week 2 and one female in week 15 due to difficulties with parturition (one dead foetus present in uterus).

F0 and F1 females
There were no mortalities observed during the study period.

Clinical observations
F0 and F1 males
There were no treatment-related clinical signs observed in the 1200 and 6000 ppm groups.
At 30000 ppm F0 and F1 parental males exhibited loose stool with incidences during the pre-mating growth and breeding periods of 3/24 and 2/24 for the F0 generation, and of 13/24 and 0/24 for the F1 generation, respectively, with a significant difference in the value for the pre-mating growth period of the F1 generation. Since this finding was not observed in other groups including control, defecation of loose stool was considered to be treatment-related.
Statistically significant differences were also observed in the incidence of hair loss during the breeding period for F0 males in all test substance groups. However, the occurrence of this

change in the treated groups was rather lower than controls, and was considered to be incidental.
During the study period, one F0 male and one F1 male in the control group and one F1 male in the 6000 ppm group showed malocclusion of the incisors, respiratory wheezing, and red sebum. The aforementioned one F1 male of the 6000 ppm group also showed distension of the abdomen. These animals were euthanatised within several days after discovery due to unfavourable prognosis. Necropsy noted a fracture of the facial bones in all cases, suggesting that the alterations were caused by an accident in the cage. Accident malocclusion of incisors was also observed in one F1 male in the 1200 ppm group. However, test substance treatment of this animal was continued until termination of the study because its condition was improved.

F0 and F1 females
There were no treatment-related clinical signs observed in the 1200 and 6000 ppm groups.
In F0 and F1 parental females, loose stool was also observed at 30000 ppm. The incidences during the pre-mating growth period and the lactation and post-weaning period were 1/24 and 6/24 for the F0 generation, and 4/24 and 2/24 for the F1 generation, respectively, with a significant difference in the value for the lactation and post-weaning period of the F0 generation.

Bodyweight
F0 and F1 males
Mean body weights of F0 and F1 males in the 30000 ppm group were consistently lower than those in the control group from treatment week 1 to the day of necropsy, and the differences from controls at treatment weeks 1-12 and 14 for the F0 generation, and treatment weeks 1-6 for the F1 generation were statistically significant. In the 1200 and 6000 ppm groups, mean body weights of F0 and F1 parental males were comparable to the controls throughout the study.

F0 and F1 females
There were no significant differences in mean body weights of F0 females in any treatment group when compared to control. In F1 females in the 30000 ppm group, mean body weight on lactation day 0 was significantly higher than that in the control group. In the 1200 and 6000 ppm groups, mean body weights of F1 parental females were comparable to the controls throughout the study.

Food consumption and test compound intake
F0 and F1 males
In F0 males, mean food consumption at treatment week 13 in the 1200 ppm group was significantly higher than that in the control group. Since there was no such increase observed in the mid- and high-dose groups throughout the study, this change was not thought to be treatment-related.
In F1 males in the 30000 ppm group, mean food consumption at treatment week 4 was significantly lower than that in the control group, but the values on the other treatment weeks in this dose group were comparable to the controls. In the 1200 and 6000 ppm groups, mean food consumption of F1 males was comparable to the controls throughout the study.

F0 and F1 females
In F0 females, the values on treatment weeks 2-4 in the 30000 ppm group were significantly higher than the controls. Inversely, the value on lactation days 7-14 in this dose group was

significantly lower than those in the control group. So it was unclear these changes were treatment-related or not. In the 1200 and 6000 ppm groups, mean food consumption of F0 females was comparable to the controls throughout the study.
In F1 females in the 1200 and 6000 groups, mean food consumption on lactation days 14-21 were significantly higher than those in the control group. However, these changes were thought to be incidental because no such increase was observed in the highest dose group. In the 30000 ppm group, mean food consumption of F1 females was comparable to the controls throughout the study.

The group mean achieved dosages are summarised in Table B.6.6-11 below.

Table B.6.6-11: Group mean achieved dose levels F0 and F1-generation

Group	**Dietary concentration**	**Mean achieved dose level**			
	(ppm)	(mg/kg bw/day)			
		Males		Females	
		F0	F1	F0	F1
Control	0	0	0	0	0
Low	1200	83.6	91.7	96.9	104.8
Intermediate	6000	417	458	485	530
High	30000	2150	2411	2532	2760

Reproductive parameters
F0 males and females
Reproductive performance of F0 parental animals was not adversely affected by test substance treatment, and no significant differences were observed in such parameters as percentage of females having normal oestrous cycle, mating index, fertility index, gestation index, duration of gestation, number of implantation sites , and number, motility and morphology of epididymal sperm between the control group and the treated groups.

F1 males and females
In F1 parental animals, reproductive parameters in the treated groups were also comparable to the controls with the exception of gestation index and number of implantation sites, on which some biases were occasionally observed.
The significant higher number of implantation sites at 1200 ppm when compared to control was considered to be unrelated to treatment, since there was no increase noted at 6000 and 30000 ppm.
A similar bias was also found in the fertility index. The fertility indices in the control, 1200, 6000 and 30000 ppm groups were 95.8 (23/24), 95.8 (23/24), 87.5 (21/24) and 79.2% (23/24), respectively, with somewhat low values in the 2 higher dose groups. However, these decreases were considered to be incidental because the differences between the control and treated groups were not statistically significant, and because, as described below, normal reproduction results were obtained in the F1 parental animals, which had failed to produce offspring in this study, after remating with untreated animals.

Among the total of ten F1 females mated with untreated males, only one female in the 30000 ppm group did not undergo pregnancy. Histopathological of this female showed no abnormalities in the reproductive organs and pituitary. So the cause of infertility of this female was not known. The other nine F1 females were proved to have normal reproductive performance. One F1 male in each of the 1200, 6000 and 30000 ppm groups could not successfully impregnate untreated females mated. These 3 males had histopathological

abnormalities in the testes and epididymides, and abnormalities in the sperm parameters, as a cause of infertility. However, the other 7 males were proved to have normal reproductive performance. Thus, the majority of F1 males and females which had failed to produce offspring were proved to have normal reproductive performance.

Litter data
Number of pups delivered
Mean number of F1 and F2 pups delivered in the 1200, 6000 and 30000 ppm groups were comparable to the controls.

Sex ratio
Sex ratios of F1 and F2 pups in the 1200, 6000 and 30000 ppm groups were comparable to the controls.

Viability index
The viability indices of F1 and F2 pups in the 1200, 6000 and 30000 ppm groups were comparable to the controls.

Body weights
F1 pups
There were no effects on mean body weight noted in the low- and mid-dose group when compared to controls. F1 pups of both sexes in the 30000 ppm group, showed significantly higher mean body weights on lactation day 0 than the controls. However, mean body weights on days 14 and 21 were significantly decreased when compared controls.

F2 pups
There were no effects on mean body weight noted in the low- and mid-dose group when compared to controls during the lactation period. In F2 pups in the 30000 ppm group, mean body weights of both sexes on day 21 of lactation were significantly lower than those in the control group.

Clinical signs
There were no treatment-related abnormalities noted in F1 and F2 pups of any dose group.

During the lactation period, deaths and loss due to maternal cannibalism occurred in several pups in all groups including the control. However, the incidences in the treated groups were comparable to the control.

Pathology
Necropsy
F0 and F1 generation
Necropsy of parental animals of both sexes noted several findings in all groups including the control group. Among these alterations, the incidences of distension of the caecum in F0 and F1 males and females of the 30000 ppm group were significantly higher than those of the controls, and were considered treatment-related. Statistically significant differences from controls were also found in the incidences of hair loss in F0 males of the 1200, 6000 and 30000 ppm groups. However, the values were rather lower than controls and were considered to be incidental. Other findings were low in their incidences and considered not treatment-related.

F1 and F2 pups
Necropsy of stillbirths found on lactation days 0, pups found dead during lactation days 1-4, and pups killed to reduce the litter size on lactation day 4 demonstrated no treatment-related abnormalities in any of the F1 and F2 pups.
During days 5-21 of lactation, only 2 F1 pups in the 1200 ppm group were found dead. Necropsy of these dead pups were not performed due to advanced autolysis.
Necropsy of F1 and F2 weanlings in the 30000 ppm group noted distension of the caecum, suggesting a treatment-related occurrence. In the 1200 and 6000 ppm groups, no treatment-related abnormalities were observed in any of the F1 and F2 weanlings.

Organ weights
F0 and F1 males:
There were no effects in the absolute and relative organ weights in F0 and F1 males of the low- and mid-dose groups. At 30000 ppm relative weights of the liver and kidneys of F0 and F1 males were significantly higher than the control values. These increases were considered treatment-related. In F1 males in the high-dose group, there was also a significant decrease noted in the absolute and relative weights of the prostate. Besides these changes, the relative brain weight of F0 males in the 30000 ppm group was significantly higher than the control value. However, this finding was considered to be the change associated with the low body weights in this group.

F0 and F1 females
In F0 females, the absolute and relative weights of all organs were comparable between the control and treated groups. In F1 females in the 30000 ppm group, the absolute and relative weights of the liver and kidneys were significantly higher than the controls, and these increases were considered treatment-related.
Significantly higher-than-control value was also observed in the absolute kidney weight in the 6000 ppm group. However, this increase was not considered treatment-related because statistical significance in the difference between the control and 6000 ppm groups disappeared when all F1 females were subjected to the weighing of the kidneys fixed in 10 % neutral buffered formalin. The significant lower relative ovarian weight observed in F1 females in the 1200 ppm group was considered to be an incidental finding because no such decrease was observed in the mid- and high-dose groups.

Histopathology
F0 and F1 generations
In all F0 and F1 males and females in the 30000 ppm group, histopathological examinations of the reproductive organs and pituitaries did not indicate any treatment-related alterations.

No treatment-related histopathological alterations were also evident in the following organs in which significant weight changes were detected: kidneys of F1 females in the 6000 ppm group; kidneys of F0 males and F1 males and females in the 30000 ppm group; and liver of F1 males and females in the 30000 ppm group.

Conclusion by the Notifiers
The oral administration of HR-001 to rats by dietary admixture at a maximum dose level of 30000 ppm for two successive generations of Sprague-Dawley rat resulted in maternal toxicity at 30000 ppm. Thus, the NOAEL for maternal toxicity is 6000 ppm, equivalent to 417 - 458 mg/kg bw/day and 485 - 530 mg/kg bw/day for males and females, respectively.

The NOAEL for reproduction is 30000 ppm, since the reproductive performance was not affected in any dose group. Based on the body weight effects and increased incidences of caecum distension the NOAEL for offspring is considered to be 6000 ppm.

Comment by RMS:
The study is considered acceptable. Parental toxicity was observed at highest dose of 30000 ppm (> 2000 mg/kg bw/d) only and consisted of loose stool (F0/F1, m/f), reduced body weight (F0/F1, m) caecum distension (F0/F1, m/f), increased liver and kidney weights (F0/F1, m/f), decreased prostate weight (F1). Histopathological alterations were not detected.
Lower gestation indices were observed at mid and high dose level, however without statistical significance. Indeed, most of the F1 animals were proved to have normal reproductive performance after re-mating with untreated animals, but this is not in accordance with current test guidelines: re-mating should be performed with treated males of the same dose group. The NOAEL for reproductie toxicity is considered 6000 ppm (417 mg/kg bw/d) based on lower gestation indices of F1 females at high dose level.
~~*Reproductive toxicity was not observed up to the highest dose level, despite lower gestation indices of F1 females at mid dose of 6000 ppm (530 mg/kg bw/d) and high dose level. This finding was considered not to be treatment related, because most of the F1 animals which failed to produce offspring were proved to have normal reproductive performance after re-mating with untreated animals.*~~ *Offspring toxicity was observed at highest dose level only and confined to reduced body weight and caecum distension in both sexes. Sexual maturation (preputial separation, vaginal opening) was not examined in this study. Based on the results the NOAEL for parental and offspring toxicity was considered to be 6000 ppm and for reproductive toxicity to be 30000 ppm (>2000 mg/kg bw/d).*

Reference:	IIA, 5.6.1/04
Report:	[redacted] (1993) Two Generation Reproduction Study in Wistar Rats. [redacted] Data owner: ADAMA Agan Ltd Study No.: TOXI 885-RP-G2 Date: 1993-08-27 GLP: yes not published TOX9300009
Guidelines:	OECD 416 (1983)
Deviations:	None
GLP:	yes
Acceptability:	*See RMS comment*

Dates of experimental work: May 1991 - April 1992 (not further specified)

Materials and methods

Test material:

Identification:	Glyphosate technical
Chemical name	N-(Phosphonomethyl) glycine

Description:	Odourless, white crystal
Batch #:	60
Purity:	96.8 %
Date of receipt	11/9/1990
Stability of test compound:	More than two years at ambient temperature
Vehicle and/ or positive control:	Plain diet
Test animals:	
Species:	Rat
Strain:	Wistar rats (Random bred)
Source:	[redacted]
Age at start of treatment (F0):	8 weeks
Sex:	Males and females
Mean body weight at initiation of dosing:	Males: 160 - 190 g; females: 141 - 160 g
Acclimation period:	7 days
Diet/Food:	Standard "Gold Mohur" brand powdered rat feed manufactured by M/s Lipton India Limited, Bangalore, India
Water:	Deep bore well water passed through activated charcoal filter and exposed to UV rays (Aquaguard on-line water filter cum-purifier manufactured by M/s Eureka Forbes Limited, Bombay, India) was provided in glass bottles *ad libitum*
Housing:	Groups of five/three rats of same sex per cage depending on the size of the animals were accommodated in standard polypropylene rat cages (size: L 430 x W 270x H 150 mm) with stainless steel top grill; bedding material (paddy husk) was changed three times per week.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 40-70 % Air changes: 10-15/hour 12 hours light/dark cycle

Study design and methods:
In life dates: May 1991 to April 1992 (not further specified)

Animal assignment and treatment:
In a two-generation reproduction study groups of 30 Wistar rats per sex of the F0 generation received daily dietary doses of 0, 100, 1000 and 10000 ppm glyphosate technical in diet.
After at least 8 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis, to produce the F1 litters. The day of proved copulation (vaginal smear) was designated Day 0 of gestation. On Day 4 *post partum*, litter sizes were reduced to a maximum of 8 pups, preferable to 4 males and 4 females, and the remaining pups were culled. Weaning was done on Day 21 of lactation and all F0 parental animals were

sacrificed. Groups of 30 male and 30 female offspring from each dose group of the F0 generation were selected to form the F1 parents.
The offspring selected for the F1 generation were paired within each dose group to produce the F2 litters. F2 litters were weaned on Day 21 of lactation and terminated together with F1 parental animals.

Diet preparation and analyses
The required quantities of test compound were weighed and mixed manually with 1.0 kg of powdered rat feed to prepare the premix. The premixes were added to the bulk of remaining quantities of feed and mixed in ribbon mixer. Prepared feed bulks were sampled at different intervals for assaying test compound concentration in experimental diet.

Clinical observations
All animals were observed daily throughout the study and any visible clinical signs were recorded with details on type, severity, time of onset and duration. Any animal found dead or sacrificed *in extremis* was necropsied and macroscopically abnormal tissues were retained.

Body weight
Males were weighed weekly until termination. Females were weighed weekly during pre-mating, on Gestational Days 0, 6, 13, and 20 and on Days 1, 4, 7, 14 and 21 of lactation.
Offspring were weighed sex-wise as litters on Days 1, 4, 7, 14 and 21 *post partum*.

Food consumption and compound intake
Food consumption for each cage of males was recorded weekly until termination. Food consumption of females was recorded weekly during pre-mating and at the following intervals: Days 0-6, 6-13, 13-20 of gestation and Days 1-4, 4-7, 7-14 and 14-21 of lactation.

Reproduction parameters
Reproductive performance
The following reproductive indices were recorded: Male and female fertility index, fecundity index, mean number of implantations, parturition percentage, percentage mortality of pregnant dams, percentage of live pups born, in females the pre-coital interval (time elapsed between initial pairing and detection of mating) and duration of gestation.

Litter data
Total number of live and dead pups, viability indices (mean viable litter size on day 0, live birth index), litter weight, individual sex and observations on individual pups (if any) were determined within 24 hours after birth. Survival indices were determined on Days 2, 4, 7, 14 and 21 of lactation. Body weights were determined on Lactation Days 0, 4, 7, 14 and 21. A check for clinical signs of toxicity and mortality was made once daily during the lactation period on all F1 and F2 pups. On Day 4 *post partum*, offsprings were culled to reduce litter size to eight.

Sacrifice and pathology
All surviving parental F0 and F1 males and females and the non-selected weanlings from F1 and all F2 weanlings were sacrificed and subjected to a gross pathological examination. Tissue collection was done for parent generation only. Animals of all generations that died, were found dead or were killed moribund during the study period were necropsied as soon as possible.

The following organs and tissues were preserved from all F0 and F1 parents of all groups: Ovaries, uterus, vagina, testes, epididymides, seminal vesicles, prostate, coagulation glands, pituitary, adrenals, liver and kidneys. The organs were examined for gross pathological changes and those found abnormal were examined histopathologically.
Females failing to get mated within 21 days and females failing to produce a viable litter by Day 25 *post coitum* were necropsied and any macroscopically abnormal tissue was retained for histopathological examination. The presence of corpora lutea, implantations and resorptions was examined in females which had failed to produce a viable litter.
On Day 4 *post partum*, offsprings were culled to reduce litter size to eight, where possible; culled offspring or found dead were necropsied. All F2 pups were sacrificed at weaning.

Statistics
One or a combination of the following statistical methods were applied for the evaluation of the measured parameters: Dunnett´s t-test (for body weight, food consumption, litter number, litter weight, gestation and lactation period), Z Test (for mating performance, fertility index, gestation index, live birth index, viability index, lactation index, pups survival data, number of dead pups at birth, survival indices, number littered) and t/r test (for dose-response relationship).

Results and discussion
Analysis of dose formulations
In-house stability study for glyphosate technical was carried out at 0, 2000 and 20000 ppm. Chemical stability was given for 30 days at room temperature with a loss of less than 7 % at 0, 2000 and 20000 ppm levels in experimental diet when stored in polyethylene lined stainless steel drums.
The mean achieved concentrations of glyphosate in the diet preparations were analysed; the achieved concentrations were in the range of 96-100 % of the nominal and therefore acceptable.

Mortality
F0 and F1 males
There were no deaths in male animals.

F0 females
In the females there were three deaths, two in the low dose group, (one dystokia and one suppurative pneumonia) and one in the high dose group (cause of death not ascertained).

F1 females
One dam in low dose group died of dystokia and no other mortalities were seen.

Clinical observations
F0 generation
Nasal discharge and snuffling and cannibalism were seen in all groups. No other treatment related changes in clinical signs were observed.

F1 generation
The incidence of clinical signs was low and not treatment or dose related.

Body weight
F0 males
Initial body weight of treatment groups was higher compared to the control group and this trend continued during the entire treatment period. The absolute weight gain (difference between initial and terminal) during entire treatment period was similar to control group in low and high dose while in mid dose it was slightly higher.

F0 females
No significant treatment related differences were noted between treated and control groups.

F1 males
Mid dose group body weight (both initial and subsequent weeks) was more than control. In high dose group initial body weight (Week 0) was higher than control but at Week 2 and 3 it was less. However in this group the body weight tended to be higher (not significant) during last seven weeks.

F1 females
The body weight of all treatment groups at selection (Week 0) was higher than in the control group and continued to be significantly higher than in the control group for up to Week 10 in mid and high dose groups. Body weights of the high dose group dams on Days 0, 6 and 13 of gestation period were significantly higher compared to controls but the body weight gain was statistically not significantly different. Another incidental significant finding was higher body weight (Gestational Day 0-20) of mid dose group dams compared to controls. Absolute body weight of mid dose group on Lactation Days 1 and 4 and that of high dose group during all periods of lactation was significantly higher than in control group. The mid dose group had lost body weight during Days 7-14, 14-21 and 1-21 of lactation period as compared to control.

Food consumption and test compound intake
F0 parents
Mean food consumption of males was comparable to the controls throughout the study. High dose female animals tended to consume significantly more food than controls during gestation. During lactation low and mid-dose females consumed significantly less than controls, especially for the Periods 7-14 and 14-21. High dose females consumed significantly more food for Lactation Days 4-7 as compared to controls.

F1 males
Treatment groups did not show consistent and dose related changes as compared to control group. However initially (Weeks 0-2) mid and high dose groups consumed significantly less feed and later on a few occasions mid dose group showed increased consumption.

F1 females
Treatment group dams did not show treatment and dose related consistent difference from control group; on a few occasions the treatment groups showed both increased/decreased food consumption over control. During gestation there was no statistically significant inter group difference in feed consumption between control and treatments during gestation period. Low dose dams consumed significantly less food than controls during different lactation periods (except for Day 7 and Period 7-14). Mid and high dose group dams did not show any treatment and dose related changes over control except for an incidental finding of increased and decreased feed consumption on Day 7, 14 and Period 7-14 and 14-21 respectively in mid dose group.

Reproductive parameters
Reproductive performance parameters of F0 parental animals such as female fertility index, number of implantations, gestation index, duration of gestation, live birth index, and duration of gestation were not significantly different between treated and control groups. Male fertility index was significantly higher in low and high dose groups over control.

F0 generation
On Day 1 of lactation, mean litter size was significantly less than control in low and mid dose groups and the mean viable litter size at birth was significantly less in low dose group; the number of live pups on Day 1 was significantly lower in the mid-dose group.

F1 generation
Reproductive performance parameters of F1 parental animals such as male and female fertility index, fecundity index, parturition percentage and mortality of pregnant dams was not different between treatment and control groups. The incidence of dams not littered tended to be higher in the mid-dose group compared to controls. A significantly decreased number of implantations was observed in low and mid dose groups; the percentage of live pups born was significantly reduced in the in mid dose group and significantly increased in the high dose group.

Table B.6.6-12: Reproductive parameters of F0- and F1-generation

	Group 1 - control		**Group 2**		**Group 3**		**Group 4**	
	0 ppm		100 ppm		1000 ppm		10000 ppm	
	F0	F1	F0	F1	F0	F1	F0	F1
Number of dams in group	30	30	30	30	30	30	30	30
Number of dams littered	29	26	26	27	28	22	30	28
Mean litter size	11.3	11.7	9.8*	10.4	9.9*	10.9	10.4	11.9
Mean viable litter size at birth	11.0	11.7	9.7*	10.4	9.9	10.9	9.9	11.9
Number of pups alive on day 1	320	305	253	281	276*	239	296	334
Mean number of implantations	12.1	13.4	11.2	11.6*	11.0	12.0*	12.3	12.9
Percentage of live pups born [%]	87.9	87.6	83.5	86.5	86.5	79.7*	80.0*	92.8**
*significantly decreased; **significantly increased								

Litter data
Number of pups delivered
Mean number of F1 and F2 pups delivered and mean litter sizes in the 100, 1000 and 10000 ppm groups were comparable to the controls.

Sex ratio
Sex ratios of F1 and F2 pups in the 100, 1000 and 10000 ppm groups were comparable to the controls.

Viability index
F1 pups
In the low dose group the pup survival index for Days 4, 14 and 21 was significantly lower than in controls. In the mid dose group the live birth index and Day 14 survival index were higher and Day 4 survival index was lower compared to controls. In the high dose group on Day 14 and 21 survival index was higher than in controls. Dose response relationship was not seen in these parameters.

F2 pups
There were no statistically significant inter group differences between control and treatment groups in parameters of F2 litters at first observation including incidence of external abnormalities in pups. The mean number of pups (combined and individual sex) during different periods of lactation did not show statistically significant differences compared to control group.
The group mean values of pup survival data parameters like: live birth index, 24 hours survival index and survival index for Days 4, 7, 14 and 21 did not show any significant inter group difference between control and treatment groups.

Body weights
F1 pups
Mean litter weight of combined sex and female pups in treatment groups were significantly more than control group on Day 1 and 4, respectively. On Day 7 combined sex litter weight and male pup weight was significantly less than control in low dose group while in high dose group it was more than control group. On Day 21 the mean body weight of complete litter and individual sex pups of mid dose group were more than control group. None of these showed any apparent dose response relationship.

F2 pups
Combined sex litter weight on day one and that of female pups of all treatment groups was higher than in controls; in addition combined sex litter weight in low and mid dose groups and that of male and female in mid dose group was higher than control on Day 4. In high dose group the male pup body weight on Day 14 and 21 was lower than control. None of these parameters showed any dose response relations.

Clinical signs
There were no treatment-related abnormalities noted in F1 and F2 pups of any dose group.
During the lactation period, deaths and loss due to maternal cannibalism occurred in several pups in all groups including the control. However, the incidences in the treated groups were comparable to the control.

Pathology
Necropsy
F0 generation
The gross pathological lesions seen were consolidated lungs with ecchymoses, chronic liver changes, kidneys with cysts and dilated pelvis, and hypoplastic testes (1 in the control group, 2 in the mid-dose and 1 in the high-dose group). The incidence was low and did not appear to be compound or dose related.

F1 generation
The gross pathological lesions seen were consolidated and collapsed lungs with emphysema, hydronephrotic kidneys, and unilateral hypoplastic testes. The lesions observed were few and appeared to be incidentally. A single incidence of unilateral testicular hypoplasia was observed in each of the three treatment groups, hydronephrosis was seen in two animals in the high dose group.

F1 pups
A higher incidence of emaciated pups was recorded for the mid and high dose groups compared to controls. A low incidence of minor developmental abnormalities like Kinky tail, rudimentary tail, kidney hydro-nephrosis and dilated pelvis occurred without dose-response relation.

F2 pups
A higher incidence of emaciation has been observed in pups of high dose group. Occasional not treatment and dose related incidence of hydronephrosis and dilated pelvis in kidney have been recorded.

Histopathology
F0 generation
Reproductive organs showing gross pathological changes were recorded as outlined in the following: testes from one control animal, two mid dose and one high dose animal. The control and high dose animals showed degenerative changes in the seminiferous tubules while the mid dose group were normal. These changes appeared to be incidental and not compound related.

F1 generation
Reproductive organs showing gross pathological changes were recorded as outlined in the following: testes from one animal in each of the three treatment groups; the testes in the low and mid dose groups showed unilateral degenerative changes and giant cell formation in the seminiferous tubules and focal chronic inflammation. The testes in the high dose were normal though unequal in size. The changes appeared to be incidental and not compound related.

Conclusion by the Notifiers
The oral administration of glyphosate to rats by dietary admixture at a maximum dose level of 10000 ppm for two successive generations of Wistar-Dawley rats resulted in no maternal toxicity.
The NOAEL for reproduction is considered to be 10000 ppm, since the reproductive performance was not affected in a dose-related manner. The NOAEL for offspring is 10000 ppm, since no treatment-related effects on offspring could be observed.

Comment by RMS (Re-evaluation):
The previous evaluation in 2001 regarding the NOAELs is confirmed. However, this study is now considered supplementary, because an effect dose was lacking. The highest dose of 10000 ppm is considered to be the NOAEL for parental, reproductive and offspring toxicitiy. This dietary level would correspond to a mean daily compound intake of 700-800 mg/kg bw/d. [The mean daily intake was not reported for all dieatry levels, but for the low level of 100 ppm a corresponding average value of 7.7 mg/kg bw/d was given in the original report].

Reference:	IIA, 5.6.1/05
Report:	[redacted] (1981) A three generation reproduction study in rats with glyphosate [redacted] Data owner: Monsanto Study No.: 77-2063; BDN 77-417 Date: 1981-03-31 not published TOX9552385
Guidelines:	None (pre-guideline)
Deviations:	Not applicable
GLP:	no, pre-GLP
Acceptability:	*See RMS comment*

Dates of experimental work: 1978-06-13 to 1980-04-09

Materials and methods

Test material:	
Identification:	Glyphosate
Description:	Fine white powder
Lot #:	XHJ-64
Purity:	considered 100 % active ingredient for dosing preparations;
Stability of test compound:	Not reported
Vehicle and/ or positive control:	Plain diet
Test animals:	
Species:	Rat
Strain:	CD® (Sprague-Dawley derived)
Source:	[redacted]
Age at treatment initiation:	43 days
Sex:	Males and females
Mean weight at initiation of dosing:	Males: 139.9 - 144.3 g; females: 118.0 - 119.2 g
Acclimation period:	7 days
Diet/Food:	Standard laboratory diet (Purina Lab Chow® 5001), *ad libitum*

Water:	Automated watering system (Elizabethtown Water Company), *ad libitum*
Housing:	Individually (except during mating and lactation), in elevated stainless steel wire mesh cages; nesting material: Litter Kleen® hardwood shavings added to cages on Day 19 of gestation and changed when wet or soiled through Day 14 of lactation
Environmental conditions:	12 hours light/dark cycle No details on temperature and humidity reported

Study design and methods:
In life dates: 1978-06-14 - 1980-04-09

Table B.6.6-13: Study design

Group	**Dose level (mg/kg bw/day)**	**No. of adults initially assigned to mate F0, F1, F2**		**No. of matings per generation F0, F1, F2**	**Gross post-mortem examination**	**Histopathology of F0, F1 and F2 parents, F3b weanlings**	
		Males	Females			Male	Female
1 Control (plain diet)	0	12	24	2	All	10	10
2	3	12	24	2		none	none
3	10	12	24	2		none	none
4	30	12	24	2		10	10

Animal assignment and treatment:
In a three generation reproduction study groups of 12 male and 24 female CD rats received beginning 63 days prior to mating of the F0 generation daily dietary doses of 0, 3, 10 and 30 mg glyphosate/kg bw in diet. Diet samples were taken at four week intervals for analysis of achieved test substance concentrations.

Mating: One male and two females of equivalent dose levels were caged together nightly until a sign of mating (sperm and/or copulation plug in the vagina) was observed or until 15 days had elapsed with no evidence of mating. The day on which evidence of mating was observed was defined as Day 0 of gestation.

In this study, the first litters (F1a, F2a and F3a) from each mating were raised to weaning and discarded. Rats produced by the second matings (F1b and F2b) were selected to become parents of succeeding generations or to be subjected to complete gross necropsy (F3b).

Diet preparation and analyses
Diets were prepared weekly during the study and were adjusted on the basis of body weight and food consumption.

Clinical observations
A check for clinical signs of toxicity and mortality was made twice daily. A detailed physical examination was performed on adult generations at weekly intervals throughout the study.

Body weight
Body weights of all animals were determined weekly during growth and rest periods of all generations. Pregnant females were weighed on Days 0, 6, 15 and 20 of gestation and lactating females were weighed on Days 0, 4, 14 and 21 of lactation.

Food consumption and compound intake
Food consumption was recorded weekly during growth and rest periods of all generations. Test substance intake was calculated from individual body weight and food consumption data and reported as a group mean value for weekly intervals during the growth and rest periods of all generations.

Reproduction parameters
The day on which evidence of mating was observed was designated as Day 0 of gestation; the day of delivery was designated as Day 0 of lactation.
Mating indices, pregnancy rates, length of gestation and male fertility indices were recorded.

Litter data
Pups of all generations were examined daily for general appearance and mortality. On Days 0, 4, 14, and 21 they were counted to record the number of live and dead pups. Body weights were determined on Days 0, 4, 14, and 21 as a litter and on Day 21 individually.
Total number of live and dead pups, and the number of males and females per litter were determined on Day 0 of lactation. The sex ratio was calculated for each group on Days 0 and 21 of lactation. Viability indices, were determined for each litter on Lactation Days 0, 4 and 21.

Sacrifice and pathology
Animals of all generations that died, were found dead or were killed moribund during the study period were necropsied as soon as possible. All adult males and females were sacrificed after pup selection of the last Fb litter (F0, F1) and after last F3b litter weaned (F2) by lethal exposure to ether. Pups that were found dead or stillborn pups were weighed and given a gross post-mortem examination including internal sex determination, presence of milk in stomach. F1a, F2a, F3a and F3b animals were sacrificed at weaning, given a gross post-mortem examination and abnormal tissues were saved. F1b and F2b animals which were not selected as future parents were sacrificed after ensuing selection of parental animals, given a gross post-mortem examination and abnormal tissues were saved.

The following organs and tissues were preserved from all parents (F0, F1, F2) and from 10/sex/group of the F3b weanlings: adrenals, aorta, bone and bone marrow (sternal), brain, colon, duodenum, eyes with optic nerve and Harderian gland, gonads (ovaries and testes), heart, ileum, kidney (2), liver (2 sections), lung with main stem bronchi, lymph nodes (mesenteric), mammary gland (right inguinal), pancreas, pituitary, salivary gland, skeletal muscle (biceps femoris with right sciatic nerve), skin, spinal cord, spleen, stomach, thyroid/parathyroid, urinary bladder, uterus/prostate, gross lesions, tissue masses, thymus.
Microscopic examination of histological sections of these tissues were done for 10 male and 10 female animals from control and high-dose groups of F0, F1 and F2 parents and of F3b offsprings.

The following organs were weighed from all parents sacrificed after weaning of the second litters and from eighty F3b weanlings (10 males and 10 females per group): adrenals, gonads, kidneys, brain, spleen, liver, heart and pituitary.

All pups of the second litter of the F2 parents (F3b) were necropsied at weaning and specified tissues were preserved for selected animals in each group.

Statistics
Body weights, body weight gain, maternal body weights, food consumption, number of offspring, offspring body weights, terminal body weights and organ weight data (absolute and relative), offspring survival, litter survival, pup viability index at birth, mating indices, pregnancy rates and male fertility indices data were compared to the control. Statistically significant differences were evaluated using several methods including Dunnett's test, ANOVA, Barlett's test, Kruskal-Wallis test and Fisher Exact Test.

Results and discussion
Analysis of dose formulations
Not reported.

Mortality
F0 adults (2 dead females in mid-dose group)
In the F0 generation, no unscheduled mortality occurred in the control, low- or high-dose groups. One female of the mid-dose group died during on Lactation Day 20 of first litter having 13 live pups at time of death. A second female of the mid-dose group died on Lactation Day 7 of second litter; this female delivered eight pups - seven live and one dead - and all pups were dead at time of death. No mid-dose F0 male died.

F1 adults (1 dead female in mid-dose group, 1 dead female in high-dose group)
In the F1 generation, no unscheduled mortality occurred in the control or low-dose groups. In the mid dose group one female was killed in a moribund condition during the post-mating period for the second litter. This female had mated during the first mating but did not deliver a litter; during the second mating this female had not mated. No other mortality occurred in the mid-dose group. In the high dose group one female died due to an accident (animal was caught in the feeder jar). A second high-dose female died on Day 21 of gestation for the second litter; the uterus of this female contained 15 term foetuses. No other mortality occurred in the high-dose group.

F2 adults (1 dead female in low-dose group, 1 dead male in mid-dose group)
In the F2 generation, no unscheduled mortality occurred in the control or high-dose groups. In the low-dose group one female died during the F3a lactation period. This female delivered a litter containing only dead pups (13 pups) and died the day after parturition. No other mortality occurred in the low-dose group. In the mid-dose group one male was killed in a moribund condition during the period between mating of the first and second litters. This male had mated and impregnated both females during the first mating period. No other mortality occurred in the mid dose group.

Clinical observations
Clinical observation data were similar between the control and treated groups for each generation interval throughout the study. No adverse treatment effects were indicated.

Body weight
Mean body weight data during the growth and rest periods were comparable between the control and treated groups for each generation, throughout the study. Likewise, mean weight gain during the growth periods were comparable between these same groups for both sexes

throughout all generations. No treatment effect on body weight data during the growth and rest periods was evident.

Food consumption and test compound intake
Mean food consumption data were considered comparable between the control and treated groups (both sexes) during the growth and rest periods for each generation, throughout the study. No adverse effect of treatment on food consumption was evident throughout the study. Mean weekly test substance intake values ranged from 2.8 to 3.3 mg/kg bw/day for the low-dose group, from 9.5 to 11.2 mg/kg bw/day for the mid-dose group and from 27.7 to 33.1 mg/kg bw/day for the high dose group for all generations including both genders.

Reproductive parameters
Male and female mating indices and male fertility indices during both mating intervals of the F0 generation were considered comparable between the control and treated groups. During the second mating interval of the F0, pregnancy rates were lower than control in each of the treated groups; however, no indication of a dose-relationship was evident as the lowest pregnancy rate was seen in the mid-dose group. This reduction in pregnancy rate for the mid-dose group was not statistically significant. In the absence of a dose-response relationship the reduction in pregnancy rate during this mating interval (F1b) in the treated groups was not considered treatment-related.

In the F1 generation, mating indices (males and females) for both litter intervals were comparable between the control and treated groups. It is note-worthy that for both mating intervals of this generation, mating indices for control and some treated groups were lower than normally encountered in multi-generation studies. The reason for the poorer mating performance in this generation was unclear but no treatment effect was indicated since mating indices were lowest in the control group. Pregnancy and male fertility indices for the first mating interval of the F1 were comparable between the control and treated groups. During the second litter interval, pregnancy rates were lower than those seen for the first interval in control and treated groups. The lowest pregnancy rate was seen in the high-dose group; however, this difference from the control value was not statistically significant. Pregnancy rates for the low- and mid-dose groups, during the second mating interval, were considered comparable to control. Male fertility indices for this same mating interval were considered comparable between the control and treated groups.

In the F2 generation mating indices for the treated groups were lower than control for each mating interval. During the first mating interval of the F2 generation, the female mating indices were lower than control in each of the treated groups; however, only in the high-dose group was this difference from control statistically significant. The female mating index for the control group at this interval was 100 % which is higher than normally encountered. The female mating indices observed for the control group in this study have shown considerable variability ranging from 70.9 to 100 % The poor mating performance for the treated groups during the first mating interval is attributed to two males in each treatment group that did not mate either female in their mating unit (each mating unit was comprised of one male and two females).

During the second mating interval of the F2 generation, male mating performance improved in the mid- and high-dose groups as both mid-dose males and one of two high-dose males that did not mate during the first mating interval, mated and impregnated at least one female. Male mating indices for the low-dose group remained unchanged as the same two males that did not

mate during the first interval, failed to mate during the second interval. Pregnancy and fertility indices for the treated groups were comparable to control for both litter intervals of the F2 generation.

Mean gestation length was comparable between the control and treated groups for each pregnancy interval in each generation. Over the entire study no consistent, dose-related effect was seen in mating, fertility or pregnancy indices to indicate an adverse effect of treatment.

Litter data
Litter size
Mean litter size data on Day 21 of lactation (weaning) was comparable between the control and treated groups for each litter interval throughout the study.

Sex ratio
Pup sex distributions ratios at Day 0 and 21 were generally comparable between the control and treated groups for each litter interval for each generation. No adverse treatment effect on sex distribution data was evident.

Viability index
The mean numbers of live, dead and total pups at birth and pup viability at birth for each pregnancy interval, were comparable between the control and treated groups for each generation. The litter survival indices were comparable between the control and treated groups for each lactation interval in the F0, F1 and F2 generation. In the F0 generation, postnatal survival indices for Days 0-4 and 4-21 were comparable between the control and treated groups for the first lactation interval (F1a). For the second litter interval of the F0, postnatal survival indices for the Day 0-4 interval were comparable between the control and treated groups. During the Day 4-21 interval, survival indices were significantly lower than control in each treatment group. The increase in pup mortality during this interval (i.e. Days 4-21) was attributed to high pup mortality within one or more litters at each treatment level. In the low-dose group the lower pup survival was attributed to one female that experienced complete litter mortality (litter contained 14 live pups at Day 4). In the mid-dose group, one female died on Day 7 of lactation and all seven pups in her litter died during the Day 4-7 lactation interval. Additionally, three mid-dose litters lost five or more pups from their litters during the Day 4-21 lactation interval. In the high-dose group, one female lost nine of l2 pups during the Day 4-21 lactation interval.
In the F1 and F2 generations postnatal survival indices for Days 0-4 and 4-21 during both litter intervals were considered comparable between the control and treated groups. Some statistically significant differences in these indices were observed between the control and treated groups; however, no trend was evident through successive generations to indicate an adverse effect of treatment.

Body weights
Maternal body weights
Mean body weight data during the gestation and lactation intervals and mean weight change during these same periods were comparable between the control and treated group for each pregnancy interval from each generation throughout the study. No treatment effect was indicated in gestation - lactation body weight data throughout the study.

Offspring body weights
Mean pup body weight data during each litter interval for each generation were comparable between the control and treated groups. No adverse effects of treatment on pup weight data was evident.

Adult animals (F0, F1 and F2)
Mean terminal body weight data were comparable between the control and treated groups for both males and females throughout the study.

Pathology
Necropsy
F0, F1 and F2 generations
Gross necropsy of parental animals of both sexes did not indicate any adverse effect of treatment.

F1, F2 and F3 offspring
Gross post-mortem observations of offspring at weaning (F1a, F2a, F3a, F3b) or post-weaning (F1b, F2b) did not demonstrate an adverse effect of treatment. Likewise, evaluation of dead pups recovered at birth and during the 21-day lactation period did not note a treatment-related effect.

Organ weights
F0, F1 and F2 generations
Mean organ weight data (absolute and relative to body weights or brain weights) were comparable between the control and treated groups for both males and females from the F0 and F1 generations. Some statistically significant differences were noted between control and treated groups both in mean organ weight data and in the relative weight data; however, no trends were evident within dose levels or through these generations.
In the F2 generation, mean organ weight data (absolute and relative) for the males were comparable between the control and treated groups. In the F2 female group, mean liver/body weight ratios were significantly lower than control in each of the treated groups; however, no clear dose-relationship was apparent. Mean liver/brain weight ratios for the treated F2 females were lower than control; however, these differences from control values were not statistically significant. Mean spleen weights (absolute and relative to brain and body weights) were significantly higher than the control value in the F2 mid-dose female group; however, mean spleen weight data for the low- and high-dose F2 females were comparable to control values. In the absence of an effect on spleen weight in the high-dose Fg female group, the change seen in spleen weight data for the mid-dose females was considered spurious and not biologically meaningful. Other mean organ weight data (absolute and relative to body weight or brain
weight) for the treated F2 female groups were considered comparable to control data.

F3b offspring
Mean organ weight data (absolute and relative to body weights or brain weights) were comparable between the control and treated groups for both males and females. No treatment-related effect was evident in organ weight data for the F3b offspring.

Histopathology
In total 160 male and female rats (40 adults of each generation F0, F1 and F2 and 40 weanlings of F3b) were examined microscopically. No microscopic findings were considered treatment related. Proliferative tissue changes diagnosed as neoplasms were few. The microscopic tissue alterations, neoplastic and non-neoplastic, were indicative of common incidental histological findings.

Conclusion by the Notifiers
The oral administration of glyphosate to rats by dietary admixture at a maximum dose level of 30 mg/kg bw/day for three successive generations of CD rats resulted in no treatment-related signs of toxicity in parental animals. The NOAEL for reproduction is 30 mg/kg bw/day, since the reproductive performance was not affected in any dose group. The NOAEL for offspring is 30 mg/kg bw/day, since no adverse effects on offspring were observed.

Comment by RMS (Re-evaluation):
This study is now considered not acceptable due to the selected dose levels that were much too low. Accordingly, an effect dose was not reached (Evaluation in 2001 not confirmed).

Reference:	IIA, 5.6.1/06
Report:	[redacted] (1992) The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat. [redacted] Data owner: Cheminova Project no.: CHV 47/911129 Date: 1992-05-14 not published TOX9552389
Guidelines:	OECD 416 (1983), US-EPA FIFRA 83-4 (1982)
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: 1990-03-29 - 1991-03-22

Materials and methods

Test material:

Identification:	Glyphosate technical
Description:	White solid
Lot/Batch #:	206-Jak-119-1
Purity:	99.2 %
Stability of test compound:	Stable during the treatment period.
Vehicle and/ or positive control:	Plain diet

Test animals:	
Species:	Rat
Strain:	Sprague-Dawley Crl:CD (SD) BR VAF/Plus
Source:	[redacted]
Age:	Approximately 6 weeks
Sex:	Males and females
Weight at dosing:	Males: 143 – 201 g; females: 106 – 175 g
Acclimation period:	At least 15 days
Diet/Food:	Biosure Laboratory Animal Diet No.2, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	During pre-mating periods, animals were housed in groups of four in metal cages with wire mesh front, floor and top. During the first week of F1A and contingency animals of F2B animals were housed in plastic cages. During mating animals were housed on an 1:1 basis in plastic cages where females stayed after mating for breeding. Males were re-housed in former metal cages.
Environmental conditions:	Temperature: 23 ± 4 °C Humidity: 45 ± 24 % Air changes: not reported 12 hours light/dark cycle

Study design and methods:
In life dates: 1990-03-14 to 1991-03-22

Animal assignment and treatment:
In a two-generation reproduction study groups of 28 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 0, 1000, 3000 and 10000 ppm glyphosate technical. The dose levels were chosen based on results of a previously conducted study. After at least 70 days of treatment pairing of animals within each dose group was undertaken on a 1:1 basis to produce the F1 litters. At Day 21 *post partum* of offspring from the F0 mating phase, groups of 24 male and 24 female offspring from each dose group were selected to form the F1A generation. The remaining pups were sacrificed. Approximately 10 days following the weaning of all F1A pups, F0 males and females were re-mated. At Day 21 *post partum* all F1B pups were sacrificed. F0 males and females were terminated shortly after weaning of F1B pups.
The selected F1A animals were dosed from approximately Week 4 of age for at least 84 days and then mated on a 1:1 basis (sibling pairings were avoided). On Day 4 *post partum* F2A litters were standardized to 8 pups per litter. The remaining pups were sacrificed. On or shortly after Day 21 *post partum* all F2A pups were sacrificed. Approximately 10 days following the weaning of all F2A pups, F1 males and females were re-mated. On Day 4 *post partum* F2B litters were standardized to 8 pups per litter. The remaining pups were sacrificed. On or shortly after Day 21 *post partum* all F2B pups were sacrificed. F1 males and females were terminated shortly after weaning of F2B pups.

Diet preparation and analyses
For the weekly preparation of diet mixtures a known amount of the test substance was mixed with a small amount of basal diet. This pre-mix was then added to larger amount of basal diet and blended for further 7 minutes in a rotary double-cone-blender.
The stability and homogeneity of the test material in diet were determined. Dietary admixtures were analysed for achieved concentration throughout the study.

Clinical observations
A check for clinical signs or ill health was made once daily and recorded daily for the first week of treatment and on a weekly basis thereafter. Rats showing marked signs of ill health or reaction to treatment were killed and subjected to necropsy.

Body weight
Individual body weights were recorded at the start of each generation (F0: Week 6 of age; F1A: Week 4 of age) and subsequent at weekly intervals. Females were weighed daily during mating and continued until parturition. Weights were reported for Days 0, 7, 14, 17 and 20 of pregnancy. Females with live litters were weighed on Days 0, 7, 14 and 21 *post partum*.

Food consumption and compound intake
Food consumption was recorded on a weekly basis from allocation throughout the first pre-mating phase of each generation. During this period food conversion ratios and achieved intake (mg/kg bw/day) were calculated.

Water consumption
Water intake was observed daily during the initial and final two weeks of the first pre-mating period for each generation and from allocation for the F0 generation.

Reproduction parameters
Vaginal smears were taken daily during the 20-day mating period to examine the oestrus cycle and median *pre-coital* time. Additionally, date of mating and duration of gestation was recorded.

Litter data
The number of offspring born and the number of offspring alive were recorded daily. Pups were weighed on Days 0 and 4 and all litters containing more than eight pups were culled to eight retaining, where possible, ideally 4 pups per sex. The remaining pups were also weighed on Days 8, 12, 16 and 21. Dead and culled young were subjected to necropsy.

Sacrifice and pathology
All adult animals were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded.

The following organs were weighed of adults: adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostrate (with seminal vesicles and coagulating gland), testes (with epididymides), thymus.

The following tissues were preserved from all adults: adrenals, aorta, bone (femur and joint), bone marrow (sternum), brain, cranial vault (for lachrymal glands, teeth, nasal turbinates, inner ear), caecum, colon, duodenum, eyes, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (cervical/mesenteric), mammary gland, macroscopically abnormal tissues,

oesophagus, ovaries*, pancreas, pituitary*, prostate with seminal vesicles (with coagulating gland)*, rectum, salivary gland, sciatic nerve, skeletal muscle, skin, spinal column (vertebral column), spleen, stomach, testes (with epididymides)*, thymus, thyroids (with parathyroids), tongue, trachea (with larynx and pharynx), urinary bladder, uterus (with cervix)* and vagina*.

Histology of the reproductive tract was restricted to adults of the control and high-dose group and any apparently infertile animals at the lower dietary concentrations and confined to tissues marked with an asterisk (*).

Statistics
Two tailed significance tests were performed on adult parameters (water consumption, food consumption, bodyweight, organ weights) and litter data. Evaluation of other parameters were found not to be useful. Significances at 1 % and 5 % were reported.

Results and discussion
Analysis of dose formulations
Stability analyses indicated that the dose preparations at nominal concentrations of 500 and 30000 ppm were stable for up to 18 days during storage under animal room conditions.
Analyses for homogeneity at nominal concentrations of 500 and 30000 ppm indicated that the dose preparations were homogeneous.
Analyses for achieved concentration performed at 4-5 weekly intervals demonstrated that the prepared dietary admixture concentrations given to the animals were within ± 15 % of the nominal concentration in all groups.

Test compound intake
The group mean intakes of glyphosate are summarised in Table B.6.6-14 and Table B.6.6-15 below.

Table B.6.6-14: Group mean achieved intakes of glyphosate - F0 generation

Group	**Dietary concentration**	**Mean intakes (Week 1 - 10)**	
	(ppm)	(mg/kg bw/day)	
		Males	Females
Control	0	0	0
Low	1000	66.4	75.3
Intermediate	3000	196.8	226.0
High	10000	668.1	752.3

Table B.6.6-15: Group mean achieved intakes of glyphosate - F1 generation

Group	**Dietary concentration**	**Mean intakes (Week 5 - 16)**	
	(ppm)	(mg/kg bw/day)	
		Males	Females
Control	0	0	0
Low	1000	76.1	82.1
Intermediate	3000	230.2	244.9
High	10000	771.3	841.1

Mortality
There were no test substance related mortalities.

Four unscheduled deaths occurred during each generation.
In the F0 generation one female of the low-dose group and one male of the high dose group were killed for humane reasons during Week 15 and 23, respectively. The female exhibited pilo-erection and thin appearance and the necropsy noted thickened forestomach, invaginated stomach and abnormal contents in the gastro-intestinal tract. The male was unable to use hind limbs, exhibiting aberrations of brain and spinal cord at necropsy. Another male of the high-dose group died during Week 3, with effects on pancreas and liver noted at necropsy. One control male was sacrificed during Week 16 following poor condition, however, the aetiology of the signs was not established.

In the F1 generation one female of the low-dose group was killed following a procedural error. In the mid-dose group one male died during Week 34 but autolytic changes precluded a valid necropsy. Moreover, one male and one female died and were sacrificed, respectively, during Week 23. Necropsies failed to identify a specific cause of death.

Clinical observations
No treatment-related clinical signs of toxicity were noted. General signs were observed in occasional animals from both generations and were not related to treatment.

Body weight
No adverse effect of bodyweight change was evident for treated animals in comparison to controls for both generations.
However, absolute mean body weights in high-dose F1 males were slightly lower as compared to control. In addition it was noted that during the first mate of each generation, body weight gains during the initial stages of pregnancy tended to be slightly lower than controls at all dietary levels. Since no consistent dose-response was apparent these effects cannot conclusively be attributed to treatment.

Food and water consumption
Apart from a slightly higher but not statistically significant food consumption of high-dose F1 females during the second half of the pre-mating period, there were no marked intergroup differences in food consumption of males or females.
Apart from a slight increase among high-dose F1 females (attaining statistical significance in Week 16), no overt intergroup differences in water intake for treated males and females from the F0 or F1 generations when compared to their concurrent controls.

Reproductive parameters
There were no treatment-related effects on mating performance, fertility and gestation length for both F0 and F1 generation animals.

Litter data
Size and Viability
No overt differences in litter viability were detected.
In the high-dose group total litter size at birth was consistently, but not significantly, lower than controls across all four matings and remained lower than controls at Day 4 in three of the four matings. Since the mean litter size at birth within each mating, was not always the lowest litter size recorded, this finding could not be clearly attributed to treatment.

Growth and Development
No adverse effects on mean offspring bodyweights, bodyweight change or development were detected for male and female offspring in comparison to their controls.

Clinical signs
No clinically observable signs of toxicity were observed for offspring from treated animals.

Pathology
Necropsy
There were no toxicologically significant macroscopic abnormalities detected in the F0 and F1 animals, or offspring.

Organ weights
There were no overt or statistically significant treatment-related changes in any organ weights analysed in either generation.

Histopathology
No treatment-related changes in tissues associated with the reproductive tract were detected in the F0 or F1 generation animals.

Examination of two previously identified target organs, the parotid and submaxillary salivary glands, was initially performed only in the control and high-dose groups. Due to effects seen in the parotid gland, examination was extended to the remaining treatment groups. For the submaxillary gland, examination was extended to only the F0 and F1 females in the low- and mid dose group. The findings are summarised in Table B.6.6-16.

Table B.6.6-16: Incidence of salivary gland findings

Observation	**Dietary concentration (ppm)**							
	Males				Females			
	0	1000	3000	10000	0	1000	3000	10000
F0 Generation								
Animals examined	27	28	28	26	28	27	28	28
Hypertrophy of acinar cells with prominent granular cytoplasm (minimal)								
parotid	2	2	3	12	0	2	5	17
submaxillary	0	-	-	0	0	1	4	14
F1 Generation								
Animals examined	24	24	23	23	24	23	24	23
Hypertrophy of acinar cells with prominent granular cytoplasm (minimal)								
parotid	1	0	4	11	0	0	4	9
submaxillary	0	-	-	0	0	0	0	3

- = not examined

Treatment-related minimal changes were apparent in the parotid salivary gland of both F0 and F1 males and females in the mid- and high-dose groups and the submaxillary salivary gland of the F0 females in the mid- and high-dose groups and F1 females in the high-dose group. This finding is similar to those seen occasionally in other subchronic and long-term dietary

studies and is considered to be an adaptive response due to oral irritation from the ingestion of glyphosate, an organic acid, in the diet and is not considered to be adverse. There were no effects on the salivary glands noted in the low-dose group.

Conclusion by the Notifiers
The oral administration of glyphosate technical to rats by dietary admixture at a maximum dose level of 10000 ppm for two successive generations resulted in minimal effects consisting of increased food and water consumption of F1 females, possibly reduced bodyweights of F1 males and minimal histological changes in the target organ (salivary glands) in F0 and F1 adults at 10000 ppm. The only findings associated with treatment at 3000 ppm were minimal histopathological changes of the salivary glands in F0 and F1 adults. No effects were apparent at 1000 ppm. Thus, the parental reproductive and offspring NOAELs are considered to be 10000 ppm, corresponding to 668 and 752 mg/kg bw/day in males and females, respectively.

Comment by RMS (Re-evaluation):
The study is considered acceptable. According to the study report, balano preputial cleavage was delayed in F1 males at all dose levels (42.5; 42.2; 42.6 d versus 39.9 d in controls). However, the calculated mean value for control animals seems to be reported incorrectly and should be calculated to be 41.8 d. Based on the revised mean value for preputial separation in control animals, no definite difference between the treatment groups was observed.
In conclusion, no evidence of reproductive effects was observed and therefore, the NOAEL on reproductive toxicity is considered 10000 ppm (668 mg/kg bw/d). The NOAEL for parental and offspring toxicity is calculated to be 3000 ppm (197 mg/kg bw/d) based on increased food and water consumption in F1 females, lower body weight of F1 males and an increased incidence of cellular alteration of the parotid (males and females) and submaxillary (females) salivary gland in both F0 and F1 adults at 10000 ppm (Evaluation in 2001 confirmed).

Reference:	IIA, 5.6.1/07
Report:	[redacted] (1990) Two Generation Reproduction Feeding Study with Glyphosate in Sprague-Dawley Rats [redacted] Data owner: Monsanto Report No.: MSL-10387 Project No.: ML-88-106/EHL 88038 Date: 1990-08-27 not published ASB2012-11496
Guidelines:	Not stated, but in general accordance with OECD 416 (1983)
Deviations:	Yes: no data on food efficiency; no details on fertility indices, number of live births and post-implantation loss, number of pups with grossly visible abnormalities,
GLP:	yes
Acceptability:	*See RMS comment*

Dates of experimental work: 1988-10-24 to 1989-10-13

Materials and methods

Test material:	
Identification:	Glyphosate (Identification code: T880068)
Description:	White powder
Lot/Batch #:	XLI-203
Purity:	97.67 %
Stability of test compound:	Not reported
Vehicle and/ or positive control:	Plain diet
Test animals:	
Species:	Albino Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Approximately 7 weeks (F0 adults)
Sex:	Males and females
Weight at study start (F0):	Males: 165 – 207.6 g; Females: 135.6 – 162.7 g
Acclimation period:	No data
Diet/Food:	Purina Mills Certified RODENT CHOW No. 5002, *ad libitum*
Water:	St. Louis public water, *ad libitum*
Housing:	Housing for premating and gestation (day 0 through 13): individual suspended stainless steel cages over paper bedding; during mating females were housed in the male´s cages Housing for gestation and lactation (from day 14 of gestation through lactation): females housed in double wide cages with solid bottoms and wood shavings for bedding
Environmental conditions:	Temperature: 18 - 26 °C Humidity: 40 - 70 % 12 hours light/dark cycle

Study design and methods:

Animal assignment and treatment:

In a two-generation reproduction study groups of 30 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 2000, 10000 and 30000 ppm (corresponding to 132-140, 666-711, 1983-2230 mg/kg bw for males and 160-163, 777-804, 2322-2536 mg/kg bw/day for females (calculated from F0 and F1a adults)) glyphosate in the diet. After 11 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis in a male´s cage for 7 days, to produce the F1a litters. If there was no evidence of mating after 7 days (copulatory plug, or vaginal smear), the female was co-housed with a male having recorded copulatory activity for additional 7 days, or until copulatory evidence was found. For F0 and F1 generation, gestation day 0 was set on the day on which copulatory evidence was found and lactation day 0 the day on which delivery of pups was completed.

At weaning of offspring from the F0 mating phase, groups of 30 males and 30 females offspring from each dose group were selected to form the F1 generation and the mating procedure for F1a adults was conducted in the same way except modifications to exclude sibling matings. The remaining surviving F0 females and unselected offspring were terminated at Day 21 *post partum*. F0 males were killed at completion of mating phase. The offspring selected for the F1a generation were dosed for at approximately 14 weeks and then mated to produce the F2a and F2b litters (a second mating of the F1 generation was performed due to reduced litter sizes in pups from F0 of the 30000 ppm dose group). At weaning of the F2 litters all surviving adults and their offspring were killed, whereas F1 males were sacrificed after completion of mating phase.

Diet preparation and analyses
Approximately each week (except in one week when diets were prepared twice the same week and not during the following week) a known amount of glyphosate was mixed with the diet for 10 minutes in a HOBART HCM-450 mixing machine to achieve a batch size of 18 kilograms at each dose level.
The stability and homogeneity of the test substance in the diet were determined by liquid chromatography of duplicate samples from top, middle and bottom of mixer from the lowest and highest dietary levels stored in an open container at ambient temperature for 6 and 14 days or when frozen in a closed container for 35 days.

Clinical observations
A detailed observation for signs of toxicity was performed once weekly for the adult animals and for the offspring on days of weight measurement.

Body weight
Adult male animals of the F0 and F1a generation were individually weighted once weekly. The same was done for the female animals until copulation was confirmed, then females were weighted on days 0, 7, 14, and 21 of gestation and lactation.
Offspring was weighted on days 0, 4 (pre- and post-culling), 14 and 21 of lactation (except F1a males approximately two weeks prior to sacrifice and F1a females for approximately three weeks prior to mating for the F2b generation).

Food consumption and compound intake
Food consumption was recorded weekly for F0 and F1a adult males, except during mating, and also weekly for adult F0 and F1a female animals until mating. After confirmed copulation, the maternal food consumption was monitored for days 0-7, 7-14 and 14-21 of gestation and lactation, but it was not determined for females approximately three weeks prior to mating for the F2b generation and generally not for female animals that did not became pregnant.
Food conversion efficiency was not calculated.

Water consumption
No data on water consumption was given in the report.

Reproduction parameters

Pregnancy and parturition
Data on total paired females, females with confirmed copulation/total paired, pregnant/total paired, pregnant/ confirmed copulation was monitored as well as precoital (for pregnant

animals) and gestational length in days. For males, the following items of interest were given: males with confirmed copulation/total paired, males impregnating females/total paired and males impregnating females/confirmed copulation.

Litter data
The following litter data were recorded: Litter size, dead pups/litter, mean pup weight (on day 0, 4 (pre-/post-cull), 14, 21) and survival (%).

Physical and sexual development
No details on physical and sexual development of the offspring was reported.

Sacrifice and pathology
All adult animals, which died or were sacrificed in moribund condition were subjected to a gross necropsy and selected tissues were sampled. Pups found dead or culled pups also underwent gross pathology, but no tissues were saved. No organ weights were determined.
All F1a weanlings, that were not selected for mating, F2a and F2b weanling pups as well as females which had littered on or after 21 of lactation were sacrificed as scheduled. Non-pregnant adult females were killed at least 5 days after last expected parturition date and adult males after completion of the mating phase.
External and internal cavities of the dead animals were opened and the organs were examined in place and then removed. Hollow organs were opened and examined. The following organs of F0 and F1a males and females from each dose group that were sacrificed at the end of the study sampled, were weighed: ovaries and testes with epididymides. When present, the following organs from the F0 and F1a adults (unscheduled deaths and scheduled sacrifice) were retained: kidneys, ovaries, prostate, seminal vesicle, skin/mammary gland, testes, epididymis, uterus/vagina and gross lesions (pituitary retained for F1a adults only). Tissues from the F1a weanlings were saved at the discretion of the necropsist. From the F2a and F2b weanlings, which where sacrificed at schedule, the kidneys of 1 pup per sex and litter were saved.

A histopathological examination was performed on all sampled tissues from all F0 and F1 control and high-dose animals, and on one F2b weanling/sex/litter (selected at random) as well as on all retained tissues from unscheduled adult deaths. For preparation, fixed tissues were washed, dehydrated, embedded in paraffin, sectioned, stained with haematoxylin and eosin and examinated under light microscopy.

Statistics
Dunnett´s multiple comparison test (two-tailed) was used to detect statistically significant differences in adult body weights and food consumption between treated animals and their respective control.
Terminal body weights, maternal body weights and food consumption during gestation and lactation, pup weights, precoital length, gestational length, litter size, dead pups/litter, pup survival, absolute organ weights and organ/body weight ratios were evaluated by decision-tree statistical analyses procedures which, depending on the results of tests for normality and homogeneity of variance [Bartlett’s Test], were chosed either parametric [Dunnett’s Test and Linear Regression] or nonparametric [Kruskal-Wallis, Donckheere’s and/or Mann-Whitney Tests] routines to detect differences and analyzed for trend.
The uncorrected Chi-Square test was used to examine fertility indices, e.g. females/males with confirmed copulation/total paired, pregnant/confirmed copulation (females) and males

impregnating females/total paired as well as males impregnating females/confirmed copulation.
Fisher´s Exact test with Bonferroni Inequality Procedure was used for statistical analysis of microscopic lesions.
Other statistical routines used for some data included: Bartlett's Test to evaluate homogeneity of variances, Analysis of Variance to determine if the sample (group) means could be considered as an estimate of a common population, and Grubb's Test to detect outliers.

Results and discussion
Analysis of dose formulations
The analysis of the test substance stability conducted over the time span of the study indicated that the test material was stable in the diet and homogeneity was adequate for study use. The stability of the test material in the diet was demonstrated at the low and high dose level, stored in an open container at ambient temperature for 6 and 14 days, or when frozen in a closed container for 35 days.
Analysis for achieved concentrations, demonstrated that the test substance-levels in the prepared diet were in the range of 95 to 96.7 % of the nominal concentration.

Test compound intake
The group mean achieved dosages are summarised in the table below.

Table B.6.6-17: Group mean compound intake levels during pre-mating periods of F0 and F1

Dose group	**Dietary concentration (ppm)**	**Mean daily test substance intake (mg/kg bw/day)***			
		F0		F1	
		Males	Females	Males	Females
control	0	0	0	0	0
low	2000	132	160	140	163
mid	10000	666	777	711	804
high	30000	1983	2322	2320	2536

* based on actual food intake and body weight data; values were calculated in the report

Mortality
There were no treatment-related mortalities.
One female of the F0 generation died early in the study. This animal was never mated and at necropsy changes in bladder in kidneys were observed. Two male animals of the 2000 and 30000 ppm dose groups (F1 generation) died. Necropsy of these animals noted thymus and respiratory changes. One female animal of the F1 generation (2000 ppm) was sacrificed in extremis and another female (same generation, same dose group) died. Kidney changes and retained foetus; pups in uterus and stomach changes, respectively, were observed in these two females.

Concerning the offspring, dead pup counts at day 0 and survival of all F1a, F2a and F2b treated pups were not adversely affected when compared to the controls.

Clinical observations
The only clinical signs that were related to the test substance were soft stool in the animals of the 30000 ppm dose group. Other clinical signs, such as red ocular discharge / laboured respiration / overgrown teeth / piloerection / abrasions / emaciated and dehydrated

appearance / misuse of limbs / focal loss of hair / swollen feed, occurred sporadically and were not considered to be treatment-related.

Body weight
At the highest exposure level of 30000 ppm, reduced body weights were observed in both sexes and in F0 and F1 generation. In the F0 generation, body weights gradually decreased within time to approximately 8 % less than controls prior to mating. F0/F1 weaning animals were lighter in weight as their corresponding controls and maintained that weight difference (approx. 10 % less than control) until the end of the study (see Table B.6.6-18).
No test-substance related body weight effects were observed in the adult animals of the 2000 and 10000 ppm dose groups prior to mating.
During gestation and lactation, maternal body weights in the highest dose group tended to remain lower than in controls, but the animals showed a rather greater body weight gain than the controls during gestation and lactation so that by the end of lactation, body weights were approximately the same as those of the controls (see Table B.6.6-19 and Table B.6.6-20).
Terminal body weights were significantly decreased for both sexes at the highest exposure level (see Table B.6.6-18).

Table B.6.6-18: Mean group body weights

Dietary concentration (ppm)	**No. of animals**		**Mean group body weight (g) at Day**					
			0	72	T#	0	72	T#
			F0 Generation					
			Males			Females		
0 (Control)	30	mean	187.9	494.6	549.56	150.5	276.7	296.31
		sd	11.65	34.86	46.76	6.86	23.85	23.63
2000	30	mean	188.1	497.6	550.19	150.5	272.6	290.64
		sd	11.35	49.87	80.72	7.03	22.86	19.50
10000	30	mean	188.1	484.4	539	150.2	273	290.71
		sd	11.57	42.13	58.13	7.04	27.92	25.35
30000	30	mean	188	455.8**	503.51**	150.3	253.8**	265.91
		sd	11.56	46.46	45.66	7.06	18.46	15.44
			F1 Generation					
			129	219	T#	128	219	T#
0 (Control)	30	mean	118.3	534.7	625.04	99.8	285.8	316.21
		sd	26.11	38.84	53.11	17.44	27.63	37.37
2000	30	mean	115.2	540.3	632.14	96.7	282.1	313.74
		sd	16.2	44.9	74.57	11.47	24.5	30.53
10000	30	mean	114.8	514.1	590.98	97.1	275.9	312.36
		sd	17.42	58.31	70.06	14.18	20.55	26.71
30000	30	mean	104.9*	483.4**	543.40**	88.8*	253.7**	284.72**
		sd	19.79	41.32	58.12	16.32	19.56	18.04

*: Dunnett´s test (two-tailed) indicates statistically significant difference ($p<0.05$)
**: Dunnett´s test (two-tailed) indicates statistically significant difference ($p<0.01$)
#T: Termination

Table B.6.6-19: Mean maternal body weights during gestation

Dietary concentration (ppm)	No. of animals		Mean group body weight (g) at Day (Gestation)			
			0	7	14	21
			F0 Generation			
0 (Control)	24	mean	274	301.83	324.41	398.26
		Sd	24.26	24.58	22.85	26.12
2000	29	mean	272.72	297.33	319.90	392.86
		Sd	20.52	21.71	19.84	24.28
10000	28	mean	271.80	299.22	323.43	395.08
		Sd	24.12	26.40	28.44	25.87
30000	28	mean	255.05**	282.44**	305.83**	375**
		Sd	16.49	16.27	17.44	24.70
			F1 Generation (First Mating)			
			0	7	14	21
0 (Control)	24	mean	285.29	308.95	328.70	392.56
		Sd	25.48	26.58	29.18	36.19
2000	29	mean	278.65	304.40	324.15	383.45
		Sd	23.42	23.48	25.06	28.18
10000	28	mean	268.89*	297.23	319.08	382.71
		Sd	19.24	18.81	19.27	21.77
30000	28	mean	251.30**	276.28**	299.48**	360.46**
		Sd	17.42	18.92	19.29	33.31
			F1 Generation (Second Mating)			
			0	7	14	21
0 (Control)	24	mean	324.22	340.99	363.44	428.99
		Sd	23.11	27.81	27.98	36.87
2000	29	mean	315.21	338.27	360.35	426.88
		Sd	26.06	28.67	28.39	33.67
10000	28	mean	305.27*	333.66	357.50	428.51
		Sd	20.26	22.45	24.49	26.17
30000	28	mean	281.46**	308.92**	330.95**	393.67**
		Sd	17.79	22.19	22.36	34.88

Table B.6.6-20: Mean maternal body weights during lactation

Dietary concentration (ppm)	No. of animals		Mean group body weight (g) at Day (Lactation)			
			0	7	14	21
			F0 Generation			
0 (Control)	24	mean	299.96	319.59	317.33	313.39
		Sd	23.21	23.58	28.96	20.01
2000	29	mean	297.48	317.91	314.53	313.96
		Sd	21.10	18.66	25.22	16.63
10000	28	mean	298.78	315.15	312.41	319.10
		Sd	20.81	22.04	22.94	18.61
30000	28	mean	285.84*	307.64	304.75	316.68
		Sd	13.91	12.48	20.68	15.43
			F1 Generation (First Mating)			
			0	7	14	21
0 (Control)	24	mean	299.29	313.60	337.68	313.49
		Sd	27.02	26.12	25.31	21.38
2000	29	mean	295.16	308.28	332.10	314.69
		Sd	23.58	22.56	23.92	23.95
10000	28	mean	296.63	310.80	328.29	313.14
		Sd	19.01	18.64	18.33	14.06
30000	28	Mean	277.91**	289.88**	315.88**	306.15
		Sd	17.89	17.23	17.47	20.18
			F1 Generation (Second Mating)			
			0	7	14	21
0 (Control)	24	mean	342.78	343.21	353.34	337.16
		Sd	32.46	27.11	21.15	17.22
2000	29	mean	340.16	336.62	348.40	331.96
		Sd	28.54	16.11	25.89	20.67
10000	28	mean	333.80	342.41	352.70	334.56
		Sd	23.35	26.93	20.43	13.82
30000	28	mean	312.39**	324.09*	337.08	329.95
		Sd	23.73	20.50	19.09	18.41

Food consumption
Overall, food intake was not notably affected during the study.
All animals of the 30000 ppm dose group consumed about 1 to 2 grams/day less than controls. This effect was mostly pronounced in the first week of exposure and also observed in the F0 dams. Subsequent dams (F1 first and second matings) tended to eat similar or larger amounts of the diet than controls.
No effects on food consumption were observed in the animals of the 2000 and 10000 ppm dose groups.

Reproductive parameters
Mating Performance, Fertility, Gestation and Lactation
No effects on mating and fertility rates were observed in the F0 and F1a dams when compared to controls and no effects were observed on precoital length at any treatment level.

Litter data
Size and Viability
Day 0 dead pup counts among treated groups were comparable to the control group for all three litters of pups (F1a, F2a and F2b generation).
A slight reduction in the average litter size was observed in the F0 dams of the 30000 ppm dose group. This effect was less pronounced in animals after the first F1 mating. Although the

difference was not statistically significant and not accompanied by an increase in dead pups/litter, a treatment-related effect could not be excluded. Therefore a second mating of the F1a adults was performed. In the resulting F2b generation, no dose-related decrease in litter size was observed.

Growth and Development
Birth weights and initial growth rate for pups from the treated dams compared well to the ones of the control, except the pups of the 30000 ppm dose group had reduced body weights on day 21 of lactation (more than 10 % difference to controls). The effect was earlier pronounced in the F1 matings (day 14). This effect was reasoned by the titrated uptake of the test substance-containing diet at the end of lactation.
In the mid dose group, slight and transient decreases in the body weights of the pups were observed. They were not evident in both sexes from all generations and therefore regarded of questionable toxicological significance.

Table B.6.6-21: Mean pup weights

Dietary concentration (ppm)	**No. of animals**		**Mean group body weight (g) at Day**			
			0	21	0	21
			Males		Females	
			F0 Generation			
0 (Control)	24	mean	6.28	53.39	6.96	50.80
		Sd	0.49	3.90	0.52	4.39
2000	29	mean	6.27	51.82	6.91	49.47
		Sd	0.48	5.26	0.48	5.05
10000	28	mean	6.43	50.42*	6.15	49.16
		Sd	0.47	3.66	0.50	3.12
30000	27	mean	6.47	46.30**	6.12	44.99**
		Sd	0.62	4.09	0.59	4.34
			F1 Generation (First Mating)			
0 (Control)	28	mean	6.33	55.11	5.95	51.93
		Sd	0.60	5.64	0.55	5.07
2000	23	mean	6.20	52.47	5.90	51.42
		Sd	0.76	9.15	0.70	4.08
10000	22	mean	6.32	51.53*	5.98	48.49*
		Sd	0.74	7.35	0.64	5.93
30000	25	mean	6.50	47.29**	6.05	44.41**
		Sd	0.84	4.62	0.74	4.90
			F1 Generation (Second Mating)			
0 (Control)	16	mean	6.48	55.03	6.04	49.35
		Sd	0.75	6.38	0.63	10.96
2000	18	mean	6.17	52.74	5.86	50.73
		Sd	0.74	6.12	0.83	5.91
10000	17	mean	6.36	52.29	5.92	49.48
		Sd	0.52	3.35	0.47	2.52
30000	23	mean	6.51	44.43**	6.04	43.10**
		Sd	0.63	6.86	0.55	3.81

Clinical signs
No clinical signs were observed in the offspring of treated animals.

Pathology
Necropsy
There were no toxicologically significant macroscopic gross lesions attributed to the test chemical administration.

Organ weights
There were no statistically significant organ weight changes, except a slight increase in testes to body weight ratios in F1a adults of the 30,000 ppm dose group. This effect was attributed to their lower terminal body weight.

Histopathology
No treatment-related changes were detected.

Conclusion by the Notifiers
The oral administration of glyphosate to rats via diet at a dose levels of 2000, 10000 and 30000 ppm for two successive generations resulted in possible treatment-related changes at the maximum dose of 30000 ppm. A high incidence of soft stools in adults was accompanied by consistent reduction of body weights of adults and pups at this dose level. Decreases in body weights of the pups obviously occurred at the end of lactation, with the beginning of consuming the test substance-containing diet. Furthermore, slightly but not statistically significant reduced average litter size was noted in F0 dams of the 30000 ppm dose group at first mating.
Therefore the NOAEL was considered to be 10000 ppm for adult toxicity for both the F0 and F1 generations (corresponding to 666-711 mg/kg bw for males and 777-804 mg/kg bw/day for females).
The NOAEL for reproductive toxicity, for both generations and offspring was considered to be 30000 ppm.
The NOAEL for developmental toxicity, for both generations and offspring was considered to be 10000 ppm.

Comment by RMS (Re-evaluation):
The study is considered acceptable. In contrast to the Notifiers, the mid dose of 10000 ppm is considered the NOAEL for parental, offspring and reproductive toxicity. This assessment is in accordance with the evaluation in the previous DAR (2001, ASB2010-10302) and based on reduced body weight gain and soft stools in high dose adults, decreased pup body weight gain and equivocally reduced litter size at highest dose level of 30000 ppm. The intermediate dietary concentration of 10000 ppm was calculated to be 722 for male rats and 757 mg/kg bw/d for females.

B.6.6.2 Separate male and female studies

Not required according to Regulation 1107/2009/EEC and Directive 91/414/EEC.

B.6.6.3 Three segment designs

Not required according to Regulation 1107/2009/EEC and Directive 91/414/EEC.

B.6.6.4 Dominant lethal assay for male fertility

Studies considered not necessary. Information provided in chapter IIA 5.4.6.

B.6.6.5 Cross-matings of treated males with untreated females and vice versa

Not required according to Regulation 1107/2009/EEC and Directive 91/414/EEC.

B.6.6.6 Effects on spermatogenesis

Studies considered not necessary. Effects on spermatogenesis are assessed in the two-generation reproductive toxicity studies (see IIA 5.6.1).

B.6.6.7 Effects on oogenesis

Studies considered not necessary. Effects on oogenesis are assessed in the two-generation reproductive toxicity studies (see IIA 5.6.1).

B.6.6.8 Sperm motility and morphology

Studies considered not necessary. Parameters are assessed in the two-generation reproductive toxicity studies (see IIA 5.6.1).

B.6.6.9 Investigation of hormonal activity

Separate studies considered not necessary. The potential hormonal activity is assessed in two-generation and developmental toxicity studies (see IIA 5.6.1, IIA 5.6.10 and IIA 5.6.11).

B.6.6.10 Teratogenicity test by the oral route in the rat

For the previous EU evaluation, a total of 5 studies in rats were reported in the DAR of which four were still considered acceptable or at least supplementary and may be used for current evaluation. The previous assessment of the studies by [redacted]. (1991, TOX9552393) and by [redacted] (1980, TOX9552392) and of the [redacted] study (1981, TOX9650160, Author's name perhaps [redacted] but not verified) were confirmed by the RMS upon re-evaluation. However, during re-evaluation the study by [redacted] (TOX9551834) was considered not acceptable due to several reporting deficiencies and therefore not reported in the present RAR.
In addition, two new studies in rats have been performed and were provided in the GTF dossier. These studies were submitted either for the first time for this evaluation or had been subject to JMPR evaluation in 2004 yet. They were considered acceptable by the RMS without restrictions.

Reference:	IIA, 5.6.10/01
Report:	[redacted] (2002) Amendment 001 to Glyphosate Acid: Developmental Toxicity Study in the Rat [redacted] Data owner: Syngenta Report No.: CTL/P/4819 Date: 2002-11-20 GLP: not published ASB2012-10080
Guidelines:	OECD 414 (2001): OPPTS 870.3700 (1998): 2004/73/EC B.31 (2004)
Deviations:	None
GLP:	yes
Acceptability:	*See RMS comment*

Dates of experimental work: 1995-05-17 to 1996-03-26

Materials and methods

Test Material:	Glyphosate acid
Description:	Technical, white solid
Lot/Batch number:	P24
Purity:	95.6 % w/wa.i
CAS#:	Not reported
Stability of test compound:	Confirmed
Vehicle and/or positive control:	Deionised water.
Test Animals:	
Species	Rat
Strain	Alpk:APfSD Wistar-derived
Age/weight on arrival	Approximately 11 weeks / 210 – 303 g
Source	[redacted]
Housing	Individually
Acclimatisation period	Not applicable
Diet	CT1 diet (Special Diet Services, Witham, Essex, UK) *ad libitum*
Water	Mains water *ad libitum*
Environmental conditions	Temperature: 21 ± 2 °C Humidity: 40 - 70 % Air changes: 25 – 30 changes / hour Photoperiod: 12 hours light / 12 hours dark

Study design and methods

In-life dates: Start: Not reported End: Not reported (QA audits conducted between May 1995 and March 1996)

Mating procedure: Virgin female rats were paired overnight (at the Breeding Unit) with males of the same strain. On the following morning, vaginal smears from these females were examined for the presence of sperm. The day when spermatozoa were detected was designated day 1 of gestation and, on this same day, successfully mated females were delivered to the experimental unit at CTL.

Animal assignment: A total of 96 mated females was supplied over a two week period. Twelve female rats were supplied on each of eight days. The study was divided into twenty four replicates (randomised blocks) with each replicate containing one rat from each group. Animals were randomly assigned to test groups as shown in the following table.

Table B.6.6-22: Animal numbers and treatment groups

Dose level of Glyphosate acid (mg/kg bw/day)			
0 (control)	250	500	1000
1 - 24	**25 – 48**	**49 - 72**	**73 - 96**

Dose selection rationale: The dose. levels selected for this study were based on a dose range finding study in the pregnant rat. The highest dose level of 1000 mg/kg/day is the limit dose for this type of study.

Dose preparation and analysis: Glyphosate acid was administered in deionised water and the concentration was adjusted to give a constant volume of 1 mL/100 g bodyweight for each dose level. An appropriate amount of deionised water was added to a weighed amount of test substance (adjusted for purity) to provide each preparation. One preparation per concentration (ie 25, 50 and 100 mg/mL) was made. Each preparation was thoroughly mixed before being subdivided into aliquots. The control substance was also dispensed into aliquots. The aliquots were stored at room temperature and fresh aliquots were used for each day of the study.

A sample of each preparation was analysed prior to the start of dosing to verify the achieved concentrations of glyphosate acid in deionised water. Samples of the lowest concentration of dosing formulation was analysed to confirm the homogeneity of glyphosate acid in deionised water. The homogeneity of the 100 mg/mL formulation was not determined as part of this study and the data have been obtained from a preliminary study (*Moxon ME, 1995, Study cited but not submitted)* for which the method of preparation of the dosing formulations was the same. The chemical stability of glyphosate acid in deionised water was determined by re-analysis of the lowest and highest concentrations of dosing formulation after an interval of 26 days.

Concentration analysis results: The achieved concentrations of glyphosate acid in deionised water were within 5 % of nominal concentrations.
Homogeneity results: The homogeneity of glyphosate acid in deionised water at concentrations of 25 mg/mL and 100 mg/mL was within 5 % of the overall mean.
Stability results: The stability of the 25 mg/mL and the 100 mg/mL formulations was satisfactory over a period of 26 days which exceeded the period of use in this study.

Dosage administration: All animals were dosed once daily from days 7 – 16 (inclusive) of gestation with 1 mL of dosing formulation per 100 g bodyweight using a disposable syringe and a plastic nelaton catheter. The volume given to each animal was adjusted daily according to body weight. Control animals received the appropriate volume of deionised water. Dosing

was performed in group order with all animals receiving the same dose level being dosed sequentially.

Observations:
Maternal observations: All animals were observed on arrival to ensure that they were physically normal externally and were subsequently observed at least twice each day. Any changes in behaviour or clinical condition were recorded daily during the study.

Bodyweight: The bodyweight of each animal was recorded on arrival and on days 4, 7-16 (inclusive) and on days 19 and 22 of gestation.

Food consumption: The amount of food consumed by each animal over three day periods was measured by giving a weighed quantity of food contained in a glass jar on days 1, 4, 7, 10, 13, 16 and 19 and calculating the amount consumed from the residue on days 4, 7, 10, 13, 16, 19 and 22, respectively.

Terminal investigations: One rat requiring euthanasia was killed by over-exposure to halothane Ph. Eur. vapour and given a macroscopic examination *post mortem*.

On day 22 of gestation the animals were killed by over-exposure to halothane-Ph. Eur. vapour and a macroscopic examination *post mortem* was performed. The uterus from any animal without clear evidence of implantation was removed and stained with ammonium polysulphide to determine whether or not implantation had occurred.

For pregnant animals the intact gravid uterus (minus ovaries and trimmed free of connective tissue) was removed and weighed. The ovaries and uterus were then examined and the following data recorded:-

Number of *corpora lutea* in each ovary
Number and position of implantations subdivided into:
a) live foetuses
b) early intra-uterine deaths (decidual or placental tissue only)
c) late intra-uterine deaths (embryonic/foetal tissue plus placental tissue)
Individual foetal weights

The implantations were assigned letters of the alphabet to identify their position *in utero* starting at the ovarian end of the left horn and ending at the ovarian end of the right horn. In addition, each foetus was weighed and individually identified within the litter by means of a cardboard tag. After weighing the foetuses were killed with an intracardiac injection of approximately 0.1 mL of 200 mg/mL pentobarbitone sodium solution.
Percentage pre-implantation loss and percentage post-implantation loss were calculated.

$$\% \text{ pre-implantation loss} = \frac{\text{number of } \textit{corpora lutea} - \text{number of implantations}}{\text{number of } \textit{corpora lutea}} \times 100$$

$$\% \text{ post-implantation loss} = \frac{\text{number of implantations} - \text{number of live foetuses}}{\text{number of implantations}} \times 100$$

Foetal observations: An external examination of each foetus was made together with an examination of the oral cavity. All foetuses were then examined internally for visceral

abnormalities, sexed, eviscerated and fixed in 70 % industrial methylated spirits. After approximately 24 hours the head of each foetus was cut along the fronto-parietal suture line and the brain was examined for macroscopic abnormalities. The carcasses were then returned to 70 % industrial methylated spirits for subsequent processing and staining with Alizarin Red S. The stained foetal skeletons were examined for abnormalities and the degree of ossification was assessed. The individual bones of the *manus* and *pes* were assessed and the result converted to a six point scale.

The observations were classified as major (permanent structural or functional deviations considered likely to be incompatible with survival or rarely seen) or minor defects or variants (small, generally transient deviations considered compatible with survival). The difference between the minor defect and variant classification is the frequency of occurrence in the control population of rats of this strain.

Statistical analyses: Data relating to animals which were non-pregnant, totally resorbed their litters or died intercurrently were excluded from the statistical analysis.

Maternal body weight during the dosing and post-dosing periods was considered by analysis of covariance on initial (day 7) body weight.

Maternal food consumption during the dosing and post-dosing periods, the numbers of implantations and live foetuses per female, gravid uterus weight, litter weight, mean foetal weights per litter and mean *manus* and *pes* scores per litter were considered by analysis of variance.

Maternal-performance data (excluding the animal with undetermined pregnancy status), the proportion of foetuses with each individual *manus* and *pes* score, the proportion of foetuses with each defect and the proportion of litters with each defect were considered by Fisher's Exact Test.

Pre-implantation loss, post-implantation loss, early intra-uterine deaths, late intra-uterine deaths, male foetuses, major external/visceral defects, minor external/visceral defects, external/visceral variants, major skeletal defects, minor skeletal defects and skeletal variants were analysed as follows:

All analyses were carried out in *SAS (1989)*. For Fisher's Exact Test the proportion in each treated group was compared to the control group proportion. Analyses of variance and covariance allowed for the replicate structure of the study design. Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a Student's t-test, based on the error mean square in the analysis.
All statistical tests were two-sided.

Results
Maternal toxicity:
Mortality and clinical signs: One control animal was killed on day 7 as a result of being misdosed. Excess watery fluid in the thoracic cavity and dark red areas on the surface of the

lung lobes were observed at examination *post mortem*. The pregnancy status of the animal was not determined.
There were no changes in the clinical condition of the animals given glyphosate acid which were considered to be treatment-related.
Body weight: There was no effect of glyphosate acid on maternal body weight.

Table B.6.6-23: Intergroup comparison of maternal body weight (g) (selected timepoints, adjusted means for days 8 and 22)

	Dose level of glyphosate acid (mg/kg/day)			
day	0 (control)	250	500	1000
1	255.6	255.5	253.5	252.8
8	288.2	288.1	288.0	287.5
22	406.4	410.1	411.1	408.6

Food consumption: There was no adverse effect of glyphosate acid on maternal food consumption. The amount of food consumed by the animals given 1000 mg glyphosate acid/kg/day was marginally lower during the dosing period but differences from the controls were not statistically significant.

Table B.6.6-24: Intergroup comparison of food consumption (g/day) (selected timepoints)

	Dose level of glyphosate acid (mg/kg/day)			
day	0 (control)	250	500	1000
1-4	23.9	24.6	24.6	23.2
13-16	33.2	33.4	33.7	31.9
19226	29.5	31.6*	30.5	30.5

* Statistically significant difference from control group mean, $p<0.05$ (Student's t-test, 2-sided)

Sacrifice and pathology:
Gross pathology: There were no macroscopic findings which were considered to be related to the administration of glyphosate acid.

Developmental Toxicity: There was-no effect of glyphosate acid on the number, growth or survival of the foetuses *in utero*.

Table B.6.6-25: Intergroup comparison of maternal performance

Observation	Glyphosate acid (mg/kg/day)			
	0 (control)	250	500	1000
# Animals Assigned (Mated)	24	24	24	24
# Animals Pregnant	22	24	23	24
#pregnancy status not determined (intercurrent death)	1	0	0	0
Gravid uterus weight (g)	89.7	87.2	91.3	89.9
#Intercurrent deaths	0	0	0	0
#aborted	0	0	0	0
# totally resorbed at termination	0	0	0	1
Corpora Lutea/Dam	15.8	15.7	15.5	15.5
Implantations/Dam	14.4	12.9*	14.1	13.6
Total # Litters (viable)	22	24	23	23
Live Foetuses/Dam	12.9	12.4	13.1	12.9
Early (Proportion of litters affected)	8.7	3.4**	6.2	5.5
Late (Proportion of litters affected)	1.3	0.5	1.6	0.3
Litter Weight (g)	62.4	61.2	64.3	63.6
Mean Foetal Weight (g)	4.86	5.02	4.95	4.96
Sex Ratio (% Males per litter)	51.9	54.1	53.3	51.0
Preimplantation Loss (%)	8.7	18.0**	8.8	12.0
Postimplantation Loss (%)	9.9	4.0**	7.8	5.8*

* Statistically significant difference from control group mean, $p<0.05$ (Student's t-test, 2-sided)
** Statistically significant difference from control group mean, $p<0.01$ (Student's t-test, 2-sided)

Major defects: The incidence of foetuses with major defects was 1/284, 1/297, 1/301 and 2/296 in the control and 250, 500 and 1000 mg glyphosate acid/kg/day groups, respectively. Neither the type nor incidence of major defects provided evidence for an adverse effect of glyphosate acid. The defects were dissimilar in type and of single incidence.

Minor defect: The proportion of foetuses with minor external/visceral defects and the proportion of foetuses with minor skeletal defects were similar for all groups. Consideration of the specific defects provided no evidence for an adverse effect of glyphosate acid.

Variants: The proportion of foetuses with external/visceral variants and the proportion of foetuses with skeletal variants were lower in the glyphosate acid treated groups than in the control group. Consideration of the specific defects provided no evidence for an adverse effect of glyphosate acid.

Manus and pes assessment: There was no effect of glyphosate acid on the ossification of the *manus* or *pes*.

Conclusion by the Notifiers
The dose level of 1000 mg glyphosate acid/kg/day was the no observed effect level in this study for both maternal and developmental effects.

Comment by RMS:
The study is considered acceptable. The conclusion is agreed.

Reference:	IIA, 5.6.10/02
Report:	[redacted] (1995) HR-001: Teratogenicity Study in Rats. [redacted] Data owner: Arysta LifeScience Study No.: IET 94-0152 Date: 1995-07-21 not published ASB2012-11497
Guidelines:	Japan MAFF Guidelines 59 NohSan No.4200, 1985 U.S. EPA FIFRA Guidelines Subdivision F, 1984
Deviations:	None
GLP:	yes
Acceptability:	*See RMS comment*

Dates of experimental work: 1995-03-23 to 1995-06-26

Materials and methods

Test material:	Glyphosate technical
Identification:	HR-001
Description:	Solid crystals
Lot/Batch #:	940908
Purity:	95.68 %
Stability of test compound:	Not mentioned in the report
Vehicle and/ or positive control:	Diet
Test animals:	
Species:	Rat
Strain:	SPF Crj:CD (SD)
Source:	[redacted]
Age:	13 weeks
Sex:	Males and females
Weight at dosing:	Males: 380 – 450 g, females: 267 – 322 g
Acclimation period:	11 days
Diet/Food:	Certified diet MF Mash (Oriental Yeast Co., Ltd.), *ad libitum*
Water:	Filtered and sterilized water, *ad libitum*
Housing:	By pair in aluminium cages with wire-mesh floors for mating period; Individually for copulated females in aluminium cages with wire-mesh floors.
Environmental conditions:	Temperature: 24 ± 2 °C Humidity: 55 ± 10 % Air changes: 12/hour 12 hours light/dark cycle

Study design and methods:
In life dates: 1995-03-23 to 1995-06-26

Animal assignment and treatment:
Vaginal smears were taken from females for microscopic examination. Females showing proestrus or estrus vaginal smears were paired overnight with males on a 1:1 basis. The females were examined next morning for the presence of vaginal plugs and sperm in vaginal smears and considered to copulate when vaginal plugs and/or sperm were observed. These mating procedures were repeated for 4 consecutive days.
Four test groups were set. The test substance was administered orally with a stomach tube to 10 copulated Crj:CD (SD) female rats per group at dose levels of 0, 300, 1000 mg/kg/day from day 6 to 15 of gestation.

Clinical observations
Each female was observed for clinical signs and mortality at least once daily during the pre-dosing and post-dosing periods and at least twice daily during the dosing period.

Body weight
Individual body weights were recorded on days 0, 6-15 (daily during the dosing period) and 20 of gestation. Adjusted body weight gains were calculated by subtracting the gravid uterine weight from the body weight value on day 20 of gestation.

Sacrifice and pathology
All surviving females were euthanized by overdosage of ether inhalation and cesarian section was performed on day 20 of gestation. Each female was necropsied. The ovaries and uterus were removed and the gravid uterine weight and numbers of copora lutea and implants were recorded. Then the uterus was opened and the numbers of live and dead foetuses were recorded with their positions in the uterine horns. Resorbed embryos or dead foetuses were classified into implantation sites, placental remnants or macerated foetuses (including dead foetuses) according to developmental stage in which resorptions or deaths occurred. When no uterine implants were grossly apparent, the uterus was stained with 10 % ammonium sulphide solution to detect very early resorptions. The weights of each live foetus and of each placenta were determined and recorded. Live foetuses were sexed and were euthanized by an intraperitoneal injection of pentobarbital sodium solution for examination of external abnormalities. The eyes were examined for alterations after removing the palpebral skin. Then the foetuses were examined for visceral and skeletal abnormalities.

Statistics
Variance analysis using Bartlett's test was evaluated for body weights, adjusted body weights, body weight gains and food consumption of maternal rats, numbers of corpora lutea, implants and live foetuses, and weights of gravid uteri, foetuses and placentas.

Results and discussion
Clinical observations
During the pre-dosing period, clinical observation revealed no abnormalities in any groups.
During the dosing period, no abnormalities were observed in maternal rats of the control group. In the 30 and 300 mg/kg groups, one or two maternal rats had hair loss or scabs on the skin which have been usually observed in the historical control rats. In the 1 000 mg/kg group, 20 out of 22 pregnant females showed slightly loose stool and the increase in its incidence was statistically significant.

During the post-dosing period, slightly loose stool was also observed on the following day of last dosing (day 16 of gestation) in 9 out of 20 females that showed this finding during the dosing period in the 1 000 mg/kg group. Another finding observed during this period was hair loss in 1-2 maternal rats in each treated group.
No deaths occurred during the study in any groups.

Body weight
No significant differences were found in the mean body weights and the mean adjusted body weights of maternal rats between the control groups and any of the treated group.
No significant differences were found in the mean body weight gains of maternal rats between the control group and any of the treated groups.

Food consumption and compound intake
No significant differences were found in the mean food consumption of maternal rats between the control group and any of the 30 and 300 mg/kg groups. In the 1 000 mg/kg group, lower and higher values were observed in the mean food consumption at intervals of days 6-9 of gestation (early dosing period) and days 15-20 of gestation (post-dosing period), respectively, and the differences from the corresponding controls were statistically significant.

Necropsy:
Gross pathology at cesarian section
Gross pathological examination of maternal rats at cesarean section revealed several findings such as hair loss and pelvic dilatation in the kidney in 1-2 animals in all groups including the control group. These findings were not considered to be due to test substance treatment.

Ovaries and uterus
Out of 24 copulated females, 23, 24, 24 and 22 were proved to be pregnant in the control, 30 mg/kg, 300 mg/kg and 1 000 mg/kg groups, respectively.
No significant differences were found in the mean gravid uterine weights and the mean numbers of corpora lutea and implants between the control group and any of the treated group.

Foetuses
Number of live foetuses and percent incidences of resorptions and foetal deaths
There were no significant differences in the mean number of live foetuses and the mean percent incidence of resorptions and foetal deaths between the control group and any of the treated groups.

Sex ratio, fetal body weights and placental weights.
There were no significant differences in the foetal sex ratio, the mean foetal body weights and the mean placental weights between the control group and any of the treated group.

Findings in external, visceral and skeletal examination
External malformations observed were short tail in a foetus of the 30 mg/kg group and microphthalmia in a foetus of the 1 000 mg/kg group.
Visceral examination revealed two types of malformations: right aortic arch in a foetus of the 300 mg/kg group and ventricular septal defects in a foetus of each of the 300 and 1 000 mg/kg groups.

Visceral variations were observed in all groups including the control group. The types and number in fetuses were thymic remnant in the neck, dilatation of the renal pelvis and left umbilical artery in 16-26, 1-2 and 0-3, respectively.
Skeletal examination revealed three types of malformations: splitting of the ossification centers of the thoracic vertebral bodies in 2, 1 and 2 foetuses in the control, 300 mg/kg and 1 000 mg/kg groups, respectively, asymmetry of the sternebrae with sterno-costal joint displacement in a foetus of the 300 mg/kg group, and fusion of the sternebrae in a foetus of the 300 mg/kg group, and fusion of the sternebrae in a foetus of the 1 000 mg/kg group.
Skeletal variations were observed in all groups including the control group. The types and the number in foetuses were cervical ribs shortening of the 13th ribs, lumber ribs, sacralization of the lumber vertebra and asymmetry and/or splitting of the sternebrae in 0-1, 0-1, 1-11, 0-1 and 3-5, respectively

Conclusion by the Notifiers
Based on these results, no observable effect level and minimal toxic level in the teratogenicity study with technical glyphosate in SD rats were established as follows.

	Maternal rats	**Foetal rat**
No observable effect level	300 mg/kg/day	1000 mg/kg/day
Minimal toxic level	1000 mg/kg/day	-

It is also concluded that the highest dose level of 1000 mg/kg/day of HR-001 is not teratogenic to SD rat foetuses.

Comment by RMS:
The study is considered acceptable. The evaluation regarding maternal toxicity is agreed, which was confined to loose stool at highest dose level. The NOAEL for developmental toxicity is not supported due to the slight increase in skeletal variations at highest dose level: lumbar ribs were observed in 11 fetuses out of 7 litters compared to only 4 fetuses out of 2 litters in control animals. Teratogenic effects were not observed. Based on findings in dams and foetuses, the NOAEL for both maternal and developmental toxicity is considered to be 300 mg/kg bw/d.

B.6.6.11 Teratogenicity test by the oral route in the rabbit

Five developmental studies in rabbits had been submitted for the previous EU evaluation of which four may be still used following re-evaluation by the RMS. For the current re-evaluation the feeding study in rabbits by [redacted] *(1981, TOX9650160) was considered not acceptable due to serious reporting deficiencies and therefore not reported in the present RAR.*
In addition, three new studies in rabbits were provided in the GTF dossier. These studies were submitted either for the first time for this evaluation or had been subject to JMPR evaluation in 2004 yet and were all considered acceptable by the RMS.

Reference:	IIA, 5.6.11/01
Report:	[redacted] (1995) HR-001: A Teratogenicity Study in Rabbits [redacted] Data owner: Arysta Life Sciences Study no.: IET 94-0153 Date: 1995-07-21 not published ASB2012-11498
Guidelines:	OECD 414 (1981), JMAFF 59 NohSan 4200 (1985), US-EPA 83-3 (1984)
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: 1995-03-31 to 1995-06-09

Materials and methods

Test material:	
Identification:	Glyphosate technical, Code: HR-001
Description:	White crystal
Lot/Batch #:	T-941209
Purity:	97.56 %
Stability of test compound:	Not reported
Vehicle and/ or positive control:	0.5 % carboxymethylcellulose
Test animals:	
Species:	Rabbit
Strain:	Japanese White rabbits Kbl:JW, SPF
Source:	[redacted]
Age:	18 weeks (females); 5-50 month (males)
Sex:	Males and females
Weight at dosing:	3.32 – 4.08 kg
Acclimation period:	10 days
Diet/Food:	GC4 (Oriental Yeast Co., Ltd.), *ad libitum* (females) / *120 g/day* (males)
Water:	Tap water, *ad libitum*
Housing:	Individually in aluminium cages with wire-mesh floors.
Environmental conditions:	Temperature: 22 ± 2 °C Humidity: 55 ± 10 % Air changes: 15/hour 12 hours light/dark cycle

Study design and methods:
In life dates: 1995-03-31 to 1995-06-09

Animal assignment and treatment:
In a teratogenicity study groups of 18 Japanese White female rabbits received doses of 0, 10, 100 and 300 mg/kg bw/day test substance in carboxymethylcellulose by gavage from Gestation Day 6-18 after artificial insemination performed on 12 or 16 females each day for 5 consecutive days. The dose levels were chosen based on results of a preliminary teratogenicity study.

Diet preparation and analyses
For each dose level, dosing solutions were prepared two times during the study by suspending the test substance in purified water with the aid of 0.5 % sodium carboxymethylcellulose. For each dose level dosing solutions were analyzed for concentration of the test substance before use.

Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily during the pre- and post-dosing periods and twice daily (before and after dosing) during the dosing period.

Body weight
Individual body weights were recorded on Day 0, 6-18, 24 and 27 of gestation. Body weight gains were calculated by subtracting the body weight value on Day 0 of gestation from each value determined on Days 6 through 27 of gestation. Adjusted weights were also calculated by subtracting the gravid uterine weight from the body weight on Day 27 of gestation.

Food consumption
Food consumption of females was determined on alternate days from Day 0 to Day 26 of gestation and on Days 26- 27 of gestation. In each interval, daily food consumption (g/rabbit/day) was calculated for each female by dividing values of total food consumption by the number of days.

Sacrifice and pathology
Females were euthanatised by an injection of an overdose of a pentobarbital sodium solution into the auricular vein on Day 27 of gestation and subjected to caesarean sectioning.
The ovaries and uteri were removed, weighed and then examined for the number of corpora lutea and for the number and position of implants and dead or live foetuses. Resorptions and foetal deaths were classified into implantation sites, placental remnants, and macerated foetuses according to the difference in developmental stage at which deaths had occurred. When uterine implants were not grossly apparent, the uteri were stained with 10 % ammonium sulfide solution to detect very early resorptions. After examination of the ovaries and conceptuses, each female was necropsied.

Developmental parameters
Live foetuses and their placentas were individually weighed. Live foetuses were uniquely identified by litters. Then they were euthanatized by an intraperitoneal injection of a pentobarbital sodium solution and examined for external abnormalities. The eyes were examined for alterations after removing the palpebral skin. The sex of the foetuses was determined by observation of the gonads.

After these examinations, each foetus was examined for visceral abnormalities. Then the thoracic and abdominal organs were removed and preserved in 10 % neutral- buffered formalin along with the ovaries and placentas. The remaining skeletons were fixed in 70 % isopropanol, stained with alizarin red S and cleared in 70 % glycerin for examination of skeletal abnormalities. After examination, skeletal specimens were stored.

Statistics
The following statistical tests were used to estimate significance of differences between the control group and the treated groups. The data on body weights, adjusted body weights, body weight gains, and food consumption of maternal rabbits, numbers of corpora lutea, implants, and live foetuses, and weights of gravid uteri, foetuses and placentas were evaluated as follows: Equality of variances was first evaluated by Bartlett's test. When group variances were homogeneous, a parametric analysis of variance in one-way classifications was used to determine if any statistical differences exist among groups. If the analysis of variance was significant, Dunnett's t-test or Scheffé's multiple comparison test was performed to detect any statistically significant differences between the treated groups and their corresponding controls. When Bartlett's test indicated that the variances were not homogeneous, Kruskal-Wallis test was used for detecting any statistical differences among groups and if significant, Dunnett-type mean rank test or Scheffé-type mean rank test was performed to detect statistical differences between the treated groups and their corresponding controls. Fisher's exact probability test was used for the data on the incidences of clinical and gross pathological findings in maternal rabbits, incidences of maternal rabbits having foetuses with malformations and variations, incidences of foetal malformations and variations, and foetal sex ratio, and Mann-Whitney's U-test for the data on the percent incidences of resorptions and foetal deaths.

Results and discussion
Analysis of dose formulations
The test substance was detected at levels of 95-105 % of the target concentrations in each dosing solution.

Food consumption
Mean food consumption in the treated groups was comparable to that in the control group throughout the study period.

Mortality
One rabbit in the high dose group died on Day 20 of gestation without showing any clinical signs.

Clinical observations
During the treatment period one animal each showed hair loss (forelimb) and scab on the auricle, respectively in low and mid dose group, respectively (see Table B.6.6-26). In the high dose group four animals showed loose stool and two showed soiled fur in the perianal region that was considered to be an alteration caused by defecation of loose stool. The incidence of loose stool was significantly high when compared with the control.
During the post-dosing period, two and one animal in the control group showed loose stool and red material on the tray, respectively. In the low dose group, hair loss (forelimb) was found in one animal and loose stool in another. Besides these findings, one dam aborted on Day 20 of gestation, and another one prematurely delivered on Day 27 of gestation. In the mid dose group only one animal showed hair loss in the lower abdominal region. In the high dose

group, two animals out of four, that had shown loose stool during the dosing period, still showed this alteration, and one animal out of these two aborted on Day 26 of gestation. Although loose stool disappeared from the two other dams, the first prematurely delivered on Day 27 of gestation and the second had hair loss (dorsal region).
Considering the results of the preliminary study, defecation of loose stool and subsequent abortion or premature delivery observed in the highest dose group were considered to be related to test substance treatment.

Table B.6.6-26: Observed clinical signs during the dosing period

Clinical sign	**Number of rabbits affected in dose group**[#]			
	Control (0 mg/kg/day)	Low (10 mg/kg/day)	Mid (100 mg/kg/day)	High (300 mg/kg/day)
No abnormalities detected	18/18 (0)	16/17 (1)	15/16 (2)	13/17 (0)
Hair loss	0/18 (0)	1/17 (0)	0/16 (0)	0/17 (0)
Scab on the auricle	0/18 (0)	0/17 (0)	1/16 (0)	0/17 (0)
Soiled fur in the perianal region	0/18 (0)	0/17 (0)	0/16 (0)	2/17 (0)
Loose stool	0/18 (0)	0/17 (0)	0/16 (0)	4/17 (1)*

[#] x/y: number affected / total number of animals in group
* Significantly different from control at $p < 0.05$.
Figures in parentheses represent the number of animals having no grossly observable conceptus. These animals were excluded from statistical evaluation.

Body weight
Mean body weights of animals in the low and mid dose group were comparable to those in the control group. In the high dose group, although differences from controls were not statistically significant, the mean values on Days 16-24 of gestation were somewhat lower than those in the control group.

Pathology
Necropsy
Necropsy of maternal animals aborted, prematurely delivered or found dead on the study noted no abnormalities in the rabbits in the low dose group. In the high dose group, the aborted rabbit had yellow-coloured adipose tissue, a hair bolus in the stomach, watery contents in the large intestine and accentuated lobular pattern in the liver. The prematurely delivered rabbit in the high dose group had soiled fur in the perianal region, erosion in the stomach, a hair bolus in the stomach, and watery contents in the caecum. In the dead rabbit, pale liver and ascites (red) in the abdominal cavity were found; however, the cause of death was not known.
Gross pathological findings observed in animals which survived to termination of the study were: hair loss in the lower abdominal or dorsal region in one animal in each of the mid and high dose groups; hair bolus in the stomach in one animal each of the control and low dose groups. The occurrence of these gross pathological findings was low, and considered to be unrelated to test substance treatment.

Observations on the ovary and uterus
In the control, low, mid and high dose groups, 18, 16, 18, and 15 females, respectively, survived to termination of the study and were proven to be pregnant. However, one, two and one females in the low, mid and high dose group, respectively, had no grossly observable conceptus while implantation sites were detected by uterine staining with a 10 % ammonium

sulfide solution, indicating very early resorptions;. all data from these females were excluded from subsequent calculations.
Examinations of uterine contents demonstrated no abnormalities in all groups including the control. Mean gravid uterine weights and mean numbers of corpora lutea and implants were comparable between the control and the treated groups.

Developmental parameters
Number and viability of foetuses
No statistically significant differences were noted in the mean number of live foetuses and mean percent incidences of resorptions and foetal deaths between the control group and the treated groups.

Sex ratio, foetal body weights and placental weights
No statistically significant differences were noted in the sex ratios, mean foetal body weights, mean placental weights, mean number of live foetuses, and mean percent incidences of resorptions and foetal deaths between the control group and the treated groups.

External, visceral and skeletal examination
No statistically significant differences were noted in the incidences of maternal animals having foetuses with external, visceral and/or skeletal malformations in the low and mid dose groups when compared with the controls. In the high dose group, the number of litters with malformations was significantly higher than that in the control group (see Table B.6.6-27). This increased malformation rate was due to an increase in skeletal malformations, as no external or visceral malformations were noted in foetuses from the high dose group. This was considered to be a sporadic alteration rather than the test substance treatment-related alteration because the types of skeletal malformations observed were inconsistent. Further, a dose-response in the number of foetuses showing skeletal malformations was not evident across dose groups.

With regard to variations, the incidence of total no. of litters with skeletal variations in the 100 mg/kg bw/day group was significantly higher than that in the control group. This high value was due to a significantly high incidence (87.5 % of litters, 27.3 % of the foetuses) of lumbar ribs in this dose group when compared with the control (72.2 % of litters, 16.4 % of foetuses). The total litter incidence for skeletal variations in the 100 mg/kg/day group was 100 %. However, the increased incidence of lumbar ribs in the 100 mg/kg/day group was considered to be a sporadic alteration because the value was within the historical control range (8.1-35.0 % of examined foetuses), and because no such increase was observed in the 300 mg/kg bw/day group (13.4 %).

Table B.6.6-27: Incidence of fetal malformations and variations in rabbits treated with HR-001

Foetal findings	**Dose level (mg/kg bw/day)**			
	0	10	100	300
Malformations				
No. of litters examined	18	15	16	14
No. of foetuses examined	140	130	150	112
No of litters with anomalous foetuses	1	3	3	5*
Percentage of litters with malformations (%)	5.5	20.0	18.8	35.7
Skeletal malformations				
Fusion of the frontal/parietal bones	0	1	0	2
Fissure of the parietal bone	0	0	3	0
Hypoplasia of the interparietal bone	0	1	0	0
Splitting of the parietal bones	0	0	3	1
Shortening of the nasal/frontal/mandibular bones	0	0	1	0
Hemivertebra	1	0	0	2
Unilateral ossification centre of the thoracic/lumbar vertebral bodies	0	1	0	0
Bifurcation of the ribs	1	0	0	0
Sternal cleft	0	0	1	0
Splitting of the sternebrae with sternocostal joint displacement	0	2	0	0
Total no. of foetuses with skeletal malformations	1	4	6	5
Percentage of foetuses with skeletal malformations (%)	0.7	3.1	4.0	4.5
Total no. of litters with skeletal malformations	1	3	2	5
Percentage of litters with skeletal malformations (%)	5.6	20.0	12.5	29.4
Variations				
No. of litters examined	18	15	16	14
No. of foetuses examined	140	130	150	112
No of litters with anomalous foetuses	16	14	16	8*
Percentage of litters with variations (%)	88.9	93.3	100	57.1
Skeletal variations				
No. of foetuses examined	140	130	150	112
27 presacral vertebrae	4	1	4	3
27 presacral vertebrae with 13th ribs	12	9	15	12
Cervical ribs	1	3	1	1
Lumbar ribs	23	19	41*	15
Extra ossification centre anterior to the 1st sternebra with costal cartilage joining	0	0	0	1
Total no. of foetuses with skeletal variations	40	32	61*	31
Total no. of litters with skeletal variations	16	12	16	8
Percentage of litters with skeletal variations (%)	88.9	80	100	57.1

* Significantly different from control at $p < 0.05$.

Conclusion by the Notifiers

The oral administration of HR-001 to artificially inseminated rabbits by gavage from Gestation Day 6-18 resulted in treatment-related changes at 300 mg/kg bw/day. Therefore the 'No Observed Adverse Effect Level' (NOAEL) was considered to be 100 mg/kg bw/day for maternal toxicity. The NOAEL for offspring was considered to be 300 mg/kg bw/day.

Comment by RMS:

The study is considered acceptable. The NOAEL for both maternal and developmental toxicity in this study conducted with artificial inseminated rabbits are agreed. No developmental toxic effects were observed.

Reference:	IIA, 5.6.11/02
Report:	[redacted] (1996) Glyphosate technical: Oral gavage teratology study in the rabbit [redacted] Data owner: Nufarm SPL project no.: 434/020 Date: 1996-07-04 GLP: yes not published ASB2012-11499
Guidelines:	OECD 414 (1981), JMAFF 59 NohSan 4200 (1985), US-EPA 83-3 (1984)
Deviations:	None
GLP:	
Acceptability:	*See RMS comment*

Dates of experimental work: 1995-10-13 - 1995-12-12

Materials and methods

Test material:	
Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	H95D161A
Purity:	95.3 %
Stability of test compound:	not reported
Vehicle and/ or positive control:	1 % carboxymethyl cellulose
Test animals:	
Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	17 - 19 weeks
Sex:	Females (time-mated)
Weight at dosing:	2.2 - 4.1 kg
Acclimation period:	At least 4 days
Diet/Food:	SQC Standard Rabbit Diet (SDS Ltd., Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel cages with grid floor
Environmental conditions:	Temperature: 20 ± 3 °C Humidity: 50 ± 20 % Air changes: 15/hour 12 hours light/dark cycle

Study design and methods:
In life dates: 1995-10-13 - 1995-12-12

Animal assignment and treatment in the preliminary study:
Twenty-four time-mated females were supplied. Sexually mature, virgin females were paired with stud males. The day of copulation was designated Day 0 of gestation. The females were delivered to Safepharm Laboratories Ltd. at or before Day 3 of gestation and were allocated randomised to treatment groups. Groups of 6 mated New Zealand white female rabbits received 0, 50, 200 or 400 mg/kg bw/day test substance in 1 % carboxymethyl cellulose by gavage (5 mL/kg bw) from gestation Day 7-19. The dose levels were chosen based on results of a preliminary dose finding study with 6 female nulliparous rabbits, where administration of 500 or 1000 mg/kg bw resulted in toxicity signs (scours, fluid filled caecum, stomach ulceration, body weight loss, reduced food consumption). Based on these findings dose levels of ≥ 500 mg/kg bw were considered to be too high for a prolonged study.

Animal assignment and treatment in the main study:
Seventy-two time-mated females were supplied as described for the preliminary study (see above). Groups of 18 mated New Zealand white female rabbits received 0, 50, 200 or 400 mg/kg bw/day test substance in 1 % carboxymethyl cellulose by gavage (5 mL/kg bw) from gestation Day 7-19.

Dose formulation and analysis
For each dose level, the test material was suspended daily in 1% carboxymethyl cellulose by weighing the required amount into a glass jar and adding vehicle to make the appropriate final volume. Homogeneity was assured by mixing the formulation with a homogeniser. The concentration, stability and homogeneity of the test material were analysed. The formulation was stable for at least 1 h.

Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily during the pre- and post-dosing periods and twice daily (before and after dosing) during the dosing period.

Body weight
Individual body weights were recorded on Day 3, 7, 10, 13, 16, 19, 22, 25 and 29 of gestation.

Food consumption
Food consumption of females was recorded on Days 3 to 7, Days 7 to 10, Days 10-13, Days 13-16, Days 16-19, Days 19-22, Days 22-25 and Days 25-29 of gestation.

Sacrifice and pathology
Females were euthanatized by an i.v. injection of an overdose of sodium pentobarbitone into the auricular vein on Day 29 of gestation, examined for macroscopic abnormalities and subjected to caesarean sectioning. The ovaries and uteri were removed, weighed and then examined for the number of corpora lutea and for the number and position of implants and dead or live foetuses. Resorptions and foetal deaths were classified into implantation sites, placental remnants, and macerated foetuses according to the difference in developmental stage at which deaths had occurred. After examination of the ovaries and conceptuses, each female was necropsied.

Developmental parameters
The foetuses were killed by intrathoracic injection of sodium pentobarbitone. All foetuses were dissected and examined for visceral abnormalities macroscopically. The heads of alternate foetuses were removed and identified using an indelible marker and placed in Bouin‘s fixative. After a minimum of 14 days, the heads were transferred to 90 % industrial methylated spirits (IMS) in distilled water and examined for visceral anomalies under a low power binocular microscope. All foetuses were identified using colour coded wires and placed in 70 % IMS in distilled water. The foetuses were eviscerated, processed and the skeletons stained with alizarin red.The foetuses were examined for skeletal development and anomalies.

Statistics in the main study
Female bodyweight change (relative to Day 7 of gestation) and food consumption were analysed statistically by one-way analysis of variance with the Bonferroni multiple comparison test followed by pair wise analysis of control values against treated group values using Students ‘t‘ test where appropriate.
All foetal parameters, skeletal development, group incidence of specific visceral and skeletal anomalies were analysed statistically by Kruskall-Wallis non parametric analysis of variance followed by pair wise analysis of control values against treated values using the Mann-Whitney U - test where appropriate.

Results and discussion
Analysis of dose formulations
The test substance was detected at the levels of 81-102% of the target concentrations in each dosing solution.

Food consumption
In the preliminary study, significantly reduced food consumption was observed while administering in the high dose level of 400 mg/kg/day (Days 7 to19 of gestation). This observation was confirmed in the main study. At the high dose level, there was a reduction in food consumption during the dosing period compared to controls (Days 10 to 13, $p < 0.05$; Days 13 to 19, $p < 0.01$). No other significant changes were observed in the remaining groups during the main study.

Mortality
In the preliminary study, two does were killed in extremis in the high dose group, one had aborted foetuses and the other was bleeding from the vagina. No mortalities occurred at any dose up to 400 mg/kg/day in the preliminary study.

In the main study, two rabbits were found dead or moribund at the high dose level. One female was found dead prior to dosing on Day 19 of treatment. One female was killed *in extremis* on Day 20 of treatment. Clinical observations noted at this time included hunched posture, lethargy, ptosis, hypothermia and blood on the litter tray. At the intermediate dose level, one female was found dead after dosing on Day 16 of treatment. Necropsy findings of reddened lungs, a fluid filled thorax and test material in thoracic cavity are consistent with mal-dosing. At the low dose level, no mortalities occurred. One female was found dead two minutes after dosing in the control group. Necropsy findings of blood in thorax, inflated appearance of lungs and a large area of congestion on the right caudal lobe are consistent with mal-dosing.

Clinical Observations

In both the preliminary and the main study, the clinical signs were in general the same. There was a toxicologically significant increase in the incidence of clinical observations, particularly scours, reduced faecal output and diarrhoea at the high dose level (400 mg/kg bw/day). Observations of lethargy, ptosis, hunched posture, hypothermia and blood on tray were noted for one animal of the main study killed *in extremis*.

At 200 mg/kg bw/day, vaginal bleeding and blood on tray were noted for one animal of the main study. Scours were also noted in animals at 200 and 50 mg/kg bw/day as well as in the control group, but the incidence and duration were not as severe as at the high dose level (see Table B.6.6-28). No other treatment-related observations were evident.

Thus, for the findings observed at doses below 400 mg/kg bw/day, a clear dose-response could not be established.

Table B.6.6-28: Observed clinical signs during the dosing period

Clinical sign	**Number of rabbits affected in dose group**[#]			
	Control (0 mg/kg/day)	Low (50 mg/kg/day)	Intermediate (200 mg/kg/day)	High (400 mg/kg/day)
Scours	5/14 (4)	10/18 (0)	7/16 (2)	16/16 (2)
Reduced faecal output	0/14 (4)	1/18 (0)	2/16 (2)	2/16 (2)
Diarrhoea	0/14 (4)	1/18 (0)	0/16 (2)	10/16 (2)
Diuresis	0/14 (4)	0/18 (0)	1/16 (2)	0/16 (2)
Blood on tray	0/14 (4)	0/18 (0)	1/16 (2)	1/16 (2)
Noisy respiration	0/14 (4)	0/18 (0)	1/16 (2)	1/16 (2)
Lethargy	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Ptosis	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Hunched posture	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Hypthermia	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Anal staining	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Subdued behaviour	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Vaginal bleeding	0/14 (4)	0/18 (0)	1/16 (2)	0/16 (2)

[#] x/y: number affected / total number of animals in group
Figures in parentheses represent the number of animals having no grossly observable conceptus.

Body weight

In the preliminary, study a toxicologically significant decrease in body weight gain from Day 13 to 19 *post coitum* was evident at the high and intermediate dose levels.

Likewise a reduction in group mean body weight gain from Days 9 to 29 *post coitum* was observed in the high dose level group during the main study. The difference in group mean bodyweight change compared to controls was statistically significant (P<0.05 to 0.01) from Days 13 to 29 *post coitum.* Also in the intermediate dose level group a slight reduction (although not statistically significant) in group mean body weight gain from Day 9 to Day 29 *post coitum* was noted. In the low dose level group body weight gain was comparable to controls throughout the study period (see Table B.6.6-29).

Table B.6.6-29: Mean body weight gain during gestation

Dose level (mg/kg bw)	No. of animals	Body weight change (g) at Day (relative to Day 7)						
		10	13	16	19	22	25	29
0 (Control)	14	29	95	202	260	314	375	409
50	18	12	75	158	223	278	325	395
200	15	-11	54	143	198	263	309	294
400	15	-33	-45*	11**	21**	96**	153**	250*

* Significantly different from control at p < 0.05.
** Significantly different from control at p < 0.01.

Pathology
Necropsy
The macroscopic necropsy findings of the two does of the high-level dose group that died or were killed *in extremis* included fluid filled large intestines, haemorrhage, ulceration and sloughing of the stomach, duodenum congested and colon, rectum and appendix gas distended. These findings indicate that the test material may affect the gastrointestinal tract. The animal killed *in extremis* at this level also had both uterine horns containing blood and dead foetuses in the uterus. This may be a result of maternal toxicity. All other necropsy findings were not treatment-related.

Observations on the ovary and uterus
No treatment related effects were evident in both the preliminary and the main study.

In the control, low, intermediate and high dose level groups 14, 18, 16, and 16 females, respectively, survived to termination of the main study and were proven to be pregnant. The number and distribution of females that were not pregnant indicate that there were no treatment-related effects on pregnancy rates. Litter size at caesarean necropsy was comparable in all treatment groups.

Developmental parameters
Number and viability of foetuses
The litter size at caesarean section was comparable in all treatment groups. In the high dose level group, there were slight, but not statistically significant, increases in late foetal deaths and post implantation loss, mainly due to one animal that had nine late deaths, resulting in a post implantation loss of 69.2 %. This was therefore considered not to be a treatment-related effect. At 200 mg/kg bw/day, there were statistically significant increases ($p<0.05$) in total foetal deaths and post implantation loss. These increases were caused by a slight, but not statistically significant, rise in early foetal deaths. As at this dose level, there was no rise in late foetal deaths, as seen at the high level; the effect on early foetal deaths was considered not to be treatment-related.

Foetal body weights
No statistically significant differences were noted in the mean foetal body weights between the control group and the treated groups. Mean total litter weights were comparable in all treatment groups.

External, visceral and skeletal examination
At the high dose level, there was one litter with one foetus with major malformations. This foetus was found to have spina bifida and clubbed and malrotated hind limbs. At the intermediate dose level, two foetuses of two different litters had major malformations. One

foetus had retinal infolding and a haemorrhage in the retinal layer, the other acephaly, small kinked tail, bilateral forelimb flexure, interrupted aorta and an intraventricular septal defect. At skeletal examination, this foetus was found to have multiple rib and vertebral column abnormalities. At the low dose level, three foetuses of two different litters had major abnormalities. In one litter, one foetus had forked ribs with a displaced vertebral centrum. In another litter, one foetus had a small eye with retinal infolding and aphakia. A second foetus from this litter had nostrils close together, and a thin nasal septum not attached at posterior pole near the front of the nasal passages. In the control group, there were two foetuses from two different litters with major abnormalities. One foetus had gastroschisis and the other foetus had an extra vertebral arch resulting in scoliosis.
These findings were considered to be within the range of normal variation for this species. There were no treatment-related effects on the degree of skeletal development.

Table B.6.6-30: Incidence of foetal malformations and variations in rabbits treated with glyphosate acid

Foetal findings	**Dose level (mg/kg bw/day)**			
	0	50	200	400
No. of litters examined	14	18	15	15
No. of foetuses examined	128	157	119	134
Skeletal malformations				
Total no. of foetuses with skeletal malformations	1	0	1	0
Total no. of litters with skeletal malformations	1	0	1	0
Percentage of litters with skeletal malformations (%)	7.1	0.0	6.7	0.0
Skeletal variations				
Total no. of foetuses with skeletal variations	43	48	39	49
Total no. of litters with skeletal variations	13	18	15	15
Percentage of litters with skeletal variations (%)	92.8	100	100	100
External and visceral findings				
No. of litters examined	14	18	15	15
No. of foetuses examined	128	157	119	134
No of litters with anomalous foetuses	2	5	2	3
Percentage of litters with anomalous foetuses (%)	14.3	27.8	13.3	20
No. of litters with major malformations	2	2	2	1
Percentage of litters with malformed foetuses (%)	14.3	11.1	13.3	6.7

Conclusion by the Notifiers
The oral administration of glyphosate technical to pregnant rabbits by gavage from gestation Day 7-19 resulted maternal toxicity at 400 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level. Therefore the 'No Observed Adverse Effect Level' (NOAEL) was considered to be 200 mg/kg bw/day for maternal toxicity. The 'No Observed Adverse Effect Level' (NOAEL) for developmental toxicity was considered to be 400 mg/kg bw/day.

Comment by RMS:
The study is considered acceptable. The NOAEL of 200 mg/kg bw/d for maternal toxicity is not supported. The NOAEL is considered to be 50 mg/kg bw/d due to slight reduction in body weight gain at 200 mg/kg bw/d.
The NOAEL of 400 mg/kg bw/d for developmental toxicity is neither supported. The NOAEL is considered to be 50 mg/kg bw/d due to significantly increased post-implantation loss at 200 mg/kg bw/d. The statement, that this increase was caused by a slight rise in early foetal deaths and not in late foetal deaths, as seen at the high dose level and therefore considered not to be treatment-related, cannot be followed, because there is no information given

regarding the mechanism behind this foetal deaths. Due to some reporting deficiencies, it remains unclear, whether the heart was part of visceral examination.

Comment by GTF on the first draft of the RAR (July 2013):
1. The GTF believes the developmental NOAEL in [redacted] is 400 mg/kg/day, with no evidence to test substance related dose-response, citing the published expert review: Kimmel et al., 2013, (ASB2013-3462) Evaluation of developmental toxicity studies of glyphosate with attention to cardiovascular development, *Crit Rev Toxicol*, 2013; 43(2): 79–95.

2. Regarding [redacted] (1996, ASB2012-11499) the RMS notes that "it remains unclear whether the heart was part of visceral examination". The study sponsor contacted the contract laboratory and the response is noted….The contract laboratory Head of reprotox at the contract notes Reprotox "The examination of the heart is conducted on the fresh fetus for rabbit developmental toxicity studies. This is mentioned in our standard operating procedure". This is reflected the draft RAR Volume 3, B.6.6.11, noting "All foetuses were dissected and examined for visceral abnormalities macroscopically"……..

RMS comment (August 2013):
1. As already stated, the NOAEL is considered to be 50 mg/kg bw/d due to significantly increased post-implantation loss at 200 mg/kg bw/d. The statement, that this increase was caused by a slight rise in early foetal deaths and not in late foetal deaths, as seen at the high dose level and therefore considered not to be treatment-related, cannot be followed, because there is no information given regarding the mechanism behind this foetal deaths (please refer to the additionally inserted table below. Considering the individual animal data: at low dose level of 50 mg/kg bw/d 4/18 pregnant animals revealed post implantation losses, which was comparable to control animals (4/14 animals). Whereas at the mid dose of 200 mg/kg bw/d 10/15 animals were affected and at 400 mg/kg bw/d 9/15 animals. Currently, RMS does not see the need to change the assessment.
[Remark: The published expert review by Kimmel et al. (2013, ASB2013-3462) was already available and considered when preparing the first draft of the RAR, please refer to B.6.6.12-Published data]

Table B.6.6-31: Group Mean Litter Data

Group	Dose Level (mg/kg)	Number of Animals	Number Pregnant at Necropsy		Number of Corpora Lutea	Number of Live Foetuses	Total Number of Implants	% Male Foetuses	Embryonic/Foetal Deaths			Implantation Loss %		Total Litter Weight (g)	Mean Foeta Weight (g)
									Early	Late	Total	Pre	Post		
1	0	18	14	Mean	10.9	9.1	9.5	56.2	0.21	0.14	0.36	12.5	3.7	372.0	41.5
				SD	2.2	2.5	2.5	10.5	0.43	0.53	0.63	18.2	6.5	92.6	5.5
2	50	18	18	Mean	10.5	8.7	9.1	51.0	0.22	0.11	0.33	13.6	3.6	334.6	39.4
				SD	2.4	2.4	2.3	11.4	0.55	0.32	0.77	9.4	8.5	75.1	5.6
3	200	18	15	Mean	10.7	7.9	8.9	53.9	0.87	0.13	1.00*	16.4	11.5*	321.5	41.7
				SD	2.1	2.5	2.5	11.3	1.06	0.35	1.00	15.5	11.4	79.2	4.5
4	400	18	15	Mean	11.5	8.9	10.3	50.8	0.47	0.93	1.40	9.3	12.1	337.0	38.2
				SD	1.8	2.6	2.3	11.7	0.92	2.28	2.35	12.5	18.6	84.4	5.2

SD = standard deviations * = significantly different from control value ($p < 0.05$)

2: The additional information is acceptable, but was not given in the study report.

Reference:	IIA, 5.6.11/03
Report:	[redacted] (1996) Glyphosate acid: Developmental toxicity study in the rabbit [redacted] Data owner: Syngenta Report No.: CTL/P/5009 Date: 1996-07-02 not published TOX2000-2002
Guidelines:	OECD 414 (1981), EEC B.31 (1988),US-EPA 83-3
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: 1996-01-01 to 1996-02-09

Materials and methods

Test material:	
Identification:	Glyphosate acid
Description:	White solid
Lot/Batch #:	Y04704/034
Purity:	95.6 %
Stability of test compound:	The stability of the test substance was confirmed for the study period.
Vehicle and/ or positive control:	Deionised water
Test animals:	
Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Not reported
Sex:	Females (time-mated)
Weight at dosing:	approximately 3.8 kg
Acclimation period:	At least 4 days
Diet/Food:	Harlan Teklad 9603TRB rabbit diet, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in mobile rabbit units
Environmental conditions:	Temperature: 17 ± 2 °C Humidity: 55 ± 15 % Air changes: 25-30/hour 12 hours light/dark cycle

Study design and methods
In life dates: 1996-01-01 to 1996-02-09

Animal assignment and treatment:
Eighty time-mated females were supplied. Sexually mature, virgin females were paired with stud males. The day of copulation was designated Day 1 of gestation. The females were delivered to CTL at or before Day 3 of gestation and were allocated randomised to treatment groups. Groups of 20 time-mated New Zealand white female rabbits received 0, 100, 175 or 300 mg/kg bw/day test substance by gavage (2 mL/kg bw) from gestation Day 8-20. The dose levels were chosen based on results of a preliminary dose finding.

Dose formulation and analysis
For each dose level an appropriate amount of deionised water was added to a weighed amount of glyphosate acid (adjusted for purity). Each preparation was thoroughly mixed and subdivided into aliquots. Fresh aliquots were used for each day of the study. Two preparations were made per concentration (i.e. 0, 50, 87.5 and 150 mg/mL). The dosing preparations were stored at room temperature.
Representative samples of each dosing preparation were analysed prior to being used for dosing to verify the achieved concentration of glyphosate acid in the vehicle. Samples were taken for the determination of homogeneity at 50 and l50 mg glyphosate acid/mL (low and high dose levels).
The chemical stability of glyphosate acid in the vehicle was determined by re-analysis of the lowest and highest concentrations of the dosing preparations after an interval of 40 days.
Dose formulations were shaken prior to dosing, and during dosing as required.

Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily during the pre- and post-dosing periods and twice daily (before and after dosing) during the dosing period.

Body weight
Individual body weights were recorded on arrival, on Day 4, prior to dosing on Days 8 to 20 and on Days 23, 26 and 30 of gestation.

Food consumption
Food consumption of females was recorded on Days 4-8, Days 8-11, Days 11-14, Days 14-17, Days 17-20, Days 20-23, Days 23-26 and Days 26-30 of gestation.

Sacrifice and pathology
All rabbits at scheduled termination on day 30 and any requiring euthanasia during the study were killed by an overdose of 200 mg/mL sodium pentobarbitone solution given as i.v. injection. All animals were subjected to an examination post mortem. This involved an external observation and an examination of the thoracic and abdominal viscera. The pregnancy status of each animal was determined. Where there was no clear evidence of implantation, the uterus was removed and stained with ammonium polysulphide to determine whether or not implantation had occurred. For pregnant animals the intact gravid uterus (minus ovaries and trimmed free of connective tissue) was removed and weighed. The ovaries, uterus and contents where then examined. Number of corpora lutea, number and position of implantations, number of live foetuses, foetus weight and early and late intrauterine deaths were determined for each sacrificed doe.

Developmental parameters
After weighing the foetuses were killed with an intracardiac injection of approximately 0.1 mL of 200 mg/mL pentobarbitone sodium solution. An external examination of each foetus was made together with an examination of the oral cavity. All foetuses were then examined internally for visceral abnormalities, sexed, eviscerated and fixed in 70 % industrial methylated spirits. After approximately 24 h the head of each foetus was cut along the fronto-parietal suture line and the brain was examined for macroscopic abnormalities. The carcasses were then returned to 70 % industrial methylated spirits for subsequent processing and staining with Alizarin Red S. The remaining stained foetal skeletons were examined for abnormalities and the degree of ossification was assessed.

Statistics
Data relating to those animals which were non-pregnant and animals that died intercurrently were excluded from the statistical analysis. Maternal bodyweight during the dosing and post dosing periods was considered by analysis of covariance on initial (Day 8) bodyweight. Maternal food consumption during the dosing and post dosing periods, the numbers of implantations and live foetuses per female, gravid uterus weight, litter weight, mean foetal weights per litter and mean *manus* and *pes* scores per litter were considered by ANOVA. Maternal performance data, the proportion of foetuses with each individual *manus* and *pes* score, the proportion of foetuses with each defect and the proportion of litters with each defect were considered by Fisher‘s Exact Test. Pre-implantation loss, post-implantation loss, early intra-uterine deaths, late intra-uterine deaths, male foetuses, major external visceral defects, minor external/visceral defects, external visceral variants, major skeletal defects, minor skeletal defects and skeletal variants were analysed as follows:
1) Percentages were analysed by ANOVA following double arcsine transformations,
2) the proportion of foetuses and, with the exception of male foetuses, the proportion of litters affected were considered by Fisher‘ s Exact Test.

All analyses were carried out in SAS (1989). For Fisher‘s Exact Tests the proportion in each treated group was compared to the control group proportion. Analyses of variance and covariance allowed for the replicate structure of the study design. Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a Student‘s t-test, based on the error mean square in the analysis.
All statistical tests were two sided.

Results and discussion
Analysis of dose formulations
The concentrations of glyphosate acid in the dosing formulations were within 12 % of the target concentrations. The homogeneity and stability of the test substance in the dosing formulations was satisfactory.

Food consumption
During the dosing period, does receiving 175 or 300 mg/kg bw/day showed reduced food consumption compared to the controls.

Mortality
The incidence of intercurrent deaths was 1, 2, 2 and 2 in the control, 100, 175 and 300 mg/kg/bw/day groups, respectively.
In the post-dosing period, one doe in the control group showed weight loss, reduced food consumption, signs of diarrhoea, mucus in the faeces, few faeces and staining in the genital area. This animal aborted on Day 30. Changes in the stomach and caecum were observed post mortem.
In the low dose level group, one doe showed slight loss of bodyweight and reduced food consumption between Days 4 and 8 (i.e. prior to the onset of dosing) and this response continued into the dosing period, until the animal aborted its litter on Day 19. Examination post mortem noted the presence of a mass in the right inguinal region of the abdominal cavity. A second animal in this group aborted its litter on Day 25 having shown weight loss and reduced food consumption from Day 14.
At the intermediate dose level, one doe was killed for humane reasons on Day 23 having shown loss of bodyweight and reduced food consumption from Day 4 on. By Day 23, the animal had become thin and subdued and all uterine implantations were found to be dead. A second animal in this group aborted its litter on Day 22 having shown slight weight loss from Day 14 and reduced food consumption from Day 4.
At the high dose level, two animals aborted their litters on Days 24 and 23, respectively. Both animals showed a reduction in food consumption from Day 11 and bodyweight loss from Day 11/13. A hair-like substance was found in the stomachs of both animals at examination post mortem.

Clinical Observations
In the high dose level group, there was an increased incidence of animals producing few faeces, with signs of diarrhoea or with staining in the genital area, in comparison with the control group. The production of few faeces and signs of diarrhoea were also of increased incidence in does of the intermediate dose group. There were no clinical effects observed in rabbits treated at a dose level of 100 mg/kg bw/day (see Table B.6.6-32).

Table B.6.6-32: Observed clinical signs during the dosing period

	Number of rabbits affected in dose group			
	Control	Low	Intermediate	High
Clinical sign	(0 mg/kg/day)	(100 mg/kg/day)	(175 mg/kg/day)	(300 mg/kg/day)
Blood on tray	0	2	2	1
Cold	0	0	1	0
Dry sores 1 or more areas	0	1	0	0
Ears torn	0	2	1	1
Eye opaque	0	1	0	0
Few faeces on tray	3	3	9	9
Mucus in faeces	1	0	0	0
No faeces on tray	0	1	2	3
Scabs in 1 or more areas	4	6	3	3
Signs of diarrhoea	4	5	11	19
Staining in genital area	2	2	3	11
Subdued behaviour	0	0	1	0
Thin	0	0	1	2
Urine coloured	0	1	1	0
Wet sores in 1 or more areas	2	0	1	0

Body weight
Administration of 300 mg/kg bw/day was associated with a reduction in maternal body weight gain. The statistical different observed body weight development at 175 mg/kg bw/day was due to differences in body weights at the begin o the study. All animals except one of the high dose group showed signs of recovery in the post-dosing period. The reduction in food consumption was therefore accompanied by a corresponding reduction in body weight. In the low dose level group, body weight gain was comparable to controls throughout the study period (see Table B.6.6-33).

Table B.6.6-33: Mean body weight development (in g) during gestation

		Dose level in mg/kg bw/day			
		0 (Control)	100	175	300
Animals per group		17	18	17	17
Day of gestation	8	3924	3771	3822	3815
	9	3845	3837	3834	3823
	10	3857	3863	3856	3830
	11	3885	3873	3874	3854
	12	3894	3879	3877	3856
	13	3917	3905	3902	3880
	14	3942	3932	3930	3875
	15	3975	3982	3939	3896
	16	4020	4031	3959	3907*
	17	4049	4053	3982	3923*
	18	4063	4051	3990	3914**
	19	4085	4061	4005	3927**
	20	4088	4059	3995	3926**
	23	4177	4118	4049*	3951**
	26	4236	4210	4169	4093*
	30	4313	4294	4256	4183

* Significantly different from control at $p < 0.05$.
** Significantly different from control at $p < 0.01$.

Pathology
Necropsy
There were no macroscopic findings that were considered to be related to the administration of glyphosate acid.

Observations on the ovary and uterus
No treatment related effects were evident in the study.

In the control, low, intermediate and high dose level groups 17, 18, 17, and 17 females, respectively, survived to termination of the main study and were proven to be pregnant. The number and distribution of females that were not pregnant indicate that there were no treatment-related effects on pregnancy rates. Litter size at caesarean necropsy was comparable in all treatment groups.

Developmental parameters
Number and viability of foetuses
The proportion of foetuses that were male was statistically significantly increased in the intermediate dose level group, in comparison with the control group. In the absence of a dose-related trend, this finding was considered incidental to the administration of glyphosate acid.

There was no adverse effect of glyphosate acid on the number or survival of the foetuses in utero.

Foetal body weights
There was a statistically significant reduction in mean foetal weight in the high dose level group, in comparison with the control group. This difference was considered attributable to two litters for which the mean pup weight was particularly low.

External, visceral and skeletal examination
The number of foetuses with major defects was 3/143 (2/17 litters), 1/147 (1/18 litters), 0/135 (0/17 litters) and 2/144 (2/17 litters) in the control, 100, 175 and 300 mg/kg bw/day groups, respectively. Neither the type nor incidence of major defects provided evidence for an adverse effect of glyphosate acid. The proportion of foetuses with minor external visceral defects was similar for all groups, including the control. There were no significant differences in litter incidences for minor external/visceral defects noted. Consideration of the specific defects provided no evidence for an adverse effect of glyphosate acid (see Table B.6.6-34 and Table B.6.6-35).
The proportion of foetuses with minor skeletal defects was statistically significantly increased in the 100 and 300 mg/kg bw/day groups, in comparison with the control group, but not in the 175 mg/kg bw/day group. Evaluation of the specific defects noted an increased incidence of foetuses in the high dose level group with partially ossified transverse processes on the 7th cervical vertebra (8 foetuses in 2 litters), unossified transverse processes on the 7th lumbar vertebra (14 foetuses in 4 litters) or partially ossified 6th sternebra (16 foetuses in 7 litters). None of the specific minor defects were statistically significantly increased in the low or intermediate dose level groups. None of the foetuses were found to have an external/visceral variant.
The proportion of foetuses with skeletal variants was statistically significantly increased in the high dose level group, in comparison with the control group. Evaluation of the specific variants noted a slight, but not statistically significant, increase in the incidence of foetuses in this group with partially ossified odontoids (62 foetuses in 15 litters) or with 27 pre-sacral vertebrae (37 foetuses in 12 litters).
The slightly higher mean *manus* score observed in the high dose level group, in comparison with the control group, was due to a slight reduction in ossification as shown by the increase in incidence of foetuses scoring 4 or 5. A similar response was apparent from the *pes* scores.

Table B.6.6-34: Summary of the type and incidence of major defects

Major foetal defects	**Number of foetuses affected in dose group***			
	Control	Low	Intermediate	High
	(0 mg/kg/day)	(100 mg/kg/day)	(175 mg/kg/day)	(300 mg/kg/day)
Heart single ventricle, ventricle walls thickened, aorta enlarged, pulmonary artery reduced	0/143	1/147	0/135	1/144
Encephalocoele (gross malformation of the skull)	0/143	0/147	0/135	1/144
Cebocephaly, internal hydrocephaly, maxillae fused and shortened, aorta enlarged, persistent truncus arteriosus	1/143	0/147	0/135	0/144
Shortened upper and lower jaws, cleft lip, cleft palate, nares absent, forepaws flexed (right extremely, left slight)	1/143	0/147	0/135	0/144
Reduced number of lumbar vertebrae (25 pre-sacral vertebrae)	1/143	0/147	0/135	0/144

* number affected / total number

Table B.6.6-35: Summary of the type and incidence of major defects (litter incidences)

Major foetal defects	**Number of litters affected in dose group***			
	Control	Low	Intermediate	High
	(0 mg/kg/day)	(100 mg/kg/day)	(175 mg/kg/day)	(300 mg/kg/day)
Heart single ventricle	0/17	1/18	0/17	1/18
aorta enlarged	1/17	1/18	0/17	1/18
pulmonary artery reduced	0/17	1/18	0/17	1/18
Encephalocoele (gross malformation of the skull)	0/17	0/18	0/17	1/18
Cebocephaly, internal hydrocephaly, maxillae fused and shortened, Shortened upper and lower jaws, cleft lip, cleft palate, nares absent	1/17	0/18	0/17	0/18
persistent truncus arteriosus	1/17	0/18	0/17	0/18
forepaws flexed (right extremely, left slight)	1/17	0/18	0/17	0/18
Reduced number of lumbar vertebrae, 25 pre-sacral vertebrae	1/17	0/18	0/17	0/18

* number affected / total number

Table B.6.6-36: Incidence of foetal malformations and variations in rabbits treated with glyphosate acid

Foetal findings	**Dose level (mg/kg bw/day)**			
	0	100	175	300
No. of litters examined	17	18	17	17
No. of foetuses examined	143	147	135	144
Skeletal malformations				
Total no. of foetuses with major defects	3	0	0	1
Total no. of litters with major defects	2	0	0	1
Percentage of litters with major defects (%)	11.8	0.0	0.0	5.9
Total no. of foetuses with minor defects	58	82*	59	79*
Total no. of litters with minor defects	16	18	16	17
Percentage of litters with minor defects (%)	94.1	100	94.1	100
Skeletal variations				
Total no. of foetuses affected	119	129	116	132*
Total no. of litters affected	17	18	17	17
Percentage of litters affected (%)	100	100	100	100
External and visceral findings				
No. of foetuses with major defects	2	1	0	2
No of litters with foetuses with major defects	2	1	0	2
Percentage of litters with foetuses with major defects (%)	11.8	5.6	0.0	11.8
No. of foetuses with minor defects	12	7	9	11
No of litters with foetuses with minor defects	8	5	8	7
Percentage of litters with foetuses with minor defects (%)	47.1	27.8	47.1	41.2

* Statistically significant from control ($p < 0.05$)

Conclusion by the Notifiers

The oral administration of glyphosate acid to time-mated rabbits by gavage at a maximum dose level of 300 mg/kg bw/day from Gestation Day 8-20 resulted maternal toxicity at 175 and 300 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level that could not be attributed to maternal toxicity. Therefore the 'No Observed Effect Level' (NOEL) was considered to be 100 mg/kg bw/day for maternal toxicity. The 'No Observed Effect Level' (NOEL) for developmental toxicity was considered to be 175 mg/kg bw/day.

Comment by RMS

The study is considered acceptable. The NOAEL for both maternal and developmental toxicity are agreed. Maternal toxicity comprised clinical signs, reduced food consumption and body weight gain at 175 mg/kg bw/d and above. Developmental toxicity consisted of reduced foetal body weight and reduced ossification at 300 mg/kg bw/d.

Reference: IIA, 5.6.11/04

Report: [redacted]. (1980)
Technical Glyphosate: Teratology study in rabbits
[redacted]
Monsanto Report No.: IR-79-016
Date: 1980-02-29
not published
TOX9552392

Guidelines:	Not stated. (pre-guideline; satisfies in general the requirements of OECD 414 (1981), but not of OECD 414 (2001))
Deviations:	Not applicable
GLP:	no (pre-GLP study)
Acceptability:	*See RMS comment*

Dates of experimental work: 1979-04-10 to 1979-05-11

Materials and methods

Test material:	
Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	XHJ-64
Purity:	98.7 %
Stability of test compound:	Not reported
Vehicle and/ or positive control:	0.5 % aqueous Methocel®
Test animals:	
Species:	Rabbit
Strain:	Dutch Belted
Source:	[redacted]
Age:	Approx. 7 month
Sex:	Females
Weight at dosing:	2.533 – 3.234 kg
Acclimation period:	At least 30 days
Diet/Food:	Purina Rabbit Chow Checkers 5301, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in suspended wire mesh cages
Environmental conditions:	Temperature: Exact values not reported Humidity: Exact values not reported Air changes: Exact values not reported Light controlled

Study design and methods
In life dates: 1979-04-10 to 1979-05-11

Animal assignment and treatment:
Sixty-four female Dutch Belted rabbits were artificially inseminated and randomly assigned to treatment groups of 16 animals. The day of insemination was designated Day 0 of gestation. The rabbits received daily doses of 0, 75, 175 or 350 mg/kg bw/day test substance by gavage (1 mL/kg bw) from gestation Day 6 to 27. Individual doses based on individual body weights determined on gestation Day 6.

Dose formulation
For each dose level an appropriate amount of grounded technical glyphosate was suspended in 0.5 % aqueous Methocel® solution and homogenised. The dose solutions were prepared daily.

Clinical observations
A check for mortality or behavioural changes was made once daily prior to treatment. During the treatment and post-treatment period all rabbits were observed once daily for clinical signs of toxicity, mortality or behavioural changes.

Body weight
Individual body weights of dams were recorded on gestation Days 0, 6, 12, 18, 24 and 28.
These time points for body weight determination differ from the requirements of the current OECD guideline 414 (2001) (i.e., body weights should be determined on gestation day 0 and at 3-day intervals thereafter). Although the time-intervals were longer than required, the time points for body weight determination are considered to be sufficient to evaluate the body weight development of the pregnant animals.

Sacrifice and pathology
Dams
All rabbits at scheduled termination on day 28 were sacrificed, the uterus was excised and weight and the foetuses were removed. The number and location of viable foetuses, early and late resorptions, the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs were examined for gross pathological changes.
Rabbits that died during the study were necropsied to determine the cause of death.

Foetuses
All foetuses were weight, sexed and examined for external malformation and variations, as well as for visceral malformations and variations. The carcasses were then fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S for skeletal examination.

Statistics
All statistical analyses compared the treatment groups to the control group with a level of significance at $p < 0.05$. Foetal sex distribution and number of litters with malformations were analysed using the Chi-square test with Yates correction and/or Fisher´s exact probability test. Ther number of early and late resorption and post-implantation losses were compared by the Mann-Whitney U-test.
Mean numbers of viable fetuses, total implantations, corpora lutea and mean fetal body weights were compared by ANOVA (one-way), Bartlett´s test for homogeneity and appropriate t-test.

Results and discussion
Mortality:
There was an increased incidence of mortalities in the high dose group (see Table B.6.6-37).

Table B.6.6-37: Mortalities of dams

	Control	**Low**	**Intermediate**	**High**
	(0 mg/kg/day)	(75 mg/kg/day)	(175 mg/kg/day)	(350 mg/kg/day)
Spontaneous deaths*	0/16	1/16	2/16	10/17
Time of death (gestation day)	--	26	22, 25	3 to 21
% mortality	0.0	6.3	12.5	58.8
Sacrificed after abortion	2	0	1	1
Sacrificed on gestation day	22	--	27	23

* dead animals / total animals in group

For five of the rabbits that died spontaneously, the cause of death was attributed to pneumonia, respiratory disease, enteritis or gastroenteritis. For one rabbit of the mid-dose group and the other 7 rabbits of the high dose group, the cause of death could not be determined.
The mortality rates in the intermediate- and especially in the high-dose groups were greater than 10 %, which exceeds the OECD guideline 414 (2001) suggestion of no more than approximately 10 % maternal mortality.

Clinical Observations
Clinical signs consisting of soft stool and diarrhea were noted in all dose groups during the treatment period. In the 175 mg/kg bw/day dose group, the incidence of this finding was slightly increased when compared with the control group. At 350 mg/kg bw/day, either soft stool, diarrhea or both were observed in each animal at least once during the treatment period. Also in the high dose group, there was an increased incidence of animals with nasal discharge in comparison with the control group.

Body weight
There were no treatment-related effects on maternal body weights and body weight gain.

Pathology
Necropsy
There were no macroscopic findings in dams that were considered to be related to the administration of glyphosate technical.

Observations on the ovary and uterus
No treatment-related effects were evident in the study.

Table B.6.6-38: Maternal observations

	Historical control	**Control**	**Low**	**Intermediate**	**High**
		(0 mg/kg/day)	(75 mg/kg/day)	(175 mg/kg/day)	(350 mg/kg/day)
Surviving dams at caesarean section*	27/28	14/16	15/16	13/16	6/17
Pregnant rabbits	24/28	12/16	15/16	11/16	6/17
Non-pregnant rabbits	3/28	2/16	0/16	2/16	0/17
Abortions	1/28	2/16	0/16	1/16	1/16

* number of surviving animals / total animals in group

Developmental parameters
There were no statistically significant differences in the mean numbers of early or late resorptions, total implantations, corpora lutea, foetal body weights or foetal sex ratio in any of

the test substance groups when compared to control. The number of viable foetuses was slightly, but statistically significantly, increased in the low-dose group at 75 mg/kg bw/day. However, this finding was considered incidental and not related to the test substance.
The mean foetal body weights were slightly decreased in the test substance groups as compared to control. However, the mean foetal body weights in all test substance groups were comparable to the historical control data (i.e. 30.9 g) (see table below).

Table B.6.6-39: Mean litter data at caesarean section

	Historical control	**Control**	**Low**	**Intermediate**	**High**
		(0 mg/kg/day)	(75 mg/kg/day)	(175 mg/kg/day)	(350 mg/kg/day)
Pregnant dams[#]	24	12	15	11	6
Viable foetuses/dam	6.7	5.3 ± 2.73	7.6* ± 1.84	5.9 ± 2.77	6.3 ± 2.25
Post implantation loss/dam[##]	0.8	0.7 ± 0.89	0.4 ± 0.63	0.2 ± 0.40	0.8 ± 1.33
Total implantations /dam[##]	7.5	5.9 ± 2.39	8.0 ± 1.81	6.1 ± 2.84	7.2 ± 2.93
Corpora lutea/dam[##]	10.1	9.0 ± 2.13	10.1 ± 1.64	10.5 ± 3.45	8.5 ± 1.87
Foetal sex distribution (males/females) [#]	83/77	28/35	53/61	32/33	17/21
Mean foetal body weight (g) [##]	30.9	33.4 ± 7.27	30.9 ± 4.43	29.9 ± 7.21	29.3 ± 4.82

[#] Total number
[##] Number ± SD; historical control without SD
* Statistically significant difference compared to control ($p < 0.05$)

It should be noted that, in all dose groups, the number of pregnant dams were less than the number of pregnant dams required by the current OECD guideline 414 (2001); i.e., 16. Therefore, the evaluation of the developmental parameters may be limited.

Skeletal and visceral examination
The percentages of foetuses with skeletal malformations were 0.0, 2.6, 3.1 and 5.3 in the control, 75, 175 and 350 mg/kg bw/day groups, respectively. Although malformations were observed in the test substance groups, neither the type nor incidence of the malformations provided evidence for an adverse effect of glyphosate acid. There were no visceral malformations observed in any of the dose groups including control. There were no statistically significance differences in the variation observed in the test substance group when compared to the control group (see Table B.6.6-40).

Table B.6.6-40: Summary of foetal malformations and variations

	Hist. contr.	**Control** (0 mg/kg/day)		**Low** (75 mg/kg/day)		**Intermediate** (175 mg/kg/day)		**High** (350 mg/kg/day)	
Number of litters examined		12		15		11		6	
	%	x/y	%	x/y	%	x/y	%	x/y	%
Skeletal malformations		0/63	0.0	3/114	2.6	2/65	3.1	2/38	5.3
Exencephaly	--	0/63	0.0	0/114	0.0	1/65 (1/11)	1.5	0/38	0.0
Acrania	--	0/63	0.0	0/114	0.0	0/65	0.0	1/38 (1/6)	2.6
Scoliosis with associated rib anomalies	0.6	0/63	0.0	2/114 (2/15)	1.8	0/65	0.0	0/38	0.0
T1 rib absent	--	0/63	0.0	0/114	0.0	1/65 (1/11)	1.5	0/38	0.0
Carpal flexure	0.6	0/63	0.0	0/114	0.0	0/65	0.0	1/38 (1/6)	2.6
Fused cervical vertebral centra	0.6	0/63	0.0	1/114 (1/15)	0.9	0/65	0.0	0/38	0.0
Visceral malformation	--	0/63	0.0	0/114	0.0	0/65	0.0	0/38	0.0
Total malformations		0/63	0.0	3/114	2.6	2/65	3.1	2/38	5.3
Variations									
27 presceral vertebrae	8.7	6/63 (5/12)	9.5	7/114 (3/15)	6.1	9/65 (4/11)	13.8	7/38 (5/6)	18.4
13th rudimentary rib(s)	3.7	5/63 (3/12)	7.9	14/114 (6/15)	12.3	3/65 (3/11)	4.6	3/38 (3/6)	7.9
13th full rib(s)	8.1	3/63 (3/12)	4.8	10/114 (4/15)	8.8	5/65 (2/11)	7.7	6/38 (3/6)	15.8
Hyoid arches bent	--	--	--	2/114 (1/15)	1.8	1/65 (1/11)	1.5	--	--
Hyoid body unossified	--	6/63 (2/12)	9.5	2/114 (2/15)	1.8	6/65 (3/11)	9.2	--	--
Parietals reduced in ossification	0.6	1/63 (1/12)	1.6	--	--	1/65 (1/11)	1.5	--	--
Sternebrae #5 and/or #6 unossified	5.6	6/63 (3/12)	9.5	13/114 (7/15)	11.4	13/65 (5/11)	20.0	4/38 (2/6)	10.5
Pubis unossified	--	4/63 (1/12)	6.3	1/114 (1/15)	0.9	4/65 (1/11)	6.2	--	--
Talus unossified	--	3/63 (1/12)	4.8	--	--	5/65 (3/11)	7.7	--	--
Extra ossification center, cervical area	--	--	--	--	--	1/65 (1/11)	1.5	--	--
Major vessel variations	8.7	11/63 (6/12)	17.5	14/114 (8/15)	12.3	14/65 (5/11)	21.5	6/38 (4/6)	15.8

x/y: number of foetuses affected / total number of foetuses examined
(a/b): number of litters affected / total number of litters

Conclusion by the Notifiers

The oral administration of glyphosate acid to pregnant rabbits by gavage from Gestation Day 6-27 resulted maternal toxicity at ≥175 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level that could not be attributed to maternal toxicity. Therefore the NOAEL was considered to be 75 mg/kg bw/day for maternal toxicity. The NOAEL for developmental toxicity was considered to be 350 mg/kg bw/day.

Comment by RMS (Re-evaluation):
The study is considered supplementary, because the highest dose level revealed only 6 does with litters for examination due to the high maternal mortality. The NOAEL of 75 mg/kg bw/d for maternal toxicity is agreed. However, the previously established NOAEL for developmental toxicity of 350 mg/kg bw/day was lowered to 175 mg/kg bw/day because of the low number of available foetuses in the high dose group avoiding meaningful evaluation.

Comment by GTF on the first draft of the RAR (July 2013):
[redacted] (1980, TOX9552390) is considered a supplementary study based on high maternal mortality and therefore an insufficient number of litters to thoroughly evaluate potential developmental effects at 350 mg/kg/day. However, the study data are still robust and applicable for establishing valid NOAEL values from the mid and low doses of 175 and 75 mg/kg/day, respectively.
Further explanation:
While a dose-response curve cannot be confirmed for adverse developmental endpoints, due to high maternal morality and thus insufficient number of litters at the high dose, a dose-response is evident for maternal mortality and toxicity. Therefore this study may be considered robust for establishing the maternal toxicity NOAEL of 75 mg/kg/day. Similarly, the mid and low doses clearly demonstrated no adverse effects to offspring, and thus the offspring NOAEL of 175 mg/kg/day in this study is unequivocal, especially as this value was also the developmental NOAEL in the Moxon study.

RMS comment (August 2013):
What is the idea behind this comment? It is only a summary of the conclusion/comment given by the RMS in the Draft RAR.

Reference:	IIA, 5.6.11/05
Report:	[redacted] (1991) The Effect of Glyphosate on Pregnancy of the Rabbit (Incorporates Preliminary Investigations) [redacted] Data owner: Cheminova Study/Project No.: CHV 45 & 39 & 40/901303 Date: 1991-10-14 not published TOX9552391
Guidelines:	OECD 414, US EPA 83-3
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment.*

Dates of experimental work: 1989-12-14 to 1990-03-02

Materials and methods

Test material:	
Identification:	Glyphosate acid
Description:	White solid
Lot/Batch #:	206-JAK-25-1
Purity:	98.6 %
Stability of test compound:	Stable over the duration of the study
Vehicle and/ or positive control:	1 % methylcellulose
Test animals:	
Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	11-24 weeks (on delivery)
Sex:	Female
Weight at dosing:	Females: 3582 – 3709 g (mean values)
Acclimation period:	7 days
Diet/Food:	SQC Standard rabbit diet (Special Diet Services Ltd., Essex, UK), *ad libitum*
Water:	Mains drinking water, *ad libitum*
Housing:	Initially in litters, sexes separately, after assignment to experimental groups in group of four rats per sex per cage
Environmental conditions:	Temperature: 19 ± 1 °C Humidity: 49 ± 15 % Air changes: Not recorded Natural lighting supplemented with artificial lighting from 07 – 21:00 hours

Study design and methods:
In life dates: 14 -12-1989 – 02-03-1990

Animal assignment and treatment:
In a developmental toxicity study, groups of 16 - 20 time-mated female New Zealand White rabbits were administered glyphosate in 1% methylcellulose (dose volume 5 mL/kg) once daily by gavage at dose levels of 0 (vehicle control), 50, 150 or 450 mg/kg bw/day from Day 7 to Day 19 of pregnancy. Dose volumes were calculated for individual animals on day 7 and adjusted according to body weight on Days 9, 11 and 15. The day of mating was considered as Day 0. Dose levels were based on the findings of a preliminary study.

Dosing formulations were prepared daily and administered within 3 hours of preparation.

Observations
All animals were regularly handled and observed daily for overt changes or signs of reaction to treatment. Animals that died or were killed for animal welfare reasons were weighed and subjected to post-mortem examination.

Body weight
Individual body weights were recorded Days 1, 7, 9, 11, 15, 20, 24 and 29 of gestation.

Food consumption and compound intake
Food consumption was recorded on days of weighing throughout gestation.

Sacrifice and pathology
On day 29 of pregnancy all surviving does were subjected to post-mortem examinations for congenital abnormalities and gross pathological changes in maternal organs.
The ovaries and uteri were examined to determine the number of corpora lutea, the number and distribution of live young, the number and distribution of embryonic and /foetal deaths, individual foetal weight and foetal abnormalities. Embryonic/foetal deaths were classified as Early, Late or Abortions.

Litter parameters
Live young were examined for external, visceral and skeletal abnormalities employing appropriate techniques. Live young were killed by intrathoracic injection of pentobarbitone sodium then weighed and dissected for examination of visceral abnormalities. Where appropriate, suspected abnormalities were further examined by alternative procedures such as microdissection and histopathology to clarify initial observations. Pups were fixed in industrial methylated spirit, the heads sliced along the line of the frontoparietal suture and the brain examined for abnormalities before clearing and staining by the modified Dawson technique of the carcasses for skeletal examination. Structural changes were presented as malformations, anomalies or variants.

Statistics
Two-tailed tests for significance were performed on litter data only, and significance at 1 % and 5 % were reported. Mean values of litter size, pre and post-implantation loss, litter weight, mean foetal weight and the incidence of anomalous offspring were analysed by the Kruskal-Wallis test. Intergroup comparisons were made by the non-parametric equivalent of the Williams' test following a significant h-statistic. Where 75 % of the values for a given variable consisted of one value, a Fisher's exact test was used.

Results and discussion
Analysis of dose formulations
The analysis of the dosing formulations taken at the first dosing showed the mid- and high-dose group to be within 6 % of the nominal dose whilst the low-dose group was 19 % below the nominal dose; however, a renalysis on Day 19 showed the concentration to be 5 % above the nominal dose.

Food consumption
During the dosing period, females receiving 150 and 450 mg/kg bw/day showed reduced food consumption compared to the controls. A slight reduction was evident from Days 11 – 19 at 150 mg/kg bw/day (approximately 12 % compared with controls) and throughout the treatment period for the 450 mg/kg bw/day dose group (6-17 % during Days 7-19) (see Table B.6.6-41).

Table B.6.6-41: Summary of mean food consumption (g/rabbit/day)

	Dose Group (mg/kg bw/day)			
	0 (control)	50	150	450
Mated females	19	19	16	20
No. of animals included in assessment	18	12	15	13
Food consumption (g/rabbit/day) during				
Days 1-6	142	143	141	152
Days 7-8	143	154	150	135
Days 9-10	146	148	148	132
Days 11-14	153	149	134	129
Days 15-19	148	151	131	123
Days 20-23	142	154	149	149
Days 24-28	131	143	153	166

Mortality
There was one death in the 450 mg/kg bw/day dose group on Day 20 following signs of abortion on Day 19 and signs of gastrointestinal disturbance, manifested as soft/liquid faeces, severe reduction in food consumption and bodyweight loss from the onset of treatment. Two other deaths (a broken hind leg and an incidence of congenital abnormality) were unrelated to the treatment and were eliminated from the study assessment.

Clinical Observations
Clinical signs included a dose-related increase in the number of females showing soft/liquid faeces (gastrointestinal disturbances) and signs of lack of appetite (off feed/reduction in food consumption) at 150 and 450 mg/kg bw/day (see Table B.6.6-41).

Table B.6.6-42: Summary of relevant clinical signs in does

Parameter	**Dose Group (mg/kg bw/day)**			
	0 (control)	50	150	450
Mated females	19	19	16	20
Not pregnant	0	6	1	5
Number of does with live young or litters at Day 29	18	12	15	13
Clinical signs#				
Off-feed	8	6	10	9
Reduced faecal output	9	8	11	12
Soft/liquid faeces	0	2	5	13

Only animals with live young included

Body weight
A slight reduction in body weight gain was noted from Day 11 of pregnancy to termination of treatment in the 150 and 450 mg/kg bw/day dose groups, which coincided with the reduction in food consumption during the same period (see Table B.6.6-42).

Table B.6.6-43: Summary of body weight data (group means)

Parameter	**Dose Group (mg/kg bw/day)**			
	0 (control)	50	150	450
Mated females	19	19	16	20
No of animals included in assessment	18	12	15	13
Bodyweights (g) at				
Day 1	3538	3524	3568	3658
Day 7	3582	3604	3624	3709
Day 9	3589	3639	3637	3732
Day 11	3601	3653	3661	3743
Day 15	3742	3804	3779	3833
Day 20	3770	3831	3775	3835
Day 24	3844	3927	3849	3965
Day 29	3999	4084	3975	4103

Pathology
Necropsy
Gross examination of does at post-mortem did not identify any treatment-related effects.

Observations on the ovary and uterus
A total of 18, 12, 15 and 13 pregnant females survived to termination and 163, 104, 112 and 95 foetuses were recorded for the 0 (control), 50, 150 and 450 mg/kg bw/day dose groups respectively. Litter size at caesarean necropsy was comparable in all treatment groups. Total litter loss was recorded for one female of the 450 mg/kg bw/day dose group which aborted on Day 19 and died and also for one female at 50 mg/kg bw/day. One female at 150 mg/kg bw/day aborted 1/9 foetuses.

There were no significant intergroup differences in the numbers of corpora lutea, implantations, pre-implantation loss, foetal sex ratios or foetal weights (see Table B.6.6-44). There was a statistically significant increase in embryo/fetal death and post-implantation loss at all exposure levels. The study investigators questioned the biological significance of these findings for several reasons: 1) No dose-response pattern was evident, 2) the control value was at the lower end of the historical control range, while those of the exposed groups were at the higher end, and 3) the values in all groups were within or slightly above the historical control range. The latter two statements are supported by the historical control data provided in the study report (page 32) (see Table B.6.6-44). Although embryo/foetal death was within the historical control range, post-implantation loss was above the historical control values in the high-dose group, and both of these parameters were statistically significant ($p<0.01$) at the high dose.

Table B.6.6-44: Summary of the maternal and litter parameters (group mean values)

Parameter	**Dose Group (mg/kg bw/day)**				**Historical control range (mean value)**
	0(control)	50	150	450	
No. of mated females	19	19	16	20	--
No. not pregnant	0	6	1	5	--
No. of does with live young or litters at Day 29	18	12	15	13	--
Corpora lutea	11.5	12.4	11.7	11.3	9.0 – 12.9 (11.2)
Implantations	9.7	10.5	9.0	9.2	7.0 – 11.1 (9.5)
Pre-implantation loss	14.6	15.4	23.4	18.8	2.3 – 26.1 (15.1)
Early embryonic deaths	0.4	0.9	0.9	0.5	0.3 – 1.1 (0.6)
Late embryonic deaths	0.2	0.9	0.5	1.3**	0.1 – 1.3 (0.7)
Abortions	0.0	0.0	0.1	0.0[#]	0.0 – 0.1 (0)
Total embryonic deaths	0.6	1.8*	1.5*	1.8**	0.6 – 2.0 (1.2)
Post-implantation loss (%)	5.7	19.5*	15.3*	21.0**	6.5 – 17.5 (12.9)
Live young	9.1	8.7	7.5	7.3	6.1 – 9.5 (8.3)
Litter weight (g)	389.5	370.6	320.5	315.0	281.9 – 402.2 (352.9)
Mean foetal weight (g)	43.9	43.3	44.0	44.5	41.4 – 47.6 (44.1)
Sex (% males)	55.3	55.8	57.6	53.8	--

* Statistically significant by Kruskal –Wallis 'H' test P < 0.05
** Statistically significant by Kruskal –Wallis 'H' test P < 0.01
[#] Fisher exact test follow-up by intergroup comparison with control was not statistically significant P > 0.05

Developmental parameters
Number and viability of foetuses
There were 18, 12, 15 and 13 viable litters at 0, 50, 150 and 450 mg/kg bw/day, respectively. The concurrent control showed low mean values for embryonic deaths and post implantation losses when compared with historical control values. When compared with these historical data as noted above, mean values in the treated groups were within the expected range; therefore, it was concluded that no adverse effect on foetal survival was attributed to glyphosate.

Foetal body weights
There was a dose-related reduction in mean foetal weight on a litter basis in all treated groups (not statistically significant) compared with the control; however, the mean individual foetal weight was not affected.

External, visceral and skeletal examination
Malformations were slightly increased in the 150 and 450 mg/kg bw/day dose groups compared to controls and appeared to be associated with an apparent increase in malformations of the thoracic region. However, neither the incidence nor the percentage of malformed foetuses was outside the historical control range and the values were not statistically different from concurrent control values. Several of the cardiovascular malformations that were observed, particularly in the high-dose group, occurred in the same animals and are related to a single morphogenetic mechanism (i.e., displacement of the developing aorticopulmonary septum), which is likely to adjust during the first two weeks of postnatal life. These related findings, which often cluster together, included dilated/narrow aorta and narrow/dilated pulmonary artery; interventricular septal defect; and disproportionately sized right and left ventricles. These findings were observed (often in clusters) in the historical control data that were provided by the conducting laboratory.

Individual presentation of these malformations in tables when the malformations occurred together in the same foetus and are due to the same mechanisms and artificially inflates the sense that there is a much stronger cardiac effect than is actually present.

The cardiac malformation observed with greatest frequency in this study was interventricular septal defect. The number of foetuses and litters with ventricular septal defects were 1, 1, 1 and 4 in the 0, 50, 150 and 450 mg/kg bw/day dose groups, respectively. Comparison of the historical control data shows that the heart findings (when presented on a percent individual and/or litter incidence basis) were slightly outside of the historical background range from 13 studies conducted during the same period. However, the disparity in values is a consequence of the small numbers of litters in the study report. If the data are displayed as a fraction (rather than a percentage), then the number of litters affected were 1/18, 1/12, 1/15, and 4/13 in the 0, 50, 150, and 450 mg/kg/day dose groups, respectively. The historical control range is 0/19 – 3/13. Thus, the findings at the high dose are barely outside of the historical control range. Further, they were observed in conjunction with clear signs of maternal toxicity (reduced food consumption, body weight gains and increased clinical signs).

The other cardiovascular finding found in this study not related to the morphogenetic mechanism involving formation of the spiral septum is retroesophageal right subclavian artery. This finding was also observed regularly throughout the historical period. It is not uncommon and is oftentimes an inconsequential anatomical difference in vascular arrangement. At autopsy this condition is found in 0.5 – 2.0 % of subjects.

The malformations of the cranial region, the lumbar and the lumbar/sacral regions did not show any treatment-related trend and are considered to be incidental. The incidences of anomalies and variants did not suggest any treatment relationship. The incidence of foetuses with reduced ossification did not show any dose-relationship; however, lower foetal weights were observed for the 450 mg/kg bw/day dose group with reduced ossification.

The observed foetal malformations and anomalies are summarised in the following (Table B.6.6-45).

Table B.6.6-45: Summary of foetal parameters

Parameter	**Dose Group (mg/kg bw/day)**				**Historical control range or x/y ◊ (mean)**
	0(control)	50	150	450	
Number of does with live young or litters at Day 29	18	12	15	13	--
Mean foetal weight (g)	43.9	43.3	44.0	44.5	41.4 – 47.6 (44.1)
Sex (% males)	55.3	55.8	57.6	53.8	--
Malformations					--
Total number of foetuses examined	163	104	112	95	1511
No. of malformed foetuses	3	3	5	6	51
%	1.9	5.8	4.3	5.9 (F)	0.7 – 5.9 (3.8)
Number of Affected Litters	3	3	3	5	43/188
%	16.67	25	20	38.5	22.9
Thoracic region malformations					--
No. of foetuses with interventricular septal defect	1	1	1	4	10/1511
%	0.6	1.0	0.9	4.2	0.66
Litter incidence	1	1	1	4	10/188
%	5.56	8.3	6.67	30.8	5.32
Foetuses with enlarged left, reduced right ventricles	0	0	0	2	2/1511
%	0.0	0.0	0.0	2.1	0.13
Litter incidence	0	0	0	2	2/188
%	0	0	0	15.4	1.10
Foetuses with retro-oesophageal right subclavian artery	0	0	3	2	7/1511
%	0.0	0.0	2.7	2.1	0.46
Litter incidence	0	0	1	1	7/188
%	0	0	6.6	7.6	3.72
Foetuses with narrow/dilated aortic arch/pulmonary trunk/arterial trunk	1	1	1	3	8/1511
%	0.6	1.0	0.9	3.2	0.52
Litter incidence	1	1	1	3	8/188
%	5.56	8.3	6.67	23.1	4.25
Anomalies					--
Total number of foetuses examined[#]	160	101	107	89	--
No. of foetuses with gross/visceral anomalies	9	14	14	6	--
%	6.4	19.5	12.9	9.6 (K)	--
No. of foetuses with skeletal anomalies	21	13	14	11	--
%	11.7	17.7	12.5	10.1 (K)	--
No. of foetuses with reduced ossification	7	4	5	4	--
%	4.4	4.0	4.7	4.5	--
Mean foetal weight of foetuses with reduced ossification (g)	37.9	43.6	37.7	26.1	--

◊ number affected / total number examined
[#] Malformed foetuses are excluded
(F) Fisher's exact test applied, not statistically significant (P > 0.05)
(K) Kruskal-Wallis 'H' statistic, not significant (P > 0.05)
-- no data

Conclusion by the Notifiers

Glyphosate was not teratogenic in this developmental toxicity study in rabbits. The NOAEL for maternal toxicity was 50 mg/kg bw/day based on clinical signs of toxicity including reduced feed consumption and body weight gain and soft/liquid faeces during the dosing

period. The NOAEL for foetotoxicity was 150 mg/kg bw/day based on statistically significantly increased embryo/foetal deaths and post-implantation loss. The NOAEL for teratogenicity was 450 mg/kg bw/day.

Comment by RMS (Re-evaluation):
The study is considered acceptable. In this study there was a significant increase in embryonic death and post-implantation loss in treated groups compared to controls, however without a clear dose-relationship. Regarding the post-implantation losss, values for the low and high dose groups are outside the historical control range.
The cardiac malformation observed with greatest frequency in this study was the interventricular septal defect. At 450 mg/kg bw/d this effect was outside the historical control range (4.2 % compared to 0.66 % in historical controls). Taken into account the high post-implantation loss at the same dose level, the incidence of additionally cardiac malformation may be covered, therefore. At mid dose level foetuses with an higher incidende of retro-oesophageal right subclavian artery were reported. However, this effect has to be considered equivocal, because no clear-dose relationship could be established.
In conclusion, the NOAEL for maternal toxicity is considered 50 mg/kg bw/d based on slightly restricted inappetence, slightly reduced body weight gain and soft/liquid faeces at 150 mg/kg bw/d. The NOAEL for developmental toxicity is considered to be 150 mg/kg bw/d based on the post-implantation loss, late embryonic death and an increase in cardiac malformations at 450 mg/kg bw/d.

Reference:	IIA, 5.6.11/06
Report:	[redacted] (1993) Teratogenicity study in rabbits – Test compound: Glyphosate technical (FSG 03090 H/05 March 1990) [redacted] Data owner: ADAMA Agan Ltd Study No.: TOXI: 884-TER-RB Date: 1993-04-17, amended 1994-06-18 not published TOX9551106
Guidelines:	OECD 414 (1981)
Deviations:	None
GLP:	
Acceptability:	*See RMS comment*

Dates of experimental work: 1991-12-24 to 1992-03-06

Materials and methods

Test material:

Identification:	Glyphosate acid
Description:	Odourless white crystals
Lot/Batch #:	60
Purity:	96.8 %
Stability of test compound:	Stable over 2 years at ambient temperature.

Vehicle and/ or positive control:	0.5 % w/v carboxymethylcellulose
Test animals:	
Species:	Rabbit
Strain:	Female New Zealand White
Source:	[redacted]
Age:	Approximately 6 months and above (at the start of study)
Sex:	Males and females
Weight at dosing:	Females: >2500 g (mean values)
Acclimation period:	At least 10 days.
Diet/Food:	Pelleted rabbit diet, supplied by M/S Lipton India Ltd, Bangalore (composition and feed analysis reports were provided) was provided *ad libitum*
Water:	Protected deep bore well drinking water, treated via activated charcoal filter and UV in Aquaguard on-line water filter-cum-purifier provided *ad libitum*
Housing:	Individually in 3-tier all aluminium cages with wire mesh bottom and common self-draining litter trays.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 40 – 70 % Air changes: 10 – 15/h Natural lighting supplemented with fluorescent lighting 12 hours light/dark cycle

Study design and methods:
In life dates: not reported

Animal assignment and treatment:
In a developmental toxicity study, groups of presumed mated female New Zealand White rabbits were administered once daily by gavage, glyphosate (batch no.: 60, purity 96.8 %) in 0.5 % carboxymethylcellulose (dose volume 2 ml/kg) at dose levels of 0 (vehicle control), 20, 100 or 500 mg/kg bw/day from Day 6 to Day 18 of pregnancy. Dosages for individual animals were calculated from day 6 and adjusted daily according to body weight. Dose levels were based on the findings of preliminary studies.

In a preliminary dose-range finding study, one male rabbit/dose group was administered by gavage glyphosate technical dissolved in 0.5 % carboxymethylcellulose (dose volume 2 mL/kg bw) at dose levels of 0 (control), 10, 20, 50, 500 or 1000 mg/kg bw/day for 13 days. Doses of ≥ 500 mg/kg bw/day resulted in loss in body weight and in feed intake and the 1000 mg/kg bw/day test animal died on Day 9 of treatment.

In a second dose-range finding study, one pregnant rabbit was administered 500 mg/kg bw/day glyphosate from day 6 to 18 of gestation and the findings compared with that of 20 historical control animals. Caesarean section and terminal necropsy was performed on day 28. There were no signs of toxicity from the treatment; body weight gain was greater (26 % more than the historical control mean) but notable apparent treatment-related changes were substantial reduction in feed intake (34 % of historical control mean) and reduced litter size in the test female (4) compared with the historical control mean (7).

Observations
All animals were observed twice daily for onset and duration of signs of toxicity and for mortality. All animals in the experiment that died, were killed moribund or killed at termination were subjected to post-mortem gross pathological examination. Tissues with gross lesions were preserved for histopathological examination as necessary.

Body weight
Individual body weights were recorded on Days 0, daily from Days 6 – 18 and on Day 28 of gestation.

Food consumption and compound intake
Food consumption was recorded on days of weighing throughout gestation.

Sacrifice and pathology
On day 28 of pregnancy, all surviving dams were subjected to post-mortem examinations and pups were delivered by Caesarean section. The ovaries and uteri were excised and weighed and maternal and foetal data were recorded. The maternal data determined were pregnant/non-pregnant, uterine weight, the number of corpora lutea, the number of implantations, the number of embryonic and foetal resorptions. The foetal data recorded were the number of dead/abnormal/live foetuses, individual foetal weight and sex.

Litter parameters
All the foetuses were examined for external, visceral and skeletal abnormalities employing appropriate techniques. Live young were euthanised with ether and visceral organs examined by a modified Wilson technique. Skeletal assessments were performed after appropriate preparation including staining in Alizarin Red. Structural changes were presented as variants, minor and major malformations.

Statistics
Statistical methods employed included the following. Maternal body weight and weight gain, feed intake, number of corpora lutea, number of implantations and mean foetal weight were analyzed by Bartlett‘s test followed by ANOVA and Dunnett’s test. Day ‘0’and absolute body weight data were compared by the Paired Student’s ´t` test. The number and percent embryonic resorptions and foetal resorptions, the number of dead foetuses, the number of abnormal foetuses and percentage pre-implantation and post-implantation loss by Mann Whitney test. Litter size was by Student ‘t‘ test. The sex ratio, number of dams with any resorptions, number of dams with all resorptions and incidence of malformations were analysed by Chi-square test.

The statistical analysis and comparison of individual treatment groups with control value were done at 5 % probability level and the results were designated as significantly higher (+) / lower (-) than control value at $P \leq 0.05$.

Results and discussion
Analysis of dose formulations
Not reported.

Food consumption

During the dosing period, feed consumption was significantly reduced (31 %) in females receiving the 500 mg/kg bw/day dose compared to the controls (see Table B.6.6-46). Feed consumption during the post-treatment period did not show significant intergroup differences.

Table B.6.6-46: Summary of food consumption

Parameter	**Historical positive control#**	**Dose Group (mg/kg bw/day)**			
		0 (control)	20	100	500
Food consumption (g/rabbit/day)					
No of dams included in assessment	7	20	13	12	6
Day 0 – 6 (Pre-treatment)	105	114	88*	125	118
Day 6 – 19 (Treatment)	70*	103	109	102	71*
Day 19 – 28 (Post treatment)	129	109	135	107	105
Day 0 - 28	96	107	113	108	92

Treatment with Acetylsalicylic acid (ASA) at 200 mg/kg bw (treatment: Day 6-18; post-treatment: Day 18-28)
* Significantly lower than controls by Dunnett's test $P \leq 0.05$

Mortality
The four and eight deaths observed in the mid- and high-dose group were considered to be treatment-related by the study director (see Table B.6.6-47 below). However, the two confirmed misdosings in the control, the absence of signs of toxicity at 100 mg/kg bw and the absence of mortality in this dose range in the considerably high number of parallel studies shed serious doubt on a relation to treatment at this dose level. Further, various findings at gross necropsy were noted in the lungs and trachea for the 100 and 500 mg/kg/day dose groups; these findings suggest possible gavage errors, which could be responsible for some of the deaths observed at these doses and and are not appropriately discussed in the report.

Table B.6.6-47: Summary of mortality in dams

Parameter	**Dose Group (mg/kg bw/day)**			
	0 (control)	20	100	500
Mated females	26	17	16	15
Dead during treatment	1*	0	4	5
Died post-treatment	1*	0	0	3
Total number of deaths	2	0	4	8
% mortality	7.7	0.0	25.0	53.3

* Animal died due to wrong gavaging

Clinical observations
Signs of toxicity were observed at the 500 mg/kg bw/day dose group and were predominantly gastrointestinal effects, which included soft stool/liquid faeces and soft stool with mucus. Further signs of toxicity were rales, weakness, dyspnoea and ocular discharge.

Table B.6.6-48: Summary of relevant clinical signs in dams

Parameter / clinical sign	**Dose Group (mg/kg bw/day)**			
	0 (control)	20	100	500
Mated females	26	17	16	15
Pregnant at termination	20	13	12	6
Rales	1	0	0	3
Soft stool with mucus	0	0	0	2
Soft stool/liquid faeces	0	0	1	12
Weak	0	0	0	2
Ocular discharge	0	0	0	1
Dyspnoea	0	0	0	1

Body weight
No treatment-related and dose-related significant changes were observed in maternal body weight and body weight gain between the control, low- and mid-dose groups. In the high-dose group, initial body weight and body weights at the different time intervals were significantly lower than in the control group.

Table B.6.6-49: Summary of maternal body weight data

Parameter	**Historical positive control#**	**Dose Group (mg/kg bw/day)**			
		0 (control)	20	100	500
Number of dams pregnant at termination		20	13	12	6
Mean body weights (kg)					
Day 0		3.1	2.8	3.0	2.6*
Day 6		3.2	3.0	3.0	2.8*
Day 18		3.2	3.1	3.1	2.8
Day 28		3.3	3.3	3.3	3.0*
Day 28 (body weight – uterine weight)	2.7*	3.0	3.0	2.9	2.7
Mean body weight gain (kg)					
Day 0 – 6 (Pre-treatment)	0.2	0.1	0.2	0.0	0.1
Day 6 – 18 (Treatment)	-0.1	0.1	0.1	0.1	0.0
Day 18 – 28 (Post treatment)	0.2	0.1	0.2	0.2	0.2
Day 0 – 28 (Throughout gestation)	0.3	0.2	0.5**	0.3	0.3

Treatment with acetylsalicylic acid (ASA) at 200 mg/kg bw/day
* Significantly lower than controls by Dunnett's test $P \leq 0.05$
** Significantly higher than controls by Dunnett's test $P \leq 0.05$

Pathology
Necropsy
Gross examination of dams at post-mortem did not identify any treatment-related effects. However, various findings were noted in the lungs and trachea for the 100 and 500 mg/kg/day dose groups which suggest possible gavage errors and issues with animal husbandry.

Observations on the ovary and uterus
A total of 20, 13, 12 and 6 pregnant females survived to termination and 134, 80, 78 and 28 foetuses were recorded for the 0 (control), 20, 100 and 500 mg/kg bw/day dose groups, respectively, and were included in the assessment. Litter size at caesarean necropsy was comparable in all treatment groups. Total litter loss (complete resorptions) was recorded for

one female in the 500 mg/kg bw/day dose group; otherwise, the incidence of dams with any resorptions did not show any treatment-related differences.

There were no significant intergroup differences in the mean numbers of corpora lutea, pre-implantation and post-implantation losses and resorptions (embryonic and foetal) (see Table B.6.6-50).

Table B.6.6-50: Summary of maternal observations

Parameter	**Historical positive control#**	**Dose group (mg/kg bw/day)**			
		0 (control)	20	100	500
Mated females	12	26	17	16	15
Total number of deaths	4	2	0	4	8
Pregnant at termination	7	20	13	12	6
Mean number of corpora lutea	9	11	10	10	9
Mean number of implantations	8	8	8	9	6
Total number of embryonic resorptions (%)	6 (11)	10 (7)	11 (11)	11 (11)	9 (24)
Total number of foetal resorptions (%)	2 (4)	8 (5)	7 (7)	13 (13)	1(3)
Total number of pre-implantation loss (%)	10 (19)	72 (48)	28 (29)	20 (20)	14 (37)
Total number of post-implantation loss (%)	8 (15)	18 (12)	18 (18)	24 (24)	10 (26)
Number of dams with any resorptions (%)	2 (29)	12 (60)	11 (85)	9 (75)	2 (33)
Dams with complete resorptions (%)	1 (14)	0 (0)	0 (0)	0 (0)	1 (17)

Treatment with Acetylsalicylic acid (ASA) at 200 mg/kg bw

Developmental parameters

Number and viability of foetuses

Because of the large number of maternal deaths at 500 mg/kg/day (and thus, the reduced number of total litters), the total number of foetuses was substantially less in this dose group compared to the other dose groups. However, the mean litter size, the mean numbers of abnormal, dead or live foetuses and the sex ratios of foetuses did not show any significant treatment-related differences. Glyphosate also did not cause an increase in the number of foetal deaths in utero (see Table B.6.6-51).

Foetal body weights

Although foetal body weights in the 20 and 100 mg/kg/day dose groups were reported to be significantly different from control, the weights were increased, the changes were less than 10 % of control values and no dose-response across treatment groups was evident. Thus, the foetal body weight differences observed in these two dose groups are biologically inconsequential with respect to adverse effects (see Table B.6.6-51).

Table B.6.6-51: Mean litter data at caesarean section

	Historical positive control#	**Dose group (mg/kg bw/day)**			
		0	20	100	500
Mated females	12	26	17	16	15
Total number of deaths	4	2	0	4	8
Pregnant at termination	7	20	13	12	6
Number of litters	6	20	13	12	5
Total number of foetuses	46	134	80	78	28
Mean litter size	8	7	6	7	6
Abnormal foetuses (%)	0 (0)	1 (1)	2 (3)	0	0
Dead foetuses (%)	0 (0)	0 (0)	1 (1)	1 (1)	0 (0)
Post-implantation loss (%)	8 (15)	18 (12)	18 (18)	24 (24)	10 (26)
Number of live foetuses	46	133	77	77	28
Mean weight of live foetuses (g ± SD)	29 ± 1.4	32 ± 5.3	35 ± 3.7*	35 ± 2.4*	33 ± 4.9
Sex ratio (Male : Female)	1 : 1.3	1 : 0.7	1 : 1.2	1 : 1.2	1 : 1.8

\# Treatment with Acetylsalicylic acid (ASA) at 200 mg/kg bw
SD = standard deviation
* Significantly higher than controls by Dunnett's test P ≤ 0.05

External, visceral and skeletal examination

The incidence of major external malformations did not identify any treatment-related differences; further, none of the external malformations occurred in the highest dose group.

Visceral examination noted no significant treatment-related incidences of minor malformations or variants. Major visceral malformations primarily affected the heart, but occurred in single incidences and showed no dose-response (see Table B.6.6-52). The exception was dilated heart, which was reported in four foetuses of 3 litters in the 20 mg/kg bw/day dose group, 4 foetuses (3 + 1) from 2 litters of the 100 mg/kg bw/day dose group and all foetuses (4) of one litter and one foetus of another litter at the 500 mg/kg bw/day (Statistically significant P≤ 0.05). The terminology used to describe the heart malformations in this study is different than that typically employed in teratology research (e.g., dilated heart, seal-shaped heart). Consequently, what is meant by the description "dilated heart" is not well defined and not documented with photographs or retained tissue sections or slides.How this malformation might relate to others reported in the heart (i.e., dilated left or right ventricle, seal-shaped heart, cardiomegaly) is not clear. Further, because too few foetuses were available for examination in the high dose group, it cannot be determined whether these defects exhibited a true dose-related increase. It is important to note, however, that only 2 litters exhibited major visceral malformations in the high dose group. Additionally, these findings were found in the presence of extensive maternal toxicity, evidenced by reduced food consumption and body weight gains in the few animals that survived this dose level, clinical signs, and substantial deaths.

Major, minor and skeletal malformations did not show any clear treatment-related findings and appeared to be incidental

Table B.6.6-52: Summary of relevant external, visceral and skeletal findings (litter data)

Foetal findings	**HC**	**Dose level (mg/kg bw/day)**			
	Data#	0	20	100	500
No. of litters examined	6	20	13	12	5
No. of foetuses examined	46	133	79	77	28
Minor external malformations					
Percentage of small foetuses (%)	--	0	0	1.3	0
Litter incidence (%)	--	0	0	8.3	0
Major external malformations					
Percentage of foetuses with upper cleft palate (%)	0	0.8	2.5	0	0
Litter incidence (%)	0	5	15	0	0
Percentage of foetuses with forelimb arthrogryposis	--	0	0	1.3	0
Litter incidence (%)	--	0	0	8.3	0
Percentage of foetuses with multiple malformations	0	0.8	2.5	0	0
Litter incidence (%)	0	5	15	0	0
Percentage of foetuses with major malformations (%)	0	1.5	2.5	1.3	0
Litter incidence (%)	--	10	7.7	8.3	0
Major visceral malformations					
Percentage of foetuses with dilated heart (%)	--	0	5.1	5.2	17.9
Litter incidence (%)	--	0	23.1	16.7	40.0
Percentage of foetuses with anencephaly (%)	0	0.8	0	0	0
Litter incidence (%)	0	5.0	0	0	0
Percentage of foetuses with heart–seal shaped (%)	0	0.8	0	0	0
Litter incidence (%)	0	5.0	0	0	0
Percentage of foetuses with cardiomegaly & sealed heart (%)	--	0	0	1.3	0
Litter incidence (%)	--	0	0	8.3	0
Percentage of foetuses with dilated ventricle (left) (%)	--	0	0	1.3	0
Litter incidence (%)	--	0	0	8.3	0
Percentage of foetuses with dilated ventricle (right) (%)	--	0	0	0	3.6
Litter incidence (%)	--	0	0	0	20
Percentage of foetuses with persistent truncus arteriousus (%)	--	0.8	0	0	0
Litter incidence (%)	--	5.0	0	0	0
Percentage of foetuses with gallbladder absent (%)	--	0	0	0	3.6
Litter incidence (%)	--	0	0	0	20
Percentage of foetuses with liver (median) haematoma (%)	--	0	0	0	3.6
Litter incidence (%)	--	0	0	0	20
Minor skeletal malformations					
No. of foetuses with extra 13th rib		0	1	2	1
Percentage of foetuses with extra 13th rib	8.7**	0	1.3	2.6	3.6*
Litter incidence (%)	--	0	7.7	16.7	20
Major skeletal malformations					
Percentage of foetuses major malformations (%)	10.9	8.3	6.3	0*	3.6
Litter incidence (%)	50	20	23.1	0	20

Historical positive control data (--: no data available)
* Significantly different from control at $p < 0.05$.
** Significantly different from control by Contingency test ($P \leq 0.05$)

Conclusion by the Notifiers

Glyphosate technical was not considered to be teratogenic in this developmental toxicity study in rabbits. The incidence of one visceral effect, dilated heart, was increased at the highest test dose and was present at lower dose levels, but there were too few foetuses present in the high dose group to corroborate a dose-response relationship. Further, foetal findings at the highest test dose were observed in the presence of extensive maternal toxicity that exceeded guideline recommendations for a high dose. The NOAEL for maternal toxicity was

100 mg/kg bw/day based on mortalities at dose levels of ≥ 100 mg/kg bw/day. Mortality and clinical signs of toxicity including reduced feed consumption and soft faeces and reduced bodyweight gain during the dosing period one incidence of complete resorptions at the 500 mg/kg bw/day dose level. The NOAEL for foetotoxicity and teratogenicity was 100 mg/kg bw/day based on occurrence of general signs of secondary toxicity (incomplete ossification and similar) at the high dose.

Comment by RMS (Re-evaluation):
The study is considered supplementary due to several weaknesses including a small number of litters for examination (low pregnancy rate at all dose levels, lethality in mid and high dose dams) and reporting deficiencies. The percentage of foetuses with 'dilated heart' was significantly increased at all dose levels. However, the absolute number of affected foetuses and litters is quite small and did not show a marked difference between the treated groups. Furthermore, the diagnosis 'dilated heart' was not defined in this study report and neither criteria used to this diagnosis nor measurements of the heart were provided.
The NOAEL for maternal toxicity is still considered 20 mg/kg bw/d, because it cannot be excluded, that the intercurrent four deaths at 100 mg/kg bw/d were substance-related.
Therefore, the previous evaluation in 2001 is confirmed: the maternal NOAEL is considered 20 mg/kg bw/d and the developmental NOAEL 100 mg/kg bw/d.

Comment by GTF on the first draft of the RAR (July 2013):
The GTF seriously questions whether the findings reported in the Suresh study were directly related to treatment. The GTF respectfully suggests that the additional safety margin for risk assessment (disregarding the 75 mg/kg/day NOAEL) as proposed by the RMS, is not necessary.
Further explanation:
Whilst an increase in mortality was observed in the Suresh study, it is uncertain if the mortalities observed were directly related to treatment, given that clinical signs of rales and dyspnoea were observed and that various findings at gross necropsy were noted in the lungs and trachea for animals in the 100 and 500 mg/kg/day dose groups. These findings suggest possible gavage errors, may well be responsible for some of the deaths observed at these doses. These findings, along with the confirmed gavage traumas in the control group call into question the technical competency of the dosing procedure in the conducting laboratory. This would likely have caused additional stress to the animals during the dosing procedure and rabbits are highly susceptible to stress induced mortality.

RMS comment (August 2013):
Not agreed. According to the study report, one gavage error was reported in the control group, but not at 100 and 500 mg/kg bw/d. Assuming technical incompetency as the main reason for lethality at mid and high dose level, one would expect the same observations at lowest dose level. Furthermore, at highest dose level 3 out of 8 animals died post-treatment, which can not be related to administration procedure, definitely.

Reference:	IIA, 5.6.11/07
Report:	[redacted] (1989) Rabbit Teratology Study with Glyphosate Technical [redacted] Data owner: Excel Study no.: IIT Project No. 1086 Date: 1989-11-03 not published TOX9551960
Guidelines:	OECD 414 (1981)
Deviations:	no uterine weight, no maternal necropsy findings
GLP:	no
Acceptability:	*See RMS comment*

Dates of experimental work: 1989-07-03 to 1989-11-02

Materials and methods

Test material:	
Identification:	Glyphosate technical
Description:	White amorphous powder
Lot #:	38
Purity:	95 %
Stability of test compound:	Not reported
Vehicle and/ or positive control:	0.1 % gum acacia in water
Test animals:	
Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	24 - 28 weeks
Sex:	Females
Weight at dosing:	1.50 – 2.00 kg
Acclimation period:	6 days
Diet/Food:	Pelleted rabbit feed supplied by Lipton India Ltd., Bangalore, India
Water:	Tap water, *ad libitum*, supplied in polypropylene bottles by Maharashtra Industrial Development Corpn., New Bombay
Housing:	Individually in stainless steel cages equipped with food and water dispensers and stainless steel grate at bottom
Environmental conditions:	Temperature: 20 ± 3 °C Humidity: 30 to 70 % Air changes: not reported 12 hours light/dark cycle

Study design and methods:
In life dates: 1989-07-03 to 1989-11-02

Animal assignment and treatment:
In a teratogenicity study groups of 15 New Zealand White female rabbits received doses of 0, 125, 250 and 500 mg/kg bw/day test substance in 0.1 % gum acacia in water by gavage from Gestation Day 6-18 after successful mating with adult vigorous males. The day of mating was taken as the 1st day of pregnancy.

Diet preparation and analyses
For each dose level, dosing solutions were prepared in 0.1 % gum acacia in water as vehicle.

Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made twice daily (before and after dosing) during the dosing period.

Body weight
Individual body weights were recorded on Days 0, 6, 12, 18, 23, and 29 (at necropsy). Changes in body weight were calculated and recorded as group maternal weight changes for the periods of Days 0-6 (pre-exposure), 6-12, 12-18, 18-23, 23-29 and 18-29 (post-exposure observation period).

Food consumption
Food consumption was recorded on Days 0, 6, 12, 18, 23, and 29 (at necropsy).

Sacrifice and pathology
Females were euthanatized by carbon dioxide asphyxiation on Day 29 of gestation and examined for any abnormalities that would affect pregnancy.
The ovaries and uteri were removed, the uteri were weighed, and the ovaries were examined for the number of corpora lutea and uteri for the number and position of implants and dead or live foetuses. Uteri from non-gravid females were placed in 10% ammonium sulfide solution for detection of early resorptions.

Developmental parameters
Each rabbit foetus was removed from the uterus and was killed by injection of pentobarbitone. All live foetuses were weighed and examined for external malformations including cleft palate and variations. All live foetuses were examined for thoracic and visceral abnormalities, and each foetus was sexed. Following visceral examination, all foetuses were eviscerated and processed for skeletal staining with Alizarin Red SAll foetuses were decapitated and heads were fixed in Bouin's solution for examination of craniofacial structures.

Statistics
Not reported.

Results and discussion
Analysis of dose formulations
The analytical purity of test substance was stated to be 95 %.

Food consumption
Mean food consumption in the low and mid dose groups was comparable to that in the control group throughout the study period. Significantly lower food consumption (~17 % lower mean food consumption compared to control, low or mid dose group) was observed in the high dose group starting with the day of treatment throughout the rest of the observation period.

Mortality
None of the rabbits died during the study period.

Clinical observations
No toxic symptoms were observed in any of the animals during the study.

Body weight
Mean body weights of animals in the low and mid dose group were comparable to those in the control group. In the high dose group, the mean maternal weight increase was lower for each of the observation periods between Days 12-29 compared to controls, but no stastical comparison was provided in the report.

Pathology
Necropsy
No abnormalities that could affect pregnancy were reported at maternal necropsy.

Observations on the ovary and uterus
Two animals of the high dose group aborted (see Table B.6.6-53).

Table B.6.6-53: Gestational parameters in rabbits treated with glyphosate

Gestational parameter	**Dose level (mg/kg bw/day)**			
	0	125	250	500
No. of pregnant females	15	15	15	15
No. of early deliveries	0	0	0	0
No. of abortions	0	0	0	2
No. of females with no live foetuses	0	0	0	2
No. nonpregnant at termination	2	1	1	3
No. of litters	13	14	14	12
Mean no. of corpora lutea per doe	10.0	10.1	10.3	9.8
Mean no. of total implants per litter	9.0	9.3	9.4	8.5
Mean % pre-implantation loss	21.3	14.9	14.7	13.1
Mean no. of viable implants per litter	7.3	8.0	8.0	5.2
Mean no. of non-viable implants per litter	0.07	0.13	0.27	1.4
Mean no. of early resorptions per litter	1.7	1.1	1.0	1.9
Sex ratio (% males)	44.4	49.2	49.7	50.1
Mean foetal body weight per litter	40.6	47.1	47.5	48.7

Developmental parameters
Number and viability of foetuses
The mean number of viable implants (foetuses) per litter was lower in the high dose group, and accordingly, the mean number of non-viable implants (foetuses) per litter was greater in the high dose group (see Table B.6.6-53), but no statistical comparisons were provided in the report.

Sex ratio, foetal body weights and placental weights
No differences were noted in the sex ratios, mean foetal body weights, mean number of corpora lutea per dose, mean number of total implants per litter, mean percentage of pre-implantation loss, and mean number of early resorptions between the control and the treated groups. In the high dose group, two dams had no live foetuses due to abortions (see Table B.6.6-53). However, stasticial analyses were provided in the report.

External, visceral and skeletal examination

No difference was noted in the incidences of maternal animals having foetuses with external, visceral and/or skeletal malformations in the low and mid dose groups when compared with the controls. In the high dose group, the incidences of external, visceral and skeletal malformations were higher than that in the control group (see Table B.6.6-54). With regard to the heart malformations, 0, 1, 1, and 2 interventricular septal defects were observed in the 0, 125, 250, and 500 mg/kg bw/day dose groups.
A similar pattern was seen in the variations observed externally, viscerally and skeletally; in the high dose group, the total number of observed variations was higher than those of the control, low or mid dose groups. The increase in malformations and variations observed in the high dose group occurred in the presence of maternal toxicity (reduced food consumption and body weight gains). Further, this was at a dose (500 mg/kg bw/day) that caused significant toxicity, including mortality, in another rabbit developmental study. However, stasticial analyses were provided in the report.

Table B.6.6-54: Incidence of foetal malformations and variations in rabbits treated with glyphosate

Foetal findings	**Dose level (mg/kg bw/day)**			
	0	125	250	500
Malformations				
No. of litters examined	13	14	14	12
No. of foetuses examined	109	113	120	78
No of litters with malformations	3	6	10	12
% of litters with malformations	23.08	42.86	71.43	100
No. of foetuses with malformations	3	6	10	20
% of foetuses with malformations	2.75	5.31	8.33	25.64
Number of foetuses (litters) with external malformations				
Tail abnormal	1 (1)	1 (1)	2 (2)	3 (2)
Low-set ears	0 (0)	1 (1)	1 (1)	2 (1)
Total external malformations	1	2	3	3
Total external malformations (%)	0.92	1.77	2.50	3.85
Number of foetuses (litters)with visceral malformations				
Ventricular septal defect	0 (0)	1 (1)	1 (1)	2 (2)
Postcaval lung lobe absent	0 (0)	1 (1)	2 (2)	4 (3)
Kidney(s) absent	1 (1)	2 (2)	2 (2)	6 (4)
Total visceral malformations	1	4	5	12
Total visceral malformations (%)	0.92	3.54	4.17	15.38
Number of foetuses (litters) with skeletal malformations				
Rudimentary rib (no. 14)	1 (1)	0 (0)	2 (2)	5 (2)
Total skeletal malformations	1	0	2	5
Total skeletal malformations (%)	0.92	0.00	1.67	6.41
Variations				
No. of foetuses examined	109	113	120	78
Total no. of observed variations	26	30	49	93
Number of foetuses (litters) with external variations				
Tail blunt tipped	1 (1)	0 (0)	3 (2)	5 (4)
Number of foetuses (litters) with visceral variations				
Irregular rugae on palate	0 (0)	2 (1)	3 (2)	2 (2)
Lateral ventricles of cerebrum dilated	0 (0)	2 (2)	2 (2)	6 (4)
Right ventricle small than normal	1 (1)	3 (2)	3 (2)	5 (3)
Globular heart	2 (2)	0 (0)	3 (2)	5 (4)
Incomplete separation of lung lobes	1 (1)	2 (1)	2 (1)	4 (2)
Parietal foetal atelectasis	0 (0)	1 (1)	1 (1)	1 (1)
Liver irregular shape	0 (0)	2 (1)	2 (2)	6 (4)
Kidney(s) globular shape	0 (0)	0 (0)	2 (1)	5 (3)

Foetal findings	**Dose level (mg/kg bw/day)**			
	0	125	250	500
Number of foetuses (litters) with skeletal variations				
Cervical centra 1-3 and/or 4 bilobed	1 (1)	0 (0)	1 (1)	2 (2)
Anterior arch of the atlas poorly ossified	2 (1)	2 (1)	1 (1)	4 (2)
Anterior arch of the atlas split	0 (0)	0 (0)	2 (1)	3 (1)
Extra thoracic centrum and arch	1 (1)	3 (2)	2 (1)	5 (3)
Thoracic centrum only one ossification centre	1 (1)	0 (0)	1 (1)	3 (2)
Thoracic centra fused	2 (1)	1 (1)	1 (1)	2 (1)
Extra ribs on thoracic centra and arch 13 bilateral	1 (1)	0 (0)	3 (2)	5 (4)
Sternebra 6 poorly ossified	2 (1)	1 (1)	2 (1)	4 (2)
Sternebra(e) split	2 (1)	2 (1)	1 (1)	5 (3)
Sternebra(e) unossified	3 (2)	1 (1)	3 (2)	6 (4)
Pubis, poorly ossified	3 (2)	2 (2)	3 (1)	4 (3)
Some ossification in knee area	1 (1)	0 (0)	3 (2)	4 (3)
Skull bones poorly ossified	1 (1)	3 (2)	2 (1)	2 (2)
Frontal, hole in bone	0 (0)	1 (1)	2 (2)	2 (2)
Reduced number of caudal segments	1 (1)	2 (2)	1 (1)	3 (2)

Conclusion by the Notifiers

The oral administration of glyphosate to mated rabbits by gavage from Gestation Day 6-18 resulted in treatment-related changes at 500 mg/kg bw/day. Therefore the NOAEL for reprotoxic and non-reprotoxic effects was considered to be 250 mg/kg bw/day. Considering the significantly reduced food consumption and gain in body weight at 500 mg/kg bw/day, the maternal NOAEL is 250 mg/kg bw/day.

Comment by RMS (Re-evaluation):

The study is considered supplementary due to serious reporting deficiencies (e.g. no individual data, no statistical analysis, no uterine weights, no results of maternal necropsy). The previous NOAEL for maternal and developmental toxicity is still considered to be 250 mg/kg bw/d based on reduced food consumption and body weight gain at 500 mg/kg bw/d in does

Developmental effects were visible as foetolethality and several malformations (external, visceral, skeletal) at high dose levels: The previous evaluation did not mentioned the external malformation in rabbits which are now reported in the present RAR (abnormal tails). Total number of foetuses per litter with malformations was higher in the groups receiving the mid and high dose lever, but without statistical significance. However, it remains unclear, whether statistical analysis of the data had been performed at all. Ventricular septal defects were noted in 2 out of 78 foetuses in the high dose group (control incidence 0/109). The higher number of further visceral malformations at the top dose level was due to absent kidneys and postcaval lung lobes. Because no individual data are provided it is not identifiable, whether the malformations described were confined to single foetuses or if the foetuses were multiple malformed.

B.6.6.12 Published data (released since 2000)

A large number of studies on developmental and reproductive toxicity (DART) was published since 2000. These studies are reported and discussed below. Furthermore, also studies on endocrine disruption (ED) have been included in this chapter because they are mainly related to developmental and reproductive toxicity.

Published studies on developmental toxicity, reproductive toxicity and an endocrine disrupting potential of glyphosate and glyphosate based formulations include *in vitro* studies, *in vivo* studies and epidemiological studies. Many studies since 2000 are specifically discussed in a comprehensive glyphosate DART review publication by Williams et al. (2012, ASB2012-12052). Further discussions of significant papers follow.

In addition, glyphosate was included on the US EPA Endocrine Disruptor Screening Program's (EDSP) first list of 67 compounds to Tier 1 Screening. The US EPA published the criteria for inclusion on List 1 was strictly based on exposure potential, not hazard, specifically stating in the Federal Register (2009, ASB2012-12041);
"This list should not be construed as a list of known or likely endocrine disruptors".

A consortium of glyphosate registrants in North America, the Joint Glyphosate Task Force, LLC (JGTF), coordinated the conduct of the glyphosate battery of Tier 1 screening assays under the EDSP and submitted these assays to the US EPA. The US EPA will evaluate the full battery of Tier 1 screening assays together using a weight of evidence approach, for glyphosate's potential to interact with the estrogen, androgen and thyroid endocrine pathways. The following below were submitted by the JGTF to the US EPA in early 2012 and are reviewed. However, the Agency has announced they will not release their Data Evaluation Records (DERs) for individual EDSP studies until a weight of evidence review has been completed for List 1 compounds.

In Vitro EDSP Glyphosate Studies submitted to the US EPA

- Androgen Receptor Binding (Rat Prostate Cytosol); OCSPP 890.1150
- Aromatase (Human Recombinant); OCSPP 890.1200
- Estrogen Receptor Binding Assay Using Rat Uterine Cytosol (ER-RUC); OCSPP 890.1250
- Estrogen Receptor Transcriptional Activation (Human cell Line, HeLa-9903); OCSPP 890.1300; OECD 455
- Published OECD Validation of the Steroidogenesis Assay (Hecker et al., 2010, ASB2012-11840)

In Vivo EDSP Glyphosate Studies submitted to the US EPA

- Amphibian Metamorphosis (Frog) OCSPP 890.1100; OECD 231
- *In Vivo* Hershberger Assay (Rat); OCSPP 890.1600; OECD 441
- Female Pubertal Assay; OCSPP 890.1450; OECD None
- Male Pubertal Assay; OCSPP 890.1500
- Uterotrophic Assay (Rat); OCSPP 890.1600; OECD 440
- Fish Short-Term Reproduction Assay; OCSPP 890.1350; OECD 229

The glyphosate Tier 1 screening assay study reports are owned by the JGTF. The European Glyphosate Task Force (GTF) is negotiating to procure access rights to the battery of glyphosate EDSP Tier 1 screening study reports. Results of the Hershberger and Uterotrophic *in vivo* rat studies, now in the public domain, as are the published results of the OECD validation of the Steroidogenesis assay, in which glyphosate clearly had no impact on steroidogenesis, are discussed below.
Recently, the first publicly data available from the glyphosate Tier 1 assays under the US EPA Endocrine Disruptor Screening Program, were reported at the 2012 Society of Toxicology meeting (Saltmiras & Tobia 2012, ASB2012-12016) for the Hershberger and

Uterotrophic assays. No effects were noted for any potential for glyphosate to interact with androgenic or estrogenic pathways under these GLP studies following the US EPA 890 Series Test Guidelines.
Bailey et al. (2013, ASB2013-3464) summarized the first results of the male and female Pubertal assay of this programm. Based on these results, glyphosate does not exhibit endocrine disruption in Male and Female Pubertal assays.
Levine et al. (2012, ASB2014-9609) published a short summary of the results of tests with glyphosate in the EPA`s Endocrine Disruptor Screening Program (EDSP). They conclude that from the weight of evidence provided by the Tier 1 assays, performed at independent labs, under the EDSP along with the higher Tier regulatory safety studies, with a high level of confidence glyphosate would not be an endocrine disruptor.

In Vitro Glyphosate DART/ED Publications

Many *in vitro* research publications have characterised pesticide formulations, including glyphosate based formulations, as toxic and endocrine disrupting products. Researchers and editorial boards did in some cases not consider the fact that surfactants (which are often components of formulated pesticide products), by their physico-chemical nature, are not suitable test substances using *in vitro* cell models. Surfactants compromise the integrity of cellular membranes, including mitochondrial membranes, and thus confound endpoint measurements considered as representative of specific toxicological modes of action or pathways.

A laboratory at the University of Caen, France, has multiple recent publications of *in vitro* research with glyphosate and glyphosate based formulations (Richard et al., 2005, ASB2009-9024; Benachour et al., 2007, ASB2009-9018; Benachour and Seralini, 2009, ASB2012-11561; Gasnier et al., 2009, ASB2012-11629; Gasnier et al., 2010, ASB2012-11628; Gasnier et al., 2011, ASB2012-11630; Clair et al., 2012, ASB2012-11592; Mesnage et al., 2012, ASB2012-11900), with proposed extrapolations to an array of *in vivo* effects including potent endocrine disruption, aromatase inhibition, estrogen synthesis, placental toxicity, foetotoxicity, embryotoxicity and bioaccumulation. These publications are in some cases replicates of earlier studies, using different cell lines or primary cell cultures and in some cases the same data are reported again in a subsequent publication. Firstly, the *in vitro* synergism claims are conjecture, because no control groups of surfactant without glyphosate were tested. Secondly, the extrapolations to *in vivo* effects are unjustifiable based on both the unsuitability of surfactants in such test systems and the supraphysiological cytotoxic concentrations at which *in vitro* effects are reported. Again often overlooked by *in vitro* researchers and editorial boards, Levine et al. (2007, ASB2009-9030) presented convincing data demonstrating a lack of *in vitro* synergism for glyphosate with other formulation ingredients. Regarding Seralini's repeated claims of glyphosate induced aromatase inhibition in mircosomes (Richard et al., 2005; TOX2005-1743, Benachour et al., 2007, ASB2009-9018; Gasnier et al., 2009, ASB2012-11629), the data are confounded and thus uninterpretable where surfactants are introduced to such *in vitro* systems. This is noted in the US EPA Aromatase Inhibition Test Guideline, OECD 890.1200, in which notes, "Microsomes can be denatured by detergents [surfactants]. Therefore, it is important to ensure that all glassware and other equipment used for microsome preparations be free of detergent residue."

Another *in vitro* publication claiming a specific developmental toxicity pathway has gained significant public attention. Paganelli et al. (2010, ASB2012-11986) conducted three *in vitro*

assays, (i) frog embryos exposed to glyphosate formulation; (ii) frog embryos directly injected without injection blank negative controls; and (iii) fertilised chicken embryos exposed directly to a glyphosate formulation through a hole cut in the egg shell. Key issues surrounding this research include irrelevant routes of exposure as well as excessively high and environmentally unrealistic doses.

Thongprakaisang et al., (2013, ASB2013-11991) submitted a study on the effects of pure glyphosate on estrogen receptors mediated transcriptional activity and their expressions. The following cell lines have been used: a hormone-dependent breast cancer, T47D, a stably EREC-luc construct tranfected hormone-dependent breast cancer T47D-KBluc and a hormone-independent human breast cancer, MDA-MB231. Glyphosate (purity ≥ 98 %) was tested in concentrations from 10^{-12} to 10^{-6} M. Glyphosate exerted proliferative effects on human hormone-dependent cell lines but not in hormone-independent cell lines. Furthermore, an additive estrogenic effect between glyphosate and genistein, a phytoestrogen, was reported. The authors conclude that these in vitro results need further investigation in an animal study. It must be emphasised that no increase in mammary tumours was reported in any of the numerous long-term studies in rats or mice (see Vol. 3, B.6.5 and Vol. 1, B.2.6).
Cavalli et al. (2013, ASB2014-7495) studied the effects of the formulation Roundup Original in rat testis and Sertoli cells *in vitro*. The authors propose that Roundup toxicity, implicated in Ca^{2+} overload, cell signalling misregulation, stress response of the endoplasmatic reticulum, and/or depleted antioxidant defenses, could contribute to Sertoli cell disruption in spermatogenesis that could have an impact on male fertility.

In Vivo Glyphosate DART/ED Publications

Relatively few *in vivo* publications on glyphosate DART and ED exist in comparison with the list of *in vitro* publications. Some lack appropriate interpretation of basic toxicology; e.g. Daruich et al. (2001, ASB2012-11601). Beuret et al. (2005, ASB2012-11564) investigated the effects of 1 % Glyphosate oral exposure (a trade product from Argentina described as "Herbicygon" was used which is a commercial herbicide formulation) on lipoperoxidation and antioxidant enzyme systems in pregnant rats and in fetuses. Lipoperoxidation was higher in both maternal and fetal livers in the glyphosate treated groups. Catalase and Superoxide dismutase activity were not altered. Both studies are reviewed in Williams et al. (2012, ASB2012-12052).

Dallegrave et al. (2003, ASB2012-11600; 2007, ASB2012-2721) published results of two non-guidelines rat developmental toxicity studies, in which a glyphosate based formulation containing POEA was evaluated. However, reporting deficiencies and inconsistencies pose difficulties in data interpretation. These studies are dicussed in detail in the Appendix on [redacted] (please refer to B.6.13).

Romano et al. (2010, ASB2012-12012) evaluated a glyphosate based formulation in a male pubertal-like assay in Wistar rats, reporting decreased preputial separation, reduced seminiferous epithelial height, increased luminal diameter of seminiferous tubules, and increased relative testicular and adrenal weights. Given the gravity of the reported findings in this publication, a review was undertaken by Kelce et al. (2010, ASB2012-11867). Most recently, Romano et al. (2012, ASB2012-12011) reported additional findings in male rats after supposed *in utero* and *post natal* exposures which include "behavioral changes and histological and endocrine problems in reproductive parameters and these changes are reflected by a hypersecretion of androgens and increased gonadal activity, sperm production

and libido". As in their first publication, Romano et al. (2012, ASB2012-12011) base their hypothesis on selectively discussed literature implicating glyphosate as an endocrine disruptor, predominantly with citations to research from the Seralini laboratory.

Kimmel et al. (2013, ASB2013-3462) analyzed the information from 7 unpublished developmental studies in rabbits and 6 developmental toxicity studies in rats to determine if glyphosate poses a risk for cardiovascular malformations. In summary, assessment of the reviewed data fails to support a potential risk for increased cardiovascular defects as a result of glyphosate exposure during pregnancy.
Chruscielska et al. (2000, ASB2013-9831) submitted a teratogenicity study in Wistar outbred rats. The used test guideline was not indicated. Doses of 0-750-1500-3000 mg/kg bw/day have been administered from day 7-14 of pregnancy to 20 females per dose group. No embryotoxic and no teratogenic effects have been administered.
Omran and Salama (2013, ASB2014-7614) report that the exposition of snails to atrazine or glyphosate resultet in signs of endocrine disruption and cellular toxicity. However, in this study only the formulation "Herfosate" was used and no pure active substance glyphosate.
Razi et al. (2012, ASB2014-9390) consider that glyphosate (125 mg/kg bw/d oral administered for 10, 20, 30 & 40 days) effects testicular tissue and sperm parameters in male Wistar rats. Clear effects were already seen after 10 days administration and thereafter, however accompanied by significant clinical symptoms (decreased movement, staggering gait, occasional trembling, diarrhea) and reduced body weight gain of 20 %. These findings are in contrast to those in rat studies submitted for EU evaluation. For comparison, the current EU evaluation of glyphosate proposes an overall subchronic (90-d) NOAEL of 414 mg/kg bw/d (rats) and for reproductive toxicity of 351 mg/kg bw/d, albeit generated from feeding studies. Similarily, after oral administration in female rats an NOAEL of 300 mg/kg bw/d for maternal and developmental effects was established, toxic effects were observed at much higher dose levels, only. The high toxicity described in the present publication is hardly to explain, because the publication does not give any information whether technical material or a glyphosated based formulation was tested. To conclude, the results of the publication does not affect the current assessment of glyphosate.
Cassault-Meyer et al. (2014, ASB2014-5615) investigated the effects of a glyphosate-based herbicide (Roundup Grand Travaux Plus) after an 8-day exposure of adult rats. Endocrine (aromatase, estrogen and androgen receptors, Gperl in testicular and sperm mRNAs) and testicular functions (organ weight, sperm parameters and expression of the blood-testis barrier markers) were monitored at day 68, 87, and 122 after treatment, spermiogenesis and spermatogenesis. A significant and differential expression of aromatase in testis and a diminution of mRNA expression of nuclear markers in spermatozoa were observed. The authors conclude that results suggest changes in androgen/estrogen balance and in sperm nuclear quality.

POEA DART Studies

Polyethoxylated alkylamine (POEA) surfactants are a class of non-ionic surfactant, containing a tertiary amine, an aliphatic group of variable carbon chain length and two separate sets of ethoxy (EO) chains of variable length. A dietary exposure assessment of POEAs was submitted by Bleeke et al. (2010, ASB2010-6123). This exposure assessment report also refers to the US EPA Alky Amine Polyalkoxylates Human Health Risk Assessment, which includes POEAs (http://www.regulations.gov/search/Regs/home.html#documentDetail?R=09000064809b983b). Williams et al. (2012, ASB2012-12052) recently evaluated and detailed the results of DART studies with two different POEA surfactants.

Furthermore, a detailed comparison of the toxicity of [redacted] and glyphosate was submitted in the appendix "Toxicological evaluation of the [redacted] surfactant (CAS no. [redacted]" which is attached to this report.

Epidemiology Glyphosate DART/ED Publications

Several epidemiology studies in which glyphosate exposure was considered have evaluated the following range of reproductive outcomes; miscarriage, fecundity, pre-term delivery, gestational diabetes mellitus, birth weights, congenital malformations, neural tube defects, attention-deficit disorder / attention-deficit hyperactive disorder (ADD/ADHD). In most instances, glyphosate and reproductive outcomes lack a statistically significant positive association, as described in a recent review of glyphosate non-cancer endpoint publications (Mink et al., 2011, ASB2012-11904). In evaluating ADD/ADHD, a positive association with glyphosate use was reported by Garry et al. (2002, ASB2012-11626), but cases were reported by parents with no clinical confirmation and the reported incidence rate of approximately 1 % for the study population was well below the general population incidence rate of approximately 7 %. Regarding *in utero* exposures, McQueen et al. (2012, ASB2012-11898) report very low measured dietary exposures, from 0.005 % to 2 % of the current glyphosate ADI in Europe. Given the low perfusion rate of glyphosate across the placenta (Mose et al., 2008, ASB2012-11914), human *in utero* exposures would be very limited.

Campana et al. (2010, ASB2013-10559) estimated the frequency of 27 birth defects in 7 geographical regions of Argentina. A sample of 21,844 newborn with birth defects was selected, ascertained from 855,220 births, between 1994 and 2007, in 59 hospitals. The study results suggested that frequencies of 14 of the 27 examined birth defects were higher in one or more regions. This study was discussed in some publications in relation to the use of glyphosate pesticides. However, Campana et al. (2010, ASB2013-10559) commented on secular trends, altitude above sea level, folic acid fortification and ethnic factors and further variables. It was not indicated that any of these variables was associated with an increased occurrence of any type of birth defects.

Two studies of residential proximity to agriculture-related pesticide applications (California) by Carmichael et al. (2013, ASB2014-9307) and Yang et al. (2013, ASB2014-9644) examined whether early gestational exposure to pesticides were associated with an increased risk of hypospadia, neural tube defects or orofacial clefts in offspring. In both studies formulated glyphosate was mentioned only as one out of five chemicals to which controls were most frequently exposed. The authors of both studies concluded the few positive findings on chemicals, but other than glyphosate, should be interpreted with caution and need to be repeated in other populations.

Manfo et al. (2010, ASB2014-9611) examined the effect of pesticides use on male reproductive function in a study on farmers in Cameroon. The farmers of Djutitsa (West Cameroon) used 25 active substances (in 57 preparations) amongst others glyphosate in different formulations and were exposed to agro-pesticides due to inappropriate handling and improper protective tools. Furthermore, the authors concluded, that male farmers, who are exposed to pesticides might have impaired reproductive function through inhibition of testosterone synthesis. Serum biochemical parameter (total testosterone, estradiol/testosterone, androstenedione) were altered compared to the unexposed control group, but these alterations of chemical parameter cannot be related to single pesticides, *e.g.* glyphosate. Moreover, the fungicides were the most used active ingredients. However, considering the obvious alterations, the authors concluded, that there is urgent need for more

training to enable improvement of equipment and efficiency of application to minimize exposure risks.

Further reviews on DART

Antoniou et al. (2012, ASB2012-15927) submitted a review article on "Teratogenic Effects of Glyphosate-Based Herbicides: Divergence of Regulatory Decisions from Scientific Evidence". According to the authors published studies "have raised concern regarding the potential for glyphosate and its commercial formulations to cause birth defects and other reproductive problems". The "draft assessment report revealed that … industry tests contained clear evidence of glyphosate-mediated teratogenicity and reproductive toxicity". The EU adopted "an acceptable daily intake (ADI) for glyphosate that is unreliable and could potentially result in exposures that cause harm to humans." The authors suggest that a "new risk assessment should be conducted with full public transparency by scientists who are independent of industry."

Lopez et al. (2012, ASB2013-10534) submitted a review article on "Pesticides used in South American GMO-Based Agriculture: a review on their effects on humans and animal models". The authors discuss the results of genetic studies in agricultural regions in the province of Cordoba, Argentina, biomarkers in agricultural regions in the province of Santa Fe, Argentina and congenital malformations and genotoxicity in populations exposed to pesticides in Paraguay. According to the authors, human health in these areas was damaged by pesticides. However, a relation to glyphosate or another substance or pesticide was not evidenced. Nevertheless, based on the results of Paganelli et al. (2010, ASB2012-11986), it was concluded that glyphosate-based herbicides) would be linked to an increased activity of the retinoic acid signaling pathways and this might explain the higher incidence of embryonic malformations and spontaneous abortions observed in populations exposed to pesticides.

Basrur (2006, ASB2014-7492) submitted a review on disrupted sex differentiation and feminization of men. In this review the studies of Arbuckle and associates are cited which report a relation between pesticide exposure (including glyphosate) and reproductive risk.

Vandenberg et al. (2012, ASB2014-9635) submitted a review on low dose effects an nonmonotonic dose responses of hormones and endocrine disrupting chemicals. The authors reviewed two major concepts on EDC studies: low dose and nonmonotonicity. They conclude that nonmonotonic responses and low-dose effects would be remarkably common in studies of natural hormones and EDSs. Whether low doses of EDCs influence certain human disorders would be no longer conjecture, because epidemiologic studies would show that environmental exposures to ECDs would be associated with human diseases and disabilities. The authors demand that fundamental changes in chemical testing and safety determination woud be needed to protect human health.

In a direct response on the article of Vandenberg et al. (2012, ASB2014-9635) a discussion paper was submitted by Rhomberg and Goodman (2012, ASB2014-9391). These authors conclude that Vandenberg et al. (2012, ASB2014-9635) presented examples as anecdotes without attempting to review all available pertinent data, selectively citing studies without evaluating most of them or examining whether their putative examples are consistent and coherent with other relevant information. Many of their examples have been questioned by many scientists. Overall, Vandenberg et al. (2012, ASB2014-9635) put forth many asserted illustrations of their two conclusions without providing sufficient evidence to make the case for either and while overlooking evidence that suggest the contrary.

Lamb et al. (2014, ASB2014-9605) submitted a review with critical comments on the WHO-UNEP state of the science of endocrine disrupting chemicals – 2012. The authors conclude

that the 2012 report does not provide a balanced perspective, nor does it accurately reflect the state fo the science on endocrine disruption.
Borgert et al. (2013, ASB2014-9292) reviewed literature on thresholds of endocrine activity. The brief review highlights how the fundamental principles governing hormonal effects – affinity, potency, and mass action – dictate the existence of thresholds and why these principles also define the potential that exogenous chemicals might have to interfere with normal endocrine functioning.
The review by Sengupta and Banerjee, (2013, ASB2014-9730) is related to impacts of pesticides on male fertility. With respect to glyphosate the authors only cited in vitro data published by Richard et al. (2005, ASB2009-9025), and these have been already reported and evaluated in the present renewal assessment report (please refer to '*In vitro* Glyphosate DART/ED Publications').
Kumar (2011, ASB2014-9725) submitted a review on occupational, environmental and lifestyle factors associated with spontaneous abortion. In this review Arbuckle et al. (2001, ASB2012-11545) was cited who reported a relation between pesticide exposure (including glyphosate) and reproductive risk. This publication was already reported and discussed under 'Epidemiology DART/ED Publication'.
The extensive review by Wigle et al. (2008, ASB2014-9637) summarised the level of epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. Several references related to glyphosate were cited by the authors [(Curtis et al.1999, cited in Arbuckle et al. (2001, ASB2012-11545), Arbuckle et al. (2001, ASB2012-11545), Savitz et al. (1997, ASB2012-12022), Garry (2002, ASB2012-11626))], which were already reported and discussed under to 'Epidemiology DART/ED Publication'.
The mechanism based short review by Jamkhande et al. (2014, ASB2014-9573) summarised common human teratogenic agents. With respect to glyphosate (-based formulations) the authors cited merely data published by Antoniou et al. (2012, ASB2012-15927); Paganelli et al. (2010, ASB2012-11986). Both publications were already reported and evaluated in the present renewal assessment report (please refer to 'Further reviews on DART').
The English abstract of a Chinese publication by Zhang et al. (2013, ASB2014-9643) give notice of a summary on reproductive and developmental toxicity studies on glyphosate and the related mechanisms on humans and animals to provide suggestions for further research.

Comparison of the active substance glyphosate and glyphosate containing formulations concerning DART and ED

For the active substance glyphosate a very comprehensive data package of guideline conform studies on developmental and reproductive toxicity is available. This data package was prepared over the last decades and updated within the last years.
In these submitted studies it was demonstrated that glyphosate is not a teratogenic substance. NOEL values for developmental toxicity and reproductive toxicity can be derived from the results of these studies. There are no relevant indications of an endocrine disrupting activity of the active substance glyphosate. Additionally, also in the further guideline conform toxicological studies (e.g. the subchronic and chronic toxicity studies) no indications of an endocrine disrupting activity of glyphosate (e.g. organ weight and histology of sexual organs, behaviour etc.) have been observed. Therefore, on basis of this comprehensive and high quality data package the active substance glyphosate is not considered to be an endocrine disruptor or a teratogenic substance.
Additionally to the studies which have been performed according to validated EU- and OECD guidelines a large number of studies has been published on DART and ED. Most of these

studies use glyphosate containing preparations instead of the pure active substance glyphosate. However, some studies directly compare the toxicity of the active substance glyphosate and glyphosate conaining preparations. Furthermore, studies have been performed on the toxicity of surfactants which are used in preparations together with glyphosate, especially [redacted] The results of these surfactant studies can be compared with the results of the above mentioned guideline conform studies on glyphosate.

In result of these comparisons it can clearly be concluded that the toxicity of preparations and the toxicity of surfactants like [redacted] / polyethoxylated alkylamine is significantly higher than the toxicity of the active substance glyphosate.

A detailed comparison of the toxicity of tallowamin and glyphosate was submitted in the appendix "Toxicological evaluation of the [redacted]e surfactant (CAS no. 61791-26-2)" which is attached to this report. In this evaluation is clearly demonstrated that there is a significantly higher toxicity of the surfactant tallowamin with regard to all of the following endpoints investigated:
- acute oral toxicity
- acute dermal toxicity
- skin irritation
- eye irritation
- skin sensitization
- short term toxicity, rat
- short term toxicity, dog
- reproduction toxicity study, parental toxicity
- reproduction toxicity study, reproductive toxicity
- reproduction toxicity study, offspring toxicity
- developmental toxicity, rat, fetal effects

Walsh et al. (2000, ASB2012-12046) published research claiming that a glyphosate based formulation, but not glyphosate alone, adversely affected the steroidogenesis pathway by inhibiting progesterone production resulting in downstream reduction in mitochondrial levels of StAR protein. Subsequent research by Levine et al. (2007, ASB2009-9030) demonstrated no synergism between glyphosate and the surfactant since the cytotoxic effects were completely independent of glyphosate. Identical dose-response curves were noted for formulated product with and without the glyphosate active ingredient.

Further research addressing the steroidogenesis pathway confirmed glyphosate lacked endocrine disruption potential specific to this pathway. Quassinti et al. (2009, ASB2012-12007) evaluated effects on gonadal steroidogenesis in frog testis and ovaries on glyphosate and another active substance, noting that glyphosate unequivocally demonstrated no effect. Forgacs et al. (2012, ASB2012-11621) also tested glyphosate alone and demonstrated no effect on testosterone levels in BLTK1 murine leydig cells *in vitro*. Furthermore, the OECD multi-laboratory validation of the Steroidogenesis Assay used for Tier 1 screening of the US EPA EDSP, evaluated glyphosate and concluded no impact on steroidogenesis (Hecker et al., 2011, ASB2012-11840). Consequently, the US EPA considered reference to the OECD validation report sufficient for meeting the glyphosate Steroidogenesis Assay Test Order in the EDSP Tier 1 screening of glyphosate.
Recently, the first publicly data available from the glyphosate Tier 1 assays under the US EPA Endocrine Disruptor Screening Program, were reported at the 2012 Society of Toxicology meeting (Saltmiras & Tobia, 2012, ASB2012-12016) for the Hershberger and

Uterotrophic assays. No effects were noted for any potential for the active substance glyphosate to interact with androgenic or estrogenic pathways under these GLP studies following the US EPA 890 Series Test Guidelines.
Richard et al. (2005, TOX2005-1743) studied effects of glyphosate and roundup on human placental cells and aromatase. Summarising their results they stated that "roundup is always more toxic than its active ingredient."
In a further study from the same institute Benachour et al. (2007, ASB2009-9018) studied time- and dose-dependent effects of roundup on human embryonic and placental cells. They summarized that "in all instances, roundup … is more efficient than its active ingredient, glyphosate…". And in a further publication by Benachour and Seralini (2009, ASB2012-11561) it was stated "this work clearly confirms that the adjuvants in roundup formulations are not inert." In a response to this publication by the French Agency for Food Safety (AFSSA, 2009, ASB2012-11532) it was answered that surfactant effects … are known to increase membrane permeability, causing cytotoxicity and induction of apoptosis. In the most recent publication from the same institute, Mesnage et al. (in press, ASB2012-13917) the potential active principle for toxicity on human cells for 9 glyphosate-based formulations was studied. The authors summarized that "ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity".

In a comprehensive analysis of the available literature in developmenat and reproductive outcomes in humans and animals after glyphosate exposure, Williams et al. (2012, ASB2012-12052) summarized: "An evaluation of this database found no consistent effects of glyphosate exposure on reproductive health or the developing offspring. Furthermore, no plausible mechanism of action for such effects were elucidated. Although toxicity was observed in studies that used glyphosate-based formulations, the data strongly suggest that such effects were due to surfactants present in the formulations and not the direct result of glyphosate exposure."

In vitro DART/ED publications

Author(s)	Year	Study title
Walsh, L.P. McCormick, C. Martin, C. Stocco, D.M.	2000	Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. Environmental Health Perspectives Volume: 108 Number: 8 Pages: 769-776 ASB2012-12046

Abstract*
Recent reports demonstrate that many currently used pesticides have the capacity to disrupt reproductive function in animals. Although this reproductive dysfunction is typically characterized by alterations in serum steroid hormone levels, disruptions in spermatogenesis, and loss of fertility, the mechanisms involved in pesticide-induced infertility remain unclear. Because testicular Leydig cells play a crucial role in male reproductive function by producing testosterone, we used the mouse MA-10 Leydig tumor cell line to study the molecular events involved in pesticide-induced alterations in steroid hormone biosynthesis. We previously showed that the organochlorine insecticide lindane and the organophosphate insecticide

Dimethoate directly inhibit steroidogenesis in Leydig cells by disrupting expression of the steroidogenic acute regulatory (StAR) protein. StAR protein mediates the rate-limiting and acutely regulated step in steroidogenesis, the transfer of cholesterol from the outer to the inner mitochondrial membrane where the cytochrome P450 side chain cleavage (P450scc) enzyme initiates the synthesis of all steroid hormones. In the present study, we screened eight currently used pesticide formulations for their ability to inhibit steroidogenesis, concentrating on their effects on StAR expression in MA-10 cells. In addition, we determined the effects of these compounds on the levels and activities of the P450scc enzyme (which converts cholesterol to pregnenolone) and the 3 β-hydroxysteroid dehydrogenase (3 β-HSD) enzyme (which converts pregnenolone to progesterone). Of the pesticides screened, only the pesticide Roundup inhibited dibutyryl [$(Bu)_2$]cAMP-stimulated progesterone production in MA-10 cells without causing cellular toxicity. Roundup inhibited steroidogenesis by disrupting StAR protein expression, further demonstrating the susceptibility of StAR to environmental pollutants.
*Quoted from article

Klimisch evaluation

Reliability of study:	Reliable with restrictions
Comment:	Non-standard test systems, but publication meets basic scientific principles. However, surfactant blend in Roundup confounds results.
Relevance of study:	Relevant with restrictions: Different effects of glyphosate alone and glyphosate formulations were observed. No conclusion can be drawn that the observed effects are result of glyphosate exposure.
Klimisch code:	2

Additional comments:
Glyphosate did not affect steroidogenesis in the test system.
Roundup formulation data was confounded by mitochondrial membrane damage, attributable to the surfactant in the tested formulation.
Roundup results were comprehensively addressed in Levine et al. (2007, ASB2009-9030):
Roundup formulation containing glyphosate and Roundup formulation blank without the active ingredient was shown to have “indistinguishable” dose response curves for reductions in progesterone production in hCG stimulated MA-10 Leydig cells. Therefore the effect on progesterone levels shown by Walsh (2000, ASB2012-12046) were independent of glyphosate and attributable to the surfactant component of the formulation.
Comparable rates of progesterone inhibition for several different surfactants suggest a common mode of action for surfactants.
Roundup formulation containing glyphosate and Roundup formulation blank without the active ingredient was shown to have almost identical concentration-dependent decreases in MTT activity in MA-10 cells, suggesting the surfactant alone was responsible for the observed cytotoxicity and effect on mitochondrial function.
The JC-1 assay demonstrated the decreased progesterone production in MA-10 Leydig cells was accompanied by loss of mitochondrial membrane potential. These results confirm StAR protein function and steroidogenesis require intact mitochondrial membrane potential.
StAR protein expression were not affected by treatments, indicating that perturbed mitochondrial membrane, not StAR protein inhibition, was responsible for the effects noted by Walsh et al. (2000, ASB2012-12046).

Author(s)	Year	Study title
Paganelli, A. Gnazzo, V. Acosta H. Lopez, S.L. Carrasco, A.E.	2010	Glyphosate-Based Herbicides Produce Teratogenic Effects on Vertebrates by Impairing Retinoic Acid Signalling Chemical Research in Toxicology Volume: 23 Pages: 1586-1595 ASB2010-11410

Abstract*
The broad spectrum herbicide glyphosate is widely used in agriculture worldwide. There has been ongoing controversy regarding the possible adverse effects of glyphosate on the environment and on human health. Reports of neural defects and craniofacial malformations from regions where glyphosatebased herbicides (GBH) are used led us to undertake an embryological approach to explore the effects of low doses of glyphosate in development. *Xenopus laevis* embryos were incubated with 1/5000 dilutions of a commercial GBH. The treated embryos were highly abnormal with marked alterations in cephalic and neural crest development and shortening of the anterior-posterior (A-P) axis. Alterations on neural crest markers were later correlated with deformities in the cranial cartilages at tadpole stages. Embryos injected with pure glyphosate showed very similar phenotypes. Moreover, GBH produced similar effects in chicken embryos, showing a gradual loss of rhombomere domains, reduction of the optic vesicles, and microcephaly. This suggests that glyphosate itself was responsible for the phenotypes observed, rather than a surfactant or other component of the commercial formulation. A reporter gene assay revealed that GBH treatment increased endogenous retinoic acid (RA) activity in *Xenopus* embryos and cotreatment with a RA antagonist rescued the teratogenic effects of the GBH. Therefore, we conclude that the phenotypes produced by GBH are mainly a consequence of the increase of endogenous retinoid activity. This is consistent with the decrease of Sonic hedgehog (Shh) signaling from the embryonic dorsal midline, with the inhibition of *otx2* expression and with the disruption of cephalic neural crest development. The direct effect of glyphosate on early mechanisms of morphogenesis in vertebrate embryos opens concerns about the clinical findings from human offspring in populations exposed to GBH in agricultural fields.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-guideline study that is not sufficiently described for assessment. Inadequate positive and negative control experiments.
Relevance of study:	Not relevant: Irrelevant routes of exposure and inappropriately high doses. Test system not adequate for human risk assessment.
Klimisch code:	3

Additional comments:

Response 1 – summarized from Williams et al. (2012, ASB2012-12052)

No pH adjustment for doses and thus effects may be in response to the acidic nature of glyphosate technical acid.
Inappropriate and irrelevant routes of exposure.
Data requires further substantiation before consideration in risk assessment.

Response 2 – Saltmiras et al. (2011, ASB2012-12015) letter to the Editor
Multiple high quality toxicological studies and expert review panels consistently agree glyphosate is not a teratogen or reproductive toxicant.
The authors' justification for this research is flawed, providing no valid basis, other than an opinion, of an increase in the rate of birth defects in Argentina.
Direct injection of frog embryos and through chicken shells do not reflect real world exposure scenarios to either environmental species or humans.
Doses were excessively high and irrelevant for risk assessment purposes. Frog embryos were also bathed in glyphosate formulation at doses 9-15 times greater than the acute LC50 same species of frog. Calculating equivalent oral doses based on pharmacokinetics studies, such doses are 150000000 times greater than worst case human exposure monitoring data.
"…. the results from this research cannot be used in isolation to reach the conclusions expressed in the publication. Instead, the type of data in this research paper must be interpreted relative to all other available data on the specific materials under study and with balanced consideration for higher tier apical studies."

Response 3 – Mulet (2011, ASB2012-11916) letter to the Editor
Notes the premise for this research is falsely based on an incorrectly cited local pediatric bulletin from Paraguay.
"…. this article refers to a study in a single hospital in Paraguay showing a correlation between pesticide use (not herbicides as mentioned by Paganelli et al., ASB2010-11410) and birth malformations. In the cited study (Benitez et al., ASB2012-11563), the authors state that the results are preliminary and must be confirmed. Is important to remark that the Benitez et al. study does not include any mention to glyphosate, so does not account for what the authors are stating in the introduction…..This journal is also wrongly cited in the discussion referring to increased malformations due to herbicides, which is not the result of the study."

Response 4 – comments from BVL (2010, ASB2012-11579)
Highly artificial experimental conditions.
Inappropriate models to replace validated mammalian reproductive and developmental toxicity testing methods for use in human health risk assessment.
Inappropriate routes of exposure.
Lack of corroborative evidence in humans.
"In spite of long-lasting use of glyphosate-based herbicides worldwide, no evidence of teratogenicity in humans has been obtained so far."

Response 5– comments from European Commission Standing Committee on the Food Chain and Animal Health (2011, ASB2012-11615)
The EU commission supports the German Authorities position, "that that there is a comprehensive and reliable toxicological database for glyphosate and the effects observed have not been revealed in mammalian studies, nor evidenced epidemiologically in humans."
"…. the Commission does not consider there is currently a solid basis to ban or impose specific restrictions on the use of glyphosate in the EU."

Response 6– Palma, G. (2010, ASB2012-11989) letter to the Editor

The author of the letter claims that the study by Paganelli et al., 2010 (ASB2010-11410), described effects of glyphosate only at unrealistic high concentrations or via unrealistic routes of exposure. The data are thought to be inconsistent with the literature, and therefore not suitable or relevant for the risk assessment for humans and wildlife. Furthermore the author asserts that findings do not support the extrapolation to human health as stated in the publication.

Author(s)	Year	Study title
Richard, S. Moslemi, S. Sipahutar, H. Benachour, N. Seralini, G.E.	2005	Differential effects of glyphosate and roundup on human placental cells and aromatase. Environmental Health Perspectives Volume: 113 Pages: 716-720 TOX2005-1743

Abstract*

Roundup is a glyphosate-based herbicide used worldwide, including on most genetically modified plants that have been designed to tolerate it. Its residues may thus enter the food chain, and glyphosate is found as a contaminant in rivers. Some agricultural workers using glyphosate have pregnancy problems, but its mechanism of action in mammals is questioned. Here we show that glyphosate is toxic to human placental JEG3 cells within 18 hr with concentrations lower than those found with agricultural use, and this effect increases with concentration and time or in the presence of Roundup adjuvants. Surprisingly, Roundup is always more toxic than its active ingredient. We tested the effects of glyphosate and Roundup at lower nontoxic concentrations on aromatase, the enzyme responsible for estrogen synthesis. The glyphosate-based herbicide disrupts aromatase activity and mRNA levels and interacts with the active site of the purified enzyme, but the effects of glyphosate are facilitated by the Roundup formulation in microsomes or in cell culture. We conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in mammals. We suggest that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Study design is insufficient for risk assessment of real exposure concentrations. Methodological deficiencies (no controls were included). Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems. EPA Test Guideline OCSPP 890.1200 specifically notes that microsomes are denatured by detergents (i.e. surfactants) and that all glassware should be thoroughly rinsed.
Relevance of study:	Not relevant: Excessive doses exceed typical *in vitro* limit doses. *In vitro* test system is inappropriate with surfactants.
Klimisch code:	3

Additional comments:

Response 1 – summarized from Williams et al. (2012, ASB2012-12052)
Glyphosate at non-cytotoxic concentrations in this test system was demonstrated to have no effects on aromatase activity.
Likewise, did not affect mRNA levels after 18 hours treatment at ≤ 0.1% glyphosate.
Roundup aromatase activity measurements are confounded by surfactant effects on microsomes.
The *in vitro* test system is non-validated
Physiologically irrelevant concentrations tested.
Testing surfactant-like substances in such systems is now recognized to be not valid.

Response 2 – summarized from the French Ministry of Agriculture and Fish, Committee for Study of Toxicity (2005, ASB2009-9025)
Major methodological gaps.
JEG3 cells, a choriocarcinoma human cell line (average of 70 chromosomes vs 46 in normal human cells).
Concentrations of Roundup used in the various experiments considered to be extremely high.
In consideration of limiting factors (oral absorption, 30 %; skin absorption, 0.3 %; rapid elimination kinetics), such levels would involve considerable human exposure, or several dozen liters of Roundup diluted at 2 %.
concentrations of Roundup that trigger an effect on aromatase (0.5 % - 2 %) are at least 1000 times more effective than those of known aromatase inhibitors, such as azole derivatives
Study design does not make it possible to show the influence of the adjuvants, nor synergism of adjuvants and glyphosate.
Multiple non-specific effects of surfactant agents on a broad range of cellular targets not discussed.
No comparison with comparable surfactant agents intended for household use.
Multiple instances of bias in its arguments and its interpretation of the data.
The authors over-interpret their results in the area of potential health consequences for humans (unsuitable references, non-sustained in vitro-in vivo extrapolation, etc.).

Author(s)	Year	Study title
Benachour, N. Sipahutar, H. Moslerni, S. Gasnier, C. Travert, C. Seralini, G. E.	2007	Time- and dose-dependent effects of roundup on human embryonic and placental cells. Archives of Environmental Contamination and Toxicology Volume: 53 Pages: 126-133 ASB2009-9018

Abstract*
Roundup® is the major herbicide used worldwide, in particular on genetically modified plants that have been designed to tolerate it. We have tested the toxicity and endocrine disruption potential of Roundup (Bioforce®) on human embryonic 293 and placental-derived JEG3 cells, but also on normal human placenta and equine testis. The cell lines have proven to be suitable to estimate hormonal activity and toxicity of pollutants. The median lethal dose (LD_{50}) of Roundup with embryonic cells is 0.3 % within 1 h in serum-free medium, and it decreases to reach 0.06 % (containing among other compounds 1.27 mM glyphosate) after 72

h in the presence of serum. In these conditions, the embryonic cells appear to be 2-4 times more sensitive than the placental ones. In all instances, Roundup (generally used in agriculture at 1 -2 %, i.e., with 21-42 mM glyphosate) is more efficient than its active ingredient, glyphosate, suggesting a synergistic effect provoked by the adjuvants present in Roundup. We demonstrated that serum-free cultures, even on a short-term basis (1 h), reveal the xenobiotic impacts that are visible 1-2 days later in serum. We also document at lower non-overtly toxic doses, from 0.01 % (with 210 µM glyphosate) in 24 h, that Roundup is an aromatase disruptor. The direct inhibition is temperature-dependent and is confirmed in different tissues and species (cell lines from placenta or embryonic kidney, equine testicular, or human fresh placental extracts). Furthermore, glyphosate acts directly as a partial inactivator on microsomal aromatase, independently of its acidity, and in a dose-dependent manner. The cytotoxic, and potentially endocrine-disrupting effects of Roundup are thus amplified with time. Taken together, these data suggest that Roundup exposure may affect human reproduction and fetal development in case of contamination. Chemical mixtures in formulations appear to be underestimated regarding their toxic or hormonal impact.
Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Study report has several reporting deficiencies in the methods section (e.g. test conditions for the pH- and temperature dependent assay not reported). There is no information on the suitability of the used HEK 293 cell line for assessment of hormonal activity. Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems.
Relevance of study:	Not relevant: Excessive doses exceed typical *in vitro* limit doses. *In vitro* test system is inappropriate with surfactants.
Klimisch code:	3

Additional comments:
Glyphosate at and above relevant concentrations for this test system was demonstrated to have no effects on aromatase activity.
Roundup aromatase activity measurements are confounded by surfactant effects on microsomes.
Comparable research to Richard et al (2005, TOX2005-1743), but with an additional cell line, HEK 293, derived from aborted human embryo kidneys, transformed by inserting adenovirus DNA.
Excessively high doses tested, not environmentally relevant for human health or environmental risk assessment.
Aromatase production within the steroidogenesis pathway. Therefore, aromatase inhibition would be detected in the steroidogenesis assay. The OECD multi-laboratory validation of the steroidogenesis assay evaluated glyphosate, demonstrating no impact on the steroidogenesis pathway (Hecker et al., 2011, ASB2012-11840).

Response – summarized from Williams et al. (2012, ASB2012-12052)

pH of test system not adjusted to physiologically appropriate levels;
Negative controls were not pH adjusted to appropriate levels.
Confounding surfactant effects due to cell membrane damage render data generated with formulated products in this test system null.

Author(s)	Year	Study title
Benachour, N. Seralini, G. E.	2009	Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. Chemical Research in toxicology Volume: 22, Pages: 97-105 ASB2012-11561

Abstract*
We have evaluated the toxicity of four glyphosate (G)-based herbicides in Roundup formulations, from 10(5) times dilutions, on three different human cell types. This dilution level is far below agricultural recommendations and corresponds to low levels of residues in food or feed. The formulations have been compared to G alone and with its main metabolite AMPA or with one known adjuvant of R formulations, POEA. HUVEC primary neonate umbilical cord vein cells have been tested with 293 embryonic kidney and JEG3 placental cell lines. All R formulations cause total cell death within 24 h, through an inhibition of the mitochondrial succinate dehydrogenase activity, and necrosis, by release of cytosolic adenylate kinase measuring membrane damage. They also induce apoptosis via activation of enzymatic caspases 3/7 activity. This is confirmed by characteristic DNA fragmentation, nuclear shrinkage (pyknosis), and nuclear fragmentation (karyorrhexis), which is demonstrated by DAPI in apoptotic round cells. G provokes only apoptosis, and HUVEC are 100 times more sensitive overall at this level. The deleterious effects are not proportional to G concentrations but rather depend on the nature of the adjuvants. AMPA and POEA separately and synergistically damage cell membranes like R but at different concentrations. Their mixtures are generally even more harmful with G. In conclusion, the R adjuvants like POEA change human cell permeability and amplify toxicity induced already by G, through apoptosis and necrosis. The real threshold of G toxicity must take into account the presence of adjuvants but also G metabolism and time-amplified effects or bioaccumulation. This should be discussed when analyzing the in vivo toxic actions of R. This work clearly confirms that the adjuvants in Roundup formulations are not inert. Moreover, the proprietary mixtures available on the market could cause cell damage and even death around residual levels to be expected, especially in food and feed derived from formulation-treated crops.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems. No positive controls were included.
Relevance of study:	Not relevant (Excessive doses exceed typical *in vitro* limit doses. *In vitro* test system is inappropriate with

	surfactants)
Klimisch code:	3

Additional comments:

Response – summarized from the French Agency for Food Safety (AFSSA, 2009, ASB2012-11532)
Cell lines used present characteristics which may be at the source of a significant bias in the interpretation of the results.
Experiments were conducted with 24 hours exposure in a medium without serum, which could lead to disturbance of the physiological state of the cells.
The glyphosate used in the study is glyphosate acid, whereas in the preparations tested it is in the form of an isopropylamine salt. No precise information is given about the pH of test concentrations except the highest dose.
No mention of any positive evidence for the apoptosis test.
Cytoxicity and induction of apoptosis may due to pH and/or variations in osmotic pressure on cell survival at the high doses tested.
Surfactant (tensoactive) effects and increased osmolality are known to increase membrane permeability, causing cytotoxicity and induction of apoptosis.
Conclusions are based on unvalidated, non-representative cell models (in particular tumour or transformed cell lines) directly exposed to extremely high product concentrations in culture conditions which do not observe normal cell physiological conditions.
No new information is presented on mechanism of action of glyphosate and preparations containing glyphosate.
The authors over-interpret their results with regard to potential health consequences for humans, based in particular on an unsupported *in vitro–in vivo* extrapolation
The cytotoxic effects of glyphosate, its metabolite AMPA, the tensioactive POAE and other glyphosate-based preparations proposed by Benachour and Seralini do not add any pertinent new facts which call into question the conclusions of the European assessment of glyphosate or those of the national assessment of the preparations.

Author(s)	Year	Study title
Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M. C., Seralini, G. E	2009	Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology Volume: 262, Number: 3, Pages: 184-191 ASB2012-11629

Abstract*
Glyphosate-based herbicides are the most widely used across the world; they are commercialised in different formulations. Their residues are frequent pollutants in the environment. In addition, these herbicides are spread on most eaten transgenic plants, modified to tolerate high levels of these compounds in their cells. Up to 400 ppm of their residues are accepted in some feed. We exposed human liver HepG2 cells, a well-known model to study xenobiotic toxicity, to four different formulations and to glyphosate, which is usually tested alone in chronic in vivo regulatory studies. We measured cytotoxicity with three assays (Alamar Blue, MTT, ToxiLight), plus genotoxicity (comet assay), anti-estrogenic (on ERα, ERβ) and anti-androgenic effects (on AR) using gene reporter tests. We also checked androgen to estrogen conversion by aromatase activity and mRNA. All parameters

were disrupted at sub-agricultural doses with all formulations within 24h. These effects were more dependent on the formulation than on the glyphosate concentration. First, we observed a human cell endocrine disruption from 0.5 ppm on the androgen receptor in MDA-MB453-kb2 cells for the most active formulation (R400), then from 2 ppm the transcriptional activities on both estrogen receptors were also inhibited on HepG2. Aromatase transcription and activity were disrupted from 10 ppm. Cytotoxic effects started at 10 ppm with Alamar Blue assay (the most sensitive), and DNA damages at 5 ppm. A real cell impact of glyphosate-based herbicides residues in food, feed or in the environment has thus to be considered, and their classifications as carcinogens/mutagens/reprotoxics is discussed.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Due to reporting deficiencies (e.g. correlation between concentration used in toxicity tests and concentrations used in comet assay) assessment of results difficult. Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems.
Relevance of study:	Not relevant: Excessive doses exceed typical *in vitro* limit doses. *In vitro* test system is inappropriate with surfactants.
Klimisch code:	3

Additional comments:

Response 1 – summarized from Williams et al. (2012, ASB2012-12052)
Glyphosate demonstrated no significant anti-estrogenic potential
Glyphosate demonstrated some anti-androgenic potential at lower concentrations, but not as doses increased and therefore results are considered unrelated to treatment
Four glyphosate based formulations demonstrated both estrogenic and androgenic activity.
Results are confounded due to surfactants within the formulated products tested, which affect cell membrane integrity and produces false findings.

Response 2 – summarized from BfR Review (2009, ASB2012-11565)
Numerous methodological flaws are noted.
Test substance(s) not characterized
Source of materials for cell culture not provided.
Dosing concentrations not well described
Serum free media only appropriate for short term (3-4 hour) *in vitro* exposures.
pH control of dilutions not clear.
Osmolality of test solutions not reported.
Electrophoresis parameters insufficiently or inaccurately reported.
Numerous reporting deficiencies are noted.
Influence of serum-free cell culturing on endpoints can not be determined
Incomplete data reporting, in cluding β-galactosirase activity, cototoxicity for select assays.
Positive control data not reported.

Confusion between maximum residue levels verses systemic concentrations in humans.

Author(s)	Year	Study title
Clair, E., Mesnage, R., Travert, C., Seralini, G.E.	2012	A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells *in vitro*, and testosterone decrease at lower levels. Toxicology in Vitro Volume: 26, Number: 2, Pages: 269-279 ASB2012-1628

Abstract*
The major herbicide used worldwide, Roundup, is a glyphosate-based pesticide with adjuvants. Glyphosate, its active ingredient in plants and its main metabolite (AMPA) are among the first contaminants of surface waters. Roundup is being used increasingly in particular on genetically modified plants grown for food and feed that contain its residues. Here we tested glyphosate and its formulation on mature rat fresh testicular cells from 1 to 10000 ppm, thus from the range in some human urine and in environment to agricultural levels. We show that from 1 to 48 h of Roundup exposure Leydig cells are damaged. Within 24–48 h this formulation is also toxic on the other cells, mainly by necrosis, by contrast to glyphosate alone which is essentially toxic on Sertoli cells. Later, it also induces apoptosis at higher doses in germ cells and in Sertoli/germ cells co-cultures. At lower non toxic concentrations of Roundup and glyphosate (1 ppm), the main endocrine disruption is a testosterone decrease by 35%. The pesticide has thus an endocrine impact at very low environmental doses, but only a high contamination appears to provoke an acute rat testicular toxicity. This does not anticipate the chronic toxicity which is insufficiently tested and only with glyphosate in regulatory tests.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-guideline *in vitro* test with methodological (i.e. no positive controls included) and reporting deficiencies (e.g. dose levels not always specified).
Relevance of study:	Not relevant (Due to reliability. In addition, *in vitro* data do not reflect real *in vivo* exposure situations, and therefore not relevant for human risk assessment purposes.)
Klimisch code:	3

Additional comments:
In vitro test with methodological (i.e. no positive controls included) and reporting deficiencies (e.g. dose levels not always specified). The concentrations used in these experiments are not relevant to human exposures to glyphosate and the experimental system used is not relevant to whole animal outcomes. Importantly, the alleged impacts on endocrine function have not been observed in animal studies of glyphosate or other components of glyphosate formulations at relevant concentrations. Authors state that the lowest concentration of glyphosate tested was 50 ppm, several orders of magnitude higher than an anticipated human intake (based on

pharmacokinetics described in Anadon et al., 2009, ASB2012-11542) following worst case dietary exposure at the ADI.

Author(s)	Year	Study title
Hokanson, R. Fudge, R. Chowdhary, R. Busbee, D.	2007	Alteration of estrogen-regulated gene expression in human cells induced by the agricultural and horticultural herbicide glyphosate. Human & Experimental Toxicology Volume: 26, Pages: 747-752, ASB2012-11846

Abstract*
Gene expression is altered in mammalian cells (MCF-7 cells), by exposure to a variety of chemicals that mimic steroid hormones or interact with endocrine receptors or their co-factors. Among those populations chronically exposed to these endocrine disruptive chemicals are persons, and their families, who are employed in agriculture or horticulture, or who use agricultural/horticultural chemicals. Among the chemicals most commonly used, both commercially and in the home, is the herbicide glyphosate. Although glyphosate is commonly considered to be relatively non-toxic, we utilized *in vitro* DNA microarray analysis of this chemical to evaluate its capacity to alter the expression of a variety of genes in human cells. We selected a group of genes, determined by DNA microarray analysis to be dysregulated, and used quantitative real-time PCR to corroborate their altered states of expression. We discussed the reported function of those genes, with emphasis on altered physiological states that are capable of initiating adverse health effects that might be anticipated if gene expression were significantly altered in either adults or embryos exposed *in utero*.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Not acceptable *in vitro* methods for test mixtures containing surfactant. Well documented study publication, but surfactants are inappropriate test substance in cell lines.
Relevance of study:	Not relevant Temporal altered gene expression is not a biomarker for toxicity, but rather, may be within the range of normal biological responses of homeostasis. *In vitro* cytotoxicity of surfactants, however, is a significant confounder in data interpretation. Data do not reflect real *in vivo* exposure situations, and therefore not relevant for human risk assessment purposes.
Klimisch code:	3

Additional comments:
In vitro cytotoxicity of surfactants is a significant confounder in data interpretation. Relevance of altered gene expression in a cell line derived from a breast cancer should not be extrapolated to reflect human health endpoints. Altered gene expression should not be confused with adverse health outcomes. Rather altered gene expression may equally be considered a biological response within the range of normal homeostasis.

In vivo DART/ED publications

Author(s)	Year	Study title
Yousef, M.I., Salem, M.H., Ibrahim, H.Z., Helmi, S., Seehy, M.A., Bertheussen, K.	1995	Toxic Effects of Carbofuran and Glyphosate on Semen Characteristics in Rabbits. Journal of Environmental Science and Health. Part B. Volume: 30, Number: 4, Pages: 513-534 ASB2012-12058

Abstract*
The present study was undertaken to investigate the effect of chronic treatment with two sublethal doses of Carbofuran (carbamate insecticide) and Glyphosate (organophosphorus herbicide) on body weight and semen characteristics in mature male New Zealand white rabbits. Pesticide treatment resulted in a decline in body weight, libido, ejaculate volume, sperm concentration, semen initial fructose and semen osmolality. This was accompanied with increases in the abnormal and dead sperm and semen methylene blue reduction time. The hazardous effect of these pesticides on semen quality continued during the recovery period, and was dose-dependent. These effects on sperm quality may be due to the direct cytotoxic effects of these pesticides on spermatogenesis and/or indirectly via hypothalami-pituitary-testis axis which control the reproductive efficiency.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-GLP, non-guideline study with major reporting deficiencies. Dose-levels poorly defined as 1/10 and 1/100 LD_{50}. Purity of the test substances, source of animals, environmental conditions, mortality and clinical signs not reported. No testis and epididymis weights were determined or reported and no histopathological examination conducted. In addition, stability and homogeneity assessment of test substance preparations were not done or not reported. Rabbits have low body weights at study start, suggesting impaired health status.
Relevance of study:	Not relevant (Due to low confidence in study conduct and the inadequacy of reporting.)
Klimisch code:	3

Additional comments:

Response – summarized from Williams et al. (2000, ASB2012-12053)
Numerous serious deficiencies in the design, conduct, and reporting of this study which make the results uninterpretable.
Only four rabbits per treatment group were used, and therefore statistics are questionable.

Rabbits appeared to be small for their age; at study start (32 weeks) tested animals had 16-25 % lower body weight than historical weights for commercially bred animals of the same age and strain.
Low body weights as study start suggest compromised health status of the animals at initiation.
Dose levels were not quantified.
Purity of glyphosate and composition of the glyphosate formulation were not reported.
Inadequate description of test material administration.
Improper semen collection technique reported.
Report is unclear whether control animal sham handling was undertaken, a critical factor in stress related outcomes in this species.
Food consumption of test and control groups not adequately reported.
Variability not adequately reported for endpoint measurements in test and control groups, preventing statistical analysis to support the author's conclusions.
Dose-responses not observed, despite the wide dose spread.
Sperm concentrations of all groups within normal ranges for this strain of rabbit.
No meaningful conclusions can be drawn from this publication.

Author(s)	Year	Study title
Daruich, J. Zirulnik, F. Gimenez, M. S.	2001	Effect of the herbicide glyphosate on enzymatic activity in pregnant rats and their fetuses Environmental Research Volume: 85 Pages: 226-231 ASB2012-11601

Abstract*

To prevent health risk from environmental chemicals, particularly for progeny, we have studied the effects of the herbicide glyphosate on several enzymes of pregnant rats. Glyphosate is an organo-phosphorated nonselective agrochemical widely used in many countries including Argentina and acts after the sprout in a systemic way. We have studied three cytosolic enzymes: isocitrate dehydrogenase-NADP dependent, glucose-6-phosphate dehydrogenase, and malic dehydrogenase in liver, heart, and brain of pregnant Wistar rats. The treatment was administered during the 21 days of pregnancy, with 1 week as an acclimation period. The results suggest that maternal exposure to agrochemicals during pregnancy induces a variety of functional abnormalities in the specific activity of the enzymes in the studied organs of the pregnant rats and their fetuses.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Basic data given, however, the study is performed with methodological and reporting deficiencies (unknown exposure levels, only cytosolic enzymes measured, inappropriate controls, lack of consistent dose-response data).

Relevance of study:	Not relevant (Due to reliability. In addition, study was performed with a glyphosate formulation (commercialised in Argentina) and not with glyphosate).
Klimisch code:	3

Additional comments:
The study was performed with a glyphosate formulation (commercialised in Argentina) and not with glyphosate. Test substance administration is poorly described, but rough calculations on approximate surfactant intake show excessively high and unrealistic exposures when compared to DART systemic parental and reproductive/developmental NOAEL values for POEA formulation surfactants.

Response summarized from Williams et al. (2012, ASB2012-12052)
Test substance and doses not adequately described.
Inappropriate control groups.
Results suggest that the effect of treatment on body and organ weights may be due to reduced food and water intakes.
A consistent effect of treatment was not observed and dose-response relationships were generally lacking
The information gathered may be misleading because the enzymes monitored are found in both the cytosol and mitochondria.
Food restriction affects the activity of many enzymes, including those examined in this study.

Author(s)	Year	Study title
Romano, R.M. Romano, M.A. Bernardi, M.M. Furtado, P.V. Oliveira, C.A.	2010	Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology. Archives of Toxicology Volume: 84, Pages: 309-317 ASB2012-12012

Abstract*
Glyphosate is a herbicide widely used to kill weeds both in agricultural and non-agricultural landscapes. Its reproductive toxicity is related to the inhibition of a StAR protein and an aromatase enzyme, which causes an in vitro reduction in testosterone and estradiol synthesis. Studies in vivo about this herbicide effects in prepubertal Wistar rats reproductive development were not performed at this moment. Evaluations included the progression of puberty, body development, the hormonal production of testosterone, estradiol and corticosterone, and the morphology of the testis. Results showed that the herbicide (1) significantly changed the progression of puberty in a dose-dependent manner; (2) reduced the testosterone production, in semineferous tubules' morphology, decreased significantly the epithelium height ($P < 0.001$; control = 85.8 ± 2.8 µm; 5 mg/kg = 71.9 ± 5.3 µm; 50 mg/kg = 69.1 ± 1.7 µm; 250 mg/kg = 65.2 ± 1.3 µm) and increased the luminal diameter ($P < 0.01$; control = 94.0 ± 5.7 µm; 5 mg/kg = 116.6 ± 6.6 µm; 50 mg/kg = 114.3 ± 3.1 µm; 250 mg kg = 130.3 ± 4.8 µm); (4) no difference in tubular diameter was observed; and (5) relative to the controls, no differences in serum corticosterone or estradiol levels were detected, but the concentrations of testosterone serum were lower in all treated groups ($P < 0.001$; control = 154.5 ± 12.9 ng/dL; 5 mg/kg = 108.6 ± 19.6 ng/dL; 50 mg/dL = 84.5 ± 12.2 ng/dL; 250

mg/kg = 76.9 ± 14.2 ng/dL). These results suggest that commercial formulation of glyphosate is a potent endocrine disruptor in vivo, causing disturbances in the reproductive development of rats when the exposure was performed during the puberty period.
Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Study with methodological and reporting deficiencies or conflicting findings (e.g., increased relative testicular weights, but decreased testosterone measurements.
Relevance of study:	Relevant study type for investigating male reproductive endpoints, but questionable relevance of this specific study based on low reliability of data and interpretation. Not relevant for glyphosate (test material was a formulated product, not glyphosate).
Klimisch code:	3

Additional comments:
Test material was a formulated product, not glyphosate. The authors failed to measure many of the key parameters in the validated pubertal male assay protocol and hence generated data that were internally inconsistent or incomplete.

Author(s)	Year	Study title
Romano, M.A. Romano, R.M. Santos, L.D. Wisniewski, P. Campos, D.A. de Souza, P.B. Viau, P. Bernardi, M.M. Nunes, M.T. de Oliviera, C.A.	2012	Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression Archives of Toxicology Volume: 86, Number: 4, Pages: 663-673 ASB2012-12011

Abstract*
Sexual differentiation in the brain takes place from late gestation to the early postnatal days. This is dependent on the conversion of circulating testosterone into estradiol by the enzyme aromatase. The glyphosate was shown to alter aromatase activity and decrease serum testosterone concentrations. Thus, the aim of this study was to investigate the effect of gestational maternal glyphosate exposure (50 mg/kg, NOAEL for reproductive toxicity) on the reproductive development of male offspring. Sixty-day-old male rat offspring were evaluated for sexual behavior and partner preference; serum testosterone concentrations, estradiol, FSH and LH; the mRNA and protein content of LH and FSH; sperm production and the morphology of the seminiferous epithelium; and the weight of the testes, epididymis and seminal vesicles. The growth, the weight and age at puberty of the animals were also recorded to evaluate the effect of the treatment. The most important findings were increases in sexual partner preference scores and the latency time to the first mount; testosterone and estradiol serum concentrations; the mRNA expression and protein content in the pituitary gland and the

serum concentration of LH; sperm production and reserves; and the height of the germinal epithelium of seminiferous tubules. We also observed an early onset of puberty but no effect on the body growth in these animals. These results suggest that maternal exposure to glyphosate disturbed the masculinization process and promoted behavioral changes and histological and endocrine problems in reproductive parameters. These changes associated with the hypersecretion of androgens increased gonadal activity and sperm production.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-guideline, non-GLP study meeting scientific principles. Unusual and short dosing regiment commencing towards the end of pregnancy (GD18, rather than GD6 as per OECD Test Guidelines 414) through post natal day 5. *In vivo* study with reporting deficiencies (detailed strain description, source of animals, housing conditions, no information if clinical signs were assessed, stability and homogeneity assessment of test substance preparations, no of male offspring evaluated in individual tests evaluated). A number of atypical endpoints evaluated.
Relevance of study:	Not relevant (due to questionable dosing regimen and atypical array of endpoints measured).
Klimisch code:	3

Additional comments:
Study with some reporting deficiencies (detailed strain description, source of animals, housing conditions, no information if clinical signs were assessed, stability and homogeneity assessment of test substance preparations, no of male offspring evaluated in individual tests evaluated). Dosing was limited to dams, starting on gestation day 18, well after organogenisis, through post natal day 5. No controls for litter effects appear to be reported, confounding interpretation of results. With the very short window of maternal exposure, biological plausibility of any test substance related effects in the mature offspring is questionable. However, the normal variability of some unusual or atypical endpoint measurements, such as "sexual partner preference" along with mRNA and protein expression, is not known. Of particular concern, however, are differences in critical endpoints for control animals reported in Romano et al. (2010, ASB2012-12012) compared to Romano et al. (2012, ASB2012-12011); these include increased day of preputional sepratarion (PPS) of control male rate (37 days in 2010; 47 days in 2012), body weight at day of PPS (146 grams in 2010; 245 grams in 2012), serum testosterone concentrations (155 ng/dL in 2010; 63 ng/dL in 2012), estradiol concentrations (32 pg/mL in 2010; 1.4 pg/mL in 2012), subular diameter (266 µm in 2010; 479 µm in 2012), epithelial height (86 µm in 2010; 92 µmin 2012) and luminal height (94 µm in 2010; 257µm in 2012). Therefore, results are difficult to interpret, particularly for relevance to human health risk assessment.

A letter to the editor by DeSesso and Williams, (2012, ASB2014-9369) concluded as follows: "Taken together, the shortcomings in this paper erode any confidence that these experiments are able to demonstrate disruption in the development or function of the male reproductive

system in offspring whose dame were treated with glyphosate". Romano and Romano (2012, ASB2014-9396) rebutted these comments and conclusions.

Epidemiology DART/ED Publications

Author(s)	Year	Study title
Arbuckle, T. E. Lin, Z. Mery, L. S.	2001	An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population Environmental Health Perspectives Volume: 109 Pages: 851-857 ASB2012-11545

Abstract*

The toxicity of pesticides on human reproduction is largely unknown—particularly how mixtures of pesticide products might affect fetal toxicity. The Ontario Farm Family Health Study collected data by questionnaire on the identity and timing of pesticide use on the farm, lifestyle factors, and a complete reproductive history from the farm operator and eligible couples living on the farm. A total of 2,110 women provided information on 3,936 pregnancies, including 395 spontaneous abortions. To explore critical windows of exposure and target sites for toxicity, we examined exposures separately for preconception (3 months before and up to month of conception) and postconception (first trimester) windows and for early (< 12 weeks) and late (12–19 weeks) spontaneous abortions. We observed moderate increases in risk of early abortions for preconception exposures to phenoxy acetic acid herbicides [odds ratio (OR) = 1.5; 95% confidence interval (CI), 1.1–2.1], triazines (OR = 1.4; 95% CI, 1.0–2.0), and any herbicide (OR = 1.4; 95% CI, 1.1–1.9). For late abortions, preconception exposure to glyphosate (OR = 1.7; 95% CI, 1.0–2.9), thiocarbamates (OR = 1.8; 95% CI, 1.1–3.0), and the miscellaneous class of pesticides (OR = 1.5; 95% CI, 1.0–2.4) was associated with elevated risks. Postconception exposures were generally associated with late spontaneous abortions. Older maternal age (> 34 years of age) was the strongest risk factor for spontaneous abortions, and we observed several interactions between pesticides in the older age group using Classification and Regression Tree analysis. This study shows that timing of exposure and restricting analyses to more homogeneous endpoints are important in characterizing the reproductive toxicity of pesticides.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	No information about exposure duration, used glyphosate products and application rates. No information, if the subjects used more than one pesticide.
Relevance of study:	Not relevant (Study design is not suitable for assessment of glyphosate exposure).
Klimisch code:	3

Additional comments:

Pre-conception glyphosate exposure odds ratio for spontaneous abortion is considered of borderline significance (OR = 1.4). Post-conception glyphosate exposure was not associated

with spontantous abortin (OR = 1.1). Authors note multiple limitations of the study relating to exposure, likely misclassification of pesticides and correct assignment of exposure window to pre- or/and post-conception
OFFHS information gathering methodology has high potential recall bias. Blair and Zahm (1993, ASB2012-11567) report 60 % accuracy when comparing self reported pesticide usage with purchasing records.
OFFHS relied exclusively on maternal self-reports of adverse pregnancy outcomes, not all of which were confirmed via medical or other records.
Three highly relevant confounding factors were not considered in the OFFHS questionnaire: history of previous spontaneous abortion(s), maternal age and smoking.

Response summarized from Williams et al. (2012, ASB2012-12052)
395 spontaneous abortions were reported out of 3936 pregnancies; rate of spontaneous aborting in Arbuckle et al. (2001, ASB2012-11545) was 10 %.
The baseline rate of spontaneous abortions in the general populations is much higher, ranging from 12 % to 25 %.
Recall bias is reflected in the recall of spontaneous abortion over the previous 5 years (64 % of all spontaneous abortions reported) being much higher than the recall of those greater than 10 years prior to the survey (34% of all spontaneous abortions reported).
Substantial exposure misclassification may have occurred (pre- versus post-conception) due to likely author extrapolation of exposure data.
Strong confounding variables are not apparent in previous data analyses published by the authors of the OFFHS, and therefore odds ratios are crude.
Published results fail to demonstrate a significant association of glyphosate exposure spontaneous abortion risk and therefore must be considered cautiously.

Author(s)	Year	Study title
Savitz, D.A. Arbuckle, T. Kaczor, D. Curtis, K.M.	1997	Male pesticide exposure and pregnancy outcome. American Journal of Epidemiology Volume: 146, Number: 12, Pages: 1025-1036 ASB2012-12022

Abstract*
Potential health effects of agricultural pesticide use include reproductive outcomes. For the Ontario Farm Family Health Study, the authors sampled Ontario farms from the 1986 Canadian Census of Agriculture, identified farm couples, and obtained questionnaire data concerning farm activities, reproductive health experience, and chemical applications. Male farm activities in the period from 3 months before conception through the month of conception were evaluated in relation to miscarriage, preterm delivery, and small-for-gestational-age births. Among the 1,898 couples with complete data (64 % response), 3,984 eligible pregnancies were identified. Miscarriage was not associated with chemical activities overall but was increased in combination with reported use of thiocarbamates, carbaryl, and unclassified pesticides on the farm. Preterm delivery was also not strongly associated with farm chemical activities overall, except for mixing or applying yard herbicides (odds ratio = 2.1, 95 % confidence interval 1.0-4.4), Combinations of activities with a variety of chemicals (atrazine, glyphosate, organophosphates, 4-[2,4-dichlorophenoxy] butyric acid, and insecticides) generated odds ratios of two or greater. No associations were found between farm chemicals and small-for-gestational-age births or altered sex ratio. Based on these data, despite limitations in exposure assessment, the authors encourage continued evaluation of male exposures, particularly in relation to miscarriage and preterm delivery.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not Reliable
Comment:	No information about exposure duration, used glyphosate products and application rates. No information, if the subjects used more than one pesticide. Due to study design and evaluation methods, study results are not reliable.
Relevance of study:	Not Relevant (Study design is not suitable for assessment of glyphosate exposure).
Klimisch code:	3

Additional comments:
Glyphosate is one of many pesticides mentioned in three epidemiological reports that examine possible links between on-farm pesticide use and reproductive outcomes. All three reports - Savitz et *al.* (1997, ASB2012-12022), Curtis et *al.* (1999, cited in ASB2012-11545) and Arbuckle et *al.* (2001, ASB2012-11545) - use data from the Ontario Farm Family Health Study (OFFHS) (Arbuckle 1994, cited in ASB2012-11545). Savitz *et al.* (1997, ASB2012-12022) investigated associations between reported pesticide use by males and pregnancy outcomes, specifically: miscarriage, pre-term delivery and small-for-gestational-age birth. Curtis *et al.* (1999, cited in ASB2012-11545) studied whether reported pesticide use by males or females was associated with delayed pregnancy, while Arbuckle *et al.* (2001, ASB2012-11545) looked for associations between reported pesticide use and spontaneous abortion.

In the study by Savitz et al. (1997, ASB2012-12022), a number of specific pesticides had weak statistical associations with miscarriages and pre-term deliveries, but pesticides tended not to be associated with small for gestational age births. There were no statistically significant findings for glyphosate. In the study by Curtis et al.(1999, cited in ASB2012-11545), for farms on which glyphosate was used, there was no significant association for women being engaged in pesticide activities. For men, glyphosate use was associated with a slight, but statistically significant, decrease in time to pregnancy. The authors dismissed this finding, which was contrary to their hypothesis that pesticide exposure delayed pregnancy, as probably due to uncontrolled factors or chance. Arbuckle *et al.* (2001, ASB2012-11545) found that reported preconception use of phenoxyacetic acids, triazines, glyphosate, and thiocarbamates were weakly, but statistically significantly, associated with spontaneous abortions. Post conception reported use was not associated with increased risk. The authors characterized the associations between pesticides and spontaneous abortions as "hypothesis generating" pending confirmation from other epidemiologic studies.
These studies are not convincing evidence of a relationship between glyphosate exposure and adverse pregnancy outcomes for a number of reasons:

There was no actual exposure data per se in these three epidemiologic studies. Exposures were assumed based on questionnaire responses by study subjects about farm activities and pesticide use. This type of information can be inaccurate. For example, according to a study by the National Cancer Institute, self-reports of pesticide usage were found to be only 60 percent accurate when compared with purchasing records (Blair & Zahm 1993, ASB2012-11567). Further increasing the potential for inaccuracy is the fact that study subjects were only asked about pesticide use for the 5 years before the OFFS survey. These responses were

assumed to be applicable to the entire farming careers of study subjects, an assumption inconsistent with changes in agricultural practice. Lastly, basing exposure estimation on questionnaire responses has the potential to be influenced by what epidemiologists call "recall bias." This refers to the likelihood that families that experienced an adverse reproductive outcome are more likely to remember use of certain pesticides than families that had only normal births.

The most widely used pesticides, like atrazine, glyphosate, and 2,4-D, are most easily recalled and most likely to be over-reported.

The OFFHS study relied exclusively on maternal self-reports of adverse pregnancy outcomes with no medical or other validation. Generally, scientists place less confidence in reports of health outcomes that are not validated with medical records.

A confounding factor is a cause of a disease that is correlated with another exposure being studied. Failure to control confounding factors, especially those that are strong causes of a disease, can create spurious associations between benign exposures and diseases. In the Arbuckle study, there were at least three important potential confounding factors that were not controlled: history of previous spontaneous abortion, maternal age, and smoking. Even a weak correlation between these factors and use (or recall of use) of pesticides would produce spurious associations. In addition, in all three studies, the authors did not control the putative effect of one pesticide for the putative effects of other pesticides. So, for example, since farmers tend to use 4 or more pesticides each year, a disease that is associated with one pesticide will likely be associated with all, since their use patterns are correlated. In the absence of an analysis that controls for multiple pesticides, the best that can be said is that the findings for any individual pesticide might be due to its correlation with another pesticide.

In summary, three publications based on data collected in the OFFHS found associations between several pesticides and various adverse reproductive outcomes. There was no actual exposure data per se in these three epidemiologic studies. Exposures were assumed based on questionnaire responses by study subjects about farm activities and pesticide use. This type of information can be inaccurate. Glyphosate was not significantly associated with adverse reproductive outcomes in two of these studies (Savitz *et al.* 1997, ASB2012-12022, Curtis *et al.* 1999, cited in ASB2012-11545). Glyphosate and other pesticides were weakly associated with spontaneous abortion in the study by Arbuckle (2001, ASB2012-11545). However, the author did not control for important personal confounding factors or for multiple exposures and no actual exposure data was used, casting doubt on the validity of the findings in this study.

Biomonitoring data for glyphosate, collected as part of the Farm Family Exposure Study (FFES), provide assurance that human health effects related to glyphosate exposure are very unlikely. In the FFES, researchers from the University of Minnesota collected 5 days of urine samples from 48 farm families before, during, and after a glyphosate application (Mandel *et al.*, 2005, ASB2012-11893, accepted for publication). Only 60% of farmers showed detectable exposure to glyphosate, with a 1 part per billion limit of detection, and the maximum estimated absorbed dose was 0.004 *mg/kg* (Acquavella *et al.*, 2004, ASB2012-11528). For farmers who apply glyphosate 10 times per year for 40 years, this maximum dose is more than 30,000-fold less than the EPA reference dose1 of 2 mg/kg/day. For spouses, only 4% showed detectable exposures and the maximum systemic dose was 0.00004 mg/kg/day. Since glyphosate is not a reproductive toxic in high dose animal studies and since actual exposures on farms are so low, it is very unlikely that glyphosate would cause adverse reproductive outcomes for farmers or their spouses.

Author(s)	Year	Study title
Garry, V. F. Harkins, M. E. Erickson, L. L. Long-Simpson, L. K. Holland, S. E. Burroughs, B. L.	2002	Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA. Environmental Health Perspectives Volume: 110 Pages: 441-449 ASB2012-11626

Abstract*
We previously demonstrated that the frequency of birth defects among children of residents of the Red River Valley (RRV), Minnesota, USA, was significantly higher than in other major agricultural regions of the state during the years 1989-1991, with children born to male pesticide applicators having the highest risk. The present, smaller cross-sectional study of 695 families and 1,532 children, conducted during 1997-1998, provides a more detailed examination of reproductive health outcomes in farm families ascertained from parent-reported birth defects. In the present study, in the first year of life, the birth defect rate was 31.3 births per 1,000, with 83% of the total reported birth defects confirmed by medical records. Inclusion of children identified with birth or developmental disorders within the first 3 years of life and later led to a rate of 47.0 per 1,000 (72 children from 1,532 live births). Conceptions in spring resulted in significantly more children with birth defects than found in any other season (7.6 vs. 3.7%). Twelve families had more than one child with a birth defect (n = 28 children). Forty-two percent of the children from families with recurrent birth defects were conceived in spring, a significantly higher rate than that for any other season. Three families in the kinships defined contributed a first-degree relative other than a sibling with the same or similar birth defect, consistent with a Mendelian inheritance pattern. The remaining nine families did not follow a Mendelian inheritance pattern. The sex ratio of children with birth defects born to applicator families shows a male predominance (1.75 to 1) across specific pesticide class use and exposure categories exclusive of fungicides. In the fungicide exposure category, normal female births significantly exceed male births (1.25 to 1). Similarly, the proportion of male to female children with birth defects is significantly lower (0.57 to 1; p = 0.02). Adverse neurologic and neurobehavioral developmental effects clustered among the children born to applicators of the fumigant phosphine (odds ratio [OR] = 2.48; confidence interval [CI], 1.2-5. 1). Use of the herbicide glyphosate yielded an OR of 3.6 (CI, 1.3-9.6) in the neurobehavioral category. Finally, these studies point out that a) herbicides applied in the spring may be a factor in the birth defects observed and b) fungicides can be a significant factor in the determination of sex of the children of the families of the RRV. Thus, two distinct classes of pesticides seem to have adverse effects on different reproductive outcomes. Biologically based confirmatory studies are needed.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Epidemiological study with some methodological / reporting deficiencies (selection of study subjects, no information about exposure duration, exposure concentration, pesticide use frequency).
Relevance of study:	Not relevant because of methodological deficiencies.
Klimisch code:	3

Additional comments:

Response 1 – summary from Mink et al. (2011) (ASB2012-11904)
Publication reports on different classes of pesticides and several birth defects and developmental outcomes.
Paternal use of glyphosate was associated with parent-reported ADD/ADHD in children (OR = 3.6). Six out of 14 children with parent reported ADD/ADHD also reported exposure to glyphosate.
Diagnoses of ADD/AHDH were not all confirmed. However, overall rate for the sample population (14/1532) was well below ADD/ADHD rates for the general population (7%).
Variables in statistical model analyses were not reported.

Response 2 – summary from Williams et al. (2012, ASB2012-12052)
Health data obtained via parent reporting for 695 families via written questionnaire and confirmed where possible.
Pesticide use information obtained initially via telephone then followed up by written questionnaire.
Reproductive health outcomes for births occurring between 1968 and 1998 were obtained for 1532 live births. Over half the births occurred prior to 1978, approximately 20 years after study initiation.
All pesticide use classes (herbicide only; herbicide and insecticide; herbicide, insecticide and fungicide; herbicide, insecticide and fumigant) were associated with birth defects.
Authors state neurobehavioral disorder would not be considered based lack consistent diagnoses. However, a detailed analysis was conducted for ADD/ADHD.
43% (6/14) parent reported children with ADD/ADHD were associated with glyphosate formulation use.
14 cases of ADD/ADHD reported out of 1532 live births, which is substantially lower that the diagnosed incidence of 7% for the general population.
No conclusions regarding glyphosate exposure and ADD/ADHD outcome can be drawn.
No other glyphosate specific data were reported.

Author(s)	Year	Study title
Garry, V.F., Holland, S.E., Erickson, L.L., Burroughs, B.L.	2003	Male Reproductive Hormones and Thyroid Function in Pesticide Applicators in the Red River Valley of Minnesota Journal of Toxicology and Environmental Health, Part A Volume: 66, Number: 11, Pages: 965-986 ASB2012-11627

Abstract*
In the present effort, 144 pesticide applicators and 49 urban control subjects who reported no chronic disease were studied. Applicators provided records of the season's pesticides used by product, volumes, dates, and methods of application. Blood specimens for examination of hormone levels were obtained in summer and fall. In the herbicide-only applicator group, significant increases in testosterone levels in fall compared to summer and also elevated levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the fall were noted. With respect to fungicide use, in an earlier cross-sectional epidemiologic study, data demonstrated that historic fungicide use was associated with a significant alteration of the sex ratio of children borne to applicators. As before, among current study subjects it was noted

that historic fungicide use was associated with increased numbers of girls being born. Lower mean total testosterone concentrations by quartile were also correlated with increased numbers of live-born female infants. A downward summer to fall seasonal shift in thyroid-stimulating hormone (TSH) concentrations occurred among applicators but not among controls. Farmers who had aerial application of fungicides to their land in the current season showed a significant shift in TSH values (from 1.75 to 1.11 mU/L). Subclinical hypothyroidism was noted in 5/144 applicators (TSH values >4.5 mU/L), but not in urban control subjects. Based on current and past studies, it was concluded that, in addition to pesticide exposure, individual susceptibility and perhaps economic factors may play a supporting role in the reported results.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Epidemiological study with some methodological / reporting deficiencies (e.g. selection of control subjects/samples, no details of exposure). Documentation is insufficient for assessment.
Relevance of study:	Not relevant for glyphosate (due to reliability; in addition, no direct assessment of glyphosate exposure was made).
Klimisch code:	3

Additional comments:
The publication brings little information on endpoints attributable to glyphosate.
Given the subjects were pesticide applicators, little can be drawn from the findings other than perhaps certain endpoints which may be associated with this specific occupation exposed to multiple chemical substances.
Of the 136 participants volunteering blood samples, only one individual (subject D) was noted with one abnormally high thyroid hormone levels associated with glyphosate use; thyroid stimulating hormone (FSH) was about double the normal range in the fall and thyroid stimulating hormone (TSH) higher than normal in the summer.
Another individual (subject E) had abnormally high TSH levels associated with multiple pesticide usage of 12 different active ingredients.

Author(s)	Year	Study title
Bell, E.M. Hertz-Picciotto, I. Beaumont, J.J.	2001	A Case-Control Study of Pesticides and Fetal Death Due to Congenital Anomalies Epidemiology Volume: 12, Number: 2, Pages: 148-156 ASB2012-11559

Abstract*
We examined the association between late fetal death due to congenital anomalies (73 cases, 611 controls) and maternal residential proximity to pesticide applications in ten California counties. A statewide database of all applications of restricted pesticides was linked to maternal address to determine daily exposure status. We examined five pesticide chemical classes. The odds ratios from logistic regression models, adjusted for maternal age and

county, showed a consistent pattern with respect to timing of exposure; the largest risks for fetal death due to congenital anomalies were from pesticide exposure during the 3rd– 8th weeks of pregnancy. For exposure either in the square mile of the maternal residence or in one of the adjacent 8 square miles, odds ratios ranged from 1.4 (95 % confidence interval = 0.8 – 2.4) for phosphates, carbamates, and endocrine disruptors to 2.2 (95 % confidence interval = 1.3 – 3.9) for halogenated hydrocarbons. Similar odds ratios were observed when a more restrictive definition of nonexposure (not exposed to any of the five pesticide classes during the 3rd– 8th weeks of pregnancy) was used. The odds ratios for all pesticide classes increased when exposure occurred within the same square mile of maternal residence.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Epidemoliogical study with methodological deficiencies (e.g. glyphosate was included in the pesticide class of phosphates, thiophosphates, phosphonates; no differentiation between single and multiple exposures).
Relevance of study:	Not relevant (No glyphosate-specific results.)
Klimisch code:	3

Additional comments:

Response – summary from Williams et al. (2012, ASB2012-12052)
Classes of pesticides were evaluated in this study, with glyphosate included as one of 47 active ingredients in the broad category of "phosphates/thiophosphates/phosphonates".
Of the 47 active ingredients, many were organophosphate insecticide with known mammalian modes of action. The glyphosate mode of action is on the EPSPS enzyme in plants, which is not present in the animal kingdom.
Given the very low volatility of glyphosate and the low potential for inhalation exposures to aerosol sprays up to two miles away from the subjects, systemic doses to glyphosate would be considered negligible.
Mose et al., (2008, ASB2012-11914) demonstrated a low perfusion rate of glyphosate across the placenta. Coupled with the known low dermal and gastrointestinal absorption of glyphosate and the rapid elimination of systemic doses of glyphosate in the urine, human *in utero* exposures would be extremely limited.
The reported congenital anomalies associated with fetal death in Bell et al. (2001, ASB2012-11559) can in no way be linked to glyphosate exposure.

Author(s)	Year	Study title
Aris, A. Leblanc, S.	2011	Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. Reproductive toxicology Volume: 31, Pages: 528-533 ASB2012-11547

Abstract*

Pesticides associated to genetically modified foods (PAGMF), are engineered to tolerate herbicides such as glyphosate (GLYP) and gluphosinate (GLUF) or insecticides such as the bacterial toxin bacillus thuringiensis (Bt). The aim of this study was to evaluate the correlation between maternal and fetal exposure, and to determine exposure levels of GLYP and its metabolite aminomethyl phosphoric acid (AMPA), GLUF and its metabolite 3-methylphosphinicopropionic acid (3-MPPA) and Cry1Ab protein (a Bt toxin) in Eastern Townships of Quebec, Canada. Blood of thirty pregnant women (PW) and thirty-nine nonpregnant women (NPW) were studied. Serum GLYP and GLUF were detected in NPW and not detected in PW. Serum 3-MPPA and CryAb1 toxin were detected in PW, their fetuses and NPW. This is the first study to reveal the presence of circulating PAGMF in women with and without pregnancy, paving the way for a new field in reproductive toxicology including nutrition and utero-placental toxicities.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Exact levels of PAGMF, glyphosate or AMPA in the diets were not determined. It is not clear if the measured concentrations could have been resulted from other exposure routes.
Relevance of study:	Relevant with restrictions (Provides real life actual exposure concentrations in humans. Data are limited due to the absence of any information on applied pesticides, application rates, etc.).
Klimisch code:	3

Author(s)	Year	Study title
Benítez-Leite, S. Macchi, ML and Acosta, M.	2009	Malformaciones congénitas asociadas a agrotóxicos. Arch Pediatr Urug Volume: 80, Number: 3, Pages: 237-247 ASB2012-11563

Abstract*
Introduction: exposure to pesticides is a known risk for human health. This paper describes the relationship between parental exposure and congenital malformations in the newborn. Objective: to study the association between exposure to pesticides and congenital malformations in neonates born in the Regional Hospital of Encarnación, in the Department of Itapúa, Paraguay. Materials and methods: a prospective case-controlled study carried out from March 2006 to February 2007. Cases included all newborns with congenital malformations, and controls were all healthy children of the same sex born immediately thereafter. Births outside the hospital were not counted. Exposure was considered to be any contact with agricultural chemicals, in addition to other known risk factors for congenital defects. Results: a total of 52 cases and 87 controls were analyzed. The average number of births each month was 216. The significantly associated risk factors were: living near treated fields (OR 2,46, CI95% 1,09-5,57, p<0,02), dwelling located less than 1 km (OR 2,66, CI95% 1,19-5,97, p<0,008), storage of pesticides in the home (OR 15,35, CI95% 1,96-701,63), p<0,003), direct or accidental contact with pesticides (OR 3,19, CI95% 0,97-11,4, p<0,04), and family history of malformation (OR 6,81, CI95% 1,94-30,56, p<0,001). Other known risk

factors for malformations did not show statistical significance. Conclusion: the results show an association between exposure to pesticides and congenital malformations. Further studies are required to confirm these findings.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Study design of epidemiological study for developmental toxicity insufficient for assessment, as well as methological and reporting deficiencies (no assessment to which pesticides / active substances the mothers were exposed, use frequency not specified, selection of control group after study period is questionable, no information on exposure situation of mother for this control group assessed, etc.).
Relevance of study:	Not relevant (The exposure to several pesticides was assessed in general, but no pesticide or active substance, including glyphosate, was specified or assessed).
Klimisch code:	3

B.6.7 Delayed neurotoxicity (Annex IIA 5.7.2)

Introduction into this chapter by the RMS
Two neurotoxicity studies in rats have been provided by the GTF for this re-evaluation of glyphosate. These studies are summarised in the table below and, subsequently, are described in detail. Some editorial changes to the descriptions in the GTF dossier have been made and redundant parts were deleted. Comments by the RMS may be found below the conclusions.

The delayed neurotoxicity studies in chicken that were reported in the original DAR (1998, ASB2010-10302) were re-evaluated by the RMS and found not acceptable from a today's point of view. Thus, these studies using either the active ingredient ([redacted] 1987, TOX9551839) or the formulation Glycel 41 SL ([redacted] 1988, TOX9551963) should not be used any longer for risk assessment. However, it was noted that a more recent delayed neurotoxicity study of superior quality had been performed by [redacted] (1996 ASB2013-9828). Unfortunately, this study was not part of the GTF dossier and was not submitted on request so far but was available to the RMS and could be evaluated for comprehensiveness of the database. Description of this study and its results has been amended. This study had been also reviewed by WHO/FAO in 2004 (JMPR, ASB2008-6266).

Since 2000, a number of publications have adressed glyphosate with respect to neurotoxicity endpoints. Three papers report two human cases of Parkinson`s desease. In further studies, effects on cells and animals (worms) are investigated and discussed in relation to Parkinson`s desease. These publications are presented below.

An overall evaluation of neurotoxicity of glyphosate is presented in Volume 1 (2.6.7).

Acute neurotoxicity study in rats (Horner, 1996)

Reference:	IIA, 5.7.1/01
Report:	[redacted] 1996 Glyphosate acid: Acute neurotoxicity study in rats [redacted] Data owner: Syngenta Report No. CTL/P/4866 Date: 1996-03-01 Unpublished, ASB2012-11500
Guidelines:	No guideline stated in the report but in general compliance with OECD 424 (1997).
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Materials and methods

Test material:

Identification:	Glyphosate acid
Description:	White solid

Lot/Batch #:	Y04707/034
Purity:	95.6% w/w
Stability of test compound:	The test substance was shown to be stable for the period of use.
Vehicle:	Deionised water
Test animals:	
Species:	Rats
Strain:	Alpk:AP_fSD (Wistar-derived)
Source:	[redacted]
Age:	At least 28 days
Sex:	Males and females
Weight at dosing:	♂ 171.4 – 175.0 g; ♀ 144.6 – 148.7 g
Acclimation period:	Approx. 2 weeks
Diet/Food:	CT1 diet (Special Diets Services Limited, Stepfield, Witham, Essex, UK), *ad libitum*, except 24 h prior dosing
Water:	Tap water, *ad libitum*
Housing:	In groups of five, separated by sex, in multiple rats racks.
Environmental conditions:	Temperature: 19 – 23 °C Humidity: 40 – 70 % Air changes: 25 – 30/hour 12 hours light/dark cycle

In life dates: Not reported. The study was conducted during May and June 1995.

Animal assignment and treatment
In an acute neurotoxicity study groups of ten male and ten female Alpk:APfSD (Wistar derived) rats were administered with a single oral dose of 0, 500, 1000 and 2000 mg/kg bw glyphosate acid by gavage.

Dosing Formulation Analysis
Verification of the achieved concentrations was done with samples of each preparation. Homogeneity was determined with samples from the low to high dose levels. The chemical stability of glyphosate acid in water was also determined for all dose formulations over a period of 10 days.

Clinical observations
Clinical observations were made prior to administration and daily thereafter. Any abnormalities together with the observation of no abnormality detected were recorded.

Body weight
The body weight of each rat was recorded on Days -7 and -1, immediately before dosing (Day 1), approximately 6 hours after dosing (Day 1) and on Days 8 and 15.

Food consumption
Food consumption for each cage of rats was recorded throughout the study and calculated on a weekly basis.

Functional Observational Battery
Prior to the start of treatment (Week -1) and on Day 1, 8 and 15, all animals were observed for signs of functional/behavioural toxicity. Detailed clinical assessments and functional performance tests were performed together with an assessment of sensory reactivity to different stimuli. Locomoter activity was also assessed at these time points.

Sacrifice and pathology
At scheduled termination, 5 rats/sex/group designated for neuropathology were sacrificed. The following tissues were submitted: brain, spinal cord (cervical and lumbar), Gasserian ganglion, dorsal root ganglia and spinal roots (cervical and lumbar), gastrocnemius muscle, sciatic nerve, sural nerve and tibial nerve. Neuropathological examination was performed on control and highest dose group animals only.

Statistics
Analyses of variance and covariance were carried out using the GLM procedure in SAS (1989). Least-squares means for each group were calculated using LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squared mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

Results and discussion
Dosing formulation analysis: The achieved concentrations of glyphosate acid in water were within 3 % of the nominal levels. The homogeneity was considered acceptable, with a deviation from the overall mean values of approximately ± 8 %. The chemical stability was considered satisfactory.

Mortality and clinical observations:
Two females receiving 2000 mg/kg bw glyphosate acid showed subdued behaviour, decreased activity, hunched posture, sides pinched in, tip-toe gait and hypothermia on the day of administration. One of these animals died on the subsequent day. The other one together with an additional female which showed diarrhoea on the day of administration regained full recovery the subsequent day.
One female receiving 500 mg/kg bw, was found dead approximately 6 h after administration.
In the absence of any treatment-related clinical signs prior to death, and because no deaths were observed at the intermediate dose level of 1000 mg/kg bw, the death of this animal was considered not to be treatment related.
Distension of the abdomen was recorded for several males from all treated groups on the day of administration. However, in the absence of any dose relationship, this was not considered to be treatment-related.

Body weight:
No treatment-related effects were observed.

Food consumption
During Week 1, mean food consumption was lower in animals receiving 2000 mg/kg bw glyphosate acid compared to controls, although the difference did attain statistical significance only in females (see Table B.6.7-1). There was no evidence of treatment-related effects in animals receiving 500 or 1000 mg/kg bw.

Table B.6.7-1: Intergroup comparison of food consumption (g/rat/day) during Week 1

Dose level of glyphosate (mg/kg bw)			
0 (control) Mean ± SD	500 Mean ± SD	1000 Mean ± SD	2000 Mean ± SD
Males			
29.9 ± 0.7	29.0 ± 0.1	30.1 ± 0.4	28.4 ± 0.2
Females			
22.4 ± 1.0	22.2 ± 0.2	22.8 ± 0.3	20.6* ± 0.3

* Statistically significant difference from the control group mean at the 5 % level (Student's t-test, two-sided)

Functional observation battery: Examinations of the functional observational battery did not identify any conclusive treatment- and dose-related effects

Necropsy: No macroscopic findings were detected.

Histopathology: No microscopic findings were considered to be treatment-related.

Conclusion by the Notifiers
Based on the study results the NOAEL for acute neurotoxicity, following single oral administration of glyphosate acid is 2000 mg/kg bw.

RMS comments:
The study is considered acceptable. Its evaluation as provided in the dossier is agreed with. There was no evidence of specific neurotoxicity up to the highest single dose of 2000 mg/kg bw. Clinical signs indicative of acute toxicity occurred in few animals at this dose and included one death. Thus, the NOAEL for systemic toxicity was 1000 mg/kg.

Subchronic neurotoxicity study in rats ([redacted] 1996)

Reference: IIA, 5.7.4/01
Report: [redacted] 1996, Glyphosate acid: Subchronic neurotoxicity study in rats
[redacted]
Data owner: Syngenta
Report No.: CTL/P/4867
Date: 1996-03-11
unpublished,
ASB2012-11501
Guidelines: Study was pre-guideline, but satisfies in general the requirements of OECD 424 (1997)
Deviations: None

GLP: yes
Acceptability: *See RMS comment*

Dates of experimental work: 1995-04-25 to August 1995

Materials and methods

Test material:	
Identification:	Glyphosate acid (technical)
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 %
Stability of test compound:	Confirmed for the study period
Vehicle:	Plain diet
Test animals:	
Species:	Rats
Strain:	Alpk:APfSD
Source:	[redacted]
Age:	At least 6 weeks
Sex:	male and female
Weight at dosing:	♂ 215.0 – 218.6 g (mean); ♀ 173.5 – 178.8 g (mean)
Acclimation period:	Approximately 2 weeks
Diet/Food:	CT1 diet (Special Diet Services Limited, Witham, Essex, UK), *ad libitum* (except up to 24 hours prior to dosing)
Water:	Tap water, *ad libitum*
Housing:	Four per cage per sex in stainless steel cages (26.5 x 50.0 x 20.7cm)
Environmental conditions:	Temperature: 19-23 °C Humidity: 40-70 % Air changes: 25-30/hour Photoperiod: 12 hours light/dark cycle

In life dates: 1995-05-09 to August 1995

Animal assignment and treatment:
In a subchronic neurotoxicity study, groups of 12 male and 12 female Alpk:APfSD (Wistar-derived) rats were fed diets containing 0, 2000, 8000 or 20000 ppm glyphosate acid for 13 weeks. (equivalent to mean achieved dose levels of 0, 155.5, 617.1 and 1546.5 mg/kg bw/day for males, and 0, 166.3, 672.1 and 1630.6 mg/kg bw/day for females) glyphosate technical.
All diets were based on CT1 diet supplied by Special Diets Services Limited, Stepfield, Witham, Essex, UK. The experimental diets were prepared in 30 kg batches by direct addition of the test substance to 30 kg of CT1 diet and mixing thoroughly. The diets were stored at room temperature until required for use.

Samples from all dietary levels (including controls) were taken at intervals throughout the study and analysed quantitatively for glyphosate acid. The homogeneity of glyphosate acid in CT1 diet was determined by analysing samples from the low and high dose levels. The chemical stability of glyphosate acid in diet, under the conditions of storage used on this study, was determined for 2000 ppm and 20000 ppm diets prepared for use on a concurrent 1 year feeding study in the rat in the same laboratory.

Clinical observations
A check for clinical signs of toxicity, ill health and behavioural changes was made once daily on all animals. All observations were recorded. A detailed physical examination was performed on each rat prior to start of treatment, and at weekly intervals thereafter.

Functional observational battery (FOB)
Prior to the start of treatment and during Weeks -1, 5, 9 and 14, all animals were observed for signs of functional/behavioural toxicity. The assessment involved observations in the home cage and/or while the rat was moving freely in a standard arena followed by manipulative/in hand tests Functional performance tests were also performed together with an assessment of sensory reactivity to different stimuli. The examinations included quantitative assessments of landing foot splay, sensory perception (tail-flick test) and muscle weakness (fore- and hind limb grip strength). The clinical observations included, but were not limited to, the following list of measures: assessment of autonomic function (e.g. lachrymation, salivation, piloerection, exophthalmus, urination, defecation, pupillary function, ptosis); description, incidence and severity of any convulsions, tremors, abnormal motor function, abnormal behaviour; reactivity to stimuli; changes in level of arousal; sensorimotor responses; alterations in respiration.

Locomotor activity
Locomotor activity was monitored by an automated activity recording apparatus. All animals were tested at weeks -1, 5, 9 and 14. Each observation period was divided into ten scans of five minute duration. Treatment groups were counter balanced across test times and across devices and when the trials were repeated each animal was returned to the same activity monitor at approximately the same time of day. Motor activity was assessed in a separate room to minimise disturbances.

Body weight
Individual body weights were recorded in week -1, immediately prior to treatment), at weekly intervals thereafter, and at necropsy.

Food consumption and compound intake
Food consumption was recorded as required for each cage group throughout the study and calculated on a weekly basis. Food utilisation and compound intake were calculated.

Water consumption
Not reported.

Ophthalmoscopic examination
Not performed. However, ophthalmological data are available from other repeated dose studies.

Sacrifice and pathology
At the scheduled termination, all main study animals not required for neuropathology, were killed by overexposure to rising concentrations of carbon dioxide gas and were discarded without examination.

At termination, the six rats/sex/group designated for neuropathology were deeply anaesthetised with intraperitoneal sodium pentobarbitone and killed by whole body perfusion fixation with modified Karnovsky's solution. The following tissues were submitted: brain, spinal cord (cervical and lumbar), Gasserian ganglion, dorsal root ganglia and spinal roots (cervical and lumbar), gastrocnemius muscle, sciatic nerve, sural nerve and tibial nerve.

Brain weight, brain length and brain width were determined.

Submitted tissues were processed as follows: brain (seven levels including the cerebral cortex, the hippocampus, the cerebellum, the pons and medulla), dorsal root ganglia and spinal roots from cervical and lumbar regions of the cord after decalcification, and gastrocnemius muscle from rats receiving either control diet or diet containing 20000 ppm glyphosate acid were routinely processed, paraffin wax embedded and 5µm thick sections were cut and then stained with haematoxylin and eosin. Sections of brain and cord were in the transverse plane.

The Gasserian ganglion, sciatic nerve, spinal cord (cervical and lumbar portions), sural and tibial nerve from control and high dose group rats were processed and then embedded in Araldite. Semi-thin sections were cut and then stained with toluidine blue. For bilateral tissues only the left was processed. All tissues were sectioned in the transverse plane except the sciatic nerve which was sectioned in both the transverse and the longitudinal plane.

Neuropathological examination was performed on control and highest dose group animals only. All sections were examined by light microscopy.

Statistics
All data were evaluated using analysis of variance and/or nalysis of covariance for each specified parameter using the GLM procedure in SAS (1989).
The levels of probability chosen as significant different from control were $p < 0.01$** and $p < 0.05$* (Student's t-test, two-sided).

Results and discussion
Dosing formulation analysis: The achieved mean concentrations of Glyphosate acid in diet were within 4 % of the nominal levels, with individual values being within 15 % of nominal. There were considered acceptable. The homogeneity of the low- and high-dose diets was considered acceptable, with a deviation from the overall mean values of ± 4 %. The chemical stability was considered satisfactory.

Mortality: No deaths occurred during the study.

Clinical observations: There were no treatment-related clinical signs of toxicity.

Functional observational battery (FOB)
There were no clinical signs that could be attributed to administration of glyphosate acid.

There was an apparent increase in the incidence of miosis and decreased pupil response to light in males receiving 20000 ppm. However, as these signs were seen for several of these males pre-experimentally and were also present at a similar incidence in females with no obvious relationship to treatment, this was considered to be incidental and unrelated to administration of glyphosate acid.

Landing Foot Splay Measurements
There was no evidence of any treatment-related effect on landing foot splay.

Time to Tail-Flick
There was no evidence of any treatment-related effect on time to tail-flick.

Grip Strength Measurements
There was no evidence of any treatment-related effect on forelimb or hind limb grip strength.

Motor activity
There was no evidence of any treatment-related effect on locomotor activity.

During week 5, slightly reduced locomotor activity was recorded on occasions for females receiving 20000 ppm. However, in the absence of any treatment-related effects on motor activity for these animals at other time points during the study, this is considered to be incidental and unrelated to administration of glyphosate acid.

Table B.6.7-2: Selected motor activity findings

		Dietary concentration (ppm)							
		Males				Females			
Week	**Assessment period (min)**	**0**	**2000**	**8000**	**20000**	**0**	**2000**	**8000**	**20000**
5	1-50	388.7	472.1	335.6	384.4	441.2	379.3	457.8	359.3
9	1-50	304.7	413.4*	298.4	327.3	512.3	488.9	555.1	557.0
14	1-50	299.4	395.1	292.2	372.8	553.0	512.7	569.3	514.7

* Statistically significant difference from control group mean at the 5% level (Student's t-test, 2-sided)
** Statistically significant difference from control group mean at the 1% level (Student's t-test, 2-sided)

Body weight: Group mean bodyweight for males receiving 20000 ppm was statistically significantly lower than that of controls throughout the study. At week 14, group mean bodyweight for these animals was 92.8 % that of controls, equating to a reduction in bodyweight gain of approximately 12 %.

Group mean body weight for males receiving 8000 ppm was also marginally lower than that of controls from weeks 6 to 14. However, these differences did not attain statistical significance and were considered too small to be of biological importance.

For males receiving 2000 ppm, and for females at all dose levels, mean body weight was essentially similar to that of concurrent controls throughout the study.

Table B.6.7-3: Intergroup comparison of body weights (g)

	Dietary concentration (ppm)							
	Males				Females			
Week	**0**	**2000**	**8000**	**20000**	**0**	**2000**	**8000**	**20000**
1	216.0	217.0	218.6	215.0	173.5	178.8	175.6	175.3
2	263.5	264.7	264.9	254.6**	192.7	200.6	196.1	194.3
4	338.2	340.7	339.6	323.7*	214.3	228.3**	224.9**	219.2
8	440.7	440.1	429.1	405.8**	253.6	262.1	260.4	255.4
14	534.7	532.8	526.5	496.1**	285.1	291.5	287.9	281.0

* Statistically significant difference from control group mean at the 5 % level (Student's t-test, 2-sided)
** Statistically significant difference from control group mean at the 1 % level (Student's t-test, 2-sided)

Food consumption and compound intake: There were no effects on food consumption. The efficiency of food utilisation for males receiving 20000 ppm was statistically significantly lower than that of concurrent controls during weeks 1 to 8. There were no changes in the efficiency of food utilisation for males receiving 2000 or 8000 ppm or for females from all treated groups.

Table B.6.7-4: Intergroup comparison of food utilisation (g growth/100 g food)

	Dietary concentration (ppm)							
	Males				Females			
Week	**0**	**2000**	**8000**	**20000**	**0**	**2000**	**8000**	**20000**
1-4	18.13	17.16	16.94	16.28*	9.42	9.73	9.36	9.61
5-8	11.52	10.69	10.35	9.93*	5.99	5.55	5.39	5.70
1-13	12.00	11.45	11.38	10.87**	6.08	6.03	6.06	5.96

* Statistically significant difference from control group mean at the 5 % level (Student's t-test, 2-sided)
** Statistically significant difference from control group mean at the 1 % level (Student's t-test, 2-sided)

The mean doses received for males and females respectively were 155.5, 617.1, 1546.5 and 166.3, 672.1, 1630.6 mg glyphosate acid/kg/day at dose levels of 2000, 8000 and 20000 ppm, respectively

Brain measurements: There was no evidence of any effects on brain weight, length or width.

Necropsy: There were no macroscopic findings that were considered to be attributable to treatment.

Histopathology: There were no microscopic findings in the peripheral or central nervous system that were considered to be attributable to treatment.

Conclusion by the Notifiers
Dietary administration of glyphosate acid to rats for a period of ninety consecutive days at dietary concentrations of up to 20000 ppm produced evidence of toxicity in the form of reduced growth and reductions in food utilisation for males. Comprehensive histopathological evaluation of the nervous system showed no evidence of any changes in the peripheral or central nervous system which could be attributed to administration of glyphosate acid.
The no observed effect level (NOEL) for neurotoxic potential, following dietary administration of glyphosate acid for at least 90 days, was 20000 ppm, corresponding to 1546.5 / 1630.6 mg/kg bw/day for males and females, respectively.

RMS comments:

The study is considered acceptable. The conclusion is agreed with. No evidence of a neurotoxic potential was obtained up to the the highest dose of 20000 ppm. The NOAEL for systemic toxicity was 8000 ppm (617 mg/kg bw/day) in males, based on lower body weight (gain) and impaired food utilisation, and 20000 ppm (1631 mg/kg bw/day) in females, i.e., no effects were noted at the top dose level.

Delayed neurotoxicity study in chicken ([redacted] 1996)

Reference:	Not applicable since study was not part of the dossier
Report:	[redacted] 1996, Glyphosate Acid: Acute delayed neurotoxicity study in the domestic hen. [redacted] Data owner: Syngenta (study was not part of the dossier but submitted to RMS for other purposes before) Report No.: CTL/C/3122 Date: 1996-08-23 unpublished, ASB2013-9828
Guidelines:	U.S. EPA Subdivision F 81-7
Deviations:	None
GLP:	Yes
Acceptability:	The study is considered acceptable by RMS.

Materials and methods

Test material:	
Identification:	Glyphosate acid (technical)
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 %
Stability of test compound:	Confirmed for the study period
Vehicle:	Distilled water
Positive control substance:	
Identification	Tri-ortho-cresylphosphate (TOCP)
Lot/Batch:	143-41C
Purity:	99.0 %
Expiry date:	November 1997
Supplier:	Chem Services Inc.
Vehicle:	Corn oil

Test animals:	
Species:	Chicken
Strain:	Lohmann Brown (a hybrid brown laying strain)
Source:	[redacted]

Age:	Approx. 12 months
Sex:	Female
Weight at dosing:	1927 – 2215 g (range)
Acclimation period:	Approximately 2 weeks
Diet/Food:	HRC layer ration in pellet form (Parker Bros. Ltd., Lark Mills, Suffolk, UK), *ad libitum* (except overnight starvation prior to dosing)
Water:	Tap water, *ad libitum*
Housing:	Floor pens (galvanised steel, concrete floor) measuring 1.8 x 1.4 m with up to (not further specified) hen from the same group
Environmental conditions:	Temperature: 15-17 °C Humidity: 79 % (mean) Air changes: not given in the report but ventilation considered "adequate" by study author Photoperiod: 12 hours light/dark cycle

In life dates: 1996-01-09 to 1996-02-14

Animal assignment and treatment:
20 hen were administered glyphosate acid as a single dose of 2000 mg/kg bw by oral gavage. 12 birds were employed as positive controls and received a single dose of 1000 mg TOCP/kg bw. The negative control group consisted also of 12 hen and received once distilled water also by gavage. The same volume of 10 mL/kg bw was applied to all chicken. Treatment was followed by an observation period of 21 or 22 days.

Clinical observations
A check for mortality, clinical signs of toxicity, ill health and behavioural changes was made twice daily on all animals.

Ataxia assessment
Following treatment, hen were examined daily for signs of (delayed) ataxia.

Body weight
Individual body weights were recorded weekly.

Food consumption and compound intake
No information given.

Water consumption
Not reported.

Clinical pathology
3 pre-determined chicken from each group were sacrificed 48 hours after dosing to determine brain cholinesterase, brain neuropathy target esterase and lumbar spinal cord neuropathy target esterase (NTE) activities

Sacrifice and pathology

At the scheduled termination, 6 hen from each group were selected for necropsy and histopathological examinations. Whereas in the negative control and glyphosate-treated groups the first six birds in numerical order (because of the absence of clinical signs) were employed, care was taken in the TOCP-treated group to include all animals that had shown clinical ataxia. The remaining hen from all three groups were killed and discarded.

At termination, after perfusion through the heart with fixative, head and spinal column (with brain and spinal cord exposed but left in place) and dissected sciatic nerves (including tibial branches) from the six hen/group designated for neuropathology were taken and stored. The following tissues were used to take samples for histological examination: brain (forebrain, mid and hindbrain), spinal cord (upper and lower cervical, mid-thoracic and lumbo-sacral parts), sciatic nerve (proximal and distal, above knee), tibial nerve. One transverse and two longitudinal sections were performed at each level.

Statistics
Apparently, no statistical analysis was necessary since the results were quite clear and number of animals limited.

Results and discussion

Mortality: There were two unscheduled deaths during the study.

- In the test group receiving glyphosate acid, one bird was found dead on day 10 after dosing. This hen had not exhibited any signs of toxicity prior to death. The cause of death was apparently not elucidated but if the singularity of this case (1/20) and the absence of clinical signs is taken into consideration, the opinion of the study author can be agreed with that mortality was unrelated to treatment.
- In the positive (TOCP) control group, one bird had been severely pecked by other hen and was sacrificed on humane grounds, apparently during the acclimation period yet.

Clinical observations: In the glyphosate-treated and negative control groups, there were no was a common finding (see below). In addition, one more bird in this group was hurt by other hen by pecking and had to be treated by applying Stockholm tar to the wounds.

Ataxia assessment
Ataxia was confined to the positive control group receiving 1000 mg TOCP/kg bw. 5 of 11 hen were affected. Signs occurred for the first time between post-observation days 11 and 21 and the severity of ataxia was variable.

Body weight
Group mean body weight increased in the glyphosate-treated and negative control groups but weight loss was observed in the positive controls receiving TOCP.
Clinical chemistry
In line with ataxia observations, NTE levels in brain and spinal cord were clearly reduced in the positive control group (by 84% for brain and by 78% for spinal cord as compared to negative control group) but no effect was seen in the group receiving glyphosate. A very low reduction of brain cholinesterase (6% less than in negative control) was seen in the hen that had received glyphosate. In the positive control group, the decrease in brain cholinesterase activity accounted for 19%. In the study report, the lack of statistical significance for the latter findings is emphasised but it is doubted if meaningful statistical analysis was possible with

only three birds per group under investigation. Taking into account the very low difference to negative control birds and the fact that glyphosate is not known to inhibit cholinesterases, a treatment-related effects is not likely.

Necropsy: There were no macroscopic findings that were attributable to treatment.

Histopathology: The evaluation of histological findings is complicated by the fact that axonal degeneration in the spinal cord and peripheral nerves were observed in all three groups in nearly all birds suggesting high background incidence. In the TOCP-treated group, the cerebellum was also affected in five out of 6 animals (as compared to only one bird in the glyphosate group). Furthermore, axonal degeneration in general was more severe in the positive control group.

Conclusion (RMS)
Based mainly on the occurrence of ataxia in the positive control group but not in chicken receiving glyphosate and the related reduction of NTE activity after dosing with TOCP but not with glyphosate, there is no potential of glyphosate to cause delayed neuropathy.

B.6.7.1 Published data on neurotoxicity

Several publications over the last decade have evaluated glyphosate with respect to neurotoxicity endpoints. Three papers report a total of two human cases of Parkinson's disease. The first case followed acute exposure to a glyphosate formulation while spraying a garden (Barbosa et al., 2001, ASB2012-11557; da Costa et al, 2003, ASB2012-11598). The second case reported chronic exposures to a factory worker in China, where a variety of pesticides including glyphosate were produced (Wang et al, 2011, ASB2012-12047). Several questions arise in attempting to link glyphosate exposures with each case of Parkinson's disease. Firstly, significant systemic exposures to glyphosate in each instance are questionable, given the poor dermal absorption and low volatility of the compound. Secondly, if glyphosate was a causative agent of this fairly common disease, a significant number of cases associated with either acute and/or chronic exposures would be evident. Glyphosate formulations are sometimes readily accessible for suicide attempts, which are usually unsuccessful, as less than 10% of glyphosate self administered ingestions result in death. No reports of Parkinson's disease in survivors following very acute ingestions of glyphosate products have been documented. Glyphosate has been manufactured and widely used in agriculture and consumer markets for approximately 40 years, so a single case of a pesticide factory worker developing Parkinson's disease, while unfortunate, does not constitute cause and effect; there is no evidence of a higher frequency of Parkinson's disease in glyphosate production workers.

Multiple long term animal studies with glyphosate have failed to demonstrate any evidence of neurotoxicity, and certainly have not shown evidence of Parkinson's-like abnormalities. While some studies have suggested statistical associations with general pesticide exposure or general insecticide or herbicide exposure (Engel et al., 2001, ASB2012-11612), there is no evidence suggesting a specific association between glyphosate and Parkinson's disease. In the largest study to date of US Farmers (Agricultural Health Study), no increased risk of Parkinson's disease was found in association with reported glyphosate use (Kamel et al., 2007, ASB2012-11862). Human non-cancer epidemiologic outcomes related to glyphosate have recently been reviewed (Mink et al. 2011, ASB2012-11904), and there is no convincing

evidence for an increased incidence of Parkinson's disease or other neurological disorders in individuals reporting glyphosate exposure.

Several publications open with the premise that pesticide exposures are linked with Parkinson's disease, and then proceed to report *a priori* research linking glyphosate with a measurable endpoint. This endpoint is then extrapolated to link with Parkinson's disease in humans. Despite the lack of compelling human associations between glyphosate exposure and Parkinson's disease, such research continues to be published. Astiz et al., (2009, ASB2012-11549), Negga et al. (2011, ASB2012-11923) and Gui et al. (2012, ASB2012-11835) all conducted glyphosate research in the above mentioned manner, all in very different test systems. Negga et al. (2011, ASB2012-11923) notes neurodegeneration in *Caenorhabditis elegans* worms following exposure to glyphosate (trimesium form, which has a different toxicology profile than glyphosate) uses concentrations equal to the LD25, LD50 and LD75, or actual concentrations of glyphosate of 3 to 10 percent, i.e.- the high concentration is approximately 10-fold higher than concentrations applied directly in the field. The relevance of such high-dose exposures to the trimesium salt in this experimental model to human Parkinson's disease is highly questionable and irrelevant to the Annex 1 renewal of glyphosate technical acid. Astiz et al. (2009, ASB2012-11549) and Gui et al. (2012, ASB2012-11835) both affirm their test models (in rats and in PC-12 cells respectively) for evaluating neurodegenerative disorders, then directly link their research results to Parkinson's disease in humans; these two studies are addressed below.

Cole et al. (2004, ASB2012-11594) evaluated 15 different pesticides for neurotoxic end points in *C. elegans* with analytical grade active ingredients, noting reduced cholinesterase for pesticides with this mode of action, but not glyphosate. Interestingly, the authors report a low pH effect resulting in reduced cholinesterase activity in the high dose of glyphosate and a plant growth promoter. Glyphosate formulations contain salt forms of glyphosate, not the technical acid and thus do not have a low pH. Additionally, human incidents of self induced glyphosate poisonings do not report the common symptoms of acute acetylcholinesterase inhibition; salivation, lacrimation, urination and defecation (SLUD).

After preparation of the original DAR in 2013, the following publications became available:

Cattani et al. (2014, ASB2014-3919) studied neurotoxic effects of the formulation Roundup in the hippocampus on immature rats following acute (30 min) and chronic (pregnancy and lactation) exposure. Results showed that acute exposure to Roundup increased CA^{2+} influx leading to oxidative stress and neural cell death. Taken together, the results demonstrate that Roundup might lead to excessive extracellular glutamate levels and to glutamate excitotoxicity and oxidative stress in rat hippocampus.

Chorfa et al. (2013, ASB2014-9328) studied the effects of four pesticides (paraquat, rotenone, maneb and glyphosate) on different molecular events in cell lines which are considered to be related to Parkinson`s disease. Three of the four pesticides triggered molecular events involved in Parkinson`s disease. However, glyphosate was not active in this study.

Seneff et al. (2013, ASB2014-9729) reviewed literature on autism. In conclusion they submit a theory on the biochemical mechanisms which could lead to autism. According to this theory, several environmental factors including pesticide exposure (glyphosate and other pesticides are mentioned, together with, *e.g.*, aluminium, mercury, intake of 'processed food', vaccinations) would promote the 'encephalopathy of autism'. The contribution of glyphosate

is ascribed to a disturbance of gut microflora rather than to a direct effect on the neuronal system. Thus, there assumptions are based on certain *in vitro* results (*e.g.*, Shehata et al., 2013, ASB2012-16301) that are discussed in depth in section B.6.8.3.3. The authors propose dietary and lifestyle changes to prevent autism.

Narayan et al. (2013, ASB2014-9620) reviewed literature on Parkinson`s disease. The authors conclude that houshold use of organophosphorus pesticides is associated with increased risk of developing Parkinson`s disease. Glyphosate is considered by these authors to be an organophosphorus pesticide.

McConnell et al. (2012, ASB2014-9615) tested multi-well microelectrode arrays for neurotoxicity screening. In result of these tests glyphosate was considered negative concerning neurotoxic effects.

LeFew et al. (2013, ASB2014-9608) evaluated microelectrode array data using Bayesian modeling as an approach to screening and prioritization for neurotoxicity testing. Glyphosate was identified to be negative in these neurotoxicity tests.

Kim et al. (2013, ASB2014-9592) studied the relation between depressive symptoms and severity of acute occupational pesticide poisoning among male farmers in South Korea. Among the pesticides causing the poisonings, paraquat dichloride was found to be a significant predictor of depressive symptoms. Glyphosate did not cause significant effects.

Kamel et al. (2012, ASB2014-9586) summarized the literature on the association of amyotrophic lateral sclerosis (ALS) with pesticides. The meta-analysis suggest that ALS risk is associated with use of pesticides. ALS was associated with aldrin, dieldrin, DDT and toxaphene. However, no relevant association was evidenced for glyphosate.

Freire and Koifmann (2012, ASB2014-9479) conducted a review of the epidemologic literature over the past decade. A significantly increased Parkinson`s disease risk was observed in 13 out of 23 case-control studies. An increased risk has been associated with different pesticides. However, no relevant increase of the risk was evidenced for glyphosate.

Faria et al. (2014, ASB2014-9477) analysed the association between occupational exposures to pesticides, nicotine and minor psychiatric disorders (MPD) among tobacco farmers in southern Brazil. The study reinforces the evidence of the association between pesticide poisoning and mental health disorders. In this study organophosphates were the only chemical group positively assciated with MPD. Glyphosate was not associated with MPD.

The study by Harrill et al. (2011, ASB2014-9558) compared the performance of two culture models, a rat primary cortical culture and a human embryonic stem cell-derived neural culture to be used as tools for screening potential developmental neurotoxicants. The authors concluded that based upon the small training set evaluated in their study, neither of the culture models performed better than the other across the determined criteria: the data demonstrated that the culture models performed differently in terms of reproducibility, dynamic range and sensitivity to neurite outgrowth inhibitors. In this study glyphosate was used as one out of six ‘negative’ chemicals and none of them inhibited neurite outgrowth in either model.

The study by Culbret et al. (2012, ASB2014-9355) compared the sensitivity of human (ReN CX) and mouse (mCNS) neuroprogenitor cell lines to chemicals using a multiplex assay for proliferation and apoptosis, endpoints critical for neural development. According to the

authors, eleven chemicals reported to affect proliferation and/or apoptosis, and 5 chemicals, amongst others glyphosate, with no reports of effects on either endpoint were examined in concentrations of 0.001 up to 100 µM. High-content screening of markers for proliferation (BrdU incorporation) and apoptosis (activated caspase 3 and p53) was used to assess the effect of chemicals in both cell lines. Under the conditions described, the hypothesis that human cells are significantly more sensitive than rodent cells to chemical insult on proliferation and apoptosis was not supported by these results. Interestingly, the 'negative' chemical, glyphosate (technical material, purity > 99 %), reached the threshold for p53 activation in mCNS at 30 µM, but not in the human cell line.

The review by Grandjean and Landrigan (2014, ASB2014-9494) on neurobehavioural effects of developmental toxicity emphasise that the total number of neurotoxic substances now recognised almost certainly represents an underestimate of the true number of developmental neurotoxicants that have been released into the global environment. In this context, the authors considered glyphosate as a human toxicant based on a case report of a 71-year old male who attempted suicide with a glyphosate formulation published by Malhotra et al. (2010, ASB2012-11890, please refer to B.6.9.2. Reports on clinical cases and poisoning incidents). This interpretation was disagreed by Goldstein and Saltmiras (2014, ASB2014-9493) and Malhotra et al. were cited, that this case raises "a suspicion of direct cerebral toxicity", but no conclusion was drawn on glyphosate to be a recognised neurotoxicant, but inquiry into other components of the ingested product was considered to be indicated.

Further studies are reported more detailed:

Author(s)	Year	Study title
Barbosa, E.R. Leiros da Costa M.D. Bacheschi, L.A. Scaff M.	2001	Parkinsonism After Glycine-Derivate Exposure Movement Disorders Volume: 16, Number: 3, Pages: 565-568 ASB2012-11557

Abstract*

This 54-year-old man accidentally sprayed himself with the chemical agent glyphosate, a herbicide derived from the amino acid glycine. He developed disseminated skin lesions 6 hours after the accident. One month later, he developed a symmetrical parkinsonian syndrome. Two years after the initial exposure to glyphosate, magnetic resonance imaging revealed hyperintense signal in the globus pallidus and substantia nigra, bilaterally, on T2-weighted images. Levodopa/benserazide 500/125 mg daily provided satisfactory clinical outcome.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not assignable
Comment:	Medical case report, single incident
Relevance of study:	Relevant with restrictions (Data are limited due to the absence of any information on purity and application concentrations of glyphosate formulation, as well as co-formulations.)
Klimisch code:	4

Author(s)	Year	Study title
Wang, G., Xiao-Ning, F., Yu-Yan, T., Qi, Ch., Shen-Di, CH.	2011	Parkinsonism after chronic occupational exposure to glyphosate. Parkinsonism and related disorders ASB2012-12047

Abstract*

Here we report a patient with parkinsonism following chronic occupational exposure to glyphosate. A previously healthy 44-year-old woman presented with rigidity, slowness and resting tremor in all four limbs with no impairment of short-term memory, after sustaining long term chemical exposure to glyphosate for 3 years as a worker in a chemical factory. The chemical plant produced a range of herbicides including: glyphosate, gibberilins, and dimethyl hydrogen phosphite; however, the patient worked exclusively in the glyphosate production division. She only wore basic protection such as gloves or a face mask for 50 h each week in the plant where glyphosate vapor was generated. She frequently felt weak. …
Physical examination revealed a parkinsonian syndrome. …
We cannot exclude the coincidence of idiopathic PD with exposure to glyphosate on our patient. …
* Quoted from article

Klimisch evaluation

Reliability of study:	Not assignable
Comment:	Medical case report, single incident
Relevance of study:	Relevant with restrictions
Klimisch code:	4

Author(s)	Year	Study title
Astiz, M. de Alaniz, M.J. Marra, C.A.	2009	Effect of pesticides on cell survival in liver and brain rat tissues Ecotoxicology and Environmental Safety Volume: 72, Pages: 2025-2032, ASB2012-11549

Abstract*
Pesticides are the main environmental factor associated with the etiology of human neurodegenerative disorders such as Parkinson's disease. Our laboratory has previously demonstrated that the treatment of rats with low doses of dimethoate, zineb or glyphosate alone or in combination induces oxidative stress (OS) in liver and brain. The aim of the present work was to investigate if the pesticide-induced OS was able to affect brain and liver cell survival. The treatment of Wistar rats with the pesticides (i.p. 1/250 LD50, three times a week for 5 weeks) caused loss of mitochondrial transmembrane potential and cardiolipin content, especially in substantia nigra (SN), with a concomitant increase of fatty acid peroxidation. The activation of calpain apoptotic cascade (instead of the caspase-dependent pathway) would be responsible for the DNA fragmentation pattern observed.
Thus, these results may contribute to understand the effect(s) of chronic and simultaneous exposure to pesticides on cell survival.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Unsuitable test system (i.p exposure route is not relevant for human exposure). No information on purities of test substances used. Small group size (4 males/dose group), reporting deficiencies
Relevance of study:	Not relevant (intraperitoneal injection is a non-relevant route of exposure for humans)
Klimisch code:	3

Additional comments
This non-guideline study utilized very small group numbers (4 rats/group) and therefore is not sufficiently robust to appropriately identify changes attributable to the test material administration.
The test materials are not well described, without indication of whether a glyphosate salt form or acid was used and purity was not reported.
The publication focuses on the post necropsy data analysis and reporting. Data on animal husbandry, clinical observations, feed and water intake, weekly body weight were not reported, but the authors note there were no adverse observations.
No statistically significant effects were noted for liver endpoints, yet the liver is in close proximity to test material administration via intraperitoneal injection.
Statistically significant effects were noted for brain tissue endpoints in the substantia nigra and cerebral cortex. However, there is a lack of biological plausibility for brain exposures to glyphosate, given the necessity to pass the blood-brain barrier and the known rapid elimination kinetics of this polar molecule via urine.

Author(s)	Year	Study title
Gui, Y.X., Fan, X.N., Wang, H.M., Wang, G., Chen, S.D.	2012	Glyphosate induced cell death through apoptotic and autophagic mechanisms. Neurotoxicology and teratology Volume: not specified (*accepted manuscript*) Pages: not specified ASB2012-11835

Abstract*
Herbicides have been recognized as the main environmental factor associated with human neurodegenerative disorders such as Parkinson's disease (PD). Previous studies indicated that the exposure to glyphosate, a widely used herbicide, is possibly linked to Parkinsonism, however the underlying mechanism remains unclear. We investigated the neurotoxic effects of glyphosate in differentiated PC12 cells and discovered that it inhibited viability of differentiated PC12 cells in dose-and time-dependent manners. Furthermore, the results showed that glyphosate induced cell death via autophagy pathways in addition to activating apoptotic pathways. Interestingly, deactivation of Beclin-1 gene attenuated both apoptosis and autophagy in glyphosate treated differentiated PC12 cells, suggesting that Beclin-1 gene is involved in the crosstalk between the two mechanisms.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Documentation insufficient for assessment (not clearly stated dose levels and duration of exposure, as well as treatment conditions for all tests. In addition, tested doses were much higher than real in vivo concentrations).
Relevance of study:	Not relevant (Due to reliability)
Klimisch code:	3

Additional comments:
In this paper, the authors apply glyphosate to adrenal cancer cells in culture at concentrations sufficient to cause cell death. Two major interacting pathways leading to cell death (autolysis and apoptosis) are evaluated, and the results are hardly surprising - the cells do indeed die via known mechanisms leading to cell death. The authors use these observations, and the fact that Parkinson's disease involves the death of certain nerve cells in the brain, to try and create a link between glyphosate and Parkinson's disease. There are, however, many problems with this extrapolation.
The cells used are not the neurons involved in Parkinson's, but rather a cell line derived from an adrenal gland cancer (pheochromocytoma), and the doses used are very high- the high dose killed nearly 50 % of cells in 72 hours, and the low dose was ¼ this level. The high dose equates to approximately 1/10 the concentration applied directly in the field, and is far higher than any internal glyphosate concentration that could ever occur following glyphosate use. A sufficiently high dose of every substance will kill cells - but this does not mean that every substance causes Parkinson's disease.
Unprotected cells in culture are highly susceptible to changes in pH and other non-specific effects, and it is not clear that the researchers assessed or accounted for these possible effects. This being said, the concentrations of glyphosate used (40 mM) are known to kill other cell

types in culture (Heu et al., 2012, ASB2012-11844) via induction of apoptosis. Thus, no particular specificity or neuronally-specific susceptibility exists for the cell line tested. While 40 mM glyphosate is toxic to cells in culture, the LD_{50} in rodents is over 5000 mg/kg and *C. elegans* will have a 25 % survival following exposure to a 10 % solution of glyphosate. *In-vitro* results do not appear to reflect *in vivo* events.

Anadon et al. (2009, ASB2012-11542) dosed rates with 400 mg/kg of glyphosate, a massive dose relative to any environmental exposure, and achieved glyphosate peak modeled plasma concentrations of approximately 5 ug/mL (5 ppm). Assuming linear kinetics, the current maximum allowable EU daily intake (0.3 mg/kg/day) would give an approximated blood concentration of 0.17 ppm (170 ppb). This is conservative, as McQueen et al (2012, ASB2012-11898) recently evaluated glyphosate exposure to pregnant women and concluded that estimated exposures based on actual measurements in food were only 0.4 % of the current European acceptable daily intake.

The lowest glyphosate concentration used in this experiment is 5mM (830 ppm), or 5000 times higher than the estimated blood concentration following the current EU maximum allowable daily exposure. It is also 166 times higher than the concentrations Anadon et al. (2009, ASB2012-11542) achieved using doses of 400 mg/kg glyphosate. In short, the concentrations used in this work are massively higher than any concentration is blood (let alone brain tissue) that can be achieved following normal human exposures.

B.6.8 Further toxicological studies

Introduction into this chapter by the RMS

The metabolite aminomethyl phosphonic acid (AMPA) was extensively investigated for acute and subchronic effects, mutagenicity and developmental toxicity. Most of these studies had been submitted for the previous EU evaluations of either glyphosate or glyphosate-trimesium yet and were re-evaluated now by the RMS for reliability and acceptability. Previously known toxicity and genotoxicity studies of insufficient quality (from a today's perspective) have been deleted ([redacted] 1973, TOX9552394; [redacted] 1978, TOX9552399; [redacted] 1991, TOX9552415; [redacted] 1980, TOX9552408; [redacted] 1991, TOX9552404). The same holds true for a brief information concerning kinetics and (absent) metabolism of AMPA ([redacted], 1973, TOX9552354). Main exclusion criteria were lacking information on purity of AMPA, severe reporting deficiencies, a too low number of animals employed or the absence of relevant examinations such as histopathology that are usually required. Range-findings studies have not been considered so far a subsequent definitive main study is available.

Meanwhile, a few more studies on acute toxicity in rats and mice, on skin sensitisation and genotoxicity have been submitted that were not subject to former EU evaluation and, therefore, are reported in detail below.

All valid studies with AMPA are compiled and summarised in Table B.6.8-1.

Other metabolites of glyphosate have not been adressed in the GTF dossier from a toxicological point of view and, indeed, may be not relevant when only the intended applications and the representative formulation are taken into consideration.

However, the metabolite N-acetyl glyphosate is newly proposed to be part of the residue definition that will occur in some genetically modified plants after application of glyphosate (see also EFSA, 2009; ASB2012-3480). Toxicological studies with this metabolite have not been submitted as part of the GTF dossier to support new approval of glyphosate in the EU but were subject to a previous (2008) evaluation by the RMS that was performed in order to set import tolerances for gyphosate in soy beans and maize from genetically modified plants. This evaluation may be included in this re-evaluation of glyphosate on request by EFSA or other MS.

Sometimes, the minor metabolite N-methyl-N-(phosphonomethyl)glycine was detected. An acute oral toxicity in rats with that metabolite was described in the 1998 DAR ([redacted] 1991, TOX9552398). In this (acceptable upon re-evaluation) study, no deaths occurred at the limit dose of 5000 mg/kg bw but some clinical signs were observed.

The second sub-section deals with possible effects on farm animals. In contrast to most other active substances, experimental toxicological studies in goats and cows are available with glyphosate and were described in detail by GTF on request of the RMS. Below each study description, the RMS conclusions and an assessment of validity/acceptability of this study may be found.

Recent findings of glyphosate in the urine of cows are reported and have been put into perspective by comparing the estimated systemic dose with proposed ADI and with the NOAELs/LOAELs in the abovementioned studies in cattle. Furthermore, a number of recent publications is discussed in which a possible impact of glyphosate on gut microflora of farm animals has been investigated.

A large number of studies on toxicity of glyphosate was published since 2000. Most of these studies are presented in the chapters on genotoxicity, carcinogenicity, reproductive toxicity and neurotoxicity of this report because they are discussed there in context with these endpoints. However, some additional studies that could not be allocated to a certain endpoint are presented at the end of this chapter.

B.6.8.1 Toxicity of the metabolite AMPA

Table B.6.8-1: Summary of toxicological studies with AMPA

Reference; study identification, owner		Type of study; Species, strain	AMPA purity [%]	Exposure conditions / test method; dose levels	Results
	[redacted] 1993; TOX9552395; Cheminova	Acute oral toxicity Rat, SD	99.2	Limit test (m/f)	LD_{50} > 5000 mg/kg bw; some general and gastrointestinal clinical signs observed
		Acute oral toxicity; Mouse, ICR	99.33	Limit test (m/f)	LD_{50} > 5000 mg/kg bw, no evidence of toxicity
	[redacted], 1988; TOX9500044; Syngenta	Acute oral toxicity Rat, Wistar	> 99	Limit test (m/f)	LD_{50} > 5000 mg/kg bw, diarrhea and some general signs of intoxication
	[redacted], 2002*; ASB2012-11503; ADAMA	Acute dermal toxicity Rat, CD	98.0	Limit test (m/f)	LD_{50} > 2000 mg/kg bw, no evidence of toxicity
	[redacted] 1993; TOX9552396; Cheminova	Acute dermal toxicity Rat, SD	99.2	Limit test (m/f)	LD_{50} > 2000 mg/kg bw, no evidence of toxicity
	[redacted] 1993; TOX9300374; Cheminova	Skin sensitisation Guinea pig (Female Dunkin-Hartley)	99.2	Maximisation test (M&K)	Not sensitising
	[redacted] 2002*, ASB2012-11506; ADAMA	Skin sensitisation Guinea pig (Male Dunkin-Hartley)	98.0	Maximisation test (M&K)	Not sensitising
	[redacted] 1993; TOX9300349; Cheminova	4-week oral toxicity, Rat, SD	99.2	Gavage; 0, 10, 100, 350, 1000 mg/kg bw/d (m/f)	NOAEL = 100 mg/kg bw/day, based on higher kidney wt in m and lower bw gain in f
	[redacted] 1993; TOX9300377; Cheminova	13-week oral toxicity, Rat, SD	99.2	Gavage; 0, 10, 100, 1000 mg/kg bw/d (m/f)	NOAEL = 1000 mg/kg bw/day

Reference; study identification, owner		**Type of study; Species, strain**	**AMPA purity [%]**	**Exposure conditions / test method; dose levels**	**Results**
	[redacted] 1979; TOX9552401; Monsanto	90-day oral toxicity, Rat, CD	99.96	Feeding (adjusted for dose levels); 0, 400, 1200, 4800 mg/kg bw/d (m/f)	NOAEL = 400 mg/kg bw/day, based on bw gain↓, urothelial hyper-plasia (bladder) and gastro-intestinal clinical signs; at top dose level in addition mortality following blood collection (f), food consumption↓, clinical chemistry findings (LDH activity↑) and hyper-plasia of renal pelvis epithelium
	[redacted], 1991; TOX9552406; Monsanto	90-day oral toxicity, Beagle dog	87.8	Capsule; 8.8, 26.3, 87,8, 263 mg/kg bw/d when adjusted for purity (m/f)	NOAEL = 263 mg/kg bw/day; no treatment-related findings
	[redacted], 1993; TOX9300378; Cheminova	Genotoxicity in bacteria; *S. typhimurium* TA100, TA98, TA1535, and TA1537	99.2	Ames test, ±S9 mix for metabolic activation, 310-5000 µg/plate, plate incorpo-ration and pre-incubation assay	Negative
	[redacted] 1993; TOX9300380; Cheminova	Genotoxicity in mammali-an cells, Mouse lymphoma (L5178Y) cells	99.2	Mouse lymphoma assay, ±S9 mix for metabolic activation, 310-5000 µg/mL	Negative
	Callander, 1988; TOX9500043; Syngenta	Genotoxicity in bacteria; *S. typhimurium* TA100, TA98, TA1535, TA1537, TA1538, and *E. coli* (WP2 *uvrA*)	> 99	Ames test, ±S9 mix for metabolic activation, 1.6 - 5000 µg/plate, plate incorpo-ration and (only with S9 mix) pre-incubation assay	Negative
	Akanuma, 1996*, ASB2012-11507; Arysta	Genotoxicity in bacteria; *S. typhimurium* TA100, TA98, TA1535, TA1537 and *E. coli* (WP2 *uvrA*)	99.33	Ames test, ±S9 mix for metabolic activation, 313 - 5000 µg/plate, pre-incubation assay	Negative (supplementary study)

Reference; study identification, owner		Type of study; Species, strain	AMPA purity [%]	Exposure conditions / test method; dose levels	Results
	Nesslany, 2002*; ASB2012-11508; Arysta	Genotoxicity *in vitro* (DNA damage and repair); primary rat hepatocytes	99.9	UDS in rat hepatocytes, 0.625-10 mM	Negative
	Bakke, 1991; TOX9552409; Monsanto	Genotoxicity *in vitro* (DNA damage and repair); primary rat hepatocytes	94.38	UDS in rat hepatocytes, 5 – 5000 µg/mL	Negative up to 2500 µg/mL, meaningful evaluation of higher concentrations not possible due to cytotoxicity
	[redacted] 1993; TOX9300379; Cheminova	Genotoxicity (clastogenicity) *in vivo*, Mouse, NMRI	99.2	Micronucleus test in bone marrow, 5000 mg/kg bw, single oral exposure (m/f)	Negative
	[redacted], 1993; TOX9552413; Monsanto	Genotoxicity (clastogenicity) *in vivo*, Mouse, CD-1	94.38	Micronucleus test in bone marrow, 100, 500, 1000 mg/kg bw, single i.p. injection (m/f)	Negative
	[redacted] 1992; TOX9300348; Cheminova	Developmental toxicity Rat, SD	99.2	0, 100, 350, 1000 mg/kg bw/d by gavage, gestation days 6-16	NOAEL = 1000 mg/kg bw/day (maternal and developmental)
	[redacted] 1991; TOX9552414 ; Monsanto	Developmental toxicity Rat, SD	94.38	0, 150, 400, 1000 mg/kg bw/d by gavage, gestation days 6-15	Maternal NOAEL 150 mg/kg bw/day, based on clinical signs, bw gain/food consumption↓; Developmental NOAEL 400 mg/kg bw/d, based on mean fetal wt↓

* Study previously not evaluated by the EU

Acute oral toxicity in the mouse ([redacted] 1996)

Reference: IIA, 5.8/01

Report: [redacted] 1996 AMPA: Acute Oral Toxicity Study In Mice.
[redacted]
Report No.: IET 96-0075
Date: 1996-11-11
not published, ASB2012-11502

Guidelines: OECD 401 (1987), JMAFF 59 NohSan 4200 (1995), US EPA (1984)

Deviations: None

GLP: Yes

Acceptability: See RMS comment

Dates of experimental work: 1996-09-24 to 1996-10-08

Materials and methods

Test material:	
Identification:	AMPA
Description:	White powder
Lot/Batch #:	A-960719
Purity:	99.33 %
Stability of test compound:	Stable for 1 year at RT.
Vehicle:	1 % carboxymethyl-cellulose (CMC)
Test animals:	
Species:	Mice
Strain:	ICR (Crj:CD-1), SPF
Source:	[redacted]
Age:	6 weeks
Sex:	Male and females
Weight at dosing:	♂ 30.5 – 34.6 g; ♀ 22.9 – 24.8 g
Acclimation period:	7 days
Diet/Food:	Pellet Diet MF (Oriental Yeast Co., Japan), *ad libitum* except for approx. 3 h before and after dosing
Water:	Tap water, *ad libitum*
Housing:	Aluminium cages with wire-mesh floors in groups of 5 animals/sex/cage.
Environmental conditions:	Temperature: 23 ± 3°C Humidity: 55 ± 15% Air changes: 12/hour 12-hour light/dark cycle

Animal assignment and treatment:
A group of five fasted mice per sex received the test material at a dose level of 5000 mg/kg bw by oral gavage (limit test). The dosing volume was 20 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made 1, 3 and 6 h after administration and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, each animal was euthanized under ether anaesthesia and subjected to gross necropsy.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: No clinical signs were observed during the study.
Body weight: No body weight losses were recorded in any animal 7 and 14 days after the administration.
Necropsy: The gross necropsy conducted at termination of the study noted no observable abnormalities.

Conclusion by the Notifiers
The oral LD_{50} of the test material (AMPA) was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, AMPA is not to be classified for acute oral toxicity.

RMS comments:
The study is considered acceptable. The conclusion is agreed with.

Acute oral toxicity in the rat (Leah, 1988)

Reference:	IIA, 5.8/02
Report:	[redacted]. 1988 Aminomethyl Phosphonic Acid: Acute Oral Toxicity to the Rat. [redacted] Data owner: Syngenta Report No.: CTL/P/2266 Date: 1988-08-10 GLP: not published, TOX9500044
Guidelines:	Not stated, but method is in accordance with OECD 401.
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: No date given in the report.

Materials and methods

Test material:	
Identification:	Aminomethyl Phosphonic Acid (AMPA)
Description:	White solid
Lot/Batch #:	Y06384/001/001 (CTL reference)
Purity:	100 % (assumed)
Stability of test compound:	No data given in the report.
Vehicle:	0.5 % (w/v) aqueous polysorbate 80
Test animals:	
Species:	Rat
Strain:	Wistar (Alpk:APfSD), SPF
Source:	[redacted]
Age:	Approx. 8-9 weeks
Sex:	Male and females
Weight at dosing:	♂ 280 – 312 g; ♀ 204 – 214 g
Acclimation period:	At least 6 days

Diet/Food:	Porton Combined Diet (Special Diets Services Ltd.), *ad libitum* except for approx. 24 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Suspended stainless steel/polycarbonate cages with stainless steel mesh floors in groups of max. 5 animals/sex/cage.
Environmental conditions:	Temperature: 15 – 24 °C Humidity: 50 ± 10 % Air changes: 20 – 30/hour 12-hour light/dark cycle

Animal assignment and treatment:
A group of five fasted rats per sex received the test material at a dose level of 5000 mg/kg bw by oral gavage (limit test). The dosing volume was 10 mL/kg bw. One animal was accidentally killed (by mis-dosing) on Day 1 and another animal was therefore substituted, but was dosed one day later. Observations for mortality and clinical/behavioural signs of toxicity were made once 30-90 minutes, 4 and 6 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded one day prior to dosing, the day of dosing (Day 1) and on Days 3, 5 or 6, 8 and 15. On Day 15 after dosing, each animal was euthanized under ether anaesthesia and subjected to gross necropsy.

Results and discussion
Mortality: There were no mortalities during the study.

Clinical observations: Signs of slight toxicity (diarrhoea, chromodacryorrhea, piloerection, stains around nose, ungroomed appearance, signs of urinary incontinence) were seen in the animals, but these did not persist and all animals had recovered by Day 3 or 4.

Body weight: All animals lost weight initially due to the pre-dose fast, but all then gained weight and had exceeded their initial bodyweight by Day 6. Moreover, one male lost weight between Day 6 and 8 and one further male and three females between Day 8 and 15. The reason was unclear as there were no associated clinical abnormalities, nor were there any abnormalities at necropsy.
Necropsy: The gross necropsy conducted at termination of the study noted no observable abnormalities.

Conclusion by the Notifiers
The oral LD_{50} of the test material (AMPA) was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, AMPA is not to be classified for acute oral toxicity.

RMS comments:
This study is considered acceptable and its conclusion is agreeed with. It should be noted that it was already evaluated by the RMS (and later on by the EU) when submitted as part of the toxicological data package for glyphosate-trimesium (DAR, 1998, ASB2010-10493)

Acute dermal toxicity in the rat (Leuschner, 2002)

Reference:	IIA, 5.8/03
Report:	[redacted] 2002 Acute Toxicity Study of AMPA (Aminomethyl Phosphonic Acid) in CD Rats by Dermal Administration – LIMIT TEST [redacted] Data owner: ADAMA Agan Ltd Report No.: 16168/02 Date: 2002-12-03 not published, ASB2012-11503
Guidelines:	OECD 402 (1987), EEC B.3 (1992)
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: 2002-10-21 to 2002-11-01

Materials and methods

Test material:	
Identification:	AMPA (Aminomethyl Phosphonic Acid)
Description:	White solid powder
Lot/Batch #:	FA005563
Purity:	98.0 %
Stability of test compound:	Stable until 2004-12-03 at RT.
Vehicle:	0.5 % aqueous hydroxypropylmethyl cellulose gel
Test animals:	
Species:	Rat
Strain:	CD / Crl:CD
Source:	[redacted]
Age:	20 - 22 days
Sex:	Male and females
Weight at dosing:	♂ 214 – 238 g; ♀ 213 – 223 g
Acclimation period:	At least 5 days.
Diet/Food:	ssniff R/M-H V1530 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Individually in MAKROLON cages (type III) with granulated textured wood as bedding.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 55 ± 15 % Air changes: not reported 12-hour light/dark cycle

Animal assignment and treatment:
The acute dermal toxicity of AMPA was tested on five male and five female CD rats. One day before treatment the administration site was clipped free of hair. A single dose of 2000 mg/kg bw test substance prepared as suspension in 0.5 % aqueous hydroxypropylmethyl cellulose gel was applied uniformly over an area of about 10 % of the total body surface. The dosing volume was 10 mL/kg bw. The application site was covered with an occlusive dressing for 24 hours. After removal of the dressing, possible residual substance was removed. All animals were observed for overt signs of toxicity or behavioural changes before and immediately, 5, 15, 30 and 60 minutes, as well as 3, 6 and 24 h after administration and subsequently once daily for 14 days. Individual body weights were recorded before administration and on Days 7 and 14. All surviving animals were killed at the end of the 14-day observation period.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: No clinical signs were observed during the study.
Body weight: No body weight losses were recorded in any animal 7 and 14 days after the administration.
Necropsy: The gross necropsy conducted at termination of the study noted no observable abnormalities.

Conclusion by the Notifiers
The dermal LD_{50} of the test material (AMPA) was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, AMPA is not to be classified for acute dermal toxicity.

RMS comments:
The study is considered acceptable and the conclusion is agreed with.

Skin sensitisation in the guinea pig by M&K test ([redacted] 2002)

Reference:	IIA, 5.8/04
Report:	[redacted] 2002 Examination of AMPA (Aminomethyl Phosphonic Acid) in the Skin Sensitisation Test in Guinea Pigs according to Magnusson And Kligman (Maximisation Test) [redacted] Data owner: ADAMA Agan Ltd Report No.: 16169/02 Date: 2002-12-03 Unpublished, ASB2012-11506
Guidelines:	OECD 406 (1992); EEC B.6 (1996)
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: 2002-10-12 to 2002-11-26

Materials and methods

Test material:	
Identification:	AMPA
Description:	White solid powder
Lot/Batch #:	FA005563
Purity:	98.0 %
Stability of test compound:	At room temperature stable until December 31, 2004.
Vehicle:	Purified water
Test animals:	
Species:	Guinea pig
Strain:	Dunkin Hartley
Source:	[redacted]
Age:	22 days
Sex:	Male
Weight at dosing:	252 - 307 g; positive control group: 228 - 341 g
Acclimation period:	At least 5 days.
Diet/Food:	ssniff Ms-H (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	In pairs in Makrolon cages (type IV) with granulated textured wood bedding.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 55 ± 15 % Air changes: no data 12 hours light/dark cycle

Animal assignment and treatment:
AMPA was tested for its sensitising effect on the skin of the guinea pig using the Maximisation test according to Magnusson and Kligman. Male Dunkin Hartley guinea pigs, young adults with body weights ranging from 228 to 341 g were used. The test substance concentrations for the main study were selected based on the results of the pre-testing performed with eight animals. The main study was performed in 10 test animals, 5 control animals and 20 positive control animals.
The induction phase consisted of an intradermal injection at Day 0 and an epidermal application on Day 7. On Day 0 the test substance was injected (0.1 mL/site) into the clipped dorsal skin of the shoulder region at a concentration of 5% either in purified water or in a 1:1 (v/v) mixture of Freund´s Complete Adjuvant and purified water.
On Day 6 the skin was shaved and coated with 0.5 mL sodium laurylsulfate 10% in vaseline in order to induce a local irritation. On Day 7 the test substance was topically applied at a concentration of 50 % to the clipped and shaved skin of the shoulder region and covered with an occlusive dressing, which was left in place for 48 hours.
The challenge was conducted on Day 21 by an occlusive patch at a concentration of 50% which was applied to the clipped and shaved left flank of each animal for 24 h. The clipped and shaved right flank of each animal was treated in the same way with the vehicle only. 24

and 48 hours after removal of the dressing skin reactions were scored according the Magnusson and Kligman grading scale.

The animals of the positive control group were treated with a 2 % benzocaine solution intracutaneously in the induction phase and with a 5 % solution topically in the induction phase and at challenge.

Body weights were determined at the first day of treatment of the main study and at termination. Mortality and clinical signs were recorded daily during the study period.

Evaluation criteria for classification as a potential skin sensitizer:
At the 24-hour and/or 48-hour reading, 30 % or more of the test animals exhibit a positive response (scores ≥ 1) in the absence of similar results in the vehicle control group.

Results and discussion
Mortality: No deaths occurred.
Clinical observations: No signs of systemic toxicity were observed.
Body weight: All animals showed the expected gain in body weight.
Skin reactions: No skin reactions were observed 24 or 48 h after the challenge treatment with AMPA in the control or test group.
Animals treated with the positive control benzocaine in 40% ethanolic 0.9% NaCl solution exhibited a sensitising reaction in all animals in form of a discrete or patchy erythema (grade 1).

Conclusion by the Notifiers
Based on the study results and according to the EU and OECD Globally Harmonized System (GHS) classification criteria, AMPA is not to be classified for skin sensitization.

RMS comments:
The study is considered acceptable. AMPA was not a skin sensitiser in the M&K test.

1st "new" mutagenicity (Ames) test in bacteria (Callander, 1988)

Reference:	IIA, 5.8/05
Report:	Callander, R.D. 1988 Aminomethyl Phosphonic Acid: An Evaluation of Mutagenic Potential Using *S. Typhimurium* and *E. Coli* ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK Data owner: Syngenta Report No.: CTL/P/2206 Date: 1988-09-21 not published, TOX9500043
Guidelines:	Comparable to OECD 471 (1997): OPPTS 870.5100 (1998): 2000/32/EEC B.13/B.14 (2000)
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: 1988-03-01 to 1988-09-21

Materials and methods

Test Material:	Aminomethyl phosphonic acid
Description:	Crop metabolite of, and an impurity in, ICIA0224, white solid
Lot/Batch number:	48F-3893
Purity:	>99 % a.i
CAS#:	Not reported
Stability of test compound:	Confirmed by Sponsor
Control Materials:	
Negative:	Water
Solvent control (final concentration):	Dimethylsulphoxide – DMSO (10 µL/plate)
Positive control:	Nonactivation: Acridine mutagen ICR191 TA1537 2-Aminoanthracene TA1537, WP2 uA Daunomycin hydrochloride TA98 4-Nitro-o-phenylene diamine TA1538 N-Methyl-N´-nitro-N-nitrosoguanidine TA1535, TA100, WP2 uA Activation: 2-Aminoanthracene TA1535, TA1537, TA1538, TA98, TA100, WP2 uA Acridine mutagen ICR191 TA1537 2-Aminothracene TA1535, TA1537, TA1538, TA98, TA100, WP2 uA N-Methyl-N´-nitro-N-nitrosoguanidine WP2 uA

Mammalian metabolic system: S9 derived

X	Induced	X	Aroclor 1254	X	Rat	X	Liver
	Non-induced		Phenobarbitol		Mouse		Lung
			None		Hamster		Other
			Other β-naphthoflavone		Other		

The metabolic activation system (S9-mix) used in this study was prepared as a 3:7:20 mixture of S9 fraction, Sucrose-tris-EDTA buffer (250:50:1 mM) and cofactor solution.

The cofactor solution was prepared in bulk as follows: Na_2HPO_4 (150 mM), KCl (49.5 mM), glucose-6-phosphate (7.5 mM), NADP (Na salt) (6 mM) and $MgCl_2$ (12 mM).

Test organisms:

***S. typhimurium* strains**									
	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537	X	TA1538		list any others		
***E. coli* strains**									
	WP2 (pKM101)	X	WP2 uvrA (pKM101)						
Properly maintained?							X	Yes		No
Checked for appropriate genetic markers (rfa mutation, R factor)?							X	Yes		No

Test compound concentrations used:
Nonactivated conditions: 5000, 1000, 200, 40, 8 and 1.6 µg/plate
Activated conditions: 5000, 1000, 200, 40, 8 and 1.6 µg/plate
For all strains triplicate plates were used for all test substance and positive control treatments. For solvent controls 5 plates were used.

Study design and methods

Preliminary Cytotoxicity Assay: Not performed.
Type of Bacterial assay:
X standard plate test (both experiments –S9, initial experiment +S9)
__ pre-incubation (60 minutes) (second experiment +S9)
__ "Prival" modification (i.e. azo-reduction method)
__ spot test
__ other

Protocol:
Bacterial cultures were prepared from frozen stocks by incubating for 10-12 hours at 37 °C.
The following materials were mixed in a test tube and poured onto the selective agar plates:
100 µL Test solution at each dose level, solvent and positive controls;
500 µL S9 mix or phosphate buffer;
100 µL Bacteria suspension;
2 mL Overlay agar containing 50 µM histidine or tryptophan as appropriate.

In this assay 100 µL aliquots of an overnight culture of each bacteria strain were stored in bijou bottles at room temperature until required (1-2 hours). 500 µL S9 mix (or Co-factor/Buffer mix) was then added by dispensing syringe to the number of bijou bottles of one strain required for one dose level, followed by 0.1 mL of the appropriate concentration of the test substance solution added by micropipette. Finally, 2.0 mL top agar was added to each bijou, the force of addition was sufficient to mix the contents. The mixture was then rapidly poured onto a prepared Vogel Banner agar plate. After the agar was set the plates were incubated upside down for 64 - 68 hours at 37 °C in the dark. For each strain and dose level including the controls, three plates were used.

Following the total incubation period the plates were examined for the lack of microbial contamination and evidence that the test was valid: i.e. there should be a background lawn on the negative control plates and on the plates for (at least) the lower doses of test substance, and that the positive controls should show at least a two-fold increase in average reversion frequency rate and there should be a dose-response relationship.

The plates were counted using an automated colony counter (AMS 40-10) with the discrimination adjusted appropriately to permit the optimal counting of mutant colonies.

Statistical analysis: None – see Evaluation Criteria below.

Evaluation criteria: A positive response in a (valid) individual experiment is achieved when one or both of the following criteria are met:
a significant, dose-related increase in the mean number of revertants is observed;
a two-fold or greater increase in the mean number of revertant colonies (over that observed for the concurrent solvent control plates) is observed at one or more concentrations
A negative result in a (valid) individual experiment is achieved when:
there is no significant dose-related increase in the mean number of revertant colonies per plate observed for the test substance; and
in the absence of any such dose response, no increase in colony numbers is observed (at any test concentration) which exceeds 2x the concurrent solvent control.
For a positive response in an individual experiment to be considered indicative of an unequivocal positive, i.e. mutagenic, result for that strain/S9 combination, then the observed effect(s) must be consistently reproducible.

Results
Mutagenicity assay: In two separate experiments, aminomethyl phosphonic acid did not induce any significant increases in the observed numbers of revertant colonies in *Salmonella typhimurium* strains TA1535,TA1538, TA98, TA100 and *Escherichia coli* WP2 *uvrA* pKM101 in either the presence or absence of an auxiliary metabolising system (S9).

In the first experiment, slight responses were observed in strain TA1537, reaching maxima of 1.9 x and 2.0 x background in the presence and absence of S9 respectively. These responses were only of limited dose-relationships, and were of limited statistical significance in both cases. In two further experiments, no significant increases in colony numbers were observed either with or without S9. This lack of reproducibility indicates that the observed effects in the first experiment are not due to compound-induced mutations.

The positive controls for each experiment induced the expected responses indicating the strains were working satisfactorily in each case.

Conclusion by the Notifiers
Under the conditions of this assay, aminomethyl phosphonic acid gave an unequivocal negative, ie non-mutagenic, response, when tested to a limit dose of 5000 µg/plate.

RMS comments:
The study is considered acceptable. No evidence of genotoxicity of AMPA in bacteria was obtained. The slight increase in revertant numbers in one strain in the first experiment was rather weak and was sufficiently contravened by subsequent trials in which the test material proved clearly negative.
It should be noted that this study was already evaluated by the RMS (and later on by the EU) when submitted as part of the toxicological data package for glyphosate-trimesium (DAR, 1998, ASB2010-10493)

2nd new mutagenicity (Ames) test in bacteria (Akanuma, 1996)

Reference: IIA, 5.8/06
Report: Akanuma M. 1996 AMPA Reverse mutation test.
The Institute of Environmental Toxicology, Tokyo, Japan
Data owner: Arysta LifeScience
Study No.: IET 96-0076
Date: 1996-12-09
not published, ASB2012-11507
Guidelines: U.S. EPA FIFRA Guidelines, Subdivision F
OECD guidelines 471, 472 (1983)
Japan MAFF guidelines 59 NohSan N° 4200 (1985)
Deviations: None
GLP: Yes
Acceptability: *See RMS comment*

Dates of experimental work: 1996-09-09 to 1996-10-11

Materials and methods

Test material: AMPA
Identification: AMPA
Description: White powder
Lot/Batch #: A-960719
Purity: 99.33 %
Stability of test compound: Stable for 1 year at room temperature
Solvent used: Sterile water
2. control materials:
Negative: Sterile water
Solvent/final concentration: Water / 50 mg/mL
Positive: non-activation and activation

Strain	Positive controls	
	Without S9 (µg/plate)	With S9 (µg/plate)
TA100	AF – 2 (0.01)	2-AA (1)
TA1535	NaN_3 (0.5)	2-AA (2)
WP2 uvra	AF-2 (0.01)	2-AA (10)
TA98	AF-2 (0.1)	2-AA (0.5)
TA1537	9-AA (80)	2-AA (2)

AF-2: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide dissolved inDMSO; NaN_3: sodium azide dissolved in sterile water
2-AA: 2-aminoanthracene dissolved in DMSO; 9-AA: 9-aminoacridine hydrochloride dissolved in sterile water

activation:	The enzyme activity measured by mutagenicity was good. S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factor. The component of S9 mix were 10 % (v/v) S9 fraction, 8 mM MgCl2, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADH, 4 mM NADPH and 100 mM sodium phosphate buffer.
test organisms:	*Escherichia coli* WP2 uvrA *Salmonella typhimurium* (TA100, TA1535, TA98 and TA1537)

test concentrations:

Preliminary cytotoxicity assay: One preliminary assay was performed:
Plate incorporation assay: Concentrations up to 5000 µg/plate were evaluated with and without S9 activation in strain TA1535, TA1537, TA98, TA100 and WP2 uvrA. A single plate was used, per dose, per condition.
Pre-incubation assay: As above.

Mutation assays:
Plate incorporation assay: 156, 313, 625, 1250, 2500 and 5000 µg/plate were evaluated in triplicate in the presence and absence of S9 activation; all test strains were used.
Pre-incubation assay: As above for the plate incorporation assay.

The independently repeated mutation assay was conducted using the pre-incubation modification to the standard plate incorporation test. The pre-incubation assay was carried out as described above with the following two exceptions: 0.5 mL of buffer were added to cultures prepared for testing under non-activated conditions; prior to the addition of top agar, reaction mixtures were incubated for 20 minutes at 37 ± 1°C.

Statistics
Results were judged without statistical analysis.

Evaluation Criteria
The test items were carried out twice. Reproducibility of results was confirmed by two independent experiments. Results were judged positive without statistical analysis when the following criteria are all satisfied:
A two-fold or greater increase above solvent control in the mean number of revertants is observed
This increase in the number of revertants is accompanied by a dose-response relationship
This increase in the number of revertants is reproducible.

Results and discussion
Analytical determinations: None

Table B.6.8-2: Results of the preliminary dose range finding test

		Concentration	**Revertants (n° colonies/plate)**				
		(µg/plate)	TA100	TA1535	WP2 *uvr A*	TA98	TA1537
- S9 mix		Solvent Control (H_2O)	150*	8*	20*	24*	7*
		200	131	11	21	12	8
		500	152	10	19	22	6
		1000	131	10	15	20	5
		2000	154	3	23	24	10
		5000	140	11	20	18	11
+ S9 mix		Solvent Control (H_2O)	106*	6*	28*	25*	10*
		200	92	7	19	16	16
		500	116	1	20	24	15
		1000	124	5	25	17	12
		2000	102	7	24	30	8
		5000	128	10	29	26	11
Positive Control	- S9 Mix	Compound	AF-2	NaN_3	AF-2	AF-2	9-AA
		µg/plate	0.01	0.5	0.01	0.1	80
		Revertants/plate	666*	673*	201*	658*	540*
	+ S9 Mix	Compound	2-AA	2-AA	2-AA	2-AA	2-AA
		µg/plate	1	2	10	0.5	2
		Revertants/plate	450*	233*	415*	456*	84*

AMPA did not show any toxicity to any strain up to the highest dose of 5000 µg/plate with and without S9 Mix.
Mutation assays: Results are shown in the following tables.

Table B.6.8-3: Reverse mutation tests without metabolic activation– Experiment 1

	Concentration (µg/plate)	**Revertants (n° colonies/plate) ***				
		TA100	TA1535	WP2 *uvr A*	TA98	TA1537
- S9 mix	Solvent Control (H_2O)	101±17	11±1	17±4	18±3	4±3
	313	84±7	10±5	17±6	14±2	5±3
	625	91±14	8±6	17±7	16±4	4±2
	1250	97±17	8±3	14±2	13±2	4±2
	2500	91±7	9±1	15±8	15±2	6±2
	5000	100±4	7±3	16±4	16±5	3±1
Positive Control (-S9)	Compound	AF-2	NaN_3	AF-2	AF-2	9-AA
	µg/plate	0.01	0.5	0.01	0.1	80
	Revertants/plate	619±57	619±45	160±22	667±60	710±73

* : Average ± SD

Table B.6.8-4: Reverse mutation tests with metabolic activation– Experiment 1

	Concentration (µg/plate)	**Revertants (n° colonies/plate) ***				
		TA100	TA1535	WP2 *uvr A*	TA98	TA1537
+ S9 mix	Solvent Control (H_2O)	105±11	10±4	19±1	30±5	10±1
	313	105±5	12±2	16±4	28±5	9±3
	625	92±6	6±1	16±1	28±7	13±2
	1250	90±3	6±1	16±2	25±7	11±3
	2500	83±9	9±4	20±4	25±8	10±3
	5000	93±10	10±4	24±6	32±10	7±1
Positive Control (+S9)	Compound	2-AA	2-AA	2-AA	2-AA	2-AA
	µg/plate	1	2	10	0.5	2
	Revertants/plate	529±33	184±5	384±20	407±11	94±2

* : Average ± SD

Table B.6.8-5: Reverse mutation tests without metabolic activation– Experiment 2

	Concentration (µg/plate)	**Revertants (n° colonies/plate) ***				
		TA100	TA1535	WP2 *uvr A*	TA98	TA1537
- S9 mix	Solvent Control (H_2O)	120±3	9±3	15±3	18±4	3±2
	313	136±9	4±1	18±3	14±4	4±3
	625	124±16	5±2	16±3	13±3	3±2
	1250	107±11	6±4	12±4	15±2	3±3
	2500	96±6	9±4	12±3	16±6	4±0
	5000	117±2	7±3	20±5	13±2	3±2
Positive Control (-S9)	Compound	AF-2	NaN_3	AF-2	AF-2	9-AA
	µg/plate	0.01	0.5	0.01	0.1	80
	Revertants/plate	668±27	696±20	182±16	650±8	698±53

* : Average ± SD

Table B.6.8-6: Reverse mutation tests with metabolic activation– Experiment 2

	Concentration (µg/plate)	**Revertants (n° colonies/plate) ***				
		TA100	TA1535	WP2 *uvr A*	TA98	TA1537
+ S9 mix	Solvent Control (H_2O)	95±3	8±2	17±3	28±5	7±2
	313	112±14	8±3	17±4	21±6	10±5
	625	84±5	7±4	16±5	21±5	7±3
	1250	106±8	7±2	17±4	28±9	7±1
	2500	97±4	11±3	16±2	21±1	6±1
	5000	115±12	9±5	22±2	22±3	6±5
Positive Control (+S9)	Compound	2-AA	2-AA	2-AA	2-AA	2-AA
	µg/plate	1	2	10	0.5	2
	Revertants/plate	584±56	169±28	461±8	334±14	82±4

* : Average ± SD

Conclusion by the Notifiers

A two-fold or greater increase in the mean number of revertant colonies was not observed in any strain at any dose of AMPA in the reverse mutation tests with or without metabolic activation. It is concluded that AMPA is non mutagenic for bacteria under the conditions used with this experiment.

RMS comments:
In contrast to the description in the dossier, it seems that testing was performed by means of the pre-incubation method only. Therefore, the study is considered supplementary only because a plate-incorporation test was not performed.
When the study description in the dossier was compared to the original study report, it was noted that the study director was Mie Akanuma. Erroneously, the first name had been mentioned in the dossier instead of the authors surname.

UDS asssay for DNA damage and repair *in vitro* (Nesslany, 2002)

Reference:	IIA, 5.8/07
Report:	Nesslany, F. 2002 Measurement of unscheduled DNA synthesis (UDS) in rat hepatocytes *in vitro* procedure with AMPA (Amino methyl phosphonic acid). The Institute of Environmental Toxicology, Tokyo, Japan Data owner: Arysta LifeScience Study No.: IPL-R-02025 Date: 2002-09-10 not published, ASB2012-11508
Guidelines:	OECD guideline n° 482
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: 2002-04-29 to 2002-07-02

Materials and methods

Test material:	AMPA
Identification:	AMPA
Description:	White crystalline powder
Lot/Batch #:	020404
Purity:	99.9 %
Stability of test compound:	Not mentioned in the report
Solvent used:	William's E medium Gibco

2. control materials:

Negative:	/
Solvent/final concentration:	See above
Positive:	2-acetamidofluprene
activation:	None
test organisms:	Rats hepatocytes

test concentrations: 5 dose level were tested: 10, 5, 2.5, 1.25 and 0.625 mM with and without S9 metabolic activation

Test principle
Hepatocytes were isolated from livers of rats. The primary hepatocyte cultures were exposed to the test article in the presence of 3H thymidine which is incorporated into the DNA, if DNA damage is occuring. DNA repair systems then stimulated UDS and increased the incorporation of thymidine which was measured by grain counting after autoradiography of hepatocytes.
The following results are presented:
The average NNG and standard deviation
The percent of cells in repair and standard deviation (>=5)
The average cytoplasmic and nuclear grain count
The number of cells in S-phase

Statistics
Results were judged without statistical analysis.

Evaluation Criteria

Results are judged positive when:
At any dose tested, group, mean NNG value greater than 0 NNG and 20 % or more of cells responding (NNG value >=5)
An increase is seen in both NNG and the percentage of the cells in repair
A dose related increase is seen in both NNG and the percentage of the cells in repair
Any induction of UDS can be reproduced in an independent experiment.

Validity Criteria
The assay is considered valid if:
Negative control slides have a group mean NNG value within the historical range.
The positive control have group mean NNG values of less than 5 NNG counts with 50% or more cells having NNG counts of 5 or more and statistically significant relative to the solvent control.

Results and discussion
Analytical determinations: None
Mutation assays: Negative control gave a group mean NNG value of less than zero with a percentage of cells in repair comparable with historical control data. In positive controls, group mean net nuclear grain count (NNG) values as well as percentage of cells in repair obtained were within the range of historical control. The sensitivity of the cell type used to a DNA damaging agent requiring metabolism for its action, 2-acetamidofluorene was demonstrated. Thus, the validity criteria of the test were fulfilled.
The findings of both experiments are summarised in the tables below.

Table B.6.8-7: UDS data of the first experiment

Concentration (mM)	**Net Nuclear Grain Count (NNG)**		**Net Nuclear Grain Count of cells in repair (NNG >5)**		**% cells in repair (NNG >5)**	
	Mean	+/- sd	Mean	+/- sd	Mean	+/- sd
Solvent control	-2.38	5.20	5.63	0.51	4.21	1.53
0.625	-3.81	5.29	6.61	1.56	3.89	1.53
1.25	-3.61	5.23	6.99	0.56	4.02	1.53
2.5	-3.93	5.24	6.83	0.91	5.18	1.53
5	-3.35	5.10	6.28	1.66	3.45	1.00
10	-2.04	4.82	6.91	0.74	5.43	1.53
Positive control: 2-acetamidofluorene 6.25 µM	30.81	18.22	31.55	4.84	96.61	2.00

Table B.6.8-8: UDS data of the second experiment *(amended by RMS because of errors)*

Concentration (mM)	**Net Nuclear Grain Count NNG**		**Net Nuclear Grain Count of cells in repair NNG >5**		**% cells in repair NNG >5**	
	Mean	+/- sd	Mean	+/- sd	Mean	+/- sd
Solvent control	-4.62	5.81	5.37	0.21	3.74	2.08
0.625	-4.77	5.46	6.90	0.60	1.78	0.00
1.25	-4.04	5.35	6.87	1.00	4.49	1.53
2.5	-3.81	5.82	7.07	1.61	6.18	2.08
5	-3.47	5.83	7.83	0.29	7.73	1.73
10	-5.58	6.06	6.97	1.29	3.76	1.53
Positive control: 2-acetamidofluorene 6.25 µM	17.57	11.18	17.73	3.29	94.94	9.45

Over the two experiments, group mean net nuclear grain count (NNG) values at the dose range tested from 10 to 0.625 mM were less than zero (-2.04 to –3.81 vs –2.38 in solvent control in the first UDS assay and –5.58 to –4.77 vs –4.62 in solvent control in the second UDS assay), that is to say below the threshold valure of 0 NNG for a positive response.
Furthermore, no significant increase in the percentage of cells in repair at any dose of AMPA tested when compared with the respective controls (5.43 to 3.89% vs. 4.21% in solvent control in the first assay and 3.76 to 1.78 % vs. 3.74% in solvent control in the second assay).
In addition, in cells in repair, group mean net nuclear grain count (NNG≥5) values were comparable with the solvent controls (6.91 at 10 mM to 6.61 at 0.625 mM vs. 5.63 in control in the first assay and 6.97 ta 10 mM to 6.90 at 0.625 mM vs. 5.37 in control in the second assay).

Conclusion by the Notifiers
Under the conditions of this experiment, AMPA did not reveal any genotoxicity activity in the Unscheduled DNA synthesis assay.

RMS comments:
The study is considered acceptable. There was no evidence of a direct interaction of AMPA with the DNA of primary rat hepatocytes.
In contrast to what was mentioned in the GTF dossier, the test assay was performed in the "Institut Pasteur de Lille" in France and not in Japan.

B.6.8.2 Mechanistic studies on certain aspects of the toxicity of glyphosate

B.6.8.2.1 Studies on mechanism of salivary gland findings

Introduction by RMS:
A few studies have been submitted to investigate the mechanism behind the salivary gland findings that were obtained in quite many subchronic, long-term and reproduction studies in rats (see sections B.6.3, B.6.5 and B.6.6) and occasionally also in mice ([redacted], 1992, TOX9551954). The first experiment to elucidate the mode of action was performed by [redacted] (1992, TOX9551954) as part of their comprehensive investigations within the U.S. NTP and is described in the original DAR (1998, ASB2010-10302) on glyphosate in detail. The study was considered acceptable upon re-evaluation. In order to provide a most comprehensive evaluation of salivary gland changes, this part from the old DAR is copied here, followed by a summary Table B.6.8-9 and the detailed description of two more recent studies by [redacted] (1996, ASB2012-11537/ASB2012-11520) and [redacted] (2010, ASB2012-11519), commented by the RMS.

Copy from the previous DAR: Possible explanation for salivary gland alterations

"Because of morphologic similarities to salivary gland changes observed with the adrenergic agonist isoproterenol, a subacute study was designed to test the hypothesis that the salivary gland effects of glyphosate were also mediated through an adrenergic mechanism ([redacted], 1992, TOX9551954). Groups of four male F344/N rats received glyphosate at a dietary level of 50000 ppm or were fed an untreated control diet. In three of the groups, the adrenergic agonist isoproterenol and/or the antagonist propranolol were administered by continuous subcutaneous infusion by osmotic minipumps. The study design was as follows:
Group 1: control diet, only vehicle (water + 0.1% ascorbate) administered by minipump;
Group 2: glyphosate diet, only vehicle administered by minipump;
Group 3: glyphosate diet, propranolol (1.2 mg/kg bw/d) administered by minipump;
Group 4: control diet, isoproterenol (1.0 mg/kg bw/d) administered by minipump;
Group 5: control diet, isoproterenol and propranolol administered by minipump.
After 14 days of treatment, the left parotid and submandibular/sublingual salivary glands were removed, weighed separately and processed for electron microscopy. The right parotid and submandibular/sublingual salivary glands were removed, sectioned and stained for histological evaluation.
Both isoproterenol and glyphosate induced significant enlargement of the salivary glands, glyphosate having much greater effect than isoproterenol. The parotid was most affected. Propranolol inhibited the effect of both substances on salivary gland weight but not completely in the case of glyphosate. Microscopically, similar changes were induced by glyphosate and isoproterenol consisting of cytoplasmic basophilic change, fine vacuolation and swelling of acinar cells resulting in a relative reduction in the number of ducts present. Glyphosate-treated animals were most severely affected. Propranolol, however, clearly protected the rats from the more severe lesions. Likewise, modest protection of histological effects caused by isoproterenol was seen. Cytoplasmic alteration of the submandibular gland

was more subtle and histologically detectable only in glyphosate-treated animals. However, electron microscopy elucidated an effect of isoproterenol on this gland, too. It could not be determined if the serous or mucous glandular acini were selectively affected by glyphosate. No changes were seen in the sublingual glands examined from any group demonstrating target specifity of glyphosate- and isoproterenol-associated lesions to those salivary glands which are mainly innervated by adrenergic fibers.
The authors assume that effects of glyphosate on salivary glands were due to an adrenergic mechanism. The biological significance of this finding is unkown ([redacted], 1992, TOX9551954)."

Table B.6.8-9: Summary of new mechanistic studies on salivary gland effects

	Reference; study identification; owner	Type of study; species, strain	Application route (dose)	Test substance	Purity [%]	Results
Studies not reviewed in the 2001 evaluation	[redacted] 2010; ASB2012-11519; Monsanto	8-week oral toxicity; Sprague-Dawley Rat, ♂	Citric acid: Oral gavage (791-1316 mg/kg bw) or via diet (14000 ppm); Trisodium citrate dehydrate: diet (21400 ppm)	Citric acid, Trisodium citrate dihydrate	99.3	Higher parotid salivary gland weights and a generally correlative increase in severity of background cytoplasmic alterations in the parotid salivary glands
	[redacted] 1996; ASB2012-11520 and 2012-115337; Syngenta	4-week oral toxicity; Sprague-Dawley (CD)/Fischer 344/Alpk:APSD (AP), Rat, ♂	Diet (20000 ppm)	Glypho-sate acid, batch P24	95.6	Marked strain differences in the severity of effect in the parotid salivary glands; most pronounced effect occurred in the F344 strain: diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells; similar but slighter an apparently reversible effects occurred in the AP and CD strains involving small foci of cells only

<u>Effects of citric acid ([redacted] 2010)</u>

Reference: IIA, 5.10/01
Report: [redacted] (2010)
An 8-Week Oral (Diet and Gavage) Toxicity Study of Citric Acid in Male Rats
[redacted]
Data owner: Monsanto/GTF
Study No.: WIL-50361
Date: 2010-01-08
Unpublished
ASB2012-11519
Guidelines: Guideline does not exist for this kind of study but data from the study report is similar to OECD 408.

Deviations: not applicable
GLP: yes
Acceptability: *See RMS comment*

Dates of experimental work: 2009-02-24 to 2009-05-15

Materials and methods

Test materials:	
Identification:	Anhydrous Citric Acid
Description:	White powder
Lot/Batch #:	XR3050
Purity:	99.9 %
Stability of test compound:	Stable at room temperature until 2010-01-06.
Identification:	Trisodium Citrate Dihydrate (TCD)
Description:	White crystalline solid
Lot/Batch #:	1387609
Purity:	99.3 %
Stability of test compound:	Stable at room temperature until 2011-03-01.
Vehicle:	Gavage: deionised water, Diet: plain diet
Test animals:	
Species:	Rats
Strain:	Sprague-Dawley (CD)
Source:	[redacted]
Age:	approx. 6 weeks upon beginning of treatment
Sex:	Males
Weight at dosing:	177 - 227 g
Acclimation period:	14 days
Diet/Food:	Certified Rodent LabDiet #5002 (PMI Nutrition International, LLC), *ad libitum*
Water:	tap water, *ad libitum*
Housing:	Upon arrival, animals were housed three per cage for approximately 3 days. Thereafter, all animals were housed individually in clean, stainless steel, wire-mesh cages suspended above cage-board.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 50 ± 20 % Air changes: at least 10/hour 12 hours light/dark cycle

Animal assignment and treatment:
In a 8 week gavage and feeding study, groups of 10 Sprague Dawley rats received the respective vehicles or test substances for 56 consecutive days via oral gavage (Groups 1 and 3) or in the diet (Groups 2, 4 and 5; see Table B.6.8-10). A low pH diet containing 14000 ppm of citric acid in basal diet was offered continuously to Group 4. A high pH diet containing 21400 ppm of trisodium citrate dihydrate in basal diet (at an equivalent citrate ion concentration to Group 4) was offered continuously to Group 5. A concurrent control group (Group 2) received the basal diet on a comparable regimen. Citric acid in the vehicle, deionised water, was administered orally by gavage at a dose level of 791-1316 mg/kg/day to Group 3.
Concentrations of the Group 3 formulations were calculated and adjusted weekly, based on the average food consumption and body weights of the Group 4 animals from the previous week of dosing in order to maintain approximately equivalent citric acid dose levels to Group 4. A concurrent gavage control group (Group 1) received the vehicle on a comparable regimen.

Table B.6.8-10: Study group assignment

Group Number	Test Substance application	Dose Level	Dose Volume	Number of animals
		(mg/kg bw/day or ppm)	(mL/kg)	
1	Gavage Vehicle	0	10	10
2	Basal Diet	0	na	10
3	Gavage Citric Acid (low pH)	791-1316	10	10
4	Diet Citric Acid (low pH)	14,000	na	10
5	Diet Trisodium Citrate (high pH)	21,400	na	10

na - not applicable

Observations
All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed weekly.

Body weight
Individual body weights were recorded weekly.

Food consumption and compound intake
Food consumption was recorded weekly.

Sacrifice and pathology
All animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined: parotid salivary glands, mandibular salivary glands and sublingual salivary glands. The mandibular and sublingual salivary glands were weighed together as one organ since they were fused and could not be adequately separated for weighing.
Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta, bone & bone marrow (sternum and femur (incl. joint)), brain (cerebrum at two levels; cerebellum with medulla/pons), caecum, colon, duodenum, epididymides, eyes with optic nerves, gross lesions, harderian glands, heart, ileum, jejunum, kidneys, lacrimal gland (exorbital), liver, lungs (incl. bronchi), mammary gland, lymph nodes (mandibular, mesenteric and axillary), nasal cavity, oesophagus, pancreas, Peyer's patches, pituitary,

prostrate, rectum, salivary glands (mandibular, parotid, sublingual), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea and urinary bladder.
Microscopic examination was performed on the parotid salivary glands and gross lesions from all animals at the scheduled necropsy.

Statistics
All statistical tests were performed using the WIL Toxicology Data Management System (WTDMS™). Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1 % and 5 %, comparing each test substance-treated group to its respective control group.
Body weight, body weight change, food consumption, and organ weight data were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA identified statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare each of the test substance-treated groups to the respective control group (Group 1 to Group 3 and Group 2 to Groups 4 and 5). Group 1 was also compared to Group 2.
Statistical analysis of the severity of histological changes was conducted. Individual animals were assigned severity scores based on parotid salivary gland changes (0=without histological change, 1=minimal change, 2=mild change, and 3=moderate change). The severity scores were then compared statistically using the Mann-Whitney U-test by comparing Group 1 to Group 3 and Group 2 to Groups 4 and 5.

Results and discussion
Mortality:
No deaths occurred during the study.

Clinical observations:
All clinical findings in the test substance-treated groups were noted with similar incidence in the control groups, were limited to single animals, and/or were common findings for laboratory rats of this age and strain.

Body weight:
There were no statistically significant differences when the respective control and test substance-treated groups were compared.

Food consumption:
Food consumption was unaffected by citric acid or trisodium citrate dihydrate administration. A statistically significant decrease in food consumption of the gavage citric acid group (Group 3, Week 7/8) was probably due to biological variability and not considered related to test substance administration.

Organ weights:
Test substance-related effects on organ weights consisted of statistically significant higher absolute and relative parotid salivary gland weights in the low pH diet group (14,000 ppm citric acid) when compared to the dietary control group; the magnitude of change was > 40% (Table B.6.8-11). Higher absolute and relative parotid salivary gland weights were also observed in the low pH gavage group (791-1316 mg/kg bw/day citric acid) and in the high pH diet group (21,400 ppm TCD) when compared to their respective control groups. However, the parotid salivary gland weight differences in the low pH gavage and high pH diet groups were not statistically significant and were of much lesser magnitude of change.

There were no other statistically significant test substance-related effects on the fused mandibular/ sublingual or parotid salivary gland weights when the control groups and test substance-treated groups were compared.

Table B.6.8-11: Organ weight differences of salivary glands

	Gavage Administration		**Dietary Administration**		
	aqueous control	791-1316 mg/kg bw/day citric acid	basal diet control	low pH diet, 14000 ppm citric acid	high pH diet, 21400 ppm trisodium citrate dihydrate
Mean Absolute Mandibular / Sublingual Fused Glands Weight (g)	0.7625 ± 0.05446	0.7873 ± 0.08397	0.7682 ± 0.08670	0.8872 ± 0.16548	0.7869 ± 0.07028
Mean Relative Mandibular / Sublingual Fused Glands Weight (g)	0.179 ± 0.0105	0.180 ± 0.0178	0.173 ± 0.0221	0.199 ± 0.0339	0.183 ± 0.0201
Mean Absolute Parotid Gland Weight (g)	0.3500 ± 0.12450	0.4082 ± 0.11990	0.2758 ± 0.08514	0.3905* ± 0.10920	0.3502 ± 0.08986
Mean Relative Parotid Gland Weight (g)	0.083 ± 0.0299	0.095 0.0304	0.062 ± 0.0194	0.088* ± 0.0236	0.082 0.0220

* - significantly different from relevant control group ($p < 0.05$) using Dunnett's test

Necropsy:
All macroscopic findings noted were considered spontaneous and/or incidental in nature and unrelated to test substance administration.

Histopathology:
Test substance-related histological effects consisted of a higher severity of cytoplasmic alterations in the parotid salivary glands of the citric acid and trisodium citrate dihydrate-treated groups when compared to their respective control groups (Table B.6.8-12). The severity of cytoplasmic alteration was increased in all dose groups; however, the cytoplasmic alteration was clearly most severe in the low pH diet group (Group 4; 14000 ppm citric acid).
Cytoplasmic alteration in the parotid salivary glands was characterized by the presence of hypertrophied acinar cells with basophilic granular cytoplasm. The severity grades ranged from minimal to moderate, displayed by increasing numbers of affected acinar cells and more pronounced hypertrophy of acinar cells with increasing severity grade.
Cytotoxicity and hyperplasia were not observed and consequently, the observed changes were considered to be adaptive responses rather than adverse effects. There were no other test substance-related histological changes.

Table B.6.8-12: Toxicologically relevant histological changes

	Gavage Administration		**Dietary Administration**		
	aqueous control	791-1316 mg/kg bw/day citric acid	basal diet control	low pH diet, 14,000 ppm citric acid	high pH diet, 21,400 ppm trisodium citrate dihydrate
Parotid salivary glands [a]	9	10	10	10	10
Incidence (%)	*100*	*100*	*70*	*100*	*90*
Minimal	8	6	5	0	4
Mild	1	3	2	6	5
Moderate	0	1	0	4	0
Average severity [b]	1.1	1.5	0.9	2.4**	1.4

[a] - number of tissues examined from each group
[b] - 1= minimal, 2= mild and 3= moderate; animals without a histological change were assigned a severity score of 0
** - significantly different from relevant control group ($p < 0.01$) using the Mann-Whitney U-Test

Conclusion by the Notifiers

Citric acid administered orally via gavage or diet and trisodium citrate dihydrate administered via the diet to Sprague Dawley rats for 56 days resulted in higher parotid salivary gland weights and a generally correlative increase in severity of background cytoplasmic alterations in the parotid salivary glands at all dose levels (791-1316 mg/kg bw/day gavage citric acid, 14000 ppm diet citric acid, and 21400 ppm diet trisodium citrate dihydrate). This effects were noted as most severe in the low pH dietary test group. In the absence of cytotoxicity and hyperplasia the noted effects are considered an adaptive response rather than an adverse effect and are consistent with the hypothesis that low pH diets result in adaptive cellular responses within the salivary glands.

RMS comments:

The study is considered supplementary because the test substance to be evaluated, i.e., glyphosate, was not included itself. However, it is suitable to demonstrate that organ weight and histological effects on salivary glands resembling very much those which were observed in some studies following glyphosate administration may be in fact due to low pH conditions in the oral cavity. Such conditions may occur when an acidic diet is administered as it was the case in feeding studies with glyphosate. It is agreed that the resulting salivary gland effects would be then rather adaptive than toxic. However, in principle, organ weight and histological changes may become adverse even if they are adaptive by nature. Furthermore, it cannot be excluded that other mechanisms might have also contributed to the observed findings in the glyphosate studies.

Comparison of sensitivity of different rat strains to salivary gland lesions (Allen, 1996)

Reference:	IIA, 5.10/02
Report:	[redacted] (1996) Glyphosate Acid: Comparison of Salivary Gland Effects in Three Strains of Rat. [redacted] Data owner: Syngenta Study No.: CTL/P/5160 Date: 1996-08-19 Unpublished ASB2012-11520/11537
Guidelines:	Guideline does not exist for this kind of study.
Deviations:	not applicable
GLP:	yes
Acceptability:	*See RMS comment*

Dates of experimental work: 1996-01-15 to 1996-05-14

Materials and methods

Test materials:	
Identification:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 %
Stability of test compound:	No data given in the report.
Vehicle and/ or positive control:	Plain diet
Test animals:	
Species:	Rats
Strain 1:	Alpk:AP_fSD
Source:	[redacted]
Weight at dosing:	175.0 – 176.1 g
Strain 2:	Sprague-Dawley
Source:	[redacted]
Weight at dosing:	179.6 – 181.5 g
Strain 3:	Fischer 344
Source:	[redacted]
Weight at dosing:	107.4 – 108.9 g
Age:	approx. 28-30 days (on delivery)
Sex:	Males
Acclimation period:	11-13 days

Diet/Food:	CT1, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were housed by strain and four per cage.
Environmental conditions:	Temperature: 21 ± 3 °C Humidity: 50 ± 20 % Air changes: at least 15/hour 12 hours light/dark cycle

Animal assignment and treatment:
In a 28 days feeding study groups of 24 male Alpk:AP_fSD (Wistar-derived; AP), Sprague-Dawley (Charles River CD; CD) and Fischer 344 (F344) rats received 0 or 20000 ppm glyphosate acid. Eight animals from each group were killed on Day 29 and the remaining animals were retained without treatment for a further 4 (8 rats/group) or 13 weeks (8 rats/group).
Two test diet batches were prepared prior to start of treatment by mixing 1255 g test substance to 58.745 kg diet and blending. Samples of both preparations were analysed to verify the achieved concentration.

Clinical observations
Clinical examinations were performed daily. A detailed physical examination was performed prior to administration and weekly thereafter.

Body weight
Individual body weights were recorded on start of administration and weekly thereafter.

Food consumption
Food consumption was recorded continuously throughout the study for each cage of rats and calculated as a weekly mean (g food/rat/day) for each cage.

Sacrifice and pathology
All animals sacrificed at scheduled termination were subjected to a gross pathological examination of the salivary glands. Thereafter the salivary glands were removed, weighed (left and right separately) and examined by light microscopy.

Statistics
All data were evaluated using analysis of variance and/or covariance by the GLM procedure in SAS (1989). Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based in the error mean square in the analysis.

Results and discussion
Analysis of dose formulations
The mean achieved concentration of glyphosate acid in both batches of diet was within 2% of the target concentration.

Mortality
There were no treatment-related deaths. One treated AP rat was killed in Week 7 following accidental damage to its snout.

Clinical observations
There were no treatment-related findings in any of the groups noted during the study period.

Body weight
AP rats: During the administration period significant reductions in group mean bodyweight compared to control were seen. At the end of the administration period the difference was approximately 7 %. The reduction in bodyweight was maintained during the 4-week recovery period (approximately 7 % at the end of Week 9) but no differences in body weight were apparent by the end of the 13-week recovery period.

CD rats: Group mean bodyweights for treated animals were significantly reduced during the administration period in comparison to controls. The reduction in body weight was approximately 7 % (after adjusting for initial body weight) at the end of the administration period. However, body weights quickly recovered and were 5 % higher than controls (after adjusting for initial body weight) by the end of the 13-week recovery period.

F344 rats: No treatment related effects were observed.

Food consumption
AP rats: Overall, food consumption in the treated group tended to be slightly lower than the control during the administration period although this did not achieve statistical significance. No effects were seen at the end of the recovery period.

CD rats: Group mean food consumption for treated animals was generally lower than controls during the administration period although this did not always attain statistical significance. Food consumption for the recovery animals returned to control levels by Week 8.

F344 rats: There was no evidence of any treatment related effects.

Organ weights
There was no evidence of any effects of glyphosate acid on the salivary gland weight at any time point in CD rats. On the contrary salivary gland weights were increased in the treated AP and F344 rats at the end of the administration period in comparison to control. While no effects were noted in the four or 13-week recovery AP animals, in F344 rats the salivary gland weights were still increased at these time points, although there was clear evidence of recovery.

Table B.6.8-13: Mean salivary gland weights at necropsy

	AP		**CD**		**F344**	
Organ	0	20000	0	20000	0	20000
	Terminal weight (g)					
Left salivary gland	0.652	0.740*	0.715	0.695	0.461	0.666**
Right salivary gland	0.523	0.659*	0.623	0.626	0.422	0.577*
	Weight after 4 week recovery					
Left salivary gland	0.748	0.703	0.844	0.742	0.488	0.555
Right salivary gland	0.639	0.623	0.701	0.637	0.428	0.505*
	Weight after 13 week recovery					
Left salivary gland	0.750	0.760	0.790	0.819	0.623	0.612
Right salivary gland	0.669	0.681	0.668	0.705	0.495	0.528

No macroscopic abnormalities were seen in salivary glands in any rat, either at the end of the administration period or after the four or 13-week recovery periods.

Treatment-related histological findings were confined to the parotid salivary gland and comprised alteration in the staining of the cytoplasm of the acinar cells. The affected cells appeared strongly basophilic and enlarged (recorded as basophilia of parotid acinar cells).
At the end of the four-week administration period this change was most prominent in F344 rats. All rats showed marked cytoplasmic basophilia that was diffuse, involving the whole of the parotid gland. However, no evidence of cell degeneration or necrosis was seen. Most of the control F344 rats also showed a minor degree of basophilia involving occasional acinar cells only.
The other two strains, AP and CD, both showed the same effect in the parotid gland after four weeks treatment but at a much reduced severity compared to the F344. In addition the distribution was different in that only small focal groups of acinar cells were affected in the AP and CD rats in contrast to the diffuse involvement seen in the F344. The effect was weakest in the CD rat.

The incidence data at the end of the administration period indicate that the background change varies in control rats in the three strains. None was seen in the AP controls, there was a single CD control rat with a minimal focal change, whereas 7 out of 8 F344 controls showed minor changes.
After four weeks recovery in the F344 strain the severity of the parotid basophilia was reduced to minimal or slight and affected small foci of acinar cells only. No changes were seen in the CD rats and only a single AP rat showed a minimal change. As an AP control rat showed changes at this time point this is considered not to be related to treatment.
After 13 weeks recovery no treatment related changes were seen in the AP and CD strains. Slightly more of the F344 rats showed minor focal changes compared to the corresponding control group but this may reflect variations in the background spontaneous change rather than a residual effect of treatment.

Table B.6.8-14: Histopathological findings in salivary glands

Finding	AP		CD		F344	
	0	20000	0	20000	0	20000
	Termination*					
Atrophy (marked)	0 / 8	0 / 8	1/8	0 / 8	0 / 8	0 / 8
Interstitial fibrosis (marked)	0 / 8	0 / 8	1/8	0 / 8	0 / 8	0 / 8
Basophilia of parotid acinar cells	0 / 8	8 / 8	1/8	7 / 8	7 / 8	8 / 8
	4 week recovery*					
Mononuclear cell infiltration (minimal)	0 / 8	1 / 8	0 / 8	0 / 8	1 / 8	0 / 8
Basophilia of parotid acinar cells	1 / 8	1 / 8	0 / 8	0 / 8	0 / 8	6 / 8
Mucous metaplasia of parotid (slight)	0 / 8	1 / 8	0 / 8	0 / 8	0 / 8	0 / 8
	13 week recovery*					
Mononuclear cell infiltration (minimal)	0 / 8	0 / 8	0 / 8	0 / 8	1 / 8	1 / 8
Atrophy (minimal)	0 / 8	0 / 8	0 / 8	0 / 8	1 / 8	0 / 8
Basophilia of parotid acinar cells	1 / 8	1 / 8	1 / 8	1 / 8	1 / 8	5 / 8

* number of animals affected / total number of animals examined

Conclusion by the Notifiers

Administration of diets containing 20000 ppm glyphosate acid to male rats for 4 weeks produced marked strain differences in the severity of effect in the parotid salivary gland. Microscopic examination of the salivary glands showed the most pronounced effect occurred in the F344 strain where there was diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells. Similar but slighter effects occurred in the AP and CD) strains involving small foci of cells only.

Complete recovery of effects was apparent in AP and CD strains following the 4-week recovery period and significant recovery had occurred in the F344 strain. It is not clear whether the slightly higher incidence of minor focal changes in the salivary glands of the F344 strain after 13-week recovery was a residual effect of treatment or represented the random variation in the background incidence in this strain.

RMS comments:

The study is considered acceptable. Clear strain differences became obvious and might, to some extent, explain why salivary gland changes were observed in some but not all rat studies. However, it is still surprising that, e.g., histological lesions were seen in a long-term study in AP rats by Milburn (1996, TOX2000-1998) but not by Brammer (2001, ASB2012-11488) in the same laboratory although nearly identical dose levels had been employed. (However, the test material was of slightly lower purity in the Milburn study.)

It cannot be excluded that similar salivary gland effects as in rats would also occur in humans if exposure to glyphosate was high enough. F344 rats were rarely used for toxicological testing of glyphosate. Thus, it cannot be argued that a rat strain of particular sensitivity was employed in studies that were used for risk assessment. Furthermore, there is no proof that particularly sensitive F344 rats, with regard to salivary gland effects, were a less suitable model for man than other rat strains.

B.6.8.1.1 Further studies

Introduction by RMS:
In this section, different studies are compiled that cover various aspects of toxicology of glyphosate. Two studies were submitted as part of the GTF dossier, are summarised in Table B.6.8-15 and described in detail and commented by the RMS below. Amendments or corrections have been made where necessary.
In the 1998 DAR, some information on mechanisms of toxicity ([redacted], 1992, TOX9552421; [redacted] 1987; TOX9552430) and a possible additive toxic effect of glyphosate with either dalapon or 2,4-D ([redacted] 1987, TOX9551964) is given. The brief descriptions of these studies with conclusions obtained during previous evaluation are copied from the old DAR, subsequent to the new studies.

Table B.6.8-15: Overview on newly submitted mechanistic studies for effects other than on salivary glands (*provided by the Notifiers*)

Reference; Study identification; Owner		Type of study Species, Strain	Application Route (Dose)	Test substance	Purity [%]	Results
	[redacted] 1996; ASB2012-12054; Nufarm	Screening study for pharmaco-logical effects, Sprague-Dawley Rat, ♂ + ♀ for *in vivo* investi-gations, isolated organs from rats and guinea pigs for *ex vivo* experiments	Gavage (5000 mg/kg bw, single dose), for *in vivo* experiments; guinea pig ileum exposed in an isolated organ bath; for examination of neuromuscular activity follo-wing injection into previously killed animals	Glypho-sate technical	95.3	No haematological, electrocardiopgraphic or behavioural/functional changes after oral administration; contractile response similar to that seen with known parasympatho-mimetic agents in isolated guinea pig ileum; no neuromuscular blocking activity on innervated rat gastrognemius muscle
	[redacted] 2012; ASB2012-11521; Monsanto	Mice, B5C3F1 ♀	Diet 0, 500, 1500, 5000 ppm (0, 150, 449, 1448 mg/kg bw/day)	Glypho-sate	95.11	No suppression of the humoral component of the immune system. No test-substance-related effects

<u>Pharmacological activity ([redacted] 1996)</u>

Reference: IIA, 5.10/03
Report: [redacted] (1996)
Glyphosate Technical: Pharmacology Screening Study in the Rat
[redacted]
Data owner: Nufarm
Study No.: 434/021
Date: 1996-06-28
Unpublished
ASB2012-12054
Guidelines: JMAFF, 59 Nohsan No. 4200 (1985)

Deviations: not applicable
GLP: Yes
Acceptability: *See RMS comment*

Dates of experimental work: 1996-02-06 to 1996-04-04

Materials and methods

Test materials:

Identification:	Glyphosate Technical	
Description:	White powder	
Lot/Batch #:	H95D161A	
Purity:	95.3 %	
Stability of test compound:	No data given in the report.	
Vehicle:	*in-vivo*	1% carboxymethyl cellulose
	ex-vivo (guinea pig)	distilled water, krebs physiological buffer solution,
	ex-vivo (guinea pig)	physiological saline

Test animals:

in-vivo	Species:	Rats
Strain:		Sprague-Dawley (CD)
Source:		[redacted]
Age:		no data
Sex:		Males and females
Weight at animal receipt (corrected by RMS):		176 - 200 g
Acclimation period:		At least 6 days
Diet/Food:		SQC Rat and Mouse Diet No.1 Expanded (Special Diets Services Ltd., Witham Essex, UK), *ad libitum*
Water:		Tap water, *ad libitum*
Housing:		By sex in groups of five in polypropylene cages with stainless steel grid floors.
Environmental conditions:		Temperature: 19 – 25 °C Humidity: 40 – 75 % Air changes: at least 15/hour 12 hours light/dark cycle
ex-vivo	Species:	Guinea pig
Strain:		Dunkin Hartley
Source:		David Hall Ltd., Staffordshire, UK
Age:		no data
Sex:		Males
Weight at animal receipt:		250 - 300 g

Acclimation period:		no data
Diet/Food:		Guinea Pig FDI Diet (Special Diets Services Ltd., Witham Essex, UK), *ad libitum*
Water:		Tap water, *ad libitum*
Housing:		By sex in groups of up to three in polypropylene cages with solid floors and sawdust bedding.
Environmental conditions:		Temperature: 17 – 23 °C Humidity: 30 – 70 % Air changes: at least 15/hour 12 hours light/dark cycle
ex vivo (amended by RMS)	Species:	Rats
Strain:		Sprague-Dawley (CD)
Source:		Charles River UK Ltd., Margate, Kent, UK
Age:		no data
Sex:		Males and females
Weight at animal receipt :		110 - 125 g
Acclimation period:		no data
Diet/Food:		SQC Rat and Mouse Diet No.1 Expanded (Special Diets Services Ltd., Witham Essex, UK), *ad libitum*
Water:		Tap water, *ad libitum*
Housing:		By sex in groups of five in polypropylene cages with stainless steel grid floors.
Environmental conditions:		Temperature: 19 – 25 °C Humidity: 30 – 70 % Air changes: at least 15/hour 12 hours light/dark cycle

Animal assignment and treatment of *in vivo* studies:
Three groups of five male and five female rats each received glyphosate technical at a dose level of 5000 mg/kg bw by oral gavage. The control group was similar sized receiving vehicle only. The dosing volume was 10 mL/kg bw. Approximately one hour after dosing control and treated animals were examined for either haematological changes, electrocardiographic changes or behavioural/functional changes.

Blood parameters
Blood samples were taken from all animals via a tail vain. The following parameters were evaluated: Haemoglobin (Hb), total erythrocyte count (RBC), haematocrit (Hct), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), total leucocyte count (WBC), platelet count (PLT) and clotting (Prothrombin) time (CT).

Cardiovascular system
After animals were anaesthetised, cardiac activity was assessed using an electrocardiograph. A limb lead was attached to each limb and connected to the electrocardiogram. The equipment was set to lead II measurement at a sensitivity of either 10 mm/mvolt or 5 mm/mvolt and a chart speed of 25 mm/second. The following parameters were evaluated: Heart rate, P-R interval, QRS interval, Q-T interval, P-amplitude, R-amplitude, T-amplitude.

Nervous system
Animals were placed individually in a purpose built arena and assessed for behaviour and response to various stimuli using a modified Irwin Screen. The following parameters were evaluated: Salivation, hypo/hyperthermia, skin colour, respiration, lacrymation, palpebral closure, pilo-erection, exophthalmia, gait, twitches, tremors, convulsions, abnormal behaviour, tail elevation, transfer arousal, urination, defaecation, vocalisation, finger approach, touch escape, tail & toe pinch, grasp response, auditory startle response, pupil response to light, palpebral reflex.

Animal assignment and treatment of *ex vivo* studies:
Guinea pig - Isolated ileum
Sections of ileum were dissected from previously untreated guinea pigs killed by cervical dislocation. and were transferred to a purpose built isolated organ bath containing Krebs buffer solution with a test substance concentration of 12 mg/mL (maximum solubility). The isolated ileum was connected to the lever arm of an isotonic transducer by a cotton ligature. The transducer was connected to a chart recorder. Contractions of the isolated ileum could then be recorded. Standard solutions of acetylcholine, a known agonist, were prepared and added to the volume of buffer solution used to bathe the isolated ileum. A maximum volume of 2 mL was used for all experiments to ensure the integrity of the tissue in the medium. The contraction response of isolated ileum was recorded for each concentration of acetylcholine to produce a standard curve. Between additions of each new concentration of acetylcholine, the buffer in the organ bath was flushed out and replaced by fresh buffer. The test material, dissolved in buffer, was added and its response compared with standards. Following initial results an antagonist (atropine) to the effects of acetylcholine was added together with the agonist. The results were then compared with the effects of an antagonist and the test material.

The following parameters were evaluated: Response to acetylcholine (agonist), test material, atropine (antagonist).

Rat - Gastrocnemius muscle
Previously untreated rats were killed by cervical dislocation. The abdomen was immediately dissected open and the dorsal aorta exposed. A butterfly needle was inserted into the dorsal aorta, near to the bifurcation in a posterior direction.
A volume of 0.3 mL of lithium heparin at a concentration of 10 mg/mL in sterile saline was injected into the dorsal aorta followed by 0.5 mL of sterile saline.

The experiment itself was performed on separate animals with doses of either the test material dissolved in sterile saline at a concentration of 12 mg/mL (maximum solubility) or of tubocurarine (positive control) that were injected into the dorsal.

The gastrocnemius muscle of the hind limb was exposed with the sciatic nerve intact. The gastrocnemius muscle was detached from the ankle joint and this area was ligated with cotton which was then attached to the lever arm of a transducer. The limb was held in place by a series of pins. An electrical stimulus of 12 volts was applied to the sciatic nerve and the muscle response was recorded. This action was repeated at approximately twelve second intervals until sufficient responses had been recorded.
The following parameters were evaluated: Response to injection of sterile saline, tubocurarine and test material.

Results and discussion

Blood parameters

There were no biologically significant differences, among the parameters measured, between treated and control animals.

Cardiovascular system

There were no biologically significant differences, among the parameters measured, between treated and control animals.

Nervous system

There were no biologically significant differences, among the parameters measured, between treated and control animals.

Guinea pig isolated ileum

The addition of acetylcholine to the medium containing the isolated guinea pig ileum resulted in contraction of the tissue in a concentration related response. Incubation with atropine sulphate immediately prior to addition of acetylcholine diminished or abolished the contraction response in a concentration related manner.

The addition of glyphosate technical at the maximum solubility in buffer also resulted in contraction of the ileum. The force of contraction was increased by an increasing volume of the test material in solution. Incubation with atropine sulphate prior to addition of glyphosate technical also resulted in the abolition of contractile response.

Rat- Gastrocnemius muscle

Injection of tubocurarine at a concentration of 25 mg/mL resulted in a significant diminution of the contractile response of the rat gastrocnemius muscle when the sciatic nerve was stimulated. There was no effect on muscle contraction when either glyphosate technical (12 mg/mL) or physiological saline was injected. The difference in force of response seen with glyphosate technical and physiological saline can be attributed to individual animal variation.

Conclusion by the Notifiers

At a maximum dose level of 5000 mg glyphosate technical/kg bw there were no effects seen from the *in vivo* screens performed. When administered to the isolated guinea pig ileum glyphosate technical caused a contractile response similar to that seen with known parasympathomimetic agents. Evaluation of innervated muscle response using showed that glyphosate technical, when administered at the maximum solubility concentration in physiological saline, did not cause any neuromuscular blocking activity.

RMS comments:

The study is considered acceptable although it will not contribute that much to risk assessment of glyphosate. The conclusion is agreed with. A parasympathicomimetic activity of glyphosate, at least at a high dose level, was shown. This seems to be a bit contradictory to the adrenergic mechanism postulated by Chan and Mahler (1992, TOX9551954) for salivary gland effects but it must be taken into account that different tissues were investigated.

Immunotoxicity in mice ([redacted] 2012)

Reference: IIA, 5.10/04

Report: [redacted] (2012)
Glyphosate – A 28-Day Oral (Dietary) Immunotoxicity Study in Female B6C3F1 Mice
[redacted]
Project No.: WI-10-460 (Study No.: WIL-50393)
Data owner: Monsanto
Date: 2012-03-21
not published
ASB2012-11521

Guidelines: US-EPA OPPTS 870.7800 (1998)

Deviations: None

GLP: Yes

Acceptability: *See RMS comment*

Dates of experimental work: 2010-10-05 - 2010-11-17

Materials and methods

Test material:	
Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	GLP-0807-19475-T
Purity:	95.11 % (dried)
Stability of test compound:	Expiry date: 2011-06-10
Vehicle and/ or positive control:	Basal diet Cyclophosphamid monohydrate
Test animals:	
Species:	Mouse
Strain:	B6C3F1/Crl
Source:	[redacted]
Age:	Approx. 37 days (on arrival)
Sex:	Female
Weight at dosing:	16.5 – 20.0 g
Acclimation period:	14 days
Diet/Food:	Certified Rodent LabDiet® # 5002 (meal) (PMI Nutrition International, LCC.), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel, wire-mesh cages suspended above cage-board.

Environmental conditions:	Temperature:	22 ± 3 °C
	Humidity:	50 ± 20 %
	Air changes:	10/hour
	12 hours light/dark cycle	

Animal assignment and treatment:
In a 28-day oral immunotoxicity study groups of 10 female B6C3F1/Crl mice received daily dietary doses of 0, 500, 1500 and 5000 ppm glyphosate (equivalent to 0, 150, 449 and 1448 mg/kg bw/day).
A further group of 10 females were used as positive immunosuppressive control group. These mice received basal diet for 28 days and were treated with an intraperitoneal (IP) injection of 50 mg/kg bw/day once daily for four consecutive days (study days 24-27).
Test diets were prepared weekly and stored at room temperature. For the negative and positive control groups an appropriate amount of basal diet was weighed into a plastic storage bag. For the test substance groups 500 g of basal diet was weighed (pre-mixture). An appropriate amount of glyphosate was weighted into a mortar, mixed with a small amount of the pre-mixture basal diet, and ground until uniform. This admixture was transferred to a Hobart mixer and mixed with the remainder of the pre-mixture basal diet for five minutes. The resultant mixture was then transferred to a V-blender with a sufficient amount of basal diet to achieve the correct diet concentration and mixed for an additional 10 minutes using an intensifier bar during the first and last three minutes of mixing to ensure a homogeneous mixture. The test diets were prepared from the lowest to highest concentration. The stability and homogeneity of the test substance in the diet was determined in an in-house stability study at 450 and 5500 ppm. Analyses for achieved concentrations on the test diets were done during study weeks 0 and 3.

Mortality
Each animal was checked for mortality or signs of morbidity twice a day during the treatment period, including weekends and public holidays.

Clinical observations
A check for clinical signs of toxicity was made once daily on all animals. In addition, a detailed clinical examination was performed at once a week during the study period, beginning one week prior to randomisation, and on the day of scheduled necropsy.

Body weight
Individual body weights were recorded twice weekly, beginning approximately one week prior to randomization, at the time of animal selection for randomization, on study day 0, and just prior to the scheduled necropsy. Mean body weights and mean body weight changes were calculated for the corresponding intervals.

Food consumption and test substance intake
The quantity of food consumed was recorded for each animal weekly, beginning approximately one week prior to randomization, and just prior to the scheduled necropsy. Food intake was calculated as g/animal/day for the corresponding body weight intervals. The mean amounts of glyphosate consumed (mg/kg/day) per dose group were calculated from the mean food consumed (g/kg of body weight/day) and the appropriate target concentration of glyphosate in the food (mg/kg of diet).

Serum collection for possible IgM antibody analysis
For determination of the possible extent of the suppression of IgM antibody production blood samples were collected from all animals at scheduled necropsy and processed to serum. Following euthanasia by carbon dioxide inhalation, approximately 0.75 mL of blood was collected from the inferior vena cava of each mouse into a tube containing no anticoagulant and allowed to clot. Serum was obtained and aliquots of approximately 150 µL (including any remainder serum) were transferred to cryovials and stored frozen (approximately -70 °C).

Sacrifice and pathology
A complete necropsy was conducted on all animals at scheduled termination or on animals that died or were sacrificed during the study period. Any macroscopic findings were recorded. The following organ weights were determined from all animals surviving to scheduled termination: spleen and thymus.
Tissue samples were taken from the spleen and thymus. Spleen samples were placed in EBSS/HEPES buffer. Thymus samples were preserved in 10% neutral-buffered formalin.

Spleen processing for immunotoxicological evaluation
For the determination of the number of specific IgM antibody-forming cells directed towards sRBC an AFC assay, as a modification of the Jerne plaque assay (Jerne et al., 1963, 1974) was conducted.

Spleens were collected from all animals at the scheduled necropsy (study day 28) immediately following blood collection. Individual spleens were placed into individual tared tubes containing EBSS with 15 mM HEPES, supplemented with gentamicin as a bacteriostat, and maintained on ice. Each tube was then weighed to provide a “wet” weight for each spleen. Spleen samples from Groups 1-4 animals were randomized and coded for antibody-forming cell (AFC) analysis. Spleen samples from Group 5 were labelled as positive control samples for analysis. The spleen samples were placed on crushed ice until procession for AFC analysis.
The spleen samples were processed into single-cell suspensions. The cell suspensions were then centrifuged and resuspended in EBSS with HEPES. Spleen cell counts were performed using a Model Z1™ Coulter Counter®. Viability of splenocytes was determined using propidium iodide and the Coulter® EPICS® XL-MCL™ Flow Cytometer

Statistics
Body weight, body weight change, and food consumption data were subjected to a parametric one way ANOVA to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare the test substance treated groups to the control group.
The positive control data were evaluated using the Student’s t-Test and compared to the basal diet control group.

Organ weight (wet spleen and thymus), final body weight, and AFC data obtained were first tested for homogeneity of variances using the Bartlett’s Chi Square test. Homogeneous data were evaluated using a parametric one-way ANOVA. When significant differences occurred, the treatment groups were compared to the basal diet control group using Dunnett’s test. Non-homogeneous data were evaluated using a non-parametric ANOVA. When significant differences occurred, the treatment groups were compared to the basal diet control group using the Gehan-Wilcoxon test when appropriate. The Jonckheere’s test was used to test for dose-related trends across the basal diet control and test substance treated groups. The

positive control data were evaluated using the Student's t Test and compared to the basal diet control group. The criteria for accepting the results of the positive control group included a statistically significant ($p \leq 0.05$) decrease in the response when compared to the response of the basal diet control group.
The AFC data were expressed as Specific Activity, IgM antibody forming cells per million spleen cells (AFC/106 spleen cells), and as IgM Total Spleen Activity (AFC/spleen).

Results and discussion

Diet analysis
The achieved concentrations of glyphosate in the dietary preparation were in the range of 85.6 - 97.5% of nominal, and therefore within the acceptable range of 85 – 115 %. The diet formulations were homogeneous and stable for 10 days when stored at room temperature with the following exception. During homogeneity/concentration acceptability testing, the 450 ppm diet formulation was 83.1% of target. The 5500 ppm diet formulation was within acceptable range (90.8 %) but was considered low, therefore, calibration standards were prepared as matrix-based samples and a cross-validation was conducted. The diet formulations were reanalyzed using matrix-based calibration standards and met the testing facilities SOP acceptance criteria for homogeneity and concentration acceptability. Based on these results, the protocol-specified doses of test substance were offered to the animals. The test substance was not detected in the basal diet that was offered to the basal diet control (Group 1) and positive control (Group 5) groups.

Mortality
There were no mortalities observed during the study period.

Clinical observations
There were no test substance-related clinical findings.

Body weight
There were no test substance related

Food consumption
There were no test substance-related effects on food consumption noted.

Gross pathology
There were no test substance-related macroscopic effects.

Treatment with the positive control CPS produced a small thymus in three of the 10 animals. These changes were consistent with the known effects of CPS in female B6C3F1 mice.

Organ weights
There were no test substance-related effects on terminal body weights or on spleen or thymus weights (absolute or relative to final body weight) when the test substance-treated groups were compared to the basal diet control group.
Treatment with the positive control CPS produced statistically significantly lower spleen and thymus weights (absolute and relative to final body weight) when compared to the basal diet control group. These changes were consistent with the known effects of CPS in female B6C3F1 mice.
The results of final body and organ weight determinations are presented in the Table B.6.8-16 below.

Table B.6.8-16: Final body weight and organ weight data

Dose group	Body weight	Spleen		Thymus	
		weight	% body weight	weight	% body weight
	(g)#	(mg) #	(%)#	(mg) #	(%)#
1 (negative control)*	20.9 ± 0.3	85.3 ± 3.5	0.41 ± 0.02	44.3 ± 3.5	0.21 ± 0.02
2 (low)	20.6 ± 0.2	82.3 ± 4.6	0.40 ± 0.02	41.5 ± 1.9	0.20 ± 0.01
3 (mid)	21.6 ± 0.3	91.6 ± 6.5	0.42 ± 0.03	45.9 ± 2.7	0.21 ± 0.01
4 (high)	21.3 ± 0.2	86.0 ± 3.6	0.40 ± 0.02	42.0 ± 2.6	0.20 ± 0.01
5 (positive control)	21.5 ± 0.3	50.2 ± 3.2**	0.23 ± 0.02**	13.3 ± 0.8**	0.06 ± 0.01**

Values presented the mean ± SD derived from the number of animals evaluated per dose group
** Statistically significant from negative control at $p \leq 0.01$

AFC assay
There were no test substance-related effects on spleen cell numbers, and in the functional evaluation of the IgM antibody-forming cell (AFC) response, treatment with glyphosate did not result in a statistically significant suppression of the humoral immune response when evaluated as either Specific Activity (AFC/106 spleen cells) or Total Spleen Activity (AFC/spleen). There were no statistically significant differences nor any dose-related trends noted when the basal diet control and test substance-treated groups were compared.
Statistically significantly lower spleen cell numbers, mean specific activity, and mean total spleen activity values were noted in the positive control (CPS treated) group when compared to the basal diet control group. These effects were consistent with the known immunosuppressant effects of CPS and validated the appropriateness of the AFC assay.
The results of the AFC assay are summarised in Table B.6.8-17 below.

Table B.6.8-17: Results of AFC assay

Dose group	Spleen cells	IgM AFC / 10^6 spleen cells	IgM AFC/spleen
	(x 10^7)#	#	(x 10^3)#
1 (negative control)*	11.29 ± 0.65	1160 ± 131	127 ± 11
2 (low)	11.45 ± 0.64	1273 ± 123	144 ± 16
3 (mid)	13.45 ± 1.24	1368 ± 163	190 ± 37
4 (high)	12.51 ± 0.66	1514 ± 204	195 ± 32
5 (positive control)	5.18 ± 0.53**	0 ± 0**	0 ± 0**

Values presented the mean ± SD derived from the number of animals evaluated per dose group
** Statistically significant from negative control at $p \leq 0.01$

Conclusion by the Notifiers
Repeated dietary administration of glyphosate to females B6C3F1 mice did not suppress the humoral component of the immune system. The no-observed-effect level (NOEL) for suppression of the humoral immune response in female B6C3F1 mice offered glyphosate in the diet for 28 days was considered to be 5000 ppm (equivalent to 1448 mg/kg bw/day), the highest dietary concentration.

RMS comments:
The study is considered acceptable. For the parameters included, there was no evidence of immunotoxicity of glyphosate in mice up to a very high dose level.

Studies on mechanisms of toxicity and additive effects (copied from 1998 DAR, ASB2010-10302)

[redacted] ***(1992, TOX9552421):*** *Ammonium salt of glyphosate (MON-8750): General pharmacological study. Institute of Environmental Toxicology, Tokyo, Japan; Study no. IET 90-0149/ET-92-15). Dates of experimental work: 04 February 1992 - 02 March 1992. GLP: yes (self-certification of the laboratory). This report was submitted as part of the joint dossier of Monsanto and Cheminova.*
The potential effects of MON 8750 (purity: 94.78%) on the nervous, respiratory and circulatory systems were assessed following single i.p. injection to male and female ICR mice at doses up to 5000 mg/kg bw and single i.v. injection to urethane-anesthetized and non-anesthetized male rabbits at doses up to 500 mg/kg bw. At the top dose levels, all mice died within 0.5 hours and all anesthetized rabbits within a few minutes after injection. Non-anesthetized rabbits survived i.v. application of 500 mg/kg bw although animals showed some neurological signs. In rabbits which died, heart rate was decreased and ECG changes have been noted. At the next lower dose levels (1250 mg/kg bw in mice or 125 mg/kg bw and 31.3 mg/kg bw in rabbits, respectively), transient symptoms like a decrease in blood pressure, reduced activity and neuromuscular signs were observed but cleared to normal values or behaviour within some hours at the latest. Respiratory rate was increased in surviving rabbits but decreased in anesthetized rabbits which died. It was concluded that an impact on cardiorespiratory functions is involved in acute toxicity. The lethal dose appears to be decreased under anesthesia.

[redacted] ***(1987, TOX9551964):*** *Synergism and potentiation in rats of glyphosate (tech.) of Excel Industries Ltd., Bombay. Study identification and dates of experimental work not given. GLP: no. This report was submitted independently by the notifiers Barclay and Luxan.*
Glyphosate was administered to groups of 10 male Wistar rats as a single oral dose of 5000 mg/kg bw at a constant dose volume of 10 ml/kg in corn oil. Simultaneously, the animals received the compound 2,4-D sodium salt at dose levels of 376, 473, 596, 750, 944 or 1189 mg/kg bw. The second compound used for a potentiation experiment was dalapon at doses of 2500 and 5000 mg/kg bw. After simultaneous dosing, the rats were observed for 14 days for toxic symptoms and mortality. According to the study authors, no potentiation has been observed with glyphosate and dalapon. However, two out of ten rats died after application of 5000 mg/kg bw glyphosate and 5000 mg/kg bw dalapon. When both compound were administered alone, no mortalities occurred.
The simultaneous administration of glyphosate and 2,4-D sodium salt caused a markedly higher mortality in all dose groups. In the groups receiving glyphosate and doses of 596 mg 2,4-D sodium salt/kg bw and above, all animals died. When the latter compound was administered alone, 100% mortality has been reached only at 1189 mg/kg bw. Hence, at least an additive acutely toxic effect of glyphosate and 2,4-D can be assumed.

[redacted]***1987, TOX9552430):*** *Irritating effect of glyphosate, surfactant and Roundup on stomach and small intestine in dogs. Dep. of clinical medicine, University of Tsukuba, Japan. The study was submitted as part of the joint dossier of Monsanto and Cheminova.*
The IPA salt of glyphosate, Roundup herbicide (41% IPA) and the surfactant MON 0818 (15 % of which is contained in Roundup) and 0.25 N hydrochloric acid solution (control) were directly administered on the gastric and small intestinal mucosa of fasted male beagle dogs. The specimens were examined microscopically and evaluated for mucosal damage in comparison with normal gastric and intestinal tissues. Direct application of Roundup®

herbicide, and the surfactant caused mild mucosal damage in the stomach and intestine. These effects were more severe with the Roundup formulation than with either the IPA salt or the surfactant. The intestine appeared to be more affected than the stomach. The severity of the damage was equivalent to that caused be 0.25 N hydrochloric acid.

Further Studies on mechanisms of toxicity and additive effects (submitted after 2000)

Song, H.-Y. et al. (2012, ASB2013-10531): *In vitro* cytotoxic effect of glyphosate mixture containing surfactants.
The authors investigated wheter glyphosate influences the cellular toxicity of the surfactants polyoxyethylene tallow amine (TN-20) and polyoxyethylene lauryl amine ether (LN-10) on the mouse fibroblast like cells, alveolar epithelial cells and a heart cell line. The cytotoxicity of TN-20 qne LN-10 (0.4-100 µM), in the presence or absence of glyphosate was determined by assessing membrane integrity. TN-20 toxicity was significantly lower in the presence of 50 µM glyphosate for the fibroblast-like cell, for the alveolar cells and for the heart cell line compared to that of TN-20 alone. The cellular toxicity of LN-10 towards the fibroblast-like cells was found to be increased in the presence of 50 µM glyphosate. The authors conclude that these results suggest that the mixture toxicity may be a factor in glyphosate-surfactant toxicity in patients with acute glyphosate herbicide intoxication.

Gress, S. et al. (2014, ASB2014-12161): Cardiotoxic electrophysiological effects of the herbicide Roundup in rat and rabbit ventricular myocardium in vitro.
The authors investigated electrophysiological actions in vitro of Roundup (36 % glyphosate) on right ventricular tissues (male Sprague-Dawley rats up to 20,000 ppm; female NZ rabbits at 25 and 50 ppm). According to the authors, Roundup concentrations were selected based on human blood ranges found after acute intoxication. Additionally, the active substance glyphosate (purity not given) was tested only in rats at 18 and 180 ppm, concentrations stimulating the content of glyphosate in Roundup at 50 and 500 ppm. All electrophysiological findings were mainly restricted to rats and only observed after Roundup superfusion. In conclusion, the *in vitro* investigations revealed no evidence for cardiotoxic electrophysiological effects of the active substance glyphosate in rats. However, for Roundup there was evidence for electrophysiological changes, conduction blocks and arrhythmia. Hence, the described results do not influence the risk assessment of the active substance glyphosate in the present Renewal Assessment Report.

B.6.8.3 Studies in farm animals

Table B.6.8-18: Summary of studies in farm animals

Reference; Study identification; Owner	Type of study Species, Sex	Application route (Dose)	Test material	Purity [%]	Results
[redacted] 1987; TOX9552422; Monsanto	Acute oral toxicity, Goat ♀	Gavage (0, 1980, 3090, 4620, and 10 000 mg/kg bw)	Glyphosate	98.7	LD_{50} = 3530 mg/kg bw
[redacted] 1987; TOX9552423; Monsanto	Acute oral toxicity, Goat ♀	Gavage (0, 1400, 4290, 5360, 6700,	Glyphosate isopropyl-amine salt	62.5 (46.2% glyphosate)	LD_{50} = 5700 mg/kg bw [CI 95%, 3.73–8.71]

Reference; Study identification; Owner	Type of study Species, Sex	Application route (Dose)	Test material	Purity [%]	Results
		and 10 000 and mg/kg bw)			
[redacted] 1987; TOX9552424 ; Monsanto	Subacute oral toxicity (7 d), Cow ♀	Gavage (540, 830, 1290, and 2000 mg/kg bw/d; 1000 mg/kg bw/d in pre-test)	Glyphosate isopropyl-amine salt	62.4 (46.2% glyphosate)	MLD = 1290 mg/kg bw/d; LOAEL = 830 mg/kg bw/d; NOAEL = 540 mg/kg bw/d
[redacted] 1987; ASB2010-8131; Monsanto	Subacute oral toxicity (7 d), Cow ♀	Gavage (400, 500, 630, and 790 mg/kg bw/d; 1000 mg/kg bw/d in pre-test)	Glyphosate isopropyl-amine as Roundup ® formulation MON2139	41.1 (30.5% glyphosate)	MLD = 790 mg/kg bw/d; LOAEL = 500 mg/kg bw/d NOAEL = 400 mg/kg bw/d

MLD: minimum lethal dose

B.6.8.3.1 Acute toxicity in goats

Study with glyphosate acid

Reference: IIA, 5.10/05
Report: [redacted] (1987) The acute toxicology of glyphosate in female goats, [redacted]
Data owner: Monsanto
Report No.: VT-80-450
Date: 1987-03-23
TOX9552422
Guidelines: Non-guideline study
Deviations: Not applicable.
GLP: No
Acceptability: *See RMS comments*

Remark: The goats used in this study were *a priory* not tested for pregnancies (in fact, at least three of the goats employed for the study were pregnant) and, thus, females of different hormonal and physiological status were used. Further, weight and age of the test animals were widely distributed. Although randomization was applied with regard to weight, the test population was quite heterogeneous.
Due to the small number of animals investigated, the relevance of the biochemical and haematological findings is questionable.

Dates of experimental work: 1980-12-22 to 1981-05-05

Material and methods
Test material: Glyphosate
Description: white crystalline solid
Lot/Batch #: XHJ-64, NBP1494248

Purity: 98.7 % (N-phosphonomethyl glycine)
Stability of test compound: Stable for > 1 year (protocol date: 1980-08-28)
Vehicle and/or positive control: Water
Test animals: Species: Goat, Strain:not applicable (Spanish goats)
Source: [redacted]
Age: 8 months–4 years
Sex: female
Weight at dosing: 26.4 ± 7.2 kg
Acclimation period: 30 days
Diet/Food: During the initial acclimatization (outdoor): bermudagrass hay and commercial goat meal containing not less than 16 % crude protein [Purina ® Goat Chow ® (Coarse) (Ralston Purina Company, Gonzales, TX, USA)]. After the initial acclimatisation (indoor) the goats were fed bermudagrass only.
Water: not specified
Housing: Outdoor covered pens during acclimation and in indoor pens 11–15 days prior to treatment until the end of the study
Environmental conditions: not stated

Study design and methods
Dose 1980, 3090, 4620, and 10 000 mg/kg bw
4 control groups of 5 goats each
Application route: Oral, gavage
Application volume: 500 mL/goat
Fasting time: not stated
Group size: 5
Post-treatment observation period: 14 days
Observations: Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology
In life dates: not stated

Animal assignment and treatment:
Groups of female Spanish goats received the test item, glyphosate, at dose levels of 10 000, 4620, 3090, and 1980 mg/kg bw by oral gavage in a sequential manner. Initially, the test item was given to a group receiving 10 000 mg/kg/bw and subsequent dosages were selected based on the observed responses. Control animals were treated with tap water. All goats were penned by treatment group and observed daily during pre-treatment and at least twice daily following treatment. Surviving animals were observed for 14–15 days after treatment. Body weights were determined prior to dosing and for surviving animals on post-dosing days 7 and 14. Blood samples were collected on 3 separate days during pre-treatment and after dosing on days 1, 3, 7, and 14. Additional samples were then taken at unscheduled times when dictated by clinical signs or evidence of impending death.

Results and discussion
Mortality
All animals treated with 10 000 and 4620 mg/kg bw glyphosate died. 1/5 animals treated with 3090 mg/kg bw also died.
The results are summarised in the following table.

Table B.6.8-19: Mortality, survival time and animals used

Dose (mg/kg bw)	Toxicological result			Onset of death after	Mortality (%)
	Dead animals	Animals with toxic signs	N		
10 000	5	5	5	3.9–19.2 h	100
4620	5	5	5	2.4–32.2 h	100
3090	1	5	5	71.7 h	20
1980	0	4	5		0
0	0	0	20		0

N = Total number of animals in group

The median lethal dose (LD_{50}) was calculated to be
LD_{50}, goat, oral = 3530 mg/kg bw.

Clinical observations
Clinical signs of toxicity included colic, diarrhea, depression, and ataxia. Most of these signs were observed at all dosage levels except the 1980 mg/kg bw level at which only diarrhea was observed.
In detail: Clinical signs of goats that died included cecession of feeding activity, abdominal distress, depression, ataxia, mild diarrhea, and, shortly prior to death, recumbency. Toxic tubular nephrosis was the only consistent histopathological lesion observed in goats that died.
Clinical signs of surviving goats included decreased feed consumption, diarrhea, and body weight loss.
Clinical signs of goats treated with the minimum dose included decreased feed consumption and diarrhea.
All clinical signs were absent at the end of the experiment (14–15 d).
One control goat gave birth to a single live kid approximately one hour before the time of treatment. Another control goat gave birth to one normal and one small and very weak kid on day 4 of the experiment.
One goat of the 4.62 g/kg bw-treated animals gave birth to two full-term kids during the night before dosing.

Table B.6.8-20: Prominent clinical observations in female Spanish goats given glyphosate

Dose (mg/kg bw)	N	Observations (Number of goats affected)
10 000	5	CFA (5), apparent colic (2), depressed demeanor (2), slight ataxia (3), recumbency (4), death (5)
4620	5	CFA (5), apparent colic (2), depressed demeanor (2), ataxia (2), labored breathing (2), recumbency (4), diarrhea (1), death (5)
3090	5	fatally poisoned goat (1): CFA, diarrhea, apparent colic, subdued demeanor, thirst, ataxia, recumbency, nystagmus, death survivors (4): decreased food consumption (4), apparent colic (2), diarrhea (4)
1980	5	Decreased food consumption (4), diarrhea (3)

CFA: cecession of feeding activity

Body weight
Most animals, which died while on study, exhibited losses in weight immediately prior to death.
Of the animals that survived until terminal sacrifice, those in the 3090 mg/kg bw group had mean body weights less than their respective control group. Goats in the 1980 mg/kg bw group had mean body weights similar to their respective control group.

Food consumption was not precisely measured, but cessation of feeding activity was observed in all treatment groups.

Table B.6.8-21: Survival time, body weights and carcass weights (control groups not pooled)

Dose (mg/kg bw)	**Mean survival time (h)**	**Mean body weight (kg)**				**Mean carcass weight**		
		N	Initial	Day 7	Day 14	N	Weight (kg)	Day
10 000	13.7	5	24.68	–	–	2	26.82	0
Control	S	5	24.36	23.22	23.68	–	–	–
4620	16.46	5	29.46	–	–	5	28.78	0–1
Control	S	5	27.76	26.81	26.63	–	–	–
3090	71.7*	5**	25.81	21.04	22.79	1	30.62	3
Control	S	5	28.58	29.89	28.12	–	–	–
1980	S	5	24.72	22.77	23.54	–	–	–
Control	S	5	25.95	24.95	25.08	–	–	–

–: not applicable
S: all animals survived
* One animal died at day 3 the others survived
** N = 4 for day 7 and 14.

Necropsy
Pulmonary edema to some degree was noted in several animals and was judged to be a terminal event.
The results of the post-mortem observations are summarised in the following table.

Table B.6.8-22: Summary of major gross pathologic diagnoses (animals affected/total)

Dose (mg/kg bw)	Died/ sacrificed	Body as a whole	Respiratory	Cardiovascular	Gastro-intestinal	Urogenital
10 000	5/0	NGPD	Pulmonary edema (4/5) Pneumonia (1/5)	NGPD	NGPD	NGPD
4620	5/0	Serous atrophy of fat (1/5)	Pulmonary edema (4/5)	NGPD	Hepatic atrophy (1/5)	Macerated fetus (1/5)
3090	1/4	Serous atrophy of fat (1/5)	Pneumonia (1/5)	Pericarditis (1/5)	Fatty liver (1/5) Rumen haemorrhage (1/5)	Pallor, kidneys (4/5) Renal hypertrophy (1/5) Endometritis (2/5) Fetal death (1/5)
1980	0/5	NGPD	Pulmonary edema (3/5) Pneumonia (1/5)	NGPD	Fatty liver (1/5) Chronic hepatitis (1/5)	Renal atrophy (1/5)
Controls	0/4*	Minimal fat stores (1/4)	Pulmonary edema (2/4)	NGPD	NGPD	Pallor kidney (2/4) Cystitis (1/4) Metritis (1/4) Endometritis (1/4)

NGPD: No gross pathologic diagnosis
* only one randomly selected goat from each of the 4 control groups subjected to *post mortem* examination

Histopathology
Histological examination was performed only on heart, liver, kidney, spleen and other tissues with grossly visible lesions from animals given glyphosate at 4620 and 3090 mg/kg bw and of 4 sacrificed control animals.
The most consistent finding in fatally poisoned animals was mild to severe tubular nephrosis. 3090 mg/kg bw-treated animals that survived lack such lesions.
4/5 fatally poisoned goats had mild fatty change in the liver.

Clinical biochemistry and haematology
Elevation of blood urea nitrogen concentration, serum creatinine concentration, and numbers of circulating segmental neutrophils were the most consistent laboratory findings in goats given glyphosate. These changes were observed at all dosage levels used in this study.
Clinical laboratory findings were almost universally within or near control limits for surviving glyphosate-treated goats at the end of the experiment (day 14).

The results are summarized in the following tables.

Table B.6.8-23: Biochemical and haematological measurements of goats that died (min–max values)

Measurement (units)	Reference value	Control[1]	Death in less than 4 h (1 goat)[2]	Death in 15–20 h (3 goats)[3]	Death in 32–72 h (2 goats)[4]	Death in 72 h (1 goat)[5]
BUN (mg/dL)	10.0–20.0	2.3–21.3	12.7	16.2–25.0[++]	16.7–35.2[++]	55.5[++]
SC (mg/dL)	1.0–1.8	0.7–1.7	1.5	2.7–3.3[++]	4.0–6.0[++]	14.1[++]
GLU (mg/dL)	50.0–75.0	43–80	407[++]	48–57	35–38	268[++]
Na (mg/dL)	327–356	311–354	303[++]	333–335	313–321	327
K (mg/dL)	13.6–26.1	12.7–21.2	44.8[++]	21.8–31.5	15.2–20.5	12.9
Ca (mg/dL)	8.9–11.7	8.0–15.7	18.9[++]	15.2–18.6[++]	8.7–10.3	6.3[++]
P (mg/dL)	2.9–7.3	3.5–11.3	16.0[++]	5.4–9.5	8.8–17.0[++]	21.6[++]
Mg (mg/dL)	2.8–3.6	1.30–3.20	4.01[++]	2.87–3.61[++]	1.98–3.07	2.38
SGOT (IU/L)	167–513	31–151	78	49–126	60–85	129
LDH (IU/L)	123–392	108–385	295	172–543[++]	268–288	694[++]
SACH (IU/L)	---	319–478	426	272–475	266–287	324
ALKP (IU/L)	93–387	21–902	124	108–430	59–255	350
CPK (IU/L)	20–42	44–878	213	124–184	59–173	3285[++]
GGT (IU/L)	---	12–42	---	23–26	20–21	39
WBC[5]	4.0–15.0	5.5–24.8	14.5	22.5–28.0[++]	13.5–27.0[++]	57.3[++]
SEGS[5]	1.6–7.5	1.4–10.4	7.3	10.8–18.2[++]	4.9–14.9[++]	38.4[++]
BANDS[5]	---	0–1.4	2.8[++]	6.4–7.6[++]	4.1–10.0[++]	11.5[++]
LYMPHS[5]	1.8–9.0	2.4–15.4	3.8	3.1–7.0	1.4–4.5	5.7
EOS[5]	0.1–1.5	0–1.2	0.1	0	0	0
MONO[5]	0.1–0.9	0–0.9	0.6	0.2–0.5	0.1–0.8	1.7
BASO[5]	0–0.2	0–0.5	0	0–0.3	0	0
PCV(%)	19–40	23–42	26.0	31.0–40.0	27.0–28.0	39.0
HGB (g/dL)	8–16	8.1–16.2	8.6	9.4–12.3	8.2–11.0	15.4
RBC[6]	7–21	13.3–29.5	13.6	19.0–24.0	14.0–15.9	21.4
MCV (fL)	15–39	13.0–21.0	19.0	14.0–17.0	16.0–18.0	18.0
MCH (pg)	5.3–8.4	4.4–7.8	6.3	4.7–5.1	5.9–6.9	7.2
MCHC (g/dL)	32–40	26.9–40.8	33.1	30.3–30.8	30.4–39.3	39.5
TSP (g/dL)	6.4–7.0	5.0–8.2	8.9[++]	5.7–6.3	5.6–6.1	6.4

[1]: Control values were obtained by pooling all pre-treatment values from all post-treatment values from untreated controls on a study-wide basis (355 measurements for each parameter)
[2]: Goat sampled at death 3.8 h after receiving 10.0 g/kg
[3]:Goats sampled 11 h after receiving 10.0 g/kg
[4]:Goats sampled 24 h after receiving 4.62 or 3.09 g/kg
[5]:Goat sampled at death 72 h after receiving 3.09 g/kg
[++]: One or more values outside of control limits

Table B.6.8-24: Biochemical and haematological measurements of surviving goats (min–max values)

Measurement (units)	Reference value	Control[1]	Sign of intoxication observed (4 goats)[2]		No signs of intoxication observed (5 goats)[3]	
			days 1 and 3	days 7 and 14	days 1 and 3	days 7 and 14
BUN (mg/dL)	10.0–20.0	2.3–21.3	15.1–45.7[++]	3.1–26.4[++]	5.2–54.5[++]	7.0–31.3
SC (mg/dL)	1.0–1.8	0.7–1.7	1.8–5.3[++]	0.8–1.6	0.8–5.0[++]	0.8–1.3
GLU (mg/dL)	50.0–75.0	43–80	36–87	51–87	55–100	59–98
Na (mg/dL)	327–356	311–354	317–331	301–332	317–347[++]	319–350[++]
K (mg/dL)	13.6–26.1	12.7–21.2	6.7–18.1	9.4–17.0	12.3–18.3	11.9–18.2
Ca (mg/dL)	8.9–11.7	8.0–15.7	6.2–10.3[++]	7.9–10.2	6.1–10.1	8.5–11.4
P (mg/dL)	2.9–7.3	3.5–11.3	4.4–22.4[++]	4.1–8.0	3.7–9.0	5.3–8.7
Mg (mg/dL)	2.8–3.6	1.30–3.20	1.53–2.48	1.53–2.38	1.98–3.22	1.29–2.38
SGOT (IU/L)	167–513	31–151	44–69	47–229[++]	40–91	46–106
LDH (IU/L)	123–392	108–385	100–233	127–415[++]	145–276	144–226
SACH (IU/L)	---	319–478	231–447	221–390	293–440	291–445
ALKP (IU/L)	93–387	21–902	23–273	27–205	41–143	40–211
CPK (IU/L)	20–42	44–878	83–187	73–245	84–264	81–158
GGT (IU/L)	---	12–42	23–34	22–36	21–31	19–34
WBC[5]	4.0–15.0	5.5–24.8	17.5–41.5[++]	8.0–19-5	11.0–22.4	8.0–17.5
SEGS[5]	1.6–7.5	1.4–10.4	11.5–30.7[++]	2-4–14-8[++]	4.1–17.5[++]	2.0–7.2
BANDS[5]	---	0–1.4	0.2–7.5[++]	0.1–0.8	0–0.7	0–1.4
LYMPHS[5]	1.8–9.0	2.4–15.4	2.4–5.4	2.9–12.2	4.0–6.9	5.0–10.2
EOS[5]	0.1–1.5	0–1.2	0–0.2	0–0.3	0–0.3	0–0.7
MONO[5]	0.1–0.9	0–0.9	0–1.0	0.1–0.5	0.1–0.2	0.1–0.4
BASO[5]	0–0.2	0–0.5	0–0.2	0–0.3	0–0.3	0–0.3
PCV(%)	19–40	23–42	30.0–37.0	24.0–38.0	30.0–39.0	28.0–39.0
HGB (g/dL)	8–16	8.1–16.2	11.0–13.0	8.9–12.8	8.3–12.6	9.3–12.2
RBC[6]	7–21	13.3–29.5	17.4–22.4	13.3–20.3	15.0–24.5	16.6–23.6
MCV (fL)	15–39	13.0–21.0	14.0–18.0	15.0–20.0	13.0–19.0	14.0–19.0
MCH (pg)	5.3–8.4	4.4–7.8	5.6–6.9	5.6–7.1	4.8–6.6	4.4–6.8
MCHC (g/dL)	32–40	26.9–40.8	34.2–40.0	29.1–39.3	31.1–35.3	26.9–34.9
TSP (g/dL)	6.4–7.0	5.0–8.2	6.5–7.5	5.5–7.5	6.0–7.3	6.0–7.4

[1]: Control values were obtained by pooling all pre-treatment values from all post-treatment values from untreated controls on a study-wide basis (355 measurements for each parameter)
[2]: Goats given 3.9 g/kg
[3]:Goats given 1.98 g/kg
[++]: One or more values outside of control limits

Conclusion (Notifier)

The median lethal dose (LD_{50}) was calculated to be LD_{50}, goat, oral = 3530 mg/kg bw.

RMS comments: The study is considered acceptable in spite of the heterogeneity of the animals used. It must be taken into account that studies of this type are usually not required in Europe for active ingredients in plant protection products and that no guideline exists. The quality of the study is good and it is suitable to provide additional information about acute oral toxicity in a ruminant species. The extent of investigations exceeded that one in acute toxicity studies in laboratory rodents. However, pathology was confined to four of a total of 20 control animals. In addition, assessment was made a bit difficult by the fact that sometimes data obtained in the four control groups were pooled and sometimes reported separately. The conclusion of the notifier is agreed with. Acute oral toxicity of glyphosate in goats was low although the goat appeared more sensitive than the rat, both with regard to dose levels causing mortality and severity of effects.

Study with glyphosate isopropylamine salt

Reference:	IIA, 5.10/06
Report:	[redacted] (1987) The acute toxicology of the isopropylamine salt of glyphosate in female goats [redacted] Data owner: Monsanto Report No.: VT-80-451 Date: 1987-04-16 TOX9552423
Guidelines:	Non-guideline study
Deviations:	Not applicable
GLP:	Yes *(Not checked by RMS. Not compulsory when the study was performed.)*
Acceptability:	*See RMS comments below.*

Remark: The goats used in this study were *a priory* not tested for pregnancies (at least two goats, one each in the low dose and the control groups, had been pregnant because they aborted) and, thus, females of different hormonal and physiological status were used.
Further, weight and age of the test animals were widely distributed. Although randomization was applied with regard to weight, the test population was quite heterogeneous.
Due to the small number of animals investigated, the relevance of the biochemical and haematological findings is questionable.

Dates of experimental work: 1980-12-22 to 1981-05-08

Material and methods
Test material: MON 0139 (isopropylamine salt of glyphosate/
N-phosphonomethyl glycine isopropylamine)
Description: amber liquid
Lot/Batch #: LURT 08020
Purity: 62.5 % (N-phosphonomethyl glycine isopropylamine)
46.2 % glyphosate/N-phosphonomethyl glycine
Stability of test compound: Stable for > 1 year (protocol date: 1980-08-28)
Vehicle and/or positive control: water
Test animals:
Species: Goat
Strain: not applicable
(Spanish goats, the term is used to distinguish range meat goats from Angoras and dairy breeds. Most of the same origin as the Mexican Criollo but they may show traces of Nubian and Toggenburg blood.)
Source: [redacted]
Age: 8 months–4 years
Sex: Female
Weight at dosing: 27.1 ± 7.0 kg
Acclimation period: 30 days
Diet/Food: During the initial acclimatisation (outdoor): bermudagrass hay and commercial goat meal containing not less than 16 % crude protein (Purina ® Goat Chow ® (Coarse)

(Ralston Purina Company, Gonzales, TX, USA). After the initial acclimatization (indoor) the goats were fed bermudagrass only.
Water: Fresh water
Housing: Outdoor covered pens during acclimation and in indoor pens 11–15 days prior to treatment until the end of the study
Environmental conditions: not stated

Study design and methods
Animal assignment and treatment
Dose 1400, 4290, 5360, 6700, and 10 000 and mg/kg bw
Application route: Oral, gavage
Application volume: 500 mL/goat
Fasting time: not stated
Group size: 5
Four control groups of five animals each included
Post-treatment observation period: 14 days
Observations: Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology
In life dates: not stated

Animal assignment and treatment:
Groups of females Spanish goats received the test item, MON 0139, at dose levels of 10 000, 6700, 5360, 4290, and 1400 mg/kg bw by oral gavage in a sequential manner. Initially, the test item was given at 10 000 mg/kg/bw and subsequent dosages were selected based on the observed responses. Control animals were treated with tap water. All goats were penned by treatment group and observed daily during pre-treatment and at least twice daily following treatment. Surviving animals were observed for 14–15 days after treatment. Body weights were determined prior to dosing and for surviving animals on post-dosing days 7 and 14. Blood samples were collected on 3 separate days during pre-treatment and after dosing on days 1, 3, 7, and 14. Additional samples were then taken at unscheduled times when dictated by clinical signs or evidence of impending death.

Results and discussion
Mortality
Treatment with 10 000, 6700, 5360, 4290, and 14000 mg/kg bw MON 0139 resulted in 5/5, 3/5, 2/5, 2/5, and 0/5 deaths, respectively. In the four control groups, there were also no deaths.
The results are summarised in the following table.

Table B.6.8-25: Mortality, survival time and animals used.

Dose (mg/kg bw)	**Toxicological result**			**Onset of death after**	**Mortality (%)**
	Dead animals	Animals with toxic signs	N		
10 000	5	5	5	15.6–42.0 h	100
0	0	0	5		0
6700	3	5	5	37.0–64.0 h	60
0	0	0	5		0
5360	2	5	5	47.5–49.0 h	40
0	0	0	5		0
4290	2	5	5	53.2–85.1 h	40
0	0	0	5		0
1400	0	2	5	--	0
0	0	0	5		0

N: Number of animals

The median lethal dose (LD_{50}) was calculated to be LD_{50}, goat, oral = 5700 mg/kg bw [CI 95 %, 3.73–8.71].

Clinical observations
Clinical signs of toxicity included colic, diarrhea, taxia and weakness. These signs of toxicity were observed at most dosage levels above 1400 mg/kg bw. Additional symptoms suggestive of central nervous system involvement were observed at dosages of 4290 mg/kg bw and above, including tremors, convulsions, and unusual behaviour.
In detail:
Clinical signs of goats that died included decreased food consumption, abdominal distress, ataxia and, shortly prior to death, recumbency.
One goat that died and one surviving goat each displayed an unusual “collapsing syndrome” of apparent neurological origin approximately 2 days after receiving MON 0139 while other goats displayed various other neurological signs.
One surviving goat developed extensive ulceration of the tongue and oral mucosa. These lesions healed completely by the end of the 14-day observation period.

Table B.6.8-26: Prominent clinical observations in female Spanish goats given glyphosate MON 0139

Dose (mg/kg bw)	N	Observations (Number of goats affected)
10 000	5	non-survivors (5): DFC (5); apparent colic (5); ataxia, shaking and jerking movements (2); depression (2); head bobbing (2); nystagmus (1); recumbency (4); diarrhea (1); paddling (2); mild convulsions (2)
6700	5	non-survivors (3): DFC (3); apparent colic (2); diarrhea (1); bloating (3); ataxia (3); "saw-horse" stance (2); tremor (1); recumbency (3); nystagmus (2); jerky movements (2); blinking (1); terminal clonic-tonic activity (3) survivors (2): DFC (2); bloating (1); abdominal distention (1); tremor (2); "collapsing syndrome" (1); diarrhea (2). (Abortion seen in one control goat)
5360	5	non-survivors (2): DFC (2); diarrha (1); ataxia (2); "saw-horse" stance (1); salivation (1); recumbency (1); tremor (1); "star-gazing" trance (1) survivors (3): DFC (3); diarrhea (3)
4290	5	non-survivors (2): DFC (2); ataxia (1); "collapsing syndrome (1); "chewing convulsions" (1); recumbency (2); salivation (1); terminal opisthotonus-like convulsions (1) survivors (3): DFC (3); diarrhea (3); apparent colic, salivation, lethargy, ulceration of oral mucosa (1)
1400	5	Minimal or no ill effects seen; reduced urination; abdominal distention (2) (Abortion seen in one control animal)

DFC: Decreased food consumption

Body weight
No statistically significant differences in body weight gain were observed between the groups treated with MON 0139 and their respective control groups.
Food consumption, although not precisely measured, was greatly reduced following treatment with MON 0139 at dosages of 4290 mg/kg bw and above. For most groups, feeding activity gradually increased, reaching normal levels by the end of the study.

Necropsy
No lesions considered to be treatment-related were noted at gross necropsy.

Table B.6.8-27: Summary of major gross pathologic diagnoses (animals affected/total)

Dose (mg/kg bw)	Died/ sacrifiecd	Body as a whole	Respiratory	Cardiovascular	Gastro-intestinal	Urogenital
10 000	5/0	NGPD	pulmonary edema (2/5)	NGPD	NGPD	NGPD
6700	3/2	NGPD	pulmonary edema (1/5)	NGPD	bloat (2/5)	pale kidneys (1/5) renal hypertrophy (1/5)
5360	2/3	minimal fat stores (1/5)	pulmonary edema (3/5)	NGPD	fatty liver (4/5)	pale kidneys (2/5)
4290	2/3	NGPD	pulmonary edema (3/5)	NGPD	fatty liver (1/5) gallbladder edema (1/5)	pale kidneys (2/5) renal hypertrophy (1/5)
1400	0/5	minimal fat stores (1/5)	pneumonia (1/5) pulmonary edema (1/5)	pericarditis (1/5)	fatty liver (1/5)	metritis (1/5) pregnancy (1/5)
Control	0/4*	minimal fat stores (1/4)	pulmonary edema (2/4)	NGPD	NGPD	pale kidneys (2/4) mild cystitis (1/4) metritis (1/4) endometritis (1/4)

NGPD: No gross pathologic diagnosis
*: 1 goats of each of the four control groups was subjected to *post mortem* investigation

Histopathology
Mild to severe tubular nephrosis was the only consistent histopathological lesion observed in goats that died. However, this tubular nephrosis was not observed in goats that survived until the end if the experiment.
Tubular nephrosis is considered to be diagnostically significant for goats that died a few days after an appropriate level of exposure.
These lesions may have contributed to the observed elevations in blood urea nitrogen (BUN) and serum creatine (SC).

Ischemic hippocampal neurons were observed in the brain of one goat that had displayed the so-called “collapsing syndrome”.

Clinical biochemistry and hematology
Slight to moderate elevations in blood urea nitrogen (BUN) and serum creatine concentrations (SC) were observed in all animals that died during the study. These findings may be related to the histopathologic kidney lesions observed in these animals.

Slight elevations in serum glutamic oxaloacetic transaminase (SGOT) and lactic dehydrogenase (LDH) activity were also observed in terminal animals immediately prior to death.
No other diagnostically or toxicologically significant changes were observed.

None of the biochemical parameters measured appeared to be involved in, or indicate the cause of, the unusual neurological manifestations seen in some goats receiving MON 0139.

The results are summarized in the following tables.

Table B.6.8-28: Biochemical and haematological measurements of goats that died (min–max values)

Measurement (units)	Reference value	Control[1]	Surviving < 49 h		Surviving > 50, < 90 h	
			Sampled 12–14 h before death (2 goats)[2]	Sampled < 4 h before death (5 goats)[3]	Sampled at 24 h post-treatment (4 goats)[4]	Sampled 6–16 h before death (3goats)[5]
BUN (mg/dL)	10.0–20.0	2.3–21.3	26.7–44.1	31.5–62.4	13.8–22.0	44.2–111.4
SC (mg/dL)	1.0–1.8	0.6–1.7	4.5–6.1	3.3–5.8	1.4–2.9	5.1–6.7
GLU (mg/dL)	50.0–75.0	37–80	120–164	17–65	74–173	57–196
Na (mg/dL)	327–356	311–349	347–376	327–396	331–343	327–345
K (mg/dL)	13.6–26.1	13.4–21.2	16.2–16.5	10.9–23.2	10.3–16.9	9.0–11.0
Ca (mg/dL)	8.9–11.7	8.0–13.4	8.7–11.5	8.5–9.8	8.6–11.3	7.2–8.6
P (mg/dL)	2.9–7.3	4.1–11.9	2.9–16.0	3.5–10.0	3.9–6.4	2.9–5.9
Mg (mg/dL)	2.8–3.6	1.3–3.4	3.0–3.9	2.3–4.1	2.1–3.5	3.0–4.0
SGOT (U/L)	167–513	31–151	99–100	128–459	58–107	105–145
LDH (U/L)	123–392	145–385	303–310	394–880	220–298	266–394
SACH (U/L)	---	240–496	405–461	388–536	344–459	409–471
ALKP (U/L)	93–387	31–902	69–283	76–282	97–422	173–476
CPK (U/L)	20–42	44–878	144–185	181–2631	110–149	183–1145
GGT (U/L)	---	12–60	19–26	23–38	30–35	30–38
WBC[5]	4.0–15.0	5.5–17.5	12.0–14.0	3.7–21.5	9.0–23.0	10.0–18.0
SEGS[5]	1.6–7.5	1.4–10.4	3.1–7.0	1.3–4.8	4.1–16.6	1.8–6.9
BANDS[5]	---	0–1.4	3.6–5.9	1.3–12.1	0–4.8	1.4–9.7
LYMPHS[5]	1.8–9.0	2.4–9.2	2.6–2.8	1.0–6.5	1.4–4.9	1.7–4.4
EOS[5]	0.1–1.5	0–1.4	0–0	0–0	0–0.1	0–0
MONO[5]	0.1–0.9	0–0.9	0.4–0.6	0–0.9	0.1–0.4	0.1–0.5
BASO[5]	0–0.2	0–0.8	0–0	0–0	0–0.1	0–0
PCV(%)	19–40	23–47	26–49	33–45	34–45	31–42
HGB (g/dL)	8–16	7.8–16.7	9.6–15.6	10.6–15.6	10.8–14.4	10.6–14.8
RBC[6]	7–21	12.8–24.2	15.6–23.8	16.0–26.9	19.0–24.5	16.7–22.9
MCV (fL)	15–39	15–21	17.0–18.0	15.0–20.0	15.0–19.0	15.0–18.0
MCH (pg)	5.3–8.4	4.6–7.8	6.2–6.6	4.8–7.3	5.5–6.5	5.4–6.5
MCHC (g/dL)	32–40	28–41	31.8–36.9	31–35	32–37	32–35
TSP (g/dL)	6.4–7.0	5.0–8.2	6.7–8.6	5.8–7.3	7.2–7.7	6.2–7.7

[1]: Control values were obtained by pooling all pre-treatment values from all post-treatment values from untreated controls on a study-wide basis (355 measurements for each parameter)
[2]: Goats treated with 10 000 and 5360 mg/kg bw
[3]: Goats treated with 10 000 (n = 3), 6700 (n = 1), and 5360 (n = 1) mg/kg bw
[4]:Goats treated with 6700 (n = 2) and 4290 (n = 2) mg/kg bw
[5]:Goats treated with 6700 (n = 2) and 4290 (n = 1) mg/kg bw

Table B.6.8-29: Biochemical and haematological measurements of surviving goats (min–max values)

Measurement (units)	Reference value	Control[1]	Definitive signs of intoxication observed (8 goats) [2]		Minimal or no signs of intoxication onserved (5 goats) [3]	
			days 1 and 3	days 7 and 14	days 1 and 3	days 7 and 14
BUN (mg/dL)	10.0–20.0	2.3–21.3	7.6–28.7	3.7–135.6	3.9–13.6	6.0–19.1
SC (mg/dL)	1.0–1.8	0.6–1.7	1.0–1.9	0.8–9.7	0.8–1.4	0.9–1.4
GLU (mg/dL)	50.0–75.0	37–80	60–139	50–90	40–59	29–58
Na (mg/dL)	327–356	311–349	309–337	319–337	323–337	317–337
K (mg/dL)	13.6–26.1	13.4–21.2	12.2–17.6	8.0–19.5	13.9–19.5	15.0–19.4
Ca (mg/dL)	8.9–11.7	8.0–13.4	8.9–11.1	8.7–10.7	9.3–10.1	9.2–10.0
P (mg/dL)	2.9–7.3	4.1–11.9	3.5–10.2	3.8–9.7	4.5–9.3	4.9–8.8
Mg (mg/dL)	2.8–3.6	1.3–3.4	1.8–2.4	1.3–2.4	1.9–2.3	1.9–2.1
SGOT (U/L)	167–513	31–151	34–77	3.7–169	53–81	50–83
LDH (U/L)	123–392	145–385	118–232	141–3.17	170–242	151–238
SACH (U/L)	---	240–496	293–528	209–450	273–386	255–407
ALKP (U/L)	93–387	31–902	58–594	52–542	31–323	30–307
CPK (U/L)	20–42	44–878	58–124	44–180	54–154	44–152
GGT (U/L)	---	12–60	23–43	21–42	15–40	11–30
WBC[5]	4.0–15.0	5.5–17.5	5.7–19.5	8.0–35.5	7.5–12.5	7.5–20.0
SEGS[5]	1.6–7.5	1.4–10.4	2.0–14.0	2.0–9.7	2.9–7.2	3.4–16.4
BANDS[5]	---	0–1.4	0–1.0	0–1.8	0–0.3	0–0.6
LYMPHS[5]	1.8–9.0	2.4–9.2	2.0–6.7	3.1–8.4	2.9–6.4	2.0–6.3
EOS[5]	0.1–1.5	0–1.4	0–0.1	0–0.7	0–0.7	0–0.4
MONO[5]	0.1–0.9	0–0.9	0–0.8	0–0.7	0–0.2	0.1–3.9
BASO[5]	0–0.2	0–0.8	0–0.3	0–0.6	0–0.5	0–0.4
PCV(%)	19–40	23–47	29–39	27–38	24–32	24–34
HGB (g/dL)	8–16	7.8–16.7	10.4–12.9	9.4–12.4	11.3–12.2	9.8–11.3
RBC[6]	7–21	12.8–24.2	15.6–23.2	15.7–21.9	18.0–19.0	15.7–17.9
MCV (fL)	15–39	15–21	15.0–19.0	13.0–19.0	14.0–21.0	15.0–210
MCH (pg)	5.3–8.4	4.6–7.8	5.1–7.5	5.3–7.0	5.0–6.9	5.2–7.2
MCHC (g/dL)	32–40	28–41	32–38	31–37	33–40	34–41
TSP (g/dL)	6.4–7.0	5.0–8.2	5.9–8.7	5.6–7.7	6.1–7.2	5.9–7.1

[1]: Control values were obtained by pooling all pre-treatment values from all post-treatment values from untreated controls on a study-wide basis (355 measurements for each parameter)
[2]: Goats treated with 6700 (n = 2), 5360 (n = 3) and 4290 (n = 3) mg/kg bw
[3]:Goats treated with14000 (n = 5) mg/kg bw

Conclusion

The median lethal dose (LD_{50}) was calculated to be LD_{50}, goat, oral = 5700 mg/kg bw [CI 95 %, 3.73–8.71].

RMS comments: The study is considered acceptable in spite of the heterogeneity of the animals used. It must be taken into account that studies of this type are usually not required in Europe for active ingredients in plant protection products and that no guideline exists. The quality of the study is good and it is suitable to provide additional information about acute oral toxicity in a ruminant species. The extent of investigations exceeded that one in acute toxicity studies in laboratory rodents. However, pathology was confined to four of a total of 20 control animals. In addition, assessment was made a bit difficult by the fact that sometimes data obtained in the four control groups were pooled and sometimes reported separately.

The conclusion of the notifier is agreed with. Acute oral toxicity of the isopropylamine salt of glyphosate in goats was low and even lower than with the acid. This finding may confirm that the salt formulations of glyphosate tend to be less toxic than the acid.

B.6.8.3.2 Subacute toxicity in cows

Subacute study with glyphosate isopropylamine salt

Reference:	IIA, 5.10/07
Report:	[redacted] (1987) The subacute toxicity of the isopropylamine salt of glyphosate (MON 0139) in female cattle [redacted] Data owner: Monsanto Report No.: VT-82-003 Date: 1987-04-16 TOX9552424
Guidelines:	None guideline study
Deviations:	Not applicable.
GLP:	Yes *(Not checked by RMS. Not compulsory when the study was performed.)*
Acceptability:	*See RMS comments below.*

Dates of experimental work: 1982-02-02 to 1982-05-05

Material and methods
Test material: MON 0139 (isopropylamine salt of glyphosate/
N-phosphonomethyl glycine isopropylamine)
Description: amber liquid
Lot/Batch #: LBRT 08023
Purity: 62.4%, (46.2% glyphosate only)
Stability of test compound: Stable for > 1 year (protocol date: 1980-01-18)
Vehicle and/or positive control: Water
Test animals:
Species: Cow
Strain: not applicable (Brahman cross)
Source: [redacted]
Age: not specified
Sex: Female (heifer/nulliparous)
Weight at dosing: 215.6 ± 23.1 kg
Acclimation period: at least 30 days
Diet/Food: During the initial acclimatization (outdoor): bermudagrass hay and commercial giat meal containing not less than 13 % crude protein (Purina ® Commercial Creep Chow ® (Ralston Purina Company, Gonzales, TX, USA). After the initial acclimatization (indoor) the heifers were fed bermudagrass only.
Water: Fresh water
Housing: Outdoor covered pens during acclimation and in indoor pens 11–15 days prior to treatment until the end of the study
Environmental conditions: not stated

Study design and methods
Animal assignment and treatment
Dose (based on a preliminary trial with 1000 mg/kg bw)
540, 830, 1290, and 2000 mg/kg bw
Application route: Oral, gavage
Application volume: approx. 500 mL/animal
Fasting time: not stated
Group size: 3/group or 2/group
4 control groups of 2 cows each
Post-treatment observation period: 14 days
Observations: Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology
In life dates: not stated

Animal assignment and treatment:
Brahman-cross heifers were treated in a sequential manner with MON 0139 by stomach tube in 7 consecutive daily doses of 540, 830, 1000 (pre-test, n = 2), 1290, and 2000 mg/kg bw (n = 3/group). Initially, the test item was given at 2000 mg/kg/bw/day and subsequent dosages were selected based on the observed responses. Control animals were treated with tap water. All animals were penned by treatment group and observed daily during pre-treatment and at least twice daily following treatment. Surviving animals were observed for 14–15 days after treatment. Body weights were determined prior to dosing and for surviving animals on post-dosing days 7 and 14. Blood samples were collected on 3 separate days during pre-treatment and after dosing on days 1, 3, 7, and 14. Additional samples were then taken at unscheduled times when dictated by clinical signs or evidence of impending death.

Results and discussion
Mortality
In the preliminary study neither heifer exposed to 1000 mg/kg bw died.
All animals treated with 2000 mg/kg bw MON 0139 died. 1/3 animals treated with 1290 mg/kg bw also died.
The results are summarised in the following table.

Table B.6.8-30: Mortality, survival time and animals used.

Dose (mg/kg bw)	**Toxicological result**			**Onset of death after**	**Mortality (%)**
	Dead animals	Animals with toxic signs	N		
2000	3	3	3	6.2–7.5	100
1290	1	3	3	7.5	33.3
1000*	0	2	2	--	0
830	0	3	3	--	0
540	0	0	3	--	0
0	0	0	8**		0

N: Number of animals
*: Preliminary test
**: All four control groups compiled

The minimum lethal dose (MLD) was 1290 mg/kg bw.
The minimum toxic dose (MTD) was 830 mg/kg bw.

Clinical observations
In the preliminary study, treatment with 1000 mg/kg bw MON 0139 decreased food intake and induced diarrhea by the second day of treatment which continued throughout the seven day treatment period. The signs ceased until the end of the study.
Treatment with 2000, 1290, and 830 mg/kg bw induced diarrhea.
Animals exposed to 2000 mg/kg bw MON 0139 additionally showed nervous system effects including head tremors, convulsions, ataxia, and possible visual impairment and sternal recumbancy.

Table B.6.8-31: Prominent clinical observations in female cows given MON 0139 (numbers of animals affected)

Dose (mg/kg bw)	N	Observations (Number of goats affected)
2000	3	diarrhea (3), decreased feed intake (3), nasal discharge (3), foamy salivation (1), head tremors (3), belligerency (1), whole-body tremors (3), ataxoa (3), head pressing (1), kicking at imaginary objects (1), apparent visual impairment (1), convulsions (1), falling (1), depression (1), recumbency (3), increased respiratory effort (2), death (3)
1290	3	diarrhea (3), decreased feed intake (3), depression (3), weakness (2), death (1)
1000	2	diarrhea (2); decreased feed intake (2)
830	3	diarrhea (3), decreased feed intake (3)
540	3	no signs of toxicosis (3)

CFA: cecession of feeding activity

Body weight and food consumption:
Treatment decreased body weight and food intake, as follows. However, the food intake was not precisely measured.
In the preliminary study, treatment with 1000 mg/kg bw MON 0139 decreased food intake.
Treatment with 2000 and 1290 mg/kg bw decreased food intake.
Treatment with 1290 mg/kg bw induced severe weight loss and depression for the first two weeks. While food consumption and fecal consistency returned to normal during the third week after treatment, the animals remained thin and weakened.
The results are summarized in the following table.

Table B.6.8-32: Mean percent body weight changes (from initial body weight) in cows (n) treated with MON 0139

Dose (mg/kg bw/day)	Study day	MON 0139-treated	Controls
2000	6	−1.2 (3)	−1.9 (2)
1290	6	−6.5[a] (3)	−2.1 (2)
	14	−12.0[b] (2)	−1.6 (2)
	21	−9.6 (2)	−3.4 (2)
830	6	−5.2[a] (3)	−1.5 (2)
	14	−4.6 (3)	−0.4 (2)
	21	−2.4[a] (3)	−5.1 (2)
540	6	0.3 (3)	−0.7 (2)
	14	1.0 (3)	2.7 (2)
	21	0.4 (3)	1.5 (2)

[a]:P < 0.083
[b]:P < 0.121

Necropsy
Few significant gross lesions were noted in fatally poisoned heifers other than dehydration, loss of weight and signs indicative of gastrointestinal irritation.
Kidney and liver to brain weight ratios were elevated at 2000 and 1290 mg/kg bw/day.

Table B.6.8-33: Mean kidney and liver weight/brain weight ratios

Dose	N	Kidney/brain	Liver/brain
2000	3	1.86[a]	7.57 [a]
0*	2	1.25	6.45
1290	3	1.49 [a]	5.96
0*	2	0.96	4.81
830	3	1.14 [a]	5.75
0*	2	1.27	6.08
540	3	1.18	6.14
0*	2	1.19	5.92

*: Concurrent control for preceding MON 0139-treated group
[a]: P < 0.83

Major gross pathologic findings are summarized in the following table.

Table B.6.8-34: Major gross pathologic findings (number)

Dose	Skin	Cardiovascular	Hemic & lymphatic	Hemic & lymphatic	Digestive	Urogenital
2000 (n = 3)	Dermatitis (3)	Aspiration (1) Nodules (1) Pulmonary edema (2)	Epicardial petechiae (3)	Petechiae, capsule of spleen (1)	Errosions, abomasal mucos a(1) Congestion intestine (3) Diarrhea (3) HLBR (3) Distension, gall bladder (1) Congestion liver (1)	HKBR (3)
0* (n = 2)	Dermatitis (2)					Infarct, rt kidney (1)
1290** (n = 3)	Dermatitis (2)	Petechiae, pleura (1)	Epicardial petechiae (1) Hemocyst, heart valve (1)		Scars, capsule of liver (1) Errosions, abomasal mucos a(1) Congestion intestine (1)	HKBR (2)
0* (n = 2)	Dermatitis (2)	Petechiae, trachea mucosa and pleura (1)			Petechiae, duodenum, congestion, peyer's patches (1) Erosion, esophagus (1)	Petechiae, urinary bladder (1)
830 (n = 3)	Dermatitis (1)				Congestion intestine (1) Scars, liver capsule (1)	
0* (n = 2)	Dermatitis (1)		Cogestion, spleen (1)		Petechiae, abomasal mucosa Congestion intestine (2)	Congestion, vulvar mucosa (1)
540 (n = 3)		Subpleural focus (1) Red foci, subpleural (1)			Erosions, esophagus (1) HLBR (1) Congestion intestine (1) Congestion peyer's patches (1) mottling, liver (1)	HKBR (1)
0* (n = 2)	Dermatitis (1)			Lymphoid hyperplasia pharyngeal nodes (1)	Lipoma (1) Parasitism (1)	

*: Concurrent control for preceding MON 0139-treated group
**: 1/3 bilateral hyphema
NGL: no gross lesions
HLBR: high liver weight to brain weight ratio relative to controls
HKBR: high kidney weight to brain weight ratio relative to controls
No findings for nervous, endocrine, muscular skeletal organs or systems, or the body as a whole

Histopathology
Microscopic examination of kidney from the dead heifer treated with 1290 mg/kg bw/day revealed marked renal tubular vacuolization.
Histologic examination of the abmomasum revealed multifocal superficial mucosal erosions, which appeared to be of recent development based on the minimal extent of the associated cellular reaction.
Segmental congestion of the ileum was characterized microscopically by focal necrosis and inflammation of the mucosa overlying Peyer's patches.
The two surviving heifers treated with 1290 mg/kg bw/day each had mild renal tubular vacuolization.
No treatment-related microscopic lesions were observed at dosages of below 1290 mg/kg bw/day or in the 6 of eight control animals that had been subjected to histopathology.

Clinical biochemistry and hematology
Treatment with 1290 and 2000 mg/kg bw/day MON 0139 increased haematocrit, haemoglobin, red blood cells and increased serum levels of total protein, urea nitrogen (BUN), creatinine and serum glutamic oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH), and creatome phosphokinase (CPK) activities.
The hematologic alterations were considered to be due to hemo-concentration secondary to fluid shifts resulting from diarrhea.
Elevations in CPK, SGOT, and LDH activities were attributed to muscle damage resulting from convulsions and/or prolonged sternal recumbancy.
Slight elevations of BUN and creatinine may have been due to decreased renal perfusion produced by dehydration secondary to diarrhea. However, the presence of histopathological kidney lesions at 1290 mg/kg bw/day and changes in serum electrolyte levels at several dosages suggest that these changes may have been partly due to some renal impairment.
An increase in the number of neutrophils and a decrease in the number of lymphocytes observed at 1290 and 2000 mg/kg bw/day probably represented a response to stress-induced release of corticosteroids.

Remark: Due to the small number of animals investigated, the relevance of the biochemical and haematological findings is questionable.

The results are summarized in the following tables.

Table B.6.8-35: Days after treatment on which values for clinical laboratory measurements performed on cows treated with MON 0139 were significantly different from concurrent median control values

Measurement	**Dose (mg/kg/bw/day)**			
	2000	1290[a]	830	540
	Days after treatment			
BUN	6	2, 6, 7, 8, 14	---	8⁻
CREAT	6	7, 8	---	8⁻
GLU	---	7	---	---
Na	6	6⁻, 14⁻	---	---
K	6⁻	6⁻, 8⁻, 14⁻	6⁻	14⁻
Ca	6⁻	---	8⁻	---
P	6	7, 21⁻	2	---
Mg	6	7, 8	2	---

Measurement	**Dose (mg/kg/bw/day)**			
	2000	1290[a]	830	540
	Days after treatment			
SGOT	6[§]	2, 6, 7, 8, 14, 21	---	---
LDH	6[§]	6, 7	---	---
SACH	---	14[–]	21[–]	---
ALKP	---	7	---	---
CKN	6	2, 6, 7, 8	---	---
GGT	---	---	---	8,14 ,20
WBC	---	---	---	---
SEGSA	6	---	---	---
BANDSA	6	---	---	---
LYMPHSA	6[–]	6[–]	---	---
EOSA	2[&], 6[&]	6[&],8[&]	---	14[–], 21[–]
MONOSA	---	---	---	---
BASOA	---	---	---	---
PCV	6	6[§], 7, 8	---	---
HBG	6	6, 7, 8	---	---
RBC	6[§]	6, 7, 8	---	---
MCV	---	---	---	---
MCH	---	---	---	---
MCHC	---	6, 21	---	---
STP	6	6, 7, 8	---	---

[a]: represents data from 3 animals on days 2 and 6 and 2 animals on days 8, 14, and 21. Entries for day 7 represent one animal, and the value is outside the range of study-wide control values.
[–]: Medians significantly lower than the concurrent median control value, entries without a symbol represent medians significantly greater than the concurrent median control values
[§]: noteworthy non-significant increase
[&]:noteworthy non-significant decrease

Conclusion

The minimum lethal dose (MLD) was MLD, cow, oral = 1290 mg/kg bw.
The minimum toxic dose (MTD) was MTD, cow, oral = 830 mg/kg bw.

<u>RMS comments:</u> The study is considered acceptable despite the low number of animals per dose group. However, studies of this type are usually not required in Europe for active ingredients in plant protection products and no guideline exists. The quality of the study is good and it is suitable to provide additional information about subacute oral toxicity in a ruminant species. The conclusion of the notifier is agreed with but the "MTD" should be rather considered the LOAEL to make it more comparable to routine toxicological studies and to avoid misunderstandings because "MTD" is often read as "maximum tolerated dose". The low dose of 540 mg/kg bw/day may be considered the NOAEL in this study. Subacute oral toxicity of the isopropylamine salt of glyphosate in young cattle (heifers) was low but signs and findings were more pronounced than in subacute studies (of longer duration) with glyphosate acid in rats and dogs.

Subacute study with a plant protection product

Reference: IIA, 5.10/08
Report: [redacted] (1987) The subacute toxicity of Roundup ® herbicide (MON-2139) in female cattle
[redacted]
Data owner: Monsanto
Report No.: VT-82-002
Date: 1987-03-23
ASB2010-8131
Guidelines: Non-guideline study
Deviations: Not applicable.
GLP: Yes *(Not checked by RMS. Not compulsory when the study was performed.)*
Acceptability: *See RMS comments below.*

Remark (Notifier): The animals were of different hormonal status (observed pregnancy) and partly infested with parasites. This heterogenicity of the study population combined with the small number of animals tested renders in particular the relevance of the clinical biochemistry and hematology findings questionable.

(Comment by RMS: It must be clarified that, according to the original study report, only in one control animal some evidence of parasitic infestation was obtained, based on eosinophilic perivascular infiltrates in the liver. In contrast, many animals on study were suffering from trichophytosis, i.e., a mycotic dermatitis.)

Dates of experimental work: 1982-02-02 to 1982-05-05

Material and methods
Test material: MON 2139 (Roundup ® formulation,
N-phosphonomethyl glycine isopropylamine)
Description: amber liquid
Lot/Batch #: LBRP 01002
Purity: 41.1%, (N-phosphonomethyl glycine isopropylamine, 30.5% glyphosate only)
Stability of test compound: Stable for > 1 year (protocol date: 1980-01-18)
Vehicle and/or positive control: Water
Test animals: Species: Cow
Strain: not applicable (Brahman cross)
Source: [redacted]
Age: not specified
Sex: Female (heifer/nulliparous)
Weight at dosing: 211.6 ± 21.5 kg
Acclimation period: at least 30 days
Diet/Food: During the initial acclimatization (outdoor): bermudagrass hay and commercial giat meal containing not less than 13% crude protein (Purina ® Commercial Creep Chow ® (Ralston Purina Company, Gonzales, TX, USA). After the initial acclimatization (indoor) the heifers were fed bermudagrass only.
Water: Fresh water

Housing: Outdoor covered pens during acclimation and in indoor pens 11–15 days prior to treatment until the end of the study
Environmental conditions: not stated

Study design and methods
Animal assignment and treatment
Dose (based on a preliminary trial with 1000 mg/kg bw)
400, 500, 630, and 790 mg/kg bw
4 control groups included
Application route: oral, rumen intubation
Application volume: approx. 500 mL/animal
Fasting time: not stated
Group size: 3/group or 2/group
Post-treatment observation period: 14 days
Observations: Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology
In life dates: not stated

Animal assignment and treatment:
Brahman-cross heifers were treated in a sequential manner with MON 2139 by intubation in 7 consecutive daily doses of 400, 500, 630,790 (n = 3/group), and 1000 mg/kg bw (n = 2/group, preliminary test). Initially, the test item was given at 1000 mg/kg/bw/day and subsequent dosages were selected based on the observed responses. Control animals (n = 2/group) were treated with fresh water. All animals were penned by treatment group and observed daily during pre-treatment and at least twice daily following treatment. Surviving animals were observed for 14–15 days after treatment.
Body weights were determined prior to dosing and for surviving animals on post-dosing days 6, 14, and 21. Blood samples were collected on 3 separate days during pre-treatment and after dosing on days 2, 6, 8, 14, and 21. Additional samples were then taken at unscheduled times when dictated by clinical signs or evidence of impending death.

Results and discussion
Mortality
In the preliminary study both heifers exposed to 1000 mg/kg bw died.
1/3 animals treated with 790 mg/kg bw MON 2139 died.
The results are summarized in the following table.

Table B.6.8-36: Mortality, survival time and animals used.

Dose (mg/kg bw)	**Toxicological result**			**Onset of death after**	**Mortality (%)**
	Dead animals	Animals with toxic signs	N		
1000*	2	2	2	3.3–6-3 days	100
790	1	3	3	8.8 days	33.3
630	0	3	3	---	0
500	0	2	3	---	0
400	0	0	3	---	0
0	0	0	8**	---	0

N: Number of animals
*: Preliminary test
**: Control groups compiled

The minimum lethal dose (MLD) was MLD, cow, oral = 790 mg/kg bw.

The minimum toxic dose (MTD) was MTD, cow, oral = 500 mg/kg bw.
The no effect level (NOEL) was NOEL, cow, oral = 400 mg/kg bw.

Clinical observations
In the preliminary study, treatment with 1000 mg/kg bw MON 2139 induced severe watery diarrhea, cessation of feed intake, and, prior to death, labored respiratory movements.
Treatment with 790 mg/kg bw decreased food intake and induced diarrhea. The two surviving animals had normal stool by day 16 and were eating normally by day 20.
Treatment with 630 mg/kg bw decreased food intake and induced diarrhea.
Treatment with 500 mg/kg bw induced only diarrhea while treatment with 400 mg/kg bw had no effect.

Table B.6.8-37: Prominent clinical observations in female cows given MON 2139 (numbers of animals affected)

Dose (mg/kg bw)	N	Observations (Number of goats affected)
1000*	2	decreased feed intake (2), diarrhea (2), depression (1), expulsion of rumen ingest through the nose (1), labored breathing (2), death (2)
790	3	decreased feed intake (3), diarrhea (2), labored breathing (1), death (1)
630	3	decreased feed intake (3), diarrhea (2), soft feces (1)
500	3	soft feces (2), no signs of toxicosis (1)
400	3	no signs of toxicosis (3)

*: Preliminary test

Body weight and food consumption
Treatment 630 and 400 mg/kg bw MON-2139 decreased body weight on day 14 and treatment with 500 mg/kg bw increased body weight on day 6 compared to controls. However, statistical significance was not reached.
The results are summarised in the following table.

Table B.6.8-38: Mean percent body weight changes (from initial body weight) in cows (n) treated with MON 2139

Dose (mg/kg bw/day)	Study day	MON 2139-treated	Controls
790	6	−1.3 (3)	−2.1 (2)
	14	−7.6 (2)	−1.6 (2)
	21	−9.0 (2)	−3.4 (2)
630	6	1.4 (3)	−0.7 (2)
	14	−0.5[a] (3)	2.7 (2)
	21	− 0.5 (3)	1.5 (2)
500	6	5.3[b] (3)	−1.9 (2)
	14	−0.2 (3)	−0.8 (1)
	21	1.9 (3)	1.2 (1)
400	6	−1.2 (3)	−1.4 (2)
	14	−2.5[a] (3)	−0.4 (2)
	21	−0.3 (3)	5.1 (2)

[a]:approaches being significantly less than control mean ($0.05 < P < 0.10$)
[b]:approaches being significantly greater than control mean ($0.05 < P < 0.1$)

Necropsy
No consistent gross post-mortem lesions were observed in any of the MON 2139- treated or control animals.

The only treatment-related finding was aspiration pneumonia in the 790 mg/kg bw-treated animal that died. One of the animals in the preliminary study also exhibited gross lung lesions consistent with broncho-pneumonia.
Mean kidney and liver weights are presented in the following table.

Table B.6.8-39: Mean kidney and liver weight/brain weight ratios after treatment with MON 2139

Dose (mg/kg bw)	N	Kidney/brain	Liver/brain
790	3	1.44[a]	5.80[a]
0*	2	0.96	4.81
630	3	1.28	5.91
0*	2	1.19	5.92
500	3	1.35[a]	5.88
0*	2	1.25	6.45
400	3	1.31	5.53
0*	2	1.27	6.08

*: Concurrent control for preceding MON 2139-treated group
[a]: P < 0.83

Major gross pathologic findings are summarized in the following table.

Table B.6.8-40: Major gross pathologic findings (number)

Body as a whole	Skin	Respiratory	Cardiovascular	Hemic & Lymphatic	Digestive	Urogenital
790 mg/kg bw (n = 3)						
Autolysis (1/3) Weight loss (1/3)	Dermatitis (3/3)	Congestion, thracheal mucosa, broncho-pneumonia (1/3)	Petechiae, epicardial (1/3) Pallor, heart (1/3)	Congestions, bronchial lymph nodes (1/3)	Abomastitis, congestion colon (1/3) Discoloration, rumen mucosa (1/3) Hyperkeratosis, tongue (1/3) erosions esophagus (1/3) darkening, rumen (1/3) petechiae, abomasum 1/3)	Congestion, kidneys (1/3) Fetus (1/3) HKBR (2/3)
Concurrent control for 790 mg/kg bw (n = 2)						
	Dermatitis (2/2)	Petechia, trachea & pleura (1/2)			Erosion, esophagus (1/2) Petechia, tuodenum (1/2) congestions, Peyer's patches (1/2)	

Body as a whole	**Skin**	**Respiratory**	**Cardiovascular**	**Hemic & Lymphatic**	**Digestive**	**Urogenital**
630mg/kg bw (n = 3)						
	Dermatitis (1/3)			Lymphoid hyperplasia, pharyngeal nodes (1/3) Slight congestion, spleen (1/3)	Focal fibrosis, liver (1/2) Parasitism (2/3)	HKBR (1/3)
Concurrent control for 630mg/kg bw (n = 2)						
	Dermatitis (1/2)			Lymphoid hyperplasia, pharyngeal nodes (mild) (1/2)	Parasitism (1/2) Lipoma (1/2)	
500 mg/kg bw (n = 3)						
Weight loss (3/3)	Dermatitis (3/3)	Petechiae, tracheal mucosa (1/3)		Congestion (1/3)	Erosion, esophagus (1/3) Petechiae, gall bladder (1/3)	HKBR (3/3)
Concurrent control for 500 mg/kg bw (n = 2)						
Weight loss (2/2)	Dermatitis (2/2)				Infarct, right kidney (1/2)	
400 mg/kg bw (n = 3)[a]						
	Dermatitis (1/3)	Adhesions, pleura (1/2)			Congestion, ileum (1/3) Parasitism (1/3)	HKBR (2/3)
Concurrent control for 500 mg/kg bw (n = 2)						
	Dermatitis (1/2)				Congestion ileum & cecum (1/2) Congestion, illeal mucosa (1/2) Petechia, abomasal mucosa (1/2)	Congestion, vulvar mucosa (1/3)

[a]: 1/3 animals showed melanosis in the adrenal capsule and 1/3 animals showed hydrocephalus
NGL: no gross lesions
HKBR: high kidney weight to brain weight ratio relative to controls
No findings for special senses or the skeletal muscle.

Histopathology
Microscopic tissue examination confirmed the presence of aspiration pneumonia in the 790 mg/kg bw-treated animal that died. One of the 790 mg/kg bw-treated animals was found to be carrying a live, apparently normal fetus when sacrificed at study termination.
There were no other treatment-related microscopic findings.

Clinical biochemistry and hematology
Treatment with 790 mg/kg bw/day MON 2139 increased serum urea nitrogen (BUN) and electrolytes, serum enzyme activities (SECT, GGT), hemoglobin concentration, and red blood cell counts.

Significant changes in group median and individual clinical laboratory values at lower dose levels were not considered to be treatment-related since no dose-response relationship was evident.
The results are summarized in the following tables.

Table B.6.8-41: Days after treatment on which values for clinical laboratory measurements performed on cows treated with MON 2139 were significantly different from concurrent median control values

Measurement	Dose (mg/kg/bw/day)			
	790	630	500	400
	Days after treatment			
BUN	8, 14	---	---	21[a]
CREAT	---	---	---	2[a], 6[a], 8[a], 14[a]
GLU	21[a]	---	2[a], 8[a]	---
Na	14[a]	---	---	---
K	14[a] , 21[a]	---	---	---
Ca	---	---	---	---
P	14[a] , 21[a]	6[a]	---	---
Mg	---	---	---	---
SGOT	14 , 21	---	---	---
LDH	14[a]	---	8[a], 14[a]	---
SACH	---	---	---	---
ALKP	8[a], 14[a] , 21[a]	---	2[a], 6[a], 8[a]	---
CKN	---	---	---	---
GGT	21	---	---	---
WBC	---	---	---	---
SEGSA	---	---	---	---
BANDSA	8	---	---	6, 14
LYMPHSA	---	---	---	---
EOSA	14[a]	---	---	---
MONOSA	---	---	---	---
BASOA	---	---	---	---
PCV	---	---	---	---
HBG	6, 8	---	---	---
RBC	6, 8	---	---	---
MCV	---	---	---	---
MCH	---	---	---	---
MCHC	6, 14	2	---	---
STP	---	---	8[a], 14[a]	---

[a]: medians significantly lower than the concurrent median control value; entries without this symbol represent medians significantly greater than the concurrent median control values.

Conclusion

The minimum lethal dose (MLD) was MLD, cow, oral = 790 mg/kg bw.
The minimum toxic dose (MTD) was MTD, cow, oral = 500 mg/kg bw.
The no effect level (NOEL) was NOEL, cow, oral = 400 mg/kg bw.

RMS comments: The study is considered supplementary because of the uncertainty with regard to parallel parasitic infestation and dermal mycosis that might have weakened the animals. Nontheless, the study is suitable to provide additional information about subacute oral toxicity of a Roundup formulation in a ruminant species and for comparison with effects of the isopropylamine salt. The conclusion of the notifier is agreed with but the "MTD" should be rather considered the LOAEL to make it more comparable to routine toxicological studies and to avoid misunderstandings because "MTD" is often read as "maximum tolerated

dose". The low dose of 400 mg/kg bw/day was the NOAEL in this study. Toxic signs and mortality occurred at dose levels that were lower than in the similar study with the isopropylamine salt of glyphosate suggesting a higher toxicity of MON 2139. This finding is in line with information from other sources pointing to a higher toxicity of certain formulations as compared to glyphosate and its salts.

B.6.8.3.3 Published data

Urinary concentrations of glyphosate in cattle and other species

Krüger *et al.* (2013, ASB2013-11599) reported the abundance of glyphosate in the urine of a total of 240 cows from Danmark. From each of eight dairy farms, the same number of 30 cows (15 fresh calving, 15 high yielding cows, *i.e.*, at the top level of milk production) was selected. All these 240 cows excreted glyphosate in the urine, however, at very different concentrations. Urine samples were diluted 1:20 with distilled water and tested for glyphosate by means of an ELISA kit (Abraxis, USA). The limit of detection (LOD) or a limit of quantification (LOQ) were, unfortunately, not mentioned. However, it is stated in the paper that validation of test results had been done by a comparison with GC-MS which is considered a more suitable method. It was mentioned that the correlation coefficient between the two methods was 0.96 and, thus, sufficiently high but this validation data was not shown in this paper. However, for further interpretation of the results, it is assumed that the method was in fact valid and that the measured values were reliable.
It is worth mentioning that the cows (breed not given) were 4 to 7 years old and had an average body weight between 550 – 600 kg and that the total number of cows in the farms ranged from 140 to 400 animals. The average daily milk yield in the different farms ranged from 8.6 to 11.2 kg~~, for *i.e.*, not very high was representative for countries with modern and efficient agriculture~~.

Mean urinary glyphosate concentrations differed very much among the eight farms, ranging from 10 ~~ng/mL~~ µg/L up to 103.3 ~~ng/mL~~ µg/L. It is a reasonable assumption that urinary excretion of glyphoate was due to dietary exposure and, thus, detection of glyphosate in the urine of cattle ist not surprising. Residues of glyphosate may occur in feedstuffs for ruminants and, so far the maximum residue limits (MRLs) are not exceeded, are allowed by law and of no concern. (Therefore, the word "contamination" as used by the authors is not correct and somehow misleading.) The reason for the remarkable differences between the farms is unknown but most probably the diet was different, including a high variability in glyphosate residues. It is one of the main deficiencies of the publication that no details on feeding regime have been given and that the diets were not analysed for glyphosate content.
It is interesting to note that the mean urinary concentrations in cows exceeded the mean value in human urines of 0.2 µg/L as found by Hoppe (2013, ASB2013-8037, see B.6.9.3) by ~~at least~~ 50 ~~(up to more than 500)~~ to more than 500 times suggesting higher residues of glyphosate in cattle rations than in human diet. This big difference is also confirmed by comparison to further data on glyphosate findings in human urine samples (see B.6.9.3)

The maximum mean value of *ca* 103 µg/L can be used to calculate a systemic dose to which the cows in that farm had been exposed to since glyphosate does not accumulate but is rapidly excreted. The study authors have estimated a maximal glyphosate excretion via urine of 3.1 mg/day. If an oral absorption rate of only 20 % is assumed instead of 30 % as used by the study authors (for justification, see B.6.1), the maximum daily oral intake should have been in the magnitude of 15 – 16 mg. This dose might be compared ~~either~~ to the ADI ~~(even though~~

but it is not clear if a reference dose established for humans is *per se* also applicable to farm animals~~)~~ and in particular to ruminants. ~~or to Thus, the caclulated intake was compared to the NOAELs in the subacute studies in cattle mentioned above.~~

~~If the first approach is taken, the use of the proposed ADI of 0.5 mg/kg body weight for glyphosate (see Vol. 1, B.6.12) would result in a tolerable intake of 225 mg for a cow of 550 kg body weight. This amount is by about 14 – 15 times higher than the expected maximal exposure of the Danish cows on study or, in other words, the systemic dose in the cow with highest urinary excretion of glyphosate would account for not more than about 7 % of the ADI.~~

~~Using the second approach,~~ Thus, the maximum expected systemic dose was better compared to the NOAELs in subacute studies with either the isopropylamine salt of glyphosate (540 mg/kg bw/day according to Rowe *et al.*, 1987, TOX9552424) or a Roundup formulation (400 mg/kg bw/day according to Rowe *et al.*, 1987, ASB2010-8131). For a cow of 550 kg, the latter NOAEL would correspond to a daily intake of 220 g. In the study description (see above), a glyphosate content of 30.5% is mentioned. Thus, a daily glyphosate intake of 67 g can be calculated. If this amount of 67000 mg is compared to the expected maximum systemic exposure of 15 or 16 mg per day in the study in Danish cows, a margin of safety of about 1:4200 would result. Based on these considerations, an impairment of animal health in the~~se~~ Danish cows is very unlikely.
In contrast, the authors reported increased activities of the enzymes creatine kinase (CK), glutamate oxaloacetate transaminase (GOT, synonymous to ASAT), and glutamate dehydrogenase (GLDH) in blood serum but also changes in cholesterol levels and an increase in blood urea concentrations. ~~when compared to (mostly not precisely mentioned) reference values of unknown origin.~~ For this comparison, they used reference values of unknown origin with the most of them not precisely mentioned. ~~They postulated a~~ A relationship with the detection of glyphosate in urine was postulated interpreting the alterations in laboratory parameters as indicative for liver damage or nephrotoxicity.
Leaving aside the serious methodical deficiencies of the study (*e.g.*, the absence of a control group with no glyphosate residues in the urine), there is no evidence that the altered clinical chemistry parameters in cows were indicative of any health deterioration. There could be many different reasons of such alterations and, taking into account the very low glyphosate concentrations (see above), exposure to glyphosate is not a likely one. Moreover, the statistical correlation between glyphosate excretion in urine and changed clinical chemistry parameters as claimed by the autors was in fact rather poor. (Thus, for the comparison of glyphosate with creatine kinase, an R value of 0.135 was considered in the article as indicative of "positive correlation" which in fact is not the case. In contrast, higher R values of up to 0.809 were obtained when two different clinical chemistry parameters such as zinc and cobalt concentrations were measured and may indicate some correlation between them but apparently not with glyphosate excretion. Unfortunately, due to the way of reporting, the reader may be misleaded here to assume correlation with glyphosate.)
Since, in parallel, the authors claimed very low serum levels of several trace elements such as cobalt or manganese, a possible chelating mode of action of glyphosate was suspected. However, these considerations appear purely speculative, in particular against the background of the very low exposure. Even if glyphosate would have chelating properties, the ingested and absorbed amount is not expected to bind trace elements to such an extent that clinical signs might be expected to occur even though such an effect was suspected by scientists from the Aarhus university uin Danmark (Sørensen et al., 2014, ASB2014-5761).

Again, statistical correlation was rather weak. Indeed, the almost complete absence of the two elements in cattle from all farms rather points to either an analytical problem or to a deficiency in the diet.
Thus, to conclude, the urinary levels in Danish cows might well reflect the abundance of glyphosate residues in their feed. The systemic exposure that may be calculated is very low and no health concern is anticipated. Final conclusion on the clinical chemistry findings is not possible but, on one hand, it seems not proven that they were adverse and, on the other, it is very unlikely that they might be related to glyphosate.

In a second paper dealing with determinations of glyphosate in the urine of cattle, Krüger et al. (2014, ASB2014-5024) reported, again, data from Danmark. The sample number was 242. It is not clearly stated but can be assumed that the 240 cows mentioned in the first paper were completely or at least partly included. These urine measurements were compared to samples from Germany. The German urine samples were collected from 343 cows from "conventional husbandry" and from 32 cows of which husbandry was not described but which were kept in so-called "GM free" regions. (There are regions in Germany where farmers try to avoid feed that is or might be produced abroad on the basis of genetically modified crops.) The samples were analysed by means of a not further specified ELISA (Abraxis, USA) but, again, an LOD or LOQ were not mentioned. Instead, a comparison between values obtained by this ELISA and GC-MS was provided and, this time, explained in greater detail. The comparison revealed a very good correlation (R^2 of 0.96 for cattle urine, based on 21 samples that were measured in parallel by both methods). Neither individual numeric values for urine concentrations nor statistical parameters such as the mean, median or standard deviation are given in this very brief paper but only figures. It seems from these printed figures that the mean urinary concentration in Danish cows was slighly above 40 µg/L as compared to only ca 20 µg/L in the German cows from conventional husbandry. This difference was statistically significant. The German cows from "GM free" regions had hardly any glyphosate in their urines. In contrast to what is said in the article, this finding is not surprising since imported feedstuffs will most certainly contain higher residues of glyphosate.
As discussed above with regard to Krüger et al. (2013, ASB2013-11599), urinary concentrations in this magnitude are of no health concern for cattle.

Furthermore, glyphosate was found in the urine of fattening rabbits (n = 77) in the magnitude of about 60 µg/L (mean value, standard deviation showing values ouf up to ca 120 µg/L). Less glyphosate (mean of 20-30 µg/L) was determined in the urine of hares (n = 193). Nothing is known on the origin of these samples and no conclusions can be drawn.
(In human samples that were analysed by the same group, the mean concentration was nearly 2 µg/L with a maximum in the magnitude of 5 µg/L. For details, see section B.6.9.3.) Information on glyphosate residues in the organs of slaughtered cows was also given in this paper and is reported in chapter B.7.

Possible impact on the microflora in ruminant's GIT

A number of papers has been published recently in which a possible causal link of glyphosate exposure and subsequent *Clostridium botulinum (C. botulinum)* overgrowth with a new disease in cattle is suggested. The scientific background of this assumption is the herbicidal mode of action of glyphosate. In plants, the enzyme 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSP synthase) is inhibited resulting in a lack of formation of aromatic amino acids by the shikimate pathway that is common in the plant kingdom but not does not occur in

animals. However, this pathway is operative in most bacteria and yeast and many protozoan species. Thus, an impact of glyphosate on microflora, *e.g.*, in the intestines, is at least conceivable. In line with that, concern on this issue was expressed by scientists from the Aarhus University in Denmark (Sørensen et al., 2014, ASB2014-5761).

Rodloff and Krüger (2012, ASB2013-13311) hypothesised that an emerging new disease in cattle but also symptoms in a small number of farmers might be caused by *Clostridium botulinum*. This animal disease of so far unknown etiology and pathogenesis was reported to have occurred from the late 1990ies onwards in cattle mainly from some parts of Germany but, according to the authors, cases had been observed also in France, the Netherlands, and the U.K. even though references were not given. Clinical signs in cattle are predominately seen in the perinatal period and comprise indigestion with alternating constipation and diarrhea, apathy, ataxia, paralysis, retracted abdomen, breathing difficulties, a decrease in milk yield, and death. In some farmers taking care of affected herds, symptoms such as dizziness, weakness, fatigue, blurred vision, nausea, and difficulties to speak, to swallow and to breathe have been occasionally reported.
The authors suggested a causal link to *Clostridium botulinum* because these signs and symptoms appear similar to what is known from the rarely occurring cases of botulism in animals and man that are not food-borne (*i.e.*, not caused by acute intoxication with the bacterial toxin). By means of an ELISA (no details given), they have detected *Clostridium botulinum* neurotoxin (BoNT) in fecal samples (in total in 16 out of 33) obtained from cattle on six German farms where the suspicious clinical signs or even fatalities had been noted but not in a total of 10 samples from two farms without any evidence of this disease. Toxin types were different (A, B, C, D, and E, in various combinations). Health state of the donor animals was not reported. In addition, neurotoxin of different types was found in various organs such as the rumen and the liver in 15 sick cows after slaughter. However, it is not known how many cows were examined in total *post mortem* and by which method and if they belonged to the group of which fecal samples had been taken before.
In man, 16 out of 77 fecal samples were positive for BoNT that were taken from humans who were reported to have been in close contact with diseased cows. It is not clear if one or more samples were obtained from the same person and, thus, the total number of humans under investigation is not known. Again, health status of the involved people was not reported. With regard to neurotoxin types, mainly type E had been found.
Unfortunately, these numbers do not completely match with a table in the paper in which 12 BoNT-positive human fecal samples in a total number of 33 and 29 BoNT-positive bovine fecal samples out of 118 under examination were mentioned to be obtained from 7 “selected” German cattle farms.

Conclusion by RMS:
This paper does not contribute anything to risk assessment of glyphosate and, in fact, this herbicidal active ingredient was not mentioned therein. However, it is important for understanding of subsequent publications and, therefore, is referred to in this RAR.
In itself, this paper is adequate to suggest a scientific hypothesis, based on some data that might require further research. However, no causal relationship between a new disease in cattle and C. botulinum has been established. C. botulinum neurotoxin could not be quantified. Qualitative detection of the neurotoxin might be also a random co-incidence. The publication is flawed by many reporting deficiencies. In particular, the method by which the neurotoxin was detected and its different types were distinguished, is not described.

In a similar paper, Krüger *et al.* (2012, ASB2013-13312) reported the abundance of (different types) of the micro-organism *C. botulinum* itself in 44 out of 196 bovine fecal samples (22.5%) and in 17 out of 77 human fecal samples (22%) but also in silages (9 / 21 = 47%), concentrate feed specimens (4 / 14 = 28.6%), and in all 7 tested house dust specimens from a total of 41 dairy farms in the German Federal State of Schleswig-Holstein. This finding was based on the immunological detection of antigens of *C. botulinum* by an ELISA technique using polyclonal antibodies. All the cattle had been reported to have shown clinical signs as described above. In addition, in four of the involved humans, symptoms were claimed to have occurred but without further specification or medical confirmation.

Conclusion by RMS:
Again, this paper, in principle, is not relevant for risk assessment of glyphosate but it is mentioned therein that ingested spores might germinate in the intestinal tract if protective indigenous bacterial flora is lacking (as observed in cases of infant botulism) or altered and this might be linked to the glyphosate hypothesis explained below. Therefore, it is mentioned here despite its many reporting deficiencies. The findings rather point to ubiquitious occurrence of C. botulinum but are not suitable to prove a causal relationship of its abundance to clinical signs or symptoms.

By the same group, it was published that different bacteria such as *Enterococcus faecalis*, *Enterococcus faecium* or *Bacillus badius* were able to inhibit growth of *C. botulinum* and/or the production of its neurotoxins *in vitro* whereas other bacterial species did not exhibit such an effect (Shehata *et al.*, 2012, ASB2013-8529). Subsequently, Krüger *et al.* (2013, ASB2013-8527) reported that glyphosate (analytical grade) and the herbicide Roundup UltraMax® containing 450 glyphosate/mL was able to suppress this antagonizing of effect of *Enterococcus* species on *C. botulinum in vitro*. What they actually observed was a growth inhibition of both *C. botulinum* (and a reduction in neurotoxin type B production) and *Enterococcus faecalis* by glyphosate and Roundup herbicide but at different concentrations. While growth of *E. faecalis* was completely inhibited by 0.1 mg glyphosate or Roundup/mL, the same effect on *C. botulinum* was seen only at a concentration of 1 mg Roundup/mL or of 10 mg glyphosate/mL.

Conclusion by RMS:
This data suggests a different susceptibility of E. faecalis and C. botulinum to cytotoxic effects of glyphosate and a glyphosate-based herbicide in vitro. With regard to C. botulinum, the Roundup UltraMax formulation was more toxic than the active ingredient confirming similar evidence from various fields of toxicological testing. No conclusions can be drawn if similar effects might occur in vivo because the situation in, e.g., the GITs of ruminants or monogastric animals is different with hundreds or thousands of mirobial species co-existing. In any case, even the lowest tested concentration of 0.1 mg/mL appears extremely high if a (maximum) systemic dose of 15 or 16 mg per cow is assumed to result from feeding the animals with a ration containing glyphosate residues (based on [redacted] 2013, ASB2013-11599, see above). Thus, the possible impact of glyphosate (herbicides) on bacteria due to inhibition of the enzyme EPSP was somehow confirmed in vitro but there is no health concern and no impact on realistic risk assessment.

A different toxicity of Roundup UltraMax® to various microbial species was also observed by Shehata *et al.* (2013, ASB2012-16301) who measured the effect of different concentrations on 23 bacterial species and strains mostly of chicken origin and also on sporulated *Eimeria tenella* (*i.e.*, a protozoon in poulty) oocytes *in vitro*. It is not clear whether the mentioned

concentratios ranging from 0.075 mg to up to 5 (bacteria) or 1.2 (*Eimeria tenella*) mg per mL are related to the herbicide formulation or had been adjusted to the active ingredient. In general, the authours found lower minimum inhibitory concentrations (0.075 – 0.6 mg/mL) for beneficial bacteria whereas, in contrast, some pathogenic germs such as *Clostridium perfringens* or several *Salmonella* species appeared much less sensitive with growth inhition seen only at the highest tested concentration of 5 mg/mL. With regard to *Eimeria tenella*, the threshold for an effects was around 0.3 mg/mL with a clear effect to be seen at 0.6 mg/mL.

Conclusion by RMS:
Different cytototoxicity of a glyphosate-based herbicide to micro-organisms was confirmed once more and might be due to either the active ingredient or, e.g., a surfactant. (It is not known whether a [redacted] surfactant was contained.) However, antibiotic activity of the herbicide (expressed in the minimum inhibitory concentrations) was lower than that of known antibiotics that are used in veterinary medicine. Even the lowest effect concentrations in this study were by far higher than the expected glyphosate concentrations in poultry feed (GTF, 2013; ASB2013-11007) and, thus, must be considered unrealistically high. Furthermore, in vitro exposure of selected indivdual species and strains to a herbicide might be not a good model for complex interactions in GIT of poultry when residues are ingested.

To conclude, a link of glyphosate residues in ruminants diet to a new disease in cattle has not been established and is not likely. Furthermore, there is no convincing proof that clinical signs in cattle (of which the occurrence cannot be doubted) were indeed caused by *C. botulinum* or its toxins. Meanwhile, a comprehensive case-control study on a possible causal relationship between *C. botulinum* and that chronic disease in cattle has been conducted in Germany. Preliminary results suggest that this was not the case even though further investigations have been considered necessary. In addition, use of glyphosate on the included farms had no impact on the occurrence of clincial signs (Seyboldt and Hoedemaker, 2014, ASB2014-10736).

However, because of the growing public concern about this cattle disease especially in Germany and because an effect on micro-organisms due to EPSP inhibition cannot be excluded, the German Federal Institute for Risk Assessment (BfR) has commisioned a study with a glyphosate-based herbicide (containing a [redacted] surfactant) in an artifical rumen system (RUSITEC) to investigate wheter (1) quantitative composition of ruminal microflora might be compromised and (2) there is evidence of *C. botulinum* overgrowth. Unfortunately, results of this project ~~were not available when this RAR was finalised (October, 2013) but it is hoped that they will be published in 2014~~ have not been published so far. However, an internal research report of the Veterinary High School in Hannover (Riede *et al.*, 2013; ASB2013-14684) has been submitted to the Federal Institute for Risk Assessment and is reported here in brief. Two different experiments were performed. In the first one, the effects of a glyphosate-based herbicide (Plantaclean® XL; 360 g/l glyphosate, containing a [redacted] surfactant) on rumen fermentative parameters were studied. Total glyphosate doses per day were 0.26 or 2.31 mg per fermentation vessel. No major changes in rumen parameters were detected except slight decreases in NH_3-N concentrations and increases in isovalerate production in response to the high dosage. There was an increase in (beneficial) *Bifidobacterium spp.* but, in general, the microbial communities were not affected. In the second trial, no effects of the herbicide on the growth of *C. sporogenes* was found that had been artificially added to serve as a surrogate for *C. botulinum*.

Malformations in piglets with suspected correlation with glyphosate

Krüger *et al.* (2014, ASB2014-8935) reported glyphosate residues in different organs/tissues (brain, gut wall, heart, kidneys, liver, lungs, and muscle tissue) from a total of 38 malformed one-day old piglets (breed not specified) which had been brought in by a Danish farmer. The same farmer had complained about reproduction and developmental problems already in the past, *e.g.*, in an Internet publication by the NGO "GM-Free Cymru (Wales)" in which health effects in pigs including malformations were mainly ascribed to the feeding of genetically modified soy (Anon., 2012, ASB2014-3921) and, apparently, his observations have been also referred to by Sørensen *et al.* (2014, ASB2014-5761). The malformations were very much different including craniofacial but also visceral and leg anomalies. For determination of glyphosate, apparently the same ELISA as for urine measurements (Abraxis, USA) was used after mincing and diluting tissue samples from the various organs. Mean glyphosate concentrations between 2.1 ppm (liver) and 12.9 ppm (heart) were found. In most organs, standard deviation was extremely large and individual values in single animals ranged from 0 (liver) and 0.1 ppm (kidney) to occasional findings as high as 80 ppm (in lung and heart). The authors speculated if there was a correlation between the malformations and intake of glyphosate residues to which the piglets might have become exposed via the placenta. The farmer claimed that the rate of malformed piglets had increased from 1:1432 when the sows had been fed a diet containing 0.25 ppm glyphosate to 1:260 when the sows received a diet with a glyhosate content of 0.87-1.13 ppm during the first 40 days of pregnancy.

Conclusion by RMS:

This publication cannot be considered to describe a reliable scientific study. First, the analytical data obtained from the piglets appear questionable since no information was given whether the ELISA had been modified for these investigations or validated for analysing tissue samples. No LOD or LOQ was mentioned. For the changing glyphosate content in the diet as claimed by the farmer, there is no confirmation in the publication.

The second main weakness of the study is that only malformed piglets had been investigated for glyphosate concentrations in their organs. Thus, there was no control group to (possibly) prove the hypothesis of a potential correlation.

For the following considerations, such a correlation is unlikely:

- *In a multitude of developmental studies and multi-generation studies in rats, no evidence of teratogenicity was obtained. Even in rabbits which proved more vulnerable, developmental effects were confined to exaggerated dose levels causing also clear maternal toxicity (see sction B.6.6). It is very unlikely that pigs, receiving much lower amounts of glyphosate by ingestion of residues in the diet, should be that much more sensitive and, if so, it is hardly conceivable that such effects would not have become apparent before and also in other countries and on other farms.*
- *Many different malformations were reported. However, most chemical teratogens produce a specific teratogenic effect or a certain pattern of findings. Moreover, teratogenic effects usually follow a dose response. In this case, the glyphosate concentrations in the organs and tissues were so variable that such a dose response may be excluded.*
- *Malformations in piglets are quiet frequent and have often a genetic background. Infectious diseases may also play a role. There is no indication in the paper that a differential diagnosis has been considered.*

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B.6.8.4 Further published data (released since 2000)

Introduction by RMS:
A large number of studies on toxicity of glyphosate and its formulations was published since 2000. Most of these studies are presented in the chapters on genotoxicity, carcinogenicity, reproductive toxicity and neurotoxicity of this report because they are discussed there in context with these endpoints. However, some additional studies are presented below that could not be allocated to these endpoints.

Additionally to the reviews reported above in the chapters on different end points of this RAR some further toxicological reviews on glyphosate have been published since 2000. The content of these reviews is a broad range of possible effects which are attributed by the authors to glyphosate, glyphosate formulations or generally to pesticides. However, some reviewers summarised effects described in the original studies without to differentiate between clearly evidenced effects and questionable or highly doubtful effects. The quality of the reviewed literature is in many cases not sufficiently discussed. On this basis some autors come to conclusions which deviate from conclusions of the rapporteur of this RAR.

Samsel and Seneff, 2013 (ASB2013-8535) reviewed toxicological literature on glyphosate and concluded that glyphosate inhibits cytochrome P450 enzymes. The authors believe that this activity would result in nearly all diseases as inflammatory diseases, obesity, depression, ADHD, autism, Alzheimer`s disease, Parkinson`s disease, ALS, multiple sclerosis, cancer, cachxia, infertility, developmental diseases, gastrointestinal disorders, heart disease, diabetes. Antoniou et al. (2011, ASB2011-7202) reviewed toxicological literature on Roundup and glyphosate. The authors conclude that Roundup and glyphosate would cause endocrine disruption, damage to DNA, reproductive developmental toxicity, neurotoxicity and cancer as well as birth defects. Many of these effects would be found at very low doses, comparable to levels of pesticide residues found in food and the environment. Mostafalou and Abdollahi (2013, ASB2014-9618) published a review on the relation between pesticides and elevated ranges of a broad range of different diseases. According to the authors pesticides cause diseases as different types of cancers, diabetes, neurodegenerative disorders like Parkinson, Alzheimer and amyotrophic lateral sclerosis, birth defects, and reproductive disorders, resipiratory problems, cardiovascular diseases, chronic nephropaties, autoimmune diseases, chronic fatique syndrome and aging. Mesnage and Seralini (2014, ASB2014-9616) submitted a review on pesticide toxicity and genetically modified organisms (GMOs) which are used in agriculture. The authors propose to pay more attention on the mixtures of pesticides with further substances and to test relevant combinations of pesticides at levels which occur in genetically modified plants. NABU (2011, ASB2012-8016) reviewed some ecological and toxicological literature on glyphosate and formulations. The active substance, metabolites and further substances in the formulations are considered toxic especially for aquatic organisms. They would disturb human cells and the development of vertebrates. In result of resistance of wild plants the amount of glyphosate products is expected to grow in the future. PANAP (2009, ASB2012-8017) reviewed literature on toxicity, environmental effects and environmental fate of glyphosate. The authors conclude that independent scientific studies and poisonings in Latin America are beginning to reveal that use of glyphosate would not be safe.

Antoniou et al. (2010, ASB2012-803) reviewed toxicological and ecological literature on glyphosate and genetically modified Soya. The authors conclude that the toxic activity of glyphosate is increased by the combination with further substances in the formulations. Toxicity was already observed at concentrations which occur in agriculture and environment. The authors conclude that there would be a relation between glyphosate and increased malformations. Furthermore, epidemiologicas studies would demonstrate a relation between glyphosate use and carcinogenicity and genotoxicity.
Brändli and Reinacher (2012, ASB2012-804) submitted a short survey on use and health effects of glyphosate. The authors conclude that the use of glyphosate for siccation would be a scandal and would be considered to be bodily injury by negligence.
Greenpeace (2011, ASB2012-810) reviewed literature on ecological and health effects of glyphosate. The authors conclude that the submitted evidence in this report demonstrates that glyphosate-based products can have advers impacts on human and animal health, and that a review of their safety for human and animal health is urgently needed. The authors demand that no genetically modified glyphosate-tolerant crops should be authorised. They would be linked to unsustainable farming practices that damage the basic natural resources food production is based upon, and their cultivation should be banned.
Altenburger et al. (2012, ASB2014-9176) submitted a review that provides an overview on experimental studies from the past decade that address diagnostic and/or mechanistic questions regarding the combined effects of chemical mixtures using toxicogenomic techniques. By joining established mixture effect models with toxicokinetic and –dynamic thinking the authors suggest a conceptual framework that may help to overcome the current limitation of providing mainly anecdotal evidence on mixture effects.

Furthermore, some studies have been published in which the authors investigate the activity of glyphosate and/or glyphosate formulations on selected biochemical or morphological structures. However, based on the provided information the impact of these results for the in vivo situation of the whole organism of animals or humans with autoregulation and feedback mechanisms is questionable. The dose dependency of the described effects and their importance for real life situations is often not sufficiently discussed by the authors. Many authors conclude that further studies would be necessary.
In some cases the authors compare the toxicity of glyphosate with the toxicity of glyphosate formulations or of surfactants. A frequent result in these cases is a higher toxicity of the formulations or the surfactants. In some further cases only glyphosate formulations have been used in the studies. Some authors use these results for a conclusion concerning the toxicity of the active substance glyphosate but do not consider the activity of surfactants or further substances in the formulation.

Chaufan et al., 2014 (ASB2014-7616) examined the effects of glyphosate, AMPA and the formulation Roundup Ultra Max on oxidative balance and cellular endpoints in HepG2 cells. Only the formulation Roundup Ultra Max had toxic effects while no effects were found with glyphosate and AMPA. The formulation produced an increase in reactive oxygen species, nitrotyrosine formulation, superoxide dismutase activity and glutathione levels.
In an in in vivo study (Larsen et al., 2012, ASB2014-6905) Wistar rats were exposed during 30 or 90 days to low levels of 0.7 and 7 mg/l glyphosate in drinking water. Only 4 animals per dose and sex were used. Levels of glutathione and glutathione peroxidase have been increased which was considered as a protective mechanism by the authors.
George et al., 2013 (ASB2014-8034) studied the effects of glyphosate on HaCaT cell proliferation. The authors concluded that glyphosate promotes proliferation in HaCaT cells probably by disrupting the balance between Ca^{2+}-levels and oxidative stress. However, in the

study the commercial formulation Roundup was used. Therefore, the results can not be attributed to the substance glyphosate only.
Hedberg et Wallin, 2010 (ASB2014-7494) studied the effects of glyphosate, Roundup and further substances on intracellular transport in Xenopus laevis. The chemicals inhibited retrograde transport of melanosomes in the range of 0.5 – 5 mM. Cellular morphology and localization of microtubules and actin filamentes were affected. The effects are pH-dependent.
El-Shenawy, 2009 (ASB2012-11611) compared the cytotoxicity of Roundup and the active substance glyphosate. Male rats were i. p. treated with Roundup or glyphosate. The results characterize Roundup as a stronger antioxidant than the active substance glyphosate itself.
Caglar and Kolankaya, 2008 (ASB2012-11580) treated rats with formulation Roundup orally during 5 and 13 weeks and studied hepatotoxicity. The authors concluded that high doses of Roundup can be a potential riks for human health.
Modesto and Martinez (2010, ASB2012-811) studied effects of Roundup Transorb on fish. They observed haematologic alterations and effects on antioxidant defenses and on acetylcholinesterase activity.
Zhao et al. (2013, ASB2014-9645) investigated the effect of different doses of glyphosate on apoptosis and expression of androgen-binding protein and vimentin mRNA in mouse Sertoli cells. The authors conclude that glyphosate can cause cellular damages, inhibit cell proliferation, induce cell apoptosis, and decrease expression of ABP and vimentin mRNAs in mouse Sertoli cells in vitro.
Xia et al. (2013, ASB2014-9642) studied the induction of vitellogenin gene expression in the fish medaka exposed to glyphosate and potential molecular mechanism. While glyphosate markedly up-regulated VTG transcription levels in both female and male fish, the upward trend was inhibited at the high glyphosate concentrations.
Wunnapak et al. (2014, ASB2014-9638) used Roundup to induce nephrotoxicity in rats. A panel of kidney injury biomarkers was evaluated in terms of suitability to detect acute kidney injury and dysfunction.
Martini et al. (2012, ASB2014-9613) used 3Z3-L1 fibroblasts to investigate the effect of a commercial formulation of glyphosate on proliferation, survival and differentiation. According to the results, a glyphosate-based herbicide inhibits proliferation and differentiation in this mammalian cell line and induces apoptosis suggesting GF-mediated cellular damage.

Larsen et al. (2014, ASB2014-9606) evaluated the activities of different xenobiotic-metabolizing enzymes in liver subcellular fractions from Wistar rats exposed to a glyphosate based herbicide . The results demonstrated certain biochemical modifications after exposure to a GLP-based herbicide. The authors conclude that the pharmacotoxicological significance of these findings remains to be clarified.
Belle et al. (2012, ASB2014-9251) write in a letter to the editor that Williams et al. (2012, ASB2012-12052) analyzed five of their articles. Williams et al. (2012, ASB2012-12052) would minimize the experimental evidence that glyphosate plays a role in toxicity and would discredit their findings.The article of Williams et al. (2012, ASB2012-12052) would contain several errors. Williams et al. (2012, ASB2012-12052) have not evidenced that the experiments of Belle et al. have been incorrect or biased.

Kim et al. (2013, ASB2014-9591) investigated the mechanism of the additive effect of glyphostate and TN-20, a common surfactant in glyphosate herbicides. The results support the possibility that mixtures of glyphosate and TN-20 aggrevate mitochondrial damage and induce apoptosis and necrosis. Throughout this process, TN-20 seems to disrupt the integrity of the cellular barrier to glyphosate uptake, promoting glyphosate-mediated toxicity.

Kilinc et al. (2013, ASB2014-9588) studied the influence of pesticide exposure on carbonic anhydrase II from sheep stomach. The authors conclude that both glyphosate isopropylamine and dichlorvos inhibited CA-II isoenzym in a noncompetetive manner.

Jasper et al. (2012, ASB2014-9583) evaluated the toxicity of hepatic, haematological, and oxidative effects of glyphosate Roundup on male and female albino Swiss mice. The results of this study indicate that glyphosate-Roundup can promote haematological and hepatic alterations, even at subacute exposure, which could be related to the induction of reactive oxygen species.
Chaufan et al. (2014, ASB2014-9314) studied the effects on oxidative formulation in HepG2 cells. The authors conclude that the results confirm that G formulations have adjuvants working together with the active ingredient and causing tolxic effects that are not seen with acid glyphosate.
Gencer et al. (2012, ASB2014-9481) studied in vitro effects of Imazethapyr, 2,4-D, glyphosate and propanocarb on human erythrocyte carbonic anhydrase activity. Imazethapyr was the most effective inhibitor for CA-H isoencyme. The lowest inhibition was caused by glyphosate.
Campo et al. (2009, ASB2014-9281) evaluated the toxicity of ten pesticides used in the municipality of Popayan., Colombia, using bioassay with Bacillus subtilis. Glyphosate was slightly toxic in this test.
Kwiatkowska et al. (2014, ASB2014-9603) published a study that was undertaken to evaluate toxic potential of glyphosate, ist metabolites AMPA, methylphosphonic acid and its impurities N-(phosphonomethyl)iminodiacetic acid (PMIDA), N-methylglyphosate, hydroxymethylphosphonic acid and bis-(phosphonomethyl)amine. The authors evaluated the effect of those compounds on haemolysis, haemoglobin oxidation, reactive oxygen species (ROS) formation and changes in morphology of human eythrocytes. Glyphosate, its metabolites and impurities induced a little haemolysis and haemoglobin oxidation. All changes were very low, even after 24 h incubation. Most of the investigated compounds induced reactive oxygen species formation from 0.25 mM, except the N-methylglyphosate wich caused an increase in ROS formation from 0,5 mM. Moreover, the investigated xenobiotics did no change the size and shape (except bis-(phosphonomethyl)amine) of the human erythrocytes. Changes in human erythrocytes were observed only when high concentrations of the compounds were applied. Some investigated metabolites and impurities caused a slight stronger damage to human erythrocytes than glyphosate.

Mesnage et al. (2013, ASB2014-1755) studied the toxicity on human cells in vitro for glyphosate-based formulations and also for polyethoxylated tallowamine POE-15, glyphosate alone and a formulation without glyphosate. Hepatic (HEPG2), embryonic (HEK293) and placental (JEG3) cells were exposed 24 h. POE-15 was the most toxic of the tested substances and combinations. The authors conclude that pesticide formulations should be studied as mixtures for toxic effect with mammals over a 2-year period. They question the use of ethoxylated substances in herbicide formulations, since they appear as active principle for human sell toxicity. The results would challenge guidance values as the ADI because they are only derived from results of studies with the active substance.

Mesnage et al. (2012, ASB2012-13917) tested the toxicity of 9 active pesticide substances including glyphosate, comparing with the toxicity of their formulations, including Roundup. The tests have been performed in vitro with 3 human cell lines. The authors conclude that Roundup was found in this experiments to be 125 times more toxic than glyphosate. These results would challenge the relevance of the ADI because this is only based on the toxicity of the active substance. Therefore, an additional adjuvant factor of at least 100 could be applied

to the present calculation of the ADI. However, this calculation would never replace the direct study of the commercial formulation with its adjuvants in regulatory tests. Coalova et al. (2014, ASB2014-7615) studied the influence of spray adjuvant on the toxicity effects of a glyphosate formulation in Hep-2-cell line. They determined the median lethal concentration of Atanor (glyphosate formulation), Impacto (spray adjuvant) and a mixture of both agrochemicals. The substances and mixtures induced dose- and time-dependent cytotoxicity. The toxicity of a mixture of Atanor and Impacto was additive in Hep2-cells. The authors conclude that the addition of adjuvant to glyphosate formulation would increase the toxicity of the mixture in cell culture.
Kwiatkowska et al. (2014, ASB2014-8085) investigated the effect of glyphosate, its metabolites and impurities on acetylcholinesterase (AchE) activity (in vitro) in human erythrocytes. The authors conclude that the compounds studied (used in concentrations that are usually determined in the environment) do not disturb function of human erythrocyte acetylcholinesterase.

Hoare (2014, ASB2014-9157) submitted a QSAR assessment on the toxicological properties of glyphosate and its impurities. To assess the toxicological properties of glyphosate and five impurities (AMPA, IBMPA, MAMPA, NMG and IDA) present in the technical grade of material, the QSAR models ACD labs, DEREK NEXUS, TOXTREE, EPA T.E.S.T. VEGA and OECD Toolbox were employed. None of the structures analysed triggered any alerts on DEREK NEXUS for carcinogenicity, chromosome damage, genotoxicity or mutagenicity. Eye and skin irritation were not anticipated in any of the QSAR models evaluated. No alert for skin sensitisation was triggered in DEREK NEXUS for any compound. Equivocal alerts for nephrotoxicity and plausible alerts for hepatotoxicity were triggered by DEREK NEXUS for glyphosate, NMG, MAMPA and AMPA.

A further study was published which describes cases of intoxication in dogs and cats:

Bates and Edwards (2013, ASB2014-9249) inform in a letter about cases of intoxication of dogs and cats by glyphosate in UK, registered by the Veterinary Poisons Information Service. Vomiting, diarrheoea and lethargy were the most common signs in dogs. Vomiting, anorexia and lethargy were the most common signs in cats.

Two further studies have been submitted which investigated ecotoxicological effects of glyphosate:

Relyea (2005, ASB2012-204) examined the impact of four globally common pesticides including glyphosate on the biodiversity of aquatic communities containing algae and animals.
In a further study Relya (2012, ASB2012-2791) created wetland communities including water plants and animals and exposed thes communities to Roundup. The author reports different effects on nontarget species.

Further studies are presented in detail below:

Author(s)	Year	Study title
Robert Bellé, Ronan Le Bouffant, Julia Morales, Bertrand Cosson, Patrick Cormier et Odile Mulner-Lorillon	2007	L'embryon d'oursin, le point de surveillance de l'ADN endommagé de la division cellulaire et les mécanismes à l'origine de la cancérisation. Journal de la Société de Biologie, Volume : 201, Number: 3, Pages: 317-327 ASB2012-11560

Abstract*
Sea urchin embryo, DNA-damaged cell cycle checkpoint and the mechanisms initiating cancer development (translation from original article)
Cell division is an essential process for heredity, maintenance and evolution of the whole living kingdom. Sea urchin early development represents an excellent experimental model for the analysis of cell cycle checkpoint mechanisms since embryonic cells contain a functional DNA-damage checkpoint and since the whole sea urchin genome is sequenced. The DNA-damaged checkpoint is responsible for an arrest in the cell cycle when DNA is damaged or incorrectly replicated, for activation of the DNA repair mechanism, and for commitment to cell death by apoptosis in the case of failure to repair. New insights in cancer biology lead to two fundamental concepts about the very first origin of cancerogenesis. Cancers result from dysfunction of DNA-damaged checkpoints and cancers appear as a result of normal stem cell (NCS) transformation into a cancer stem cell (CSC). The second aspect suggests a new definition of "cancer", since CSC can be detected well before any clinical evidence. Since early development starts from the zygote, which is a primary stem cell, sea urchin early development allows analysis of the early steps of the cancerization process. Although sea urchins do not develop cancers, the model is alternative and complementary to stem cells which are not easy to isolate, do not divide in a short time and do not divide synchronously. In the field of toxicology and incidence on human health, the sea urchin experimental model allows assessment of cancer risk from single or combined molecules long before any epidemiologic evidence is available. Sea urchin embryos were used to test the worldwide used pesticide Roundup that contains glyphosate as the active herbicide agent; it was shown to activate the DNA-damage checkpoint of the first cell cycle of development. The model therefore allows considerable increase in risk evaluation of new products in the field of cancer and offers a tool for the discovery of molecular markers for early diagnostic in cancer biology. Prevention and early diagnosis are two decisive elements of human cancer therapy.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not assignable
Comment:	Documentation insufficient for evaluation. The publication overview provides information on the general application of the sea urchin embryo model for the prediction of “cancerogenicity”. Only a short reference to another study with a glyphosate-containing herbicide is given. Details of the glyphosate product are not provided. Common surfactants have previously shown the same effects in this model. This model is not appropriate for testing materials containing surfactants because surfactant induced cytotoxicity via membrane disruption is well documented using *in vitro* systems.
Relevance of study:	Not relevant (Prevention of cell cycle transition was determined

for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants.)

Klimisch code: 4

Author(s)	Year	Study title
Marc J., Mulner-Lorillon, O., Boulben, S, Hureau, D. Durand, G., Belle, R.	2002	Pesticide Roundup Provokes Cell Division Dysfunction at the level of CDK1/Cyclin B Activation.. Chem. Res. Toxicol. 2002, 15, 326-331 ASB2013-9838

Abstract*

To assess human health risk from environmental chemicals,we have studied the effect on cell cycle regulation of the widely used glyphosate-conaining pesicide Roundup. As a model system we have used sea urchin embryonic first divisions following fertilization., which are appropriate for the study of universal cell cycle regulation without interference with transcription. We show that 0.8 % Roundup (containing 8 mM glyphosate) induces a delay in the kinetic of the first cell cleavage of sea urchin embryos. The delay is dependent on the concentration of Roundup. The delay in the cell cycle could be induced using increasing glyphosate concentrations (1-10 mM) in the presence of a subthreshold concentration of Roundup 0.2 %, while glyphosate alone was ineffective, thus indicating synergy between glyphosate and Roundup formulation products. The effect of Roundup was not lethal and involved a delay in entry into M-phase of the cell cycle, we analysed CDK1/cyclin B activation during the first division of early development. Roundup delayed the activation of CDK1/cyclin B *in vivo*. Roundup inhibited also the global protein synthetic rate without preventing the accumulation of cyclin B. In summary, Roundup affects cell cycle regulation by delaying activation of the CDK1/cyclin B complex, by synergic effect of glyphosate and formulation products. Considering the universality among species of the CDK1/cyclin B regulator, our results question the safety of glyphosate and Roundup on human health.
* Quoted from article

Author(s)	Year	Study title
Marc J., Mulner-Lorillon, O., Durand, G., Belle, R.	2003	Embryonic cell cycle for risk assessment of pesticides at the molecular level. Environmental Chemistry Letters Volume: 1, Number: 1, Pages: 8-12 ASB2009-9013

Abstract*

Cell cycle mechanisms are highly conserved from unicellular eukaryotes to complex metazoans including humans. Abnormalities in the regulation of the cell cycle result in death or diseases such as cancer. Early development of sea urchin has proved to be a powerful model for cell division studies and offers the opportunity to study synchronous cell divisions in the absence of transcriptional control. We have analyzed pesticide induced dysfunctions in the first cell division following fertilization in sea urchin embryos, using Roundup, a widely used pesticide formulation containing isopropylamine glyphosate as the active substance. The pesticide induced cell cycle dysfunction by preventing the *in vivo* activation of the universal

cell cycle regulator CDK1/cyclin B. We further show that synthesis of the regulator protein, cyclin B, as well as its association to the catalytic protein, CDK1, were not affected by the pesticide. Therefore, our results suggest that the pollutant impedes the processing of the CDK1/cyclin B complex, which is required in its physiological activation. Our studies demonstrate the relevance of sea urchin embryonic cells as a sensitive model to assess pesticide toxicity at the level of the universal cell cycle checkpoints.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Mechanistic study. Outcome with little additional information compared to the authors' previously published work. Non-standard, non-guideline. Commonly used surfactants have previously shown the same effects in this model.
Relevance of study:	Not relevant (Prevention of cell cycle transition was determined for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants because surfactant induced cytotoxicity via membrane disruption is well documented using *in vitro* systems.)
Klimisch code:	3

Author(s)	Year	Study title
Marc, J. Mulner-Lorillon, O. Belle, R.	2004	Glyphosate-based pesticides affect cell cycle regulation Biology of the Cell Volume: 96, Pages: 245-249, ASB2009-9014

Abstract*
Cell-cycle dys-regulation is a hallmark of tumor cells and human cancers. Failure in the cell-cycle checkpoints leads to genomic instability and subsequent development of cancers from the initial affected cell. A worldwide used product Roundup 3plus, based on glyphosate as the active herbicide, was suggested to be of human health concern since it induced cell cycle dysfunction as judged from analysis of the first cell division of sea urchin embryos, a recognized model for cell cycle studies. Several glyphosate-based pesticides from different manufacturers were assayed in comparison with Roundup 3plus for their ability to interfere with the cell cycle regulation. All the tested products, Amega, Cargly, Cosmic, and Roundup Biovert induced cell cycle dysfunction. The threshold concentration for induction of cell cycle dysfunction was evaluated for each product and suggests high risk by inhalation for people in the vicinity of the pesticide handling sprayed at 500 to 4000 times higher dose than the cell-cycle adverse concentration.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not Reliable
Comment:	Non-standard, non-guideline study. Commonly used

	surfactants have previously shown the same effects in this model.
Relevance of study:	Not relevant (Prevention of cell cycle transition was determined for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants because surfactant induced cytotoxicity via membrane disruption is well documented using *in vitro* systems.
Klimisch code:	3

Author(s)	Year	Study title
Marc, J. Belle, R. Morales, J. Cormier, P. Mulner-Lorillon, O.	2004	Formulated glyphosate activates the DNA-response checkpoint of the cell cycle leading to the prevention of G2/M transition. Toxicological Sciences, Volume: 82, Pages: 436-442 ASB2012-11894

Abstract*
A glyphosate containing pesticide impedes at 10 mM glyphosate the G2/M transition as judged from analysis of the first cell cycle of sea urchin development. We show that formulated glyphosate prevented dephosphorylation of Tyr 15 of the cell cycle regulator CDK1/cyclin B in vivo, the end point target of the G2/M cell cycle checkpoint. Formulated glyphosate had no direct effect on the dual specific cdc25 phosphatase activity responsible for Tyr 15 dephosphorylation. At a concentration that efficiently impeded the cell cycle, formulated glyphosate inhibited the synthesis of DNA occurring in S phase of the cell cycle. The extent of the inhibition of DNA synthesis by formulated glyphosate was correlated with the effect on the cell cycle. We conclude that formulated glyphosate's effect on the cell cycle is exerted at the level of the DNA-response checkpoint of S phase. The resulting inhibition of CDK1 cyclin B Tyr 15 dephosphorylation leads to prevention of the G2/M transition and cell cycle progression.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-standard, non-guideline study. Commonly used surfactants have previously shown the same effects in this model.
Relevance of study:	Not relevant (Prevention of cell cycle transition was determined for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants because surfactant induced cytotoxicity via membrane disruption is well documented using *in vitro* systems.
Klimisch code:	3

Additional comments:
Comments of the notifier are submitted on the web site of Monsanto (2006, ASB2013-5455):

http://www.monsanto.com/products/Documents/glyphosate-background-materials/Response_ISIS_apr_06.pdf

The following two recent publications, by Heu *et al.* (2012, ASB2012-11843 and ASB2012-11844) are commented on collectively after the second summary/Klimisch rating, below.

Author(s)	Year	Study title
Heu, C., Berquand, A., Elie-Caille, C., Nicod, L.	2012	Glyphosate-induced stiffening of HaCat keratinocytes, a Peak Force Tapping study on living cells. Journal of Structural Biology Volume: 178, Number: 1, Pages: 1-7 ASB2012-11843

Abstract*
The skin is the first physiological barrier, with a complex constitution, that provides defensive functions against multiple physical and chemical aggressions. Glyphosate is an extensively used herbicide that has been shown to increase the risk of cancer. Moreover there is increasing evidence suggesting that the mechanical phenotype plays an important role in malignant transformation. Atomic force microscopy (AFM) has emerged within the last decade as a powerful tool for providing a nanometer-scale resolution imaging of biological samples. Peak Force Tapping (PFT) is a newly released AFM-based investigation technique allowing extraction of chemical and mechanical properties from a wide range of samples at a relatively high speed and a high resolution. The present work uses the PFT technology to investigate HaCaT keratinocytes, a human epidermal cell line, and offers an original approach to study chemically-induced changes in the cellular mechanical properties under near-physiological conditions. These experiments indicate glyphosate induces cell membrane stiffening, and the appearance of cytoskeleton structures at a subcellular level, for low cytotoxic concentrations whereas cells exposed to IC_{50} (inhibitory concentration 50 %) treatment exhibit control-like mechanical behavior despite obvious membrane damages. Quercetin, a well-known antioxidant, reverses the glyphosate-induced mechanical phenotype.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-guideline *in vitro* tests with no control for low pH effects. Minor reporting deficiencies (source and purity of glyphosate, replicates per dose level)
Relevance of study:	Not relevant (*in vitro* data on the effects on an immortalised epidetmal cell-line does consider low exposure potential due to *stratum cornea* protection. Inappropriate test substance if not adjusted for pH; low pH glyphosate acid is not in formulated glyphosate based products)
Klimisch code:	3

Author(s)	Year	Study title
Heu, C., Elie-Caille, C., Mougey, V., Launay, S., Nicod, L.	2012	A step further towards glyphosate-induced epidermal cell death: Involvement of mitochondrial and oxidative mechanisms. Environmental Toxicology and Pharmacology Volume: 34, Number: 2, Pages: 144-153 ASB2012-11844

Abstract*
A deregulation of programmed cell death mechanisms in human epidermis leads to skin pathologies. We previously showed that glyphosate, an extensively used herbicide, provoked cytotoxic effects on cultured human keratinocytes, affecting their antioxidant capacities and impairing morphological and functional cell characteristics. The aim of the present study, carried out on the human epidermal cell line HaCaT, was to examine the part of apoptosis plays in the cytotoxic effects of glyphosate and the intracellular mechanisms involved in the apoptotic events. We have conducted different incubation periods to reveal the specific events in glyphosate-induced cell death. We observed an increase in the number of early apoptotic cells at a low cytotoxicity level (15%), and then, a decrease, in favour of late apoptotic and necrotic cell rates for more severe cytotoxicity conditions. At the same time, we showed that the glyphosate-induced mitochondrial membrane potential disruption could be a cause of apoptosis in keratinocyte cultures.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-guideline *in vitro* tests with no control for low pH effects. Minor reporting deficiencies (source and purity of glyphosate, replicates per dose level)
Relevance of study:	Not relevant (*in vitro* data on the effects on an immortalized epidermal cell-line does consider low exposure potential due to *stratum cornea* protection. Inappropriate test substance if not adjusted for pH; low pH glyphosate acid is not contained in formulated glyphosate based products)
Klimisch code:	3

Additional comments:
Glyphosate technical acid evaluated was not reported to be pH adjusted and therefore does not reflect real world exposures to the more neutral pH formulations, which contain glyphosate salts, not glyphosate acid
The pH range of test concentrations (850-1150 mg/L) is very acidic, approximately 1.7-2-2 pH units. Keeping in mind the pH scale is logarithmic, these values are substantially lower than those of viable skin and *in vitro* cell cultures.
Exposure potential to live human epidermal skin cells in the field is likely to be considerably lower than the authors have considered. The epidermis is protected by the *stratum cornea*. Human *in vitro* dermal absorption studies for a range glyphosate formulated products are presented in the chapter on dermal absorption, showing a very low dermal absorption of glyphosate; nearly all of the glyphosate is washed off the skin surface after 24 hour exposures (88% to >99 % before *stratum cornea* removal). Therefore, the studies of Heu et al., while

representative of glyphosate spray concentrations, are approximately two or more orders of magnitude higher of those which may result for 8-24 hour dermal exposures.

Author(s)	Year	Study title
Axelrad, J.C. Howard, C.V. McLean, W.G.	2003	The effects of acute pesticide exposure on neuroblastoma cells chronically exposed to diazinon Toxicology, Volume: 185, Pages: 67-78 ASB2012-11553

Abstract*
Speculation about potential neurotoxicity due to chronic exposure to low doses of organophosphate (OP) pesticides is not yet supported by experimental evidence. The objective of this work was to use a cell culture model of chronic OP exposure to determine if such exposure can alter the sensitivity of nerve cells to subsequent acute exposure to OPs or other compounds. NB2a neuroblastoma cells were grown in the presence of 25 µM diazinon for 8 weeks. The OP was then withdrawn and the cells were induced to differentiate in the presence of various other pesticides or herbicides, including OPs and OP-containing formulations. The resulting outgrowth of neurite-like structures was measured by light microscopy and quantitative image analysis and the IC50 for each OP or formulation was calculated. The IC50 values in diazinon-pre-exposed cells were compared with the equivalent values in cells not pre-exposed to diazinon. The IC50 for inhibition of neurite outgrowth by acute application of diazinon, pyrethrum, glyphosate or a commercial formulation of glyphosate was decreased by between 20 and 90 % after pre-treatment with diazinon. In contrast, the IC50 for pirimiphos methyl was unaffected and those for phosmet or chlorpyrifos were increased by between 1.5- and 3-fold. Treatment of cells with chlorpyrifos or with a second glyphosate-containing formulation led to the formation of abnormal neurite-like structures in diazinon-pre-exposed cells. The data support the view that chronic exposure to an OP may reduce the threshold for toxicity of some, but by no means all, environmental agents.
* Quoted from article

Klimisch Evaluation

Reliability of study:	Not reliable
Comment:	Incorrect characterisation of glyphosate as an organophosphate pesticide. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactant are well known for in vitro test systems. Exposure route not relevant for human risk assessment. Rationale for chosen test substance concentration not given.
Relevance of study:	Not relevant (*in vitro* data, do not reflect real *in vivo* exposure situations. Pre-exposure to diazinon is not relevant for this submission).
Klimisch code:	3

Author(s)	Year	Study title
Benedetti, A. L.	2004	The effects of sub-chronic exposure of Wistar rats to the

Vituri, C.D. Trentin, A.G. Domingues, M.A.C. Alvarez-Silva, M.		herbicide Glyphosate-Biocarb® Toxicology Letters, Volume: 153, Pages: 227-232 ASB2012-11562

Abstract*
The object of this study was to analyze the hepatic effects of the herbicide Glyphosate-Biocarbo (as commercialized in Brazil) in Wistar rats. Animals were treated orally with water or 4.87, 48.7, or 487 mg/kg of glyphosate each 2 days, during 75 days. Sub-chronic treatment of animals starting from the lowest dose of glyphosate induced the leakage of hepatic intracellular enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), suggesting irreversible damage in hepatocytes. We observed the increase of Kupffer cells in hepatic sinusoid of glyphosate-treated animals. This was followed by large deposition of reticulin fibers, composed mainly of collagen type III. We may conclude that Glyphosate-Biocarbo may induce hepatic histological changes as well as AST and ALT leaking from liver to serum in experimental models.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comments:	Study report meets basic scientific principles. Study design and documentation is insufficient for assessment.
Relevance of study:	Not relevant because study design not sufficient for assessment of toxicity of the active substance Glyphosate. Toxicity is attributable to high oral dosing of surfactant component. There are several reporting deficiencies.
Klimisch code:	3

Author(s)	Year	Study title
Mesnage, R. Clair, E. Gress, S. Then, C. Szekacs, A. Seralini, G.E.	2012	Cytotoxicity on human cells of Cry1Ab and Cry 1Ac *Bt* insecticidal toxins alone or with a glyphosate-based herbicide. Journal of Applied Toxicology doi: 10.1002/jat.2712. [Epub ahead of print] ASB2012-11900

Abstract*
The study of combined effects of pesticides represents a challenge for toxicology. In the case of the new growing generation of genetically modified (GM) plants with stacked traits, glyphosate-based herbicides (like Roundup) residues are present in the Roundup-tolerant edible plants (especially corns) and mixed with modified *Bt* insecticidal toxins that are produced by the GM plants themselves. The potential side effects of these combined pesticides on human cells are investigated in this work. Here we have tested for the very first time Cry1Ab and Cry1Ac *Bt* toxins (10 ppb to 100 ppm) on the human embryonic kidney cell line 293, as well as their combined actions with Roundup, within 24 h, on three biomarkers of cell death: measurements of mitochondrial succinate dehydrogenase, adenylate kinase release

by membrane alterations and caspase 3/7 inductions. Cry1Ab caused cell death from 100 ppm. For Cry1Ac, under such conditions, no effects were detected. The Roundup tested alone from 1 to 20 000ppm is necrotic and apoptotic from 50ppm, far below agricultural dilutions (50% lethal concentration 57.5ppm). The only measured significant combined effect was that Cry1Ab and Cry1Ac reduced caspases 3/7 activations induced by Roundup; this could delay the activation of apoptosis. There was the same tendency for the other markers. In these results, we argue that modified *Bt* toxins are not inert on non-target human cells, and that they can present combined side-effects with other residues of pesticides specific to GM plants.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-guideline, non-GLP *in vitro* tests meeting scientific principles. Deficiencies: No positive controls were specified, test conditions not described (referenced to a description elsewhere). Exceedingly high doses and an inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems.
Relevance of study:	Relevant with restrictions (Due to reliability. The assessed combinatory effects are of limited relevance)
Klimisch code:	3

Additional comments:
Direct exposure to cells in culture bypasses normal processes limiting absorption and cellular exposure and avoids normal metabolism, excretion, serum protein binding, and other factors that would protect cells in the intact organism.
Anadon et al. (2009, ASB2012-11542) dosed rates with 400 mg/kg of glyphosate, a massive dose relative to any environmental exposure, and achieved peak modeled plasma concentrations of glyphosate of approximately 5 ug/mL (5mg/L or 5 ppm). Assuming linear kinetics, the maximum allowable US daily intake (2 mg/kg/day) would give an approximated blood concentration of 0.025 ppm (25 ppb). McQueen et al. (2012, ASB2012-11898) recently evaluated glyphosate exposure to pregnant women and concluded that estimated exposures based on actual measurements in food were only 0.4 % of the acceptable daily intake.
The “Roundup” LC_{50} concentration used (57.5 ppm) is more than 2000-fold higher than the anticipated concentration (based on Anadon et al., 2009, ASB2012-11542) following maximum allowable intake.

The co-application of Cry protein with the glyphosate-surfactant reduces the apparent degree of cellular injury (as measured by induction of caspase levels). This occurs even at concentrations of Cry1Ab which the authors report to cause cellular injury and membrane disruption. This is worth noting for several reasons:
First, it brings into question the toxicity observations with Cry1Ab, as the argument that membrane disruption and impaired mitochondrial function should be protective seems to be highly untenable, especially in view of the studies (Levine et al, 2007, ASB2009-9030) demonstrating the mitochondrial membrane activity of surfactants.
Second, it should take off the table any implications of a “synergistic effect” of Cry proteins and glyphosate-surfactant herbicides. (The direction is, if anything, antagonistic, but the entire system is fundamentally irrelevant.)

Third, this probably is demonstrating the artificiality of the system itself. As noted above, this is a protein-free medium. Protein protects cells in culture by multiple mechanisms- binding to toxic materials, binding to potential receptor sites, or other non-specific surface-stabilisation effects. It appears from Mesnage's own data that simple addition of protein to their system, even at low concentrations (and even if that protein is a Cry protein) protects from toxicity.

Author(s)	Year	Study title
Clair E., Linn, L., Travert, C., Amiel, C., Seralini, G.E	2012	Effects of Roundup® and Glyphosate on Three Food Microorganisms: *Geotrichum candidum*, *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus delbrueckii* subsp. *bulgaricus* Current Microbiology, Volume: 64, Number: 5, Pages: 486-491 ASB2012-11592

Abstract*

Use of many pesticide products poses the problem of their effects on environment and health. Amongst them, the effects of glyphosate with its adjuvants and its by-products are regularly discussed. The aim of the present study was to shed light on the real impact on biodiversity and ecosystems of Roundup®, a major herbicide used worldwide, and the glyphosate it contains, by the study of their effects on growth and viability of microbial models, namely, on three food microorganisms (*Geotrichum candidum*, *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) widely used as starters in traditional and industrial dairy technologies. The presented results evidence that Roundup® has an inhibitory effect on microbial growth and a microbicide effect at lower concentrations than those recommended in agriculture. Interestingly, glyphosate at these levels has no significant effect on the three studied microorganisms. Our work is consistent with previous studies which demonstrated that the toxic effect of glyphosate was amplified by its formulation adjuvants on different human cells and other eukaryotic models. Moreover, these results should be considered in the understanding of the loss of microbiodiversity and microbial concentration observed in raw milk for many years.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-validated, non-guideline test with methodological and reporting deficiencies (e.g. dose concentrations in media not specified, no positive controls or controls that show the validity of the test system /and concentration range tested). Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems.
Relevance of study:	Not relevant (Due to reliability)
Klimisch code:	3

Additional Comments

Glyphosate at 1 % had no effect on lactobacilli but did impair *Geotrichum*, which is unsurprising as glyphosate at herbicidal concentrations will impact metabolism of many fungi, which (like plants) use the shikimate pathway for aromatic amino acid production.
Surfactants are known to be bacteriostatic, with (for example) quaternary ammonium compounds typically being active in the 30-150 ppm range.
Clair et al. demonstrate that surfactants are bacteriostatic for 3 microorganisms at concentration ranges well within the range of concentrations generally found to be useful for sanitation purposes. However, surfactant solutions are routinely used to sanitize food processing equipment at concentrations at or above those tested by Clair et al. (2012, ASB2012-11592).

B.6.9 Medical data and information (Annex IIA 5.9)

B.6.9.1 Report on medical surveillance on manufacturing plant personnel

Industrial hygiene air monitoring data for glyphosate with workers at the Monsanto Luling, Louisiana manufacturing facility are available for the years 1981-1998 and have been submitted as part of the GTF dossier (no particular reference available). No such data are available from a Monsanto European manufacturing facility. Based on the measured low exposures to glyphosate in the manufacturing setting (well below the ADI) and low toxicological concern, glyphosate specific medical monitoring was not considered necessary by Monsanto. The following data are air concentration measurements which are conservatively applied as 100 % bioavailable to calculations of mean and maximum daily exposures.

Table B.6.9-1: Particulate exposures from glyphosate technical acid operations involving wetcake, e.g., supersack or container filling operations. Values are time weighted averages

Glyphosate Technical Dust (mg/m^3)					**Mean Daily Exposure* (mg/kg/day)**	**Maximum Daily Exposure* (mg/kg/day)**
Sample Type	# Samples	Range	Mean	SD		
All	179	0.0003-0.2594	0.00647	0.0218	0.00108	0.04323
Personal	176	0.0003-0.2549	0.00655	0.022	0.00109	0.04248
Area	3	0.0008-0.024	0.00153	0.00081	0.00026	0.00400
Operator	158	0.0008-0.2594	0.00727	0.0235	0.00121	0.00393
Maintenance	16	0.0005-0.0053	0.00206	0.00144	0.00034	0.00088
Lab	2	0.0003-0.0004	0.00035	N/A	0.00006	0.00007

* based on breathing 10 m^3 air/shift and 60 kg worker

Table B.6.9-2: Glyphosate isopropylamine salt liquid formulation bottling, drumming and tote filling operations. Values are time weighted averages

Glyphosate IPA Salt- Liquid Formulations (mg/m^3)					**Mean Daily Exposure** (mg/kg/day)**	**Maximum Daily Exposure** (mg/kg/day)**
Sample Type	# Samples	Range	Mean	SD		
All	72	0.0001-0.47	0.085	0.105	0.01050	0.05804
Personal	58	0.0001-0.47	0.0251	0.106	0.00310	0.05804
Area	14	0.004-0.28	0.0932	0.105	0.01151	0.03458
Operator	54	0.0001-0.47	0.0966	0.11	0.01193	0.05804
Maintenance	4	0.0041-0.0088	0.00792	0.00187	0.00098	0.00099

** based on breathing 10 m^3 air/shift and 60 kg worker and divided by 1.3496 to convert IPA salt to technical acid

Improvements in manufacturing facility containment and ventilation systems over recent years further reduce the likelihood of operator exposures within glyphosate manufacturing facilities.

B.6.9.2 Reports on clinical cases and poisoning incidents

Glyphosate is worldwide used extensively as herbicide. According to this extensive use a large number of poisoning incidents happened and was published. An extensive review of clinical cases was submitted by [redacted] 2004, ASB2012-11576. Another review of cases was published by [redacted]., 2008, ASB2012-11879.

[redacted] (2009, ASB2013-11831) briefly mentioned a total of 60 reports by physicians from Germany on cases of poisoning with glyphosate herbicides since 1990. Without further specification, in the vast majority of 52 cases only slight health impairment was reported. In four cases, health disturbances were considered "moderate" whereas the only one actually life-threatening case was the result of ingestion of 200 mL of a herbicide containing glyphosate and a [redacted] surfactant with suicidal intent. In the three remaining cases, no symptoms were reported or their severity could not be evaluated.

More than 650 cases of intoxication/irritation ascribed to ingestion of/contact to glyphosate-based herbicides are mentioned in an overview on poisoning incidents from Brazil that was just recently kindly provided to the RMS by the Brazilian National Health Surveillance Agency (Paumgartten/ANVISA, 2012, ASB2013-13413). This data was collected by up to 10 Brazilian poison centers between 2010 and 2012. It is not clear if it is representative for the whole huge country in which the agricultural conditions in general and also those of pesticide use are extremely different. In addition, there is even a higher number of poison information centers in Brazil that have not provided data and, thus, the number of incidents might underestimate the real incidence. On the first glance, the exposure routes, ingested amounts, circumstances (accident, suicidal attempt?), clinical signs and medical treatment are similar to what is known from Germany and from the literature. The much higher total number of cases (as compared to Germany) seems to reflect the applied amount of glyphosate and its formulations that is by orders of magnitude higher in Brazil. Unfortunately, severity of symptoms is not graded and vey often the final outcome was not reported. Another problem is that the product to which exposure was claimed is not specified. Frequently, "Roundup" is mentioned but different formulations are marketed under this name. In many other cases, the incident is ascribed to"glyphosate". However, this is not credible because there is no simple access to the active ingredient and a certain product must have been applied. Furthermore, it is not clear whether a causal relationship had been actually confirmed. Thus, this potentially most interesting data require further thorough analysis before it may be used for evaluation of health risks.

Jayasumana et al. (2014, ASB2014-3085) published a hypothesis on an association between glyphosate exposition, hard water and nephrotoxic metals in an epidemic of chronic kidney disease in Sri Lanka. The authors conclude that although glyphostate alone does not cause an epidemic of chronic kidney disease, it seems to have acquired the ability to destroy the renal tissues when forms complexes with localized geo environmental factor (hardness) and nephrotoxic metals. This conclusion is mainly based on a metal chelating property of glyphosate and the extensive use of glyphosate in Sri Lanka, and the occurance of hard water in the concerned areas. However, the hypothesis was not experimentally evidenced up to now. Sirinathsinghji (2014, ASB2014-10742) report that Sri Lanka is set to partially ban glyphosate-based herbicide use following a study linking it to a fatal chronic kidney disease epidemic affecting the country. This decision is also based on the above described publication of Jayasumana et al. (2014, ASB2014-3085)

Zouaoui et al. (2012, ASB2014-9734) reported 13 cases of acute intoxication with glyphosate (mostly suicidal). The most common symptoms were oropharyngeal ulceration, nausea and vomiting. The main altered biological parameters were high lactate and acidosis. We also noted respiratory distress, cardiac arrhythmia, hypercaleamia, impaired renal function, hepatic toxicity and altered consciousness. In fatalities, the common symptoms were cardiovascular shock, cardiorespiratory arrest, haemodynamic disturbance, intravascular disseminated coagulation and multiple organ failure. Concentrations of glyphosate and AMPA have been determined in blood and urine.

Sribanditmongkol et al. (2012, ASB2014-9731) report a case of a woman who died after ingestion of approximately 500 ml Roundup formulation. Toxic effects of the pesticide were caused by the abilitiy to erode tissues including mucous membranes and linings of the gastrointestinal and respiratory tracts. A mild degree of pulmonary congestion and edema was observed in both lungs.

Mariager et al. (2013, ASB2014-9612) report the case of a 43-year-old man with a history of alcohol abuse. He had used a concentrated glyphosate herbicide and accidentally sprayed the liquid on himself. The patient developed local swelling, bullae and exuding wounds. Neurological impairment followed affecting finger flexion and sensation with reduced nerve conduction. Imaging revealed oedema of the soft tissue and juxta-articular osteopenia.

Lee et al. (2012, ASB2014-9607) report the case of a 60-year orld patient who intentionally ingested 450 ml Roundup. He experienced cardiac arrest but was successfully resuscitated and treated with continuous venovenous haemodiafiltration.

Knezevic et al. (2012, ASB2014-9593) report the case of a 36 year old male patient who attemted suicide by drinking approximately 300 ml of glyphosate formulation. The patient became hypotensive, hypoxic with oliguric acute renal failure. After a single 27,5 hour treatment, clinical condition and renal function parameters did not require further dialysis.

Hour at al. (2012, ASB2014-9571) describe the case of a 66 year old man with a history of alcohol abuse who ingested 500 ml of rum and 350 ml of Roundup. He was hypotensive, diaphoretic and hypoxic. After veno-venous hemodiafiltration his condition improved within 24 hours.

Hinojosa et al. (2011, ASB2014-9566) submitted a retrospective study to identify substances involved in poisonings at Lariboisiere hospital. 315 patients were included with 891 announced substances. Only 1 case of glyphosate was identified.

Garlich et al. (2014, ASB2014-9480) report the case of a 62-year old man who drank a bottle of 41% glyphosae formulation. He was bradycardic and obtunded with respiratory depression. The patient underwent haemodialysis 16 h post ingestion after which he demonstrated improvement in clinical status.

A case of an inhalative intoxication by glyphosate is reported by BfR (2007, ASB2014-9290). A 59 years old farmer sprayed a glyphosate formulation without protective equipment over approximately 3 hours. He suffered from laboured breathing, cough and fever. A biopsy showed alveolitis and bronchiolitis.

Beswick and Millo (2011, ASB2014-9283) describe a fatal poisoning with a glyphosate surfactant herbicide. A 29-year old man was admitted following deliberate ingestion of approximatly 300 ml of Roundup Ace. He developed severe and persistent lactic acidosis, hyperkalaemia, hypotension, torrential watery diarrhoea and abdominal distension in the first 24 hours. The clinical course was complicated by cardiac arrhythmia and an episode of cardiac arrest. On day three following poisoning, the patient died.
Malhotra et al. (2010, ASB2012-11890) report a case of a 71-year old male who attempted suicide with a glyphosate formulation and developed a prolonged but reversible encephalopathy suggestive of acute CNS toxicity. He was in cardiogenic shock with severe metabolic acidosis. Neurologic investigations were performed to exclude structural pathology. CT brain scan was normal. An EEG reading on day 8 demonstrated generalised slow wave acitivity with triphasic sharp and slow wave complex consistent with an encephalopathy although non convulsive seizures could not be excluded.

B.6.9.3 Observations on exposure of the general population and epidemiological studies

~~Two studies on concentrations of glyphosate in human urine samples (Acquavella *et al.*, 2004, ASB2012-11528) and Hoppe (2013, ASB2013-8037) are available and reported in detail in chapter B.6.1 under B.6.1.3 (Published information).~~

Human biomonitoring based on urine measurements
Systemic exposure of humans following application of glyphosate in the field or presumed dietary intake may be roughly calculated on the basis of urinary concentrations even though it is sometimes not easy to distinguish between the routes of exposure (occupational vs. dietary) and their respective contributions to the total intake. In the following, the available data is reported separately for Europe and the U.S. because agricultural conditions are different. In particular, the wide-spread plantation of glyphosate-resistant crops in the Americas must be taken into account because a generally higher exposure level of the population can be assumed there. For comprehensive evaluation and comparison of the resulting exposure or "systemic dose" estimates with the proposed reference values, see Vol. 1 (2.6.11). In parallel to preparation of this RAR, this data and its evaluation have been separately published by the German Federal Insitute for Risk Assessment in a scientific journal (Niemann *et al.*, ASB2014-11029)

For better comparability of the results, the detection/quantification limits in urine as well as the measured and calculated values are always given as µg/L here although, in the original studies, sometimes the unit "ppb" had been used instead (1 µg/L = 1 ppb).

- *Data from the United States*

Acquavella *et al.* (2004, ASB2012-11528) investigated urinary excretion in man following systemic exposure (presumed to be mainly dermal and inhalative) to glyphosate as part of a so-called "Farm Family Exposure Study". Urinary glyphosate concentrations were measured in 48 farmers, their spouses, and 79 children (4–18 years of age) from Minnesota (25 farms) and South Carolina (23 farms). 24-hr composite urine samples were collected for each family member the day before, the day of (day 0), and for 3 days after proven glyphosate application. Different glyphosate-based herbicides (sometimes also containing further active ingredients) were applied by the farmers themselves, perhaps assisted by family members. The analytical method was HPLC-based, following chelation ion exchange for concentration and isolation of

glyphosate, with a limit of detection (LOD) of 1 µg/L whereas a separate limit of quantification (LOQ) was not mentioned.
Sixty percent of the farmers had detectable levels of glyphosate in their urines on the day of application with a remarkable difference between the origin of the samples since glyphosate was detected in the urine of 87% of South Carolina farmers as compared to only 36% positive findings from Minnesota. The geometric mean of the concentrations for the whole group of farmers was 3.2 µg/L on the day of application (Minnesota: 1.4 µg/L, South Carolina: 7.9 µg/L). It seems that the explanation for this gap might be a different use that was made of personal protective equipment. Farmers who did not wear rubber gloves had higher urinary concentrations than found in the other men (nearly 10 µg/L as compared to 2 µg/L) and, in fact, use of rubber gloves was much more common in Minnesota.
In all participants, a decline over the next days was observed.
The maximum value was 233 µg/L. On post-application day 3, the urinary concentration had fallen to 68 µg/L. Based on this highest so far measured value, a" systemic dose" of 0.004 mg/kg bw was calculated by the study authors. For this purpose, they had taken into consideration the individually measured excretion for days 0 through 3 and assumed a daily urine volume of 2 L. Some corrections for incomplete excretion and pharmacokinetic recovery were made. If, however, only the extraordinarily high concentration on the day of application itself is considered, the systemically available amount of glyphosate would be at least 466 µg (rounded for 500 µg in case that not all of it had been excreted in urine the same day) giving, for a 60 kg weighing person, a systemic dose of 0.0083 mg/kg bw.
Among spouses, only very few (4 %) had detectable levels in their urine on the day of application but not later. The maximum value was 3 µg/L. In children, 12 % (all from South Carolina) had detectable glyphosate in their urine on the day of application, with a maximum concentration of 29 µg/L. (It is remarkable that this teenage boy's father was the same man displaying the highest urine concentration among all applicators.) All but one of the children with detectable concentrations had helped their fathers or were, at least, present during herbicide mixing, loading, or application.
This study is considered of good quality and reliable although Mage (2006, ASB2012-11888) claimed some methodological deficiencies with regard to urine collection and absent correction for prior glyphosate exposure. This field investigation is considered important because it was the first one to give an idea about urinary excretion of glyphosate in humans following proven occupational exposure. Of course, it is not known to which extent dietary exposure may have contributed to the measured values but it is presumed to have been low. At least the extremely high values of up to 233 µg/L that have been occasionally measured were most certainly due to direct application of glyphosate-based herbicides, most likely without the use of adequate protective equipment.

A second, similar study partly confirmed these findings but revealed also certain differences and, in addition, included a group of people for whom occupational exposure was unlikely. Curwin *et al.* (2006, ASB2012-11597) analysed urine samples that were obtained in 2001 from farm and non-farm households in Iowa for residues of four pesticides including glyphosate. 24 men ("fathers"), 24 women ("mothers") and 66 children (37 boys and 29 girls) living on a total of 25 farms were enrolled in the study. The control group comprised 23 men, 24 women and 51 children (32 boys and 19 girls) from "non-farm" households. Glyphosate analysis was performed by means of a fluorescent microbead covalent immunoassay that was claimed to have been validated before. An urine concentration of 0.9 µg glyphosate/L was mentioned as the LOD with an LOQ not beeing mentioned separately. In more than 60% of the samples taken from adults and in more than 80% of the samples obtained from children, urinary concentrations of glyphosate were above the LOD with a maximum value of 18 µg/L

measured in a "farm child". The mean values were in the range of 1.1 to 2.7 µg/L for the different groups (sex, age, farm/non-farm). Thus, in principle, mean glyphosate concentrations in urine were in a similar magnitude as determined by Acquavella *et al.* (2004, ASB2012-11528) in their study. Even the highest measures in farm children in both studies (29 and 18 µg/L, respectively) were well in line with each other even though extraordinarily high levels in individual farmers as seen in South Carolina were absent among the samples taken in Iowa. However, the more frequent detection in children was surprising. More striking, there was no statistically significant difference in glyphosate concentrations between study participants from farm and non-farm households, neither for adults nor for children. This finding was in clear contrast to what was found for atrazine, chlorpyrifos, and metolachlor in the same collection of samples since concentrations of these three substances were higher in urine samples taken from farm people.
However, in this study from Iowa, there is less precise information on actual use of glyphosate than in the study of Acquavella *et al.* (2004, ASB2012-11528). It seems at least that, on some farms, there was custom application of the pesticides instead of spraying by the farmers themselves. Residential use of glyphosate in the neighbourhood might be an additional explanation for the lacking difference between farm and non-farm households in this study. Last but not least, it cannot be excluded that dietary exposure to glyphosate residues will have also contributed to the measured urinary concentrations.

The most recent data from the U.S. were published by Honeycutt and Rowlands (2014, ASB2014-6793) on the Internet but not in a scientific journal so far. On behalf of two NGOs ("Moms Across America" and "Sustainable Pulse"), 35 urine samples obtained from women, men and children (4 to 71 years of age) from 14 Federal States were examined for glyphosate. Analysis was performed in a commercial laboratory in St. Louis (Missouri) by means of a not further specified ELISA with a rather high LOQ of 7.5 µg/L. This cut-off value was exceeded in 13 samples with individual measures ranging from 8.1 µg/L in a 6-yr old boy to 18.8 µg/L in a 26-yr old woman, *i.e.*, in a range that seems high but not unplausible against the background of the two studies mentioned above. Unfortunately, no further information on study participants is available that would allow to specify the most likely route of exposure. The authors themselves quoted that initial testing was "not meant to be a full scientifc study. Instead it was set up to inspire and initiate full peer-reviewed scientific studies on glyphosate, by regulatory bodies and independent scientists worldwide".

- *Data from Europe*

In a case study, Mesnage *et al.* (2012, ASB2014-3846) reported urine measurements in a farmer, presumably from Europe (country not mentioned), on the day before and two days after spraying an herbicide containing glyphosate. The analytical method was HPLC with ion trap mass spectrometry revealing an LOD of 1 µg/L and an LOQ of 2 µg/L. 3 hours after termination of spraying, the farmer had 9.5 µg glyphosate/L in his urine and, two days later, the concentration had fallen to about 1.9 µg/L. According to a figure in the article, no glyphosate was detected in the urine samples which were taken on the day before glyphosate use on the crops. Biphasic excretion was seen. From the description in the study, it seems that the farmer had taken adequate protective measures. Surprisingly, a similar concentration of 2 µg/L on day 2 after spraying was measured in one of his children living 1.5 km away from the treated fields while no glyphosate was found in urine samples obtained from the famer's wife and their two other children. The measured urine concentrations of glyphosate were in the magnitude of those reported by Acquavella *et al.* (2004, ASB2012-11528, see above), provided that the herbicide was applied in a responsible way. The finding of glyphosate in the

urine in one of the children cannot be explained with certainty but might be either due to dietary intake (although it would be surprising then that the mother and siblings had none in their urines) or to track-in of traces of the herbicide by the father resulting in residues, *e.g.*, in house dust or yard dirt to which children might be exposed to via the oral, dermal or inhalativ routes, respectively.

In this study, urine was also analysed for aminomethylphosphonic acid (AMPA), *i.e.*, the most important plant and soil metabolite of glyphosate. AMPA is normally found at very low levels in conventional plants, however, several genetically engineered varieties of glyphosate-tolerant plants degrade glyphosate very quickly giving higher amounts of this metabolite (see chapter B.7). In mammals, AMPA is formed only in traces, most likely due to the activity of intestinal bacteria (see section B.6.1). Thus, it is not surprising that Mesnage *et al.* (2012, ASB2014-3846) could not detect AMPA in any sample since the amount of glyphosate received was relatively small and the main exposure route, at least for the father, was certainly dermal and/or inhalative but not by ingestion.

A Europe-wide biomonitoring study (Hoppe, 2013, ASB2013-8037) was recently performed on behalf oft he NGO "Friends of the Earth" and its German partner organisation "Bund für Umwelt- und Naturschutz Deutschland" (BUND) and submitted on request to the RMS. This data has not been published in a scientific journal so far but is available in the Internet. 182 frozen urine samples from 18 European (EU and non-EU) countries (6 – 12 per country but mostly 10) were examined for glyphosate and AMPA by means of a modern and selective analytical method, *i.e.*, transformation of both compounds to two different derivatives followed by GC-MS/MS. The LOQ for both, glyphosate and AMPA, was 0.15 µg/L. As in the previous studies by Acquavella *et al.* (2004, ASB2012-11528) and Curwin *et al.* (2007, ASB2012-11597), creatinine was also measured as an internal proof for the validity of the urine measurements.

For glyphosate, nearly 44% (80 samples) and, for AMPA, more than one third (65) of the participants had urine concentrations above the LOQ. Maximum values of 1.82, 1.64 or 1.55 µg/L for glyphosate were found in samples obtained from Latvia, the UK, and Malta, respectively, but the mean value of 0.21 µg/L was much lower. (For calculation of the mean, the study author had included the samples with values below the LOQ and assumed a concentration of 0.075 µg/L, *i.e.*, half the LOQ, for them. Thus, in reality, the actual mean value might be either a bit higher or lower.) For AMPA, the maximum values of 2.63, 1.26, and 0.89 µg/L were measured in samples from Croatia, Belgium, and Malta with a mean urinary concentration of 0.18 µg/L for all involved people. It was surprising that in more than 30 cases the AMPA concentrations were higher than those of glyphosate, sometimes by 10 times or more. In a few samples, AMPA values were rather high with glyphosate concentrations below the LOQ.

Apart from this data, the author also mentioned a "reference value" of 0.8 µg/L for glyphosate in urine, based on analytical investigations in a total of 90 people from a not further described "urban collective" from the region of the German city of Bremen (where its laboratory is situated). This figure was the 95th percentile of the individual values and was established in 2012 in preparation of the main study. For AMPA, a "reference value of 0.5 µg/L was given.

The measured values themselves are considered reliable by the RMS. The results confirmed the previous assumption that there is in fact evidenc of a certain exposure of European population to glyphosate, most likely by dietary intake. This is not surprising since glyphosate is a widely used active substance worldwide. Residues in food and feed may occur (see

chapter B.6.7) and are allowed and of no health concern if below the MRLs. Systemically available glyphosate (*i.e.*, the rather low percentage that is absorbed from the GIT) is excreted via the urine, virtually unchanged. Apparently, there is also some exposure to AMPA although its origin is less clear and there must be other sources than the agricultural use of glyphosate that is hardly metabolised to AMPA in mammals (see section B.6.1). The author himself stated that the glyphosate and AMPA values would not correlate very well.
Without any doubt, as explorative data, the results of this study are interesting because they provide an idea of actual glyphosate intake throughout Europe. However, conclusions should be drawn with care. Due to the limited number of involved participants in the different countries and the absence of any information about them (such as age, gender, body weight, social background, origin from urban or rural environments, nutrition habits) and the way how they were recruited, the study cannot be regarded as representative. From the available information, it seems clear that the participants will have ingested glyphosate residues in their diets and were not exposed as operators. However, the mean dietary exposure level cannot be estimated on this basis, neither for a single country nor for Europe in its whole. Moreover, no conclusion can be drawn to which extent the apparent differences in urinary levels of glyphosate in the samples might reflect the actual use of glyphosate in the different countries. (It was reported, *e.g.*, that 8 out of 10 samples from Austria and 10 out of 12 from Switzerland were below the LOQ in contrast to only 3 of 10 from the UK or even 1 of 10 from Malta. However, this distribution might well be a random one.)

On behalf of the German Federal Environmental Agency, frozen urine samples that had been taken for other purposes in 1996 and 2012 in the city of Greifswald in the north-eastern part of Germany and its surrounding region were analysed in retrospect for glyphosate residues. This so far unpublished data was kindly submitted to the German Federal Insitute for Risk Assessment (Markard, 2014; ASB2014-2057) to support ongoing evaluation of glyphosate. In each of the two sampling years, urine analysis for glyphosate and AMPA was performed in samples from ten male and ten female students (age 20 – 29 years at the time of sampling). The LOQ of the test method (*i.e.*, gas chromatography) of 0.15 µg/L was exceeded for glyphosate in 22 of the totally 40 samples. The maximum value was 0.65 µg/L. There was a tendency towards an increase in glyphosate concentrations in urine in the 2012 samples compared to those from 1996, possibly reflecting a more frequent use of glyphosate in agriculture resulting in a higher dietary intake. The LOQ was exceeded more frequently and individual values tended to be higher.
Again, there were indications that AMPA concentrations in the urine may be higher than those of glyphosate. 10 out of 40 results were above the LOQ of 0.15 µg/L with a maximum value 1.31 µg/L. However, in contrast to glyphosate, the AMPA concentrations appeared to decrease between 1996 and 2012 suggesting that there is poor correlation between glyphosate and AMPA residues and that other routes or sources for exposure to AMPA than by (plant) metabolism of glyphosate should be considered. In addition, the stability of glyphosate in deep-frozen urine over more than 16 years was not investigated, maybe resulting in a shift of the glyphosate/AMPA ratio.

Krüger *et al.* (2014; ASB2014-5024) analysed several hundred human samples by means of a not further specified ELISA (Abraxis, USA). In the analytical part of this article, a comparison between values obtained by this ELISA and GC-MS was provided revealing a sufficient correlation (R^2 of 0.87 for human urine and even better for cattle and rabbit urine). Thus, the measured values may be taken for reliable. The mean concentration was nearly 2 µg/L with a maximum in the magnitude of 5 µg/L. Unfortunately, only figures were given in the brief publication instead of precise numbers and an LOD or LOQ were not mentioned.

A further deficiency of this paper is the lacking information how many subjects had been actually involved and how they were recruited for the study. On one hand, it is stated that glyphosate concentrations in 99 urine samples from humans on conventional diet were compared to 41 samples obtained from people who had claimed to eat "organic". As to be expected because of extensive use of glyphosate in conventional agriculture, the difference between mean values (nearly 2 µg/L with a maximum around 4 in the "conventional diet" group vs. a mean of about 1 µg/L and a maximal value below 2 for eaters of "organic diet") was statistically significant. On the other hand, urine concentrations of glyphosate in 102 "healthy" and 199 "chronically diseased" people were compared. In the "healthy" population, the mean was, again, slightly below 2 µg/L with a maximum of slightly less than 4. In the "diseased" group, mean value appeared to be a bit higher than 2 µg/L and the maximum reached a level of about 5 µg/L. The difference achieved statistical significance ($p<0.03$). However, it was not reported if the included groups (separated by nutritional preferences and health status) were overlapping or not. In addition, there was no information on the participants of the study given, neither with regard to age, gender, or the chronic diseases they suffered from nor with regard to occupational or social background or residential status (town or countryside). It is even not known when the samples were taken but it can be assumed that they were mainly from Germany. (Urine data obtained in cows, rabbits, and hares were also measured but are reported in section B.6.8.3.3. For glyphosate residues in organs of slaughtered cows, see chapter B.7).

Human biomonitoring based on breast milk

In nursing mothers and lactating animals, milk is a common route of elimination of xenobiotics but its actual relevance depends on the substances and the chemical classes they belong to. For substances that have very long half-lives and accumulate in the body, like many organochlorine compounds, systematic surveillance of excretion via breast milk may provide useful information on the exposure level of the general population and its long-term trends as well as on the intake by infants. For instance, the decrease in exposure to pesticides such as DDT or lindane after their ban but also excretion of chemicals such als dioxin-like and non dioxin-like polychlorinated biphenyls was followed by this method (Fürst, 2006, ASB2014-8168; UBA, 2008, ASB2014-8167; BfR, 2011, ASB2014-8171; Verdugo-Raab, 2013, ASB2014-8173). For glyphosate, because of its physico-chemical properties, accumulation in the body is not likely. The substance is not lipophilic and, thus, a deposit in body fat that might be released during nursing/lactation cannot arise. In line with these more theoretical considerations, the numerous kinetic studies (see section B.6.1) have clearly shown rapid and quantitative elimination from the body, no potential for accumulation and no affinity to fatty tissues. Investigations in lactating cows and goats following oral administration revealed very limited excretion via the milk accounting for not more than 0.1% of the administered total dose (see chapter B.7). There is no reason to suspect that this might be different in nursing mothers.

Measuring of pesticides and their residues in breast milk might be also useful if offspring toxicity in reproduction studies was due to exposure of the pups via the milk. There are examples for that (in humans mainly related to medical drugs) but, with glyphosate, evidence of such effects was completely lacking, despite the large number of multi-generation studies (see section B.6.6.1).

Thus, in principle, there is no need to investigate breast milk for glyphosate and, accordingly, no data was available when the first draft of this report was prepared.

Recently, Honeycutt and Rowlands (2014, ASB2014-6793) published data on glyphosate findings in breast milk that gained considerable public attention but neither are the measured values reliable nor the conclusions of the authors agreed with. Ten samples were obtained from ten women who live in different U.S. Federal States. It is clear that this low number is in no way representative and that the findings can be considered at best expolarative. In three out of the 10 samples (all three from different states), the detection limit of 75 µg/L was exceeded with individual values of 76, 99, and 166 µg glyphosate/L. Detection of a chemical in milk does not necessarily mean that the substance must have accumulated before. It may be simply widely distributed throughout the body and excreted, among other routes, also by the milk. However, in this case, the values appear extremely high, in particular if compared to the urinary concentrations mentioned above, taking into account that urine is the main excretion route for ingested and systemically absorbed glyphosate (see section B.6.1). Based on the Internet publication itself and the sample number given therein, the group of nursing mothers was different from the people of whom urine samples had been analysed (see above). Thus, it is not possible to compare excretion of glyphosate via urine and breast milk for the same woman. Taking into account the physico-chemical properties of glyphosate and its well known pattern of distribution and elimination (based on animal studies), it is simply not conceivable that breast milk concentrations might be higher than those in urine. In contrary, urinary concentrations are expected to be much higher. However, the maximum ever measured urine concentration of 233 µg/L (Acquavella *et al.*, 2004, ASB2012-11528) was due to immediately preceeding direct application of a herbicide by a farmer. Based on these considerations, the values reported for breast milk should be seriously doubted.

It was noted that apparently the same ELISA, without further modification, has been used for analysis of urine, "household water", and breast milk for glyphosate. It is well known that validation of an assay for different matrices is inevitable but was not reported to have been performed in this case. The LOD of 75 µg/L in milk is by ten times higher than in urine (see above) pointing to large differences. Thus, the obvious deficiencies in the analytical method make the results not reliable.

Even if the measured values were trusted in, they would not allow to give a rough estimate of the women's exposure since it is not known to which extent of ingested or otherwise absorbed glyphosate is excreted by this route although ruminant data suggest that it should be a very minor one. For phtalates (Fromme *et al.*, 2011, ASB2014-8169) and, more recently, for certain organochlochlorine and perfluorinated substances (Raab *et al.*, 2013, ASB2014-8170), exposure of exclusively breast-fed infants was calculated on the basis of (much more reliable) concentrations measured in breast milk and then compared to the respective reference values. If the same is tried for glyphosate, despites the doubts about the validity of the results, the highest measured value of 166 µg/L in breast milk could be taken as a provisional point of departure. Assuming a daily amount of breast milk of 700-900 mL that is produced (and consumed) to feed a baby in the first six month after giving birth, a total excretion of up to 150 µg glyphosate would result. For an exclusively breast-fed infant of 10 kg, the resulting exposure of 15 µg/kg bw would be by about 33 times lower than the (proposed) ADI of 500 µg/kg bw (0.5 mg/kg bw) and the margin would become bigger if the infant grows. Thus, even a glyphosate concentration in this unreliably high magnitude in breast milk would not be of health or developmental concern.

Epidemiology

A number of human studies on genotoxicity have been published since 2000 in which exposures of the studied populations to glyphosate-based formulations were postulated. These

publications are presented and discussed in chapter B.6.4 (Genotoxicity), in the "Published data" section under B.6.4.8.7 (Human and environmental studies).
Likewise, several epidemiology studies on a possible relationsship of exposure to glyphosate (and further pesticides) and cancer have been published since 2000 that are presented and discussed in chapter B.6.5 under "Published data" (B.6.5.3 Published data).
With regard to reproductive outcome in humans, a number of epidemiological studies in which glyphosate exposure was considered are presented and discussed in chapter B.6.6 (Reproductive toxicity).
Corsini et al. (2012, ASB2014-9352) submitted a comprehensive review on pesticide induced immunotoxicity in humans. The authors conclude that the available studies on the effects of pesticides on human immune system have several limitations including poor indications on exposure levels, multiple chemical exposures, heterogeneity of the apprach and diffuculty in giving a prognostic significance to the slight changes often observed. Further studies would be necessary.
Sugeng et al. (2013, ASB2014-9733) performed a hazard ranking of agricultural pesticides for cancer, endocrine disruption and reproductive/developmental toxicity in Yuma County, Arizona. Glyphosate was not considered relevant concerning carcinogenicity and reproductive toxicity in result of this ranking. Concerning endocrine disruption the authors concluded a low hazard.
Lesmes-Fabian et al. (2012, ASB2014-9726) reported results of dermal exposure assessment of pesticide use by sprayers in potato farms in the Colombian highlands. The authors conclude that the results would suggest that to reduce the health risk, three aspects have to be considered: avoiding to modification of nozzles, using adequate work clothing made of thick fabrics and cleaning properly the tank sprayer before the application activity.
Perry et al. (2014, ASB2014-9626) report the epidemiology of pesticide exposures reported to poison centres in the UK over a 9-year period. The authors conclude that the data from this survaillance study indicate that poison centre resources can usefully monitor pesticide exposures resulting in health care contact in the UK. The NPIS may usefully be one competent to the UK`s response to European legislation requiring survailance of complications resulting from pesticide use.
Labite and Cummins (2012, ASB2014-9604) submitted a quantitative approach for ranking human health risks from pesticides in Irish gorundwater. According to human health based risk glyphosate was ranked by this method at number 37 of 40 pesticide substances.
Horiuchi et al. (2007, ASB2014-9570) describe 394 cases of dermatitis in Saku district in Japan. Three of these 394 cases have been related to glyphosate.
Goldner at al. (2013, ASB2014-9492) evaluted the assciation between thryoid disease and use of insecticides, herbicides and fumigants/fungicides in male application in the Agricultural Health Study (AHS). The authors conclude that there is a association between hypothyroidism and specific herbicides (especially 2,4-D, 2,4,5-T and 1,4,5-TP) and insecticides in male applicators. There was no significantly increased association observed for glyphosate.
Roberts et al. (2012, ASB2014-9394) submitted a review on pesticide exposure in children. The authors conclude that childrens exposures to pesticides should be limited as much as possible. According to the authors there would also be numerous reports in the medical literature of adverse events after human exposure. Patients would have presented with signs and symptoms consistent with an aspiration pneumonia-like syndrome.
In response on this article of Roberts et al. a letter was published by Goldstein (2012, ASB2014-9493) from Monsanto company. In this letter the author is correcting some inaccuracies regarding glyphosate in the Roberts review.
Chien et al. (2012, ASB2014-9326) submitted a retrospective cohort study on risk and prognostic factors of inpatient mortality associated with unintentional insecticide and

herbicide poisonings. 3968 inpatients recruited at hospitalization between 199 and 2008 in Taiwan have been considered in this study. The authors conclude that overall survival for herbicide impatients was significantly worse than for insecticide poisoning patients. Further information on the specific type of pesticide was not available.

A study on the epidemiology of glyphosate-surfactant herbicide poisoning in Taiwan, 1986-2007 was submitted by Chen et al. (2009, ASB2014-9318). A total of 2186 patients were eligible for analysis. Most of the exposures were related to oral ingestion and attempted suicide. The authors conclude that age, ingested amount, delayed presentation and reason for exposure were likely to be determinants of the severety of GlySH exposure. Because shock is the major cause of death and usually develops early after GlySH exposure, prompt fluid replacement therapy seems critical in the initial management of such exposure.

Carroll et al. (2012, ASB2014-9308) studied diurnal variation in probability of death following self-poisoning in Sri Lanka. No evidence of diurnal variation in the outcome was observed for glyphosate.

B.6.9.4 Clinical signs and symptoms of poisoning and details of clinical tests

The summary in this section is based on well over 30 years of experience with numerous formulations of glyphosate in a wide range of situations. The extensive use of glyphosate has encouraged clinical assessment of various interventions and has resulted in reporting of alleged associations of symptoms with exposures to glyphosate products. The clinical toxicology of glyphosate and of glyphosate-surfactant formulations have been the subject of an extensive review ([redacted] 2004, ASB2012-11576), and a review of cases with assessment of clinical prognostic factors was more recently published ([redacted]. 2008, ASB2012-11879).

Animals do not have the shikimic acid pathway; and no direct target-mediated mode of action in mammalian systems has been clearly identified to date ([redacted] 2004, ASB2012-11576). Glyphosate does not inhibit the cholinesterases, and has no cholinergic effect. While incidental exposure in glyphosate-surfactant herbicide mixtures is common, review of available case reports (AAPCC 2003-2011) indicates that the vast majority of reported non-suicidal exposures involve skin and/or eye irritation or irritation of the respiratory tract by inhalation of spray mist, and that systemic symptoms are rare following non-suicidal exposures to glyphosate products. Based upon human experience and animal data, even those systemic symptoms reported following incidental exposure appear unlikely to be causally related to exposure ([redacted]., 2002, ASB2012-11831).

The following clinical effects are divided into those expected following minor and significant exposures for each category based upon expected severity of systemic symptoms. The factors which determine if the exposure was minor or significant include:

- Route of exposure.

Dermal, eye and mist inhalation exposures to any commercially formulated glyphosate products of any dilution are minor exposures for purposes of the symptom descriptions below. Ingestions more than 50 mL (one mouthful if amount unknown) of a product with >10 % glyphosate concentration may be significant.

- Concentration of the product.

Glyphosate concentrations of less than 10 % rarely if ever produce significant toxicity. Most serious illness has historically resulted from ingestion of the 41 % (glyphosate IPA) concentrate. In the absence of extensive clinical experience for the 11-40 % concentration range, any ingestion of greater than 50 mL of a glyphosate preparation having a greater than 10 % concentration of glyphosate salts should be considered potentially significant for purposes of the symptom descriptions below.

- Intent of the exposure.

Accidental ingestion rarely involves large quantities of concentrated formulations. Intentional ingestion cases may not present with a reliable history and may require observation if the amount ingested cannot be reliably determined.

Route and organ system specific syptoms of exposure:

Dermal

Minor exposures:

Contact with skin may produce a dermatitis similar to that of detergents (Bradberry *et al.*, 2004, ASB2012-11576)

It is expected that the severity of injury following skin exposure will be significantly decreased with a less concentrated product and with a reduced duration of contact.

Phototoxic reactions (sunlight or ultraviolet (UV) light induced skin reactions) have been reported. These symptoms are believed to be due to an antimicrobial additive (benzisothiazolone) which is present in selected residential use (i.e. non- agricultural) products containing 10 % glyphosate or less ([redacted] 2004, ASB2012-11576).

Significant absorption through the skin does not occur (see also B.6.12).

Significant exposures:

Skin exposures are not expected to cause systemic effects or serious cutaneous effects when the results of animals studies and the low dermal absorption are taken into account. There are no reports that would suggest the contrary. Symptoms as noted in the minor exposure may occur.

Ocular

Minor exposures:

A review of ocular exposures to US glyphosate-surfactant formulations (1513 exposures over a 5-year period), showed no permanent eye injury ([redacted] 1999, TOX2002-699).

Human eye exposures have generally resulted in temporary conjunctival irritation, clearing after irrigation or in 1-2 days and permanent eye damage is said to be “most unlikely” ([redacted] 2004, ASB2012-11576).

It is expected that the severity of injury following eye exposure will be significantly decreased with a less concentrated product or with a reduced contact time.

Significant exposures:

Eye exposures are not expected to cause systemic effects or serious ocular injury ([redacted] 1999; TOX2002-699, [redacted] 2004, ASB2012-11576).

Systemic exposure – ingestion or inhalation

- Neurologic

Minor exposures:
There is no clinical or experimental evidence that glyphosate or glyphosate-surfactant formulations cause neurological symptoms or injury after exposure by any route.

Significant exposures:
There have been no reports of primary convulsions after ingestion.
One author reports most patients present with a clear sensorium unless another substance, such as alcohol, has been co-ingested or severe hypoxemia has occurred ([redacted] 1989, TOX9552426); however "moderate disorders of consciousness" have been reported within 48 hours of suicidal ingestions of the concentrate ([redacted], 1987, Z35531; [redacted], 1988, Z35532). This has occurred in patients with significant systemic illness and is not believed to be the result of reduced organ perfusion ([redacted]., 2004, ASB2012-11576) or perhaps other factors such as metabolic disturbance but the possibility of a direct toxicological effect cannot be excluded ([redacted]., 2004, ASB2012-11576).
There are two isolated case report of Parkinson's disease developing in individuals with a history of glyphosate product exposure ([redacted] 2001, ASB2012-11557; [redacted] 2011, ASB2012-12047). These publications are reported in detail with Klimisch rating in chapter B.6.7 (Neurotoxicity) under B.6.7.2 (Published data), because they are discussed there in context with other studies on neurotoxicity and on Parkinson`s disease.

- Gastrointestinal:

Minor exposures:
Minor exposures are likely to be asymptomatic, but the patient may experience an unpleasant taste, tingling, mild self-limited nausea and vomiting.
Self-limited diarrhoea may also occur which is thought to be due to the surfactant.

Significant exposures:
A burning sensation in the mouth and throat, salivation, oral erythema, sore throat, dysphonia, dysphagia, epigastric pain, nausea, spontaneous vomiting, abdominal pain and diarrhoea are common and may last up to a week.
Serum amylase may be elevated; isoenzyme analysis done in a few cases identified a salivary gland origin ([redacted] 1989, TOX9552426).
In severe cases with large ingested doses, hematemesis, GI bleeding, melena and hematochezia may occur. Paralytic ileus has been reported as a rare event.
Endoscopy has noted erosions of the pharynx and larynx, esophagitis and gastritis with mucosal oedema, erosions and haemorrhage. However, transmural injury and perforation have not been noted ([redacted] 1999, ASB2012-11510).
In fatal cases, autopsy notes mucosal or transmural oedema and necrosis throughout the small bowel with erosion and haemorrhage; in the large bowel, mucosal oedema and focal haemorrhage was noted ([redacted], 1989, TOX9552426).
Clinical, autopsy and experimental evidence ([redacted] 1987, TOX9552430) indicate a potential for gastrointestinal damage from glyphosate components of glyphosate formulations, but the frequency of severe injury appears to be low.
Chien et al. (2013, ASB2014-9321) studied the spectrum of corrosive esophageal injury after intentional paraquat or glyphosate surfactant herbicide ingestion. They performed an observational study on 47 patients with paraquat or glyphosat ingestion. The authors conclude that paraquat and glyphosate are mild caustic agents that produce esophagea injuries of grad 1, 2a and 2b only. The data suggest a potential relationship between the degree of esophageal injury and systemic complications.

- Cardiovascular:

Minor exposures:
Dermal, eye and mist inhalation exposures to any commercially formulated glyphosate products of any dilution are minor exposures. Cardiovascular effects are not expected to result from such minor exposures and no reports are available.

Significant exposures:
Hypotension is common after ingestions of a mouthful or more of the concentrated product (not the diluted forms) and usually responds to IV fluids and pressor amines. Shock as manifested by oliguria, anuria and hypotension which was unresponsive to fluids and pressors, ultimately resulting in death, has been reported. ([redacted] 1989, TOX9552426, [redacted] 2004, ASB2012-11576). Transient hypertension may be noted.

- Upper respiratory:

Minor exposures:
Dermal, eye and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution are minor exposures. Significant upper respiratory effects are not expected from minor exposures, but minor irritation or discomfort may occur ([redacted]., 2004, ASB2012-11576).

Significant exposures:
Significant systemic exposures are not anticipated to occur via the inhalational route. However, if occurring, they would most probably also affect the lower respiratory tract (see below).

- Lower respiratory:

Minor exposures:
Because of the non-volatile nature of glyphosate and the surfactant, exposures to vapour is not possible. The spray equipment that is commonly used will produce particles that are non-respirable.

Significant exposures:
Tachypnea, dyspnea, cough and bronchospasm including cyanosis have been seen in severe ingestions (more than a mouthful of concentrated product). These effects are indiactive of systemic toxicity.
Aspiration pneumonia, pulmonary oedema and respiratory failure have been seen although the exact role of aspiration has not been fully investigated.

An isolated case report suggests the development of acute pneumonitis in a worker following his performing maintenance on non-operating spray equipment used to apply a glyphosate-surfactant formulation ([redacted] 1998, ASB2012-11513). However, actual exposure and its extent could not be really substantiated in this case. Accordingly, the occurrence of pneumonitis in this individual is more likely to be coincidental by nature although a (different) occupational origin seems plausible ([redacted] 1999, ASB2012-11511).

There is also a case report from Germany in which a glyphosate-surfactant product (tallowamine or “POEA” based) was applied by knapsack spayer in a 0.5ha forestry application at the registered application rate at 25° C for approximately 3 hours without

respiratory protection (Burger *et al.*, 2009, ASB2013-11831). About 7 hours after application he developed chest pain with rapidly increasing severe respiratory distress and fever up to approximately 38° C. On hospital admission, radiographic changes of lungs could be demonstrated. To further assess possible causes, bronchoscopy and closed lung biopsy was performed. Histopathology revealed "toxic inflammation of the lungs" that was significantly different from bacterial infection. After 7-days of drug treatment, changes in lung reversed but six months after the incident, the patient still experienced moderate respiratory complaints on exertion. In the X-ray examination, there were still detectable lung changes although some improvement had been noted. In addition, in the same reference, 20 cases of inhalative exposure among a total of 60 reports on confirmed or presumed poisoning incidents with glyphosate herbicides from Germany (since 1990) were mentioned with breathing difficulties occurring in 50 % of the affected people. No more details on clinical courses or outcomes were given but it was emphasised by the authors as "striking" that the involved products nearly always contained [redacted]

- Renal:

Minor exposures:
Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution are minor exposures. Renal effects are not expected to result from such minor exposures and no reports are available.

Significant exposures:
Hypotension and hypovolemic shock may result in oliguria and anuria, following severe ingestions ([redacted] 2004, ASB2012-11576). Abrupt rises in BUN and serum creatinine may be seen.

- Metabolic:

Minor exposures:
Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution should be considered minor exposures. Metabolic effects are not expected following minor exposures and no reports are available.

Significant exposures:
Mild fever may be noted even in the absence of infection ([redacted] 2004, ASB2012-11576)
Metabolic acidosis is often seen in a severely poisoned patient [redacted] 2004, ASB2012-11576) and this acidosis may fail to respond to bicarbonate therapy. Although the exact nature was not elucidated, a lactic acidosis was suspected.

- Hematologic:

Minor exposures:
Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution should be considered minor exposures. Haematological effects are not expected from minor exposures and no reports are available.

Significant exposures:
Leukocytosis without evidence of bacterial infection has been noted in peripheral blood after ingestion of the concentrate ([redacted], 2004, ASB2012-11576).

Hemoconcentration has been seen as a result of intravascular volume depletion and might indicate severe capillary fluid leakage ([redacted] 1989, TOX9552426).
No primary toxic effects on bone marrow or formed elements have been reported to date.

- Hepatic:

Minor exposures:
Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution should be considered minor exposures. Hepatic effects are not expected from such minor exposures and no reports are available.

Significant exposures:
No direct hepatotoxic effects have been noted; however, minor elevations in transaminases and bilirubin are reported ([redacted] 1989, TOX9552426; [redacted] 2004, ASB2012-11576).

- Clinical chemistry (electrolytes):

Minor exposures:
Severe or prolonged vomiting and diarrhoea may induce fluid and electrolyte imbalance. However, such signs are not expected following a minor exposure.

Significant exposures:
Electrolytes (Na, K, Cl and Ca) in the absence of renal failure generally remain normal. Severe or prolonged vomiting and diarrhoea may induce fluid and electrolyte imbalance.
POTASSIUM SALTS: While potentially toxic ingestions of all glyphosate products may result in fluid and electrolyte disturbances, particular attention to potassium may be important following ingestion of the potassium salt products. Close monitoring of serum potassium levels and/or electro-cardiographic monitoring (for peaked T-waves or rhythm disturbances) is recommended following significant ingestion of potassium salt products, particularly for high risk individuals. Individuals with the following may be at elevated risk following acute potassium exposure: known hyperkalemia, renal failure / renal dysfunction, use of potassium sparing diuretics, hypoaldosteronism, co-ingestion of other K+ containing materials, underlying heart disease, use of digoxin, digitoxin, oabain, or exposure to other cardiac glycosides. The quantity of potassium ingested from a glyphosate potassium salt product can be estimated from the weight percent of glyphosate potassium as:

Percent K+ salt x 5.3 = mEq potassium per 100 cc of product

Several case reports indicate that after ingestions of large amounts of glyphosate-potassium salt concentrate solutions, clinically significant hyperkalemia may occur. [redacted] (2001, ASB2012-11556) reported an intoxication in a 65 year old female who ingested a glyphosate-potassium salt (350 mL Roundup Maxload missing from container, in addition to 250 mL of another glyphosate formulation which was not a potassium salt but amount actually ingested unclear) in a suicidal attempt. On admission, serum potassium level was 9.3mEq/L (typical normal value < 5) with electrocardiographic changes consistent with hyperkalemia. The patient did have a concomitant acidosis (pH 7.272) which may account for some portion of the elevation in potassium (acidosis displaces intracellular potassium). The patient responded to medical management and survived.
[redacted] (2012, ASB2012-11863) reported the case of a 69 year old female who ingested approximately 500 mL of the same product. On arrival in the hospital, the patient had

hyperkalemia (10.7 mEq/L), pulseless ventricular tachycardia, and a severe metabolic acidosis (pH 7.005, will elevate potassium.) The patient required aggressive cardiopulmonary resuscitation and hemodialysis but did recover.
According to the GTF dossier (no particular reference given), Monsanto is aware of one additional (unpublished) case of a similar ingestion with dramatically elevated potassium level in which the patient was moribund when medical care was instituted. The patient could not be resuscitated. Because serum potassium levels rise rapidly following death (due to redistribution of intracellular potassium), it is not possible to know how much of the observed hyperkalemia was the result of the ingestion versus profound acidosis and *post mortem* redistribution (which is partially due to acidosis).

It should be noted that the issue of hyperkalemia is limited to cases involving the suicidal ingestion of glyphosate-potassium concentrates. Potassium is a normal component of the human diet, and potassium intake attributable to occupational glyphosate-surfactant herbicide exposure will be negligible compared to typical dietary intake. While the concentrate formulations may contain up to approximately 250 mEq of potassium per 100 mL, product diluted for use (1 % glyphosate concentration) will contain about 6 mEq potassium per 100 mL. By way of reference, a medium size banana contains about 10 mEq (425 mg) of potassium.
Finally, it should be noted that the apparently very large (>150 mL) ingestions of glyphosate-surfactant concentrates observed in these cases are well within the range isopropylamine salt products reported to produce fatalities, and that elevations in potassium concentrations are reported (probably due to acidosis) following ingestions of glyphosate IPA salt products. While the cases do suggest that potassium salt products likely contribute to the risk of hyperkalemia, it is not clear at this time if the use of postassium salts will increase the overall clinical severity and/or mortality associated with glyphosate concentrate product ingestions.

Specific diagnostic testing and prognostic considerations
Serum or other body fluid measurements of glyphosate will be generally not available in a time frame that would be useful for acute clinical diagnosis. As the management of symptoms associated with glyphosate-surfactant product ingestion is symptom-driven in any event, the lack of rapidly available knowledge on concentrations of glyphosate will generally not impair clinical care. Levels may be more helpful in addressing forensic issues.

Attention should be paid to electrolyte concentrations in individuals with significant ingestion exposures, particularly to glyphosate-potassium concentrate solutions.

Respiratory distress requiring intubation, pulmonary oedema, shock (systolic BP <90 mmHg), altered consciousness, abnormal chest X-ray, ingestion of over 200 cc concentrate (41 %), or renal failure necessitating dialysis have been associated with a higher risk of poor clinical outcomes including mortality ([redacted] 2008, ASB2012-11879). A prognostic index based upon these factors was developed but its use is not expected to contibute significantly to improved medical care. As symptom onset may be delayed, early use of such prognostic indicators may in fact result in under-estimation of clinical severity of a case.

B.6.9.5 First aid measures

The following, quite general measures have been proposed by notifiers but were not evaluated by RMS toxicologists because this is beyond the scope of this RAR :

Skin exposure:
Remove all contaminated clothing and flood the skin surface with water.
Wash the exposed skin twice with soap and water.
A close examination of the skin may be required if pain or irritation exist after decontamination.
All clothing that are contaminated should be laundered before they are worn again

Eye exposure:
Remove contact lens from the affected eye(s) if appropriate.
Exposed eyes should be irrigated with copious amounts of water or saline for at least 15 minutes. Pour the water from a cup or glass held 3 inches from the eye.
A close examination of the eyes may be needed if pain or irritation persists after 15 minutes of irrigation with water or saline. If symptoms persist, seek medical evaluation, preferably with an eye specialist.

Ingestion exposure:
Dilute preparations (Glyphosate <10 %): An ingestion of a dilute preparation of glyphosate (<10 %) probably does not require treatment other than dilution with milk or water, and symptomatic care. Further gastrointestinal decontamination is not needed, even if spontaneous emesis has not occurred.
Concentrated (> 10 %) preparations: Irrigate and dilute: irrigate the mouth with water. Immediate therapy should include dilution with milk or water if the patient is able to swallow. Do not exceed 5 mL/kg in a child or 250 mL in an adult.

Inhalation exposure:
No pulmonary treatment is necessary for occasional, accidental breathing of mist.
Severe, acute pulmonary injury has not been reported following inhalation exposure. Individuals with respiratory distress from any cause should be relocated (if medically stable) to fresh air and receive supplemental oxygen if available.
In the event of respiratory failure or lack of respiration, administer artificial respiration (or if pulse not detectable, cardiopulmonary resuscitation).

B.6.9.6 Therapeutic regimes

The following therapeutic regimes have been proposed by notifiers but were not evaluated by RMS toxicologists because this is beyond the scope of this RAR :

“The registrants believe that the following represent general best practices for medical management of serious ingestions of glyphosate-surfactant products.

Establish respiration and assure adequacy of ventilation.

Eye exposure:
Remove contact lens from the affected eye(s) if appropriate.
Exposed eyes should be irrigated with copious amounts of water or saline for at least 15 minutes. Pour the water from a cup or glass held 3 inches from the eye.

A close examination of the eyes may be needed if pain or irritation persists after 15 minutes of irrigation with water or saline. If symptoms persist, seek medical evaluation, preferably by an eye specialist.

Ingestion exposure:
Irrigate and dilute: irrigate the mouth with water. Immediate therapy should include dilution with milk or water if the patient is able to swallow. Do not exceed 5 mL/kg in a child or 250 mL in an adult.
patient disposition:
Concentrated preparations (Glyphosate 41 % or greater):
Any person ingesting greater than a large mouthful (50 mL in an adult, 0.5 mL/kg in a child) of a 41 % or greater glyphosate concentrate product should be admitted to a hospital and observed for 24 hours.
Any adult ingesting greater than 100 mL of a 41 % or greater glyphosate concentrate product (>1.4 mL/kg in a child) should be admitted to the intensive care unit.
Any suicide attempt by person ingesting a concentrated product should be evaluated for psychological status and should be admitted if necessary for observation with suicide precautions.
Concentrated preparations (Glyphosate 10 %-40 %):
An ingestion of concentrated glyphosate (10 % - 40 %) will usually result in spontaneous emesis. There is limited experience with glyphosate formulations in this concentration range. In view of this limited information, the registrants currently recommend managing these ingestions in a manner similar to the management of the 41 % concentrate.
Prevention of absorption *(This lists various methods for "Prevention of Absorption". These should NOT be construed as being in order of preference. Consult with Poison Center or medical personnel to determine the need for and preferred method for decontamination. In many instances, no intervention is required.)*
Gastric aspiration: If no significant spontaneous vomiting has occurred gastric aspiration may be considered. If performed soon after ingestion, gastric emptying by aspirating liquid gastric content with a lavage or standard NG tube may possibly remove some of the ingested glyphosate. The intent is to remove unabsorbed liquid by aspiration not to use lavage fluid. As absorption of liquids is likely to be relatively rapid, gastric aspiration after 1 to 2 hours is unlikely to be effective.
Emesis: Emesis is controversial at this time. Glyphosate/surfactant products are irritants. The registrants do not recommend the routine use of syrup of ipecac for glyphosate / surfactant ingestions because of the risk of exacerbating the irritant effects on the GI tract.
Activated charcoal: There are no data to support or refute the use of activated charcoal in glyphosate/surfactant product ingestions. Low molecular weight, amphoteric compounds and detergents do not always bind well to activated charcoal. In the event of a mixed ingestion, activated charcoal may be advisable.

Assessment of gastro-intestinal injury

Injury to the upper gastrointestinal tract may occur following ingestion of glyphosate concentrates. A study of upper gastrointestinal endoscopy following glyphosate–surfactant ingestions suggested that Zarger grade 2 lesions (erosions) were associated with longer hospital stay and with a higher incidence of serious complications (Chang 1999, ASB2012-11510). However, no major esophageal or gastrointestinal injury was observed, and strictures have not been reported following uncomplicated glyphosate-surfactant ingestion.

Because no serious gastrointestinal injury is reported, and because the need for hospitalisation and/or treatment of complications can be determined without endoscopic evaluation, the registrants recommend that endoscopy be reserved for patients with co-ingestions suggesting a need for endoscopy or for patients with signs and symptoms suggestive of more serious injury (serious oral burns, inability to handle secretions, clinical obstruction) regardless of clinical history.

Monitor blood pressure:
Monitor the patient closely for signs of hemodynamic instability. The insertion of a Swan-Ganz catheter may be warranted.

Hypotension:
If the patient is hypotensive, administer IV fluid boluses and place in Trendelenburg position. If the patient is unresponsive to these measures, administer a vasopressor (dopamine, epinephrine, norepinephrine, phenylephrine, isoproterinol, etc.) if needed.

Monitor blood gases and obtain chest radiograph:
Consider the use of repeat blood gases and a peripheral pulse oximeter to monitor hypoxemia. Observe closely for sign of acidosis.

Pulmonary oedema:
Closely monitor arterial blood gases. If PO2 cannot be maintained above 50 mm Hg with inspiration of 60 % oxygen by face mask or mechanical ventilation, then positive end expiratory pressure (PEEP) or continuous positive airway pressure (CPAP) may be needed. Avoid a positive fluid balance by careful administration of crystalloid solutions. Monitor fluid status through a central venous line or Swan Ganz catheter as needed.

Acidosis:
Correction of acidosis should be guided by blood gases, electrolytes and clinical judgment. Attention should be directed to volume status and correction of poor perfusion in mild cases. Sodium bicarbonate may be used to correct the acidosis in severe cases.

Hyperkalemia (from ingestion of Potassium salt formulations):
For moderate hyperkalemia (K+ of 6.0-7.0 mEq/L), administer sodium polystyrene sulfonate with sorbitol. For more severe hyperkalemia (K+ > 7 mEq/L) or serious complications of hyperkalemia, correct metabolic or respiratory acidosis if present to allow potassium to enter the intracellular space. Additional management may include a glucose/insulin drip, intravenous sodium bicarbonate or calcium, and dialysis to remove excess potassium.

Monitor renal function closely:
Assure adequate urine output. Catheterise severely ill patients. Hemodialysis may be needed in the event of renal failure or electrolyte disturbances.

Enhanced elimination:
Forced diuresis: Glyphosate is excreted very well by the kidneys. Adequate urine flow will ensure the rapid elimination of glyphosate. Although elimination may perhaps be enhanced by forced diuresis, there is no clinical evidence that this is necessary, and fluid overload may precipitate pulmonary oedema.
Hemodialysis: Hemodialysis may be useful to correct fluid, electrolyte and metabolic disturbances in the patient with renal failure. The institution of hemodialysis solely to enhance

the removal of glyphosate or other product components is not of proven benefit. Nevertheless, it is reasonable to consider the initiation of hemodialysis in the significantly ill patient who fails to respond to routine supportive management.

Serious exposure via inhalation is not expected:
Inhalation exposures are not expected due to the aerodynamics of droplet size from sprayers and because the product is not volatile. Monitor the patient for signs of respiratory compromise. Create an artificial airway if necessary. Check adequacy of tidal volume. Monitor the patient for respiratory distress; if a cough or dyspnea develop, evaluate the patient for respiratory irritation, bronchitis and/or pneumonia, but these are not expected.

Serious exposure via skin is not expected:
Significant skin exposures are not expected; however, the patient should be treated empirically if a dermal exposure is suspected. Remove all contaminated clothing and flood the skin surface with water. Wash the exposed skin twice with soap and water. A close examination of the skin may be required if pain or irritation exist after decontamination. All contaminated clothing should be laundered before wearing.

Laboratory:
Monitor electrolytes, especially if the patient is experiencing vomiting and diarrhea.15 Patients ingesting concentrated products based on the potassium salt of glyphosate may ingest large amounts of potassium (see calculations above). Observe serum potassium and/or electrocardiogram carefully. Patients experiencing pulmonary symptoms or having chest radiograph changes should have arterial blood gas monitoring. A peripheral pulse oximeter and a Swan Ganz catheter may be needed."

Gil et al. (2013, ASB2014-9488) examined the potential therapeutic effects of intravenous lipid emulsion (ILE) on the patients with acute glyphosate intoxication. The authors conclude that ILE administration was associated with lower incidence of hypotension and arrhythmia in patiens with acute glyphosate intoxication. ILE administration seems to be an effective treatment modality in patients who ingested glyphosate.
Subchronic in vivo studies have been performed with formulation Bushfire in Wistar rats (Tizhe et al., 2013 (ASB2014-6963; Tizhe et al., 2013 (ASB2014-6965) and Tizhe et al., 2013 (ASB2014-6964). The authors concluded that toxicity of glyphosate would be amelioreted by zinc supplementation. However, the conclusions are considered not clearly evidenced because of the use of a formulation instead of the active substance, low animal numbers, no clear dose dependency of effects and further limitations.
Astiz at al., 2013 (ASB2014-7493)studied the protective effect of lipoic acid (LA) against the intoxication by mixtures of pesticides including glyphosate. A mixture of different pesticides including glyphosate was i. p. injected to male rats. The results suggest that LA administration would be a promising therapeutic strategy for coping with disorders suspected to be caused by oxidative stress generators such as pesticides.
Astiz et al. (2012, ASB2014-9201) investigated the protective effects of lipoic acid as antioxidant in the case of oxidative stress caused by glyphosate or other pesticides. The authors conclude that lipoic acid displayed a protective role against pesticide-induced damage, suggesting that LA administration is a promising therapeutic strategy.

B.6.9.7 Expected effects and duration of poisoning as a function of the type, level and duration of exposure or ingestion

Dermal exposure:
Skin irritation following exposure to glyphosate-only or glyphosate-surfactant materials is generally limited to topical irritation which will resolve within 3 days to 1 week following exposure. If exposure is aggravated by occluded conditions or physical abrasion, more severe skin injury with open skin injury may rarely result and may take longer to fully resolve.

Eye exposure:
Irritant symptoms generally resolve within 3-7 days of exposure. Most irritation is minor, but exposure to concentrate or the occurrence of a foreign body or of abrasions (from rubbing the eye) may result in corneal abrasion requiring topical antimicrobial therapy, often given in conjunction with topical corticosteroids and temporary eye patching to provide symptomatic relief. As noted above, a large study of (U.S.) ocular exposures to glyphosate-surfactant products demonstrated no long term eye injury.

Inhalation exposure:
Glyphosate-surfactant products generally do not contain readily volatile ingredients and thus inhalation exposure is limited to inhalation of agricultural droplets, which will deposit primarily in the upper airway. Resulting irritant symptoms will generally resolve within hours to a few days following exposure.

Ingestion:
Following minor or incidental ingestions, or ingestion of fully diluted formulations, gastrointestinal upset with nausea, vomiting, and diarrhoea may occur. Nausea and vomiting usually resolve within a few hours of ingestion. Diarrhoea may last for several days but is generally not severe. Following a major ingestion, the onset of systemic symptoms may be delayed by several hours. Fatalities due to cardiovascular failure are generally delayed by 12 – 36 hours. For serious but non-fatal cases, primary clinical injury generally is manifest within 72 hours but secondary complications such as infection or respiratory distress syndrome may supervene. The majority of serious but surviving cases will be fully recovered within 7-10 days of ingestion. Individuals with complicated hospital courses may require a more extended and highly variable time to recover.

B.6.9.8 Expected effects and duration of poisoning as a function of varying time periods between exposure or ingestion and commencement of treatment

The outcome of eye, dermal, and inhalational exposures, which are not expected to result in serious injury in any event, will not be significantly altered by delays in medical management. Similarly, minor oral exposures are symptomatically managed and unlikely to result in severe gastrointestinal symptoms. Medical management with intravenous fluids may provide some symptomatic relief in the event of dehydration, but recovery is anticipated in any event.

For serious ingestions having major electrolyte disturbances or life threatening alterations of cardiovascular performance, medical intervention may be life saving. Fortunately, as noted above, the onset of serious symptoms following ingestion is generally delayed by at least several hours, allowing for medical transport in all but the most remote or extreme circumstances.

B.6.10 Summary of mammalian toxicology and proposed ADI, AOEL, ARfD and drinking water limit (Annex IIA 5.11)

For extensive discussion on reference values please see Volume 1.

RMS comment (August 2013):
Despite comments by GTF (please refer to commenting table, July 2013) the justification for deriving the ADI and AOEL based on developmental studies in rabbits as given under 2.6.12 in Vol.1 of the DRAR is still considered convincing and was not changed.

B.6.11 Acute toxicity including irritancy and skin sensitisation of preparations (Annex IIIA 7.1)

B.6.11.1 Summary

Table B.6.11-1: Information on MON 52276*

Product name and code	**MON 52276**
Formulation type	Soluble concentrate (SL)
Active substance (incl. content)	Glyphosate, 360 g/L as its isopropylamine salt, 486 g/L; Remark: This formulation does not contain any [redacted]
Function	Herbicide
Product already evaluated as the 'representative formulation' during the first Annex I inclusion	Yes

* Information on the detailed composition of MON 52276 can be found in the confidential part of this dossier, Doc. J (Vol.4).

MON 52276, the lead formulation of the Glyphosate Task Force submission, was one of the representative formulations supporting the 2001 Annex I inclusion of glyphosate. This formulation is still registered in Europe and its composition has not changed.

Justified proposals for classification and labelling
In accordance with Directives 67/548/EEC and 1999/45/EC and according to the criteria given in Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 the following classification and labelling with regard to toxicological data is proposed for the preparation:

Table B.6.11-2: Justified proposals for classification and labelling

C&L according to Directives 67/548/EEC and 1999/45/EC	
Hazard symbols:	None
Indications of danger:	None
Risk phrases:	None
Safety phrases:	None
Additional labelling phrases:	To avoid risks to man and the environment, comply with the instructions for use.
C&L according to Regulation (EC) No 1272/2008	
Hazard classes, categories:	None
Signal word:	None
Hazard statements:	None
Additional labelling phrases:	To avoid risks to man and the environment, comply with the instructions for use. [EUH401]
	'15.8 percent of the mixture consist of ingredients of unknown inhalation toxicity.'

Table B.6.11-3: Summary of risk assessment for operators, workers, bystanders and residents for MON 52276

	Result	**PPE / Risk mitigation measures**
Operators	Acceptable	**German model** - Avoid any unnecessary contact with the product. Misuse can lead to health damage. - Keep out of the reach of children. - Wear protective gloves when handling the undiluted product. **UK POEM *** - Avoid any unnecessary contact with the product. Misuse can lead to health damage. - Keep out of the reach of children. - Wear protective gloves when handling the undiluted product. - Wear protective gloves when handling/applying the product ready for application. ~~- Wear an impermeable coverall when applying/handling the product ready for application.~~
Workers	Acceptable	- Treated areas/crops may not be entered until the spray coating has dried.
Bystanders	Acceptable	None
Residents	Acceptable	None

* only tractor-mounted applications are acceptable

The risk assessment according to the German model has shown that the estimated exposure towards glyphosate in MON 52276 does not exceed the systemic AOEL for operators, workers, bystanders and residents, if prescribed PPE is worn by operators in the case of applications using knapsack sprayers. ~~No specific PPE is necessary for operators or for workers.~~

The risk assessment according to the UK-POEM has shown that the estimated exposure towards glyphosate in MON 52276 will not exceed the systemic AOEL for operators applying MON 52276 in field crops using tractor-mounted equipment provided that prescribed PPE is worn (gloves during mixing/loading and application). ~~Operator exposure will be below the systemic AOEL only, if prescribed PPE is worn~~.

On the other hand, as far as hand-held applications under high crops are concerned, no safe use could be demonstrated using the UK-POEM for exposure estimation irrespective of whether or not PPE is used (gloves mixing/loading and application as well as impermeable coverall during application).

B.6.11.2 Summary of evaluation of the studies on acute toxicity including irritancy and skin sensitisation for MON 52276

The conclusions of the 2001 EU evaluation of MON 52276 (acute toxicity profile) are summarised in Table B.6.11-4. All data are still relevant to this submission. However a new dermal sensitisation study was subsequently conducted under GLP conditions, following the revised OECD 406 test guideline (modified Buehler; 9 applications). The new dermal sensitisation study confirms both the results of the previously submitted non-GLP study and the 2001 EU evaluation for this end point.

Table B.6.11-4: Summary of evaluation of the studies on acute toxicity including irritancy and skin sensitisation for MON 52276

Annex point (2001 EU Monograph Annex Point)	Type of test, model system (Guideline)	Result	Acceptability	Classification (acc. to the criteria in Dir. 67/548/EEC)	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
IIIA 7.1.1 (B.5.11.1.1)	LD_{50} oral, rat (OECD 401)	> 5000 mg/kg bw	Yes	None	None	[redacted] 1991 TOX9552438
IIIA 7.1.2 (B.5.11.1.2)	LD_{50} dermal, rat (OECD 402)	> 5000 mg/kg bw	Yes	None	None	[redacted] 1991 TOX9552439
IIIA 7.1.3 (B.5.11.1.3)	LC_{50} inhalation, rat	Not submitted, not necessary. Justification presented in Vol. 3, B.6.11.5)				
IIIA 7.1.4 (B.5.11.1.4)	Skin irritation, rabbit (OECD 404)	Non-irritant	Yes	None	None	[redacted] 1991 TOX9552440
IIIA 7.1.5 (B.5.11.1.5)	Eye irritation, rabbit (OECD 405)	Non-irritant	Yes	None	None	[redacted] 1992 TOX9552441
IIIA 7.1.6/01 (B.5.11.1.6)	Skin sensitisation, guinea pig (OECD 406, Buehler (3 applications)	Non-sensitising	No	None	None	[redacted] 1992 TOX9552442
IIIA 7.1.6/02	Skin sensitisation, guinea pig (OECD 406, Buehler (9 applications)	Non-sensitising	Yes	None	None	[redacted] 2001 TOX2005-1135
	Supplementary studies for combinations of plant protection products	No data – not required				

Summaries of previously reviewed studies (Sections 7.1.1 – 7.1.5) and the new dermal sensitisation study (IIIA 7.1.6/02) are presented below.

B.6.11.3 Acute oral toxicity

Reference: OECD IIIA 7.1.1
Report: [redacted] 1991
Acute Oral Toxicity Study in Rats.,
BD-91-261,
TOX9552438
Guidelines: US EPA FIFRA guideline 81-1 (1984)
OECD 401 (1987),
EEC directive 84/449/EEC method B.1 (1984)
Deviations: No
GLP: Yes
Acceptability: Yes

Materials and methods

Test material (Lot/Batch No.)	MON 52276 (LLN-9105-3135-F)
Species	Rat, Sprangue-Dawley [CD® - Crl : CD® (SD)BR]
No. of animals (group size)	5 males and 5 females
Dose	5000 mg/kg bw
Exposure	Once by gavage
Vehicle/Dilution	None
Post exposure observation period	14 days
Remarks	None

Results and discussions

Table B.6.11-5: Results of acute oral toxicity study in rats of MON 52276

Dose (mg/kg bw)	Toxicological results [1)]	Duration of signs	Time of death	LD_{50} (mg/kg bw) (14 days)
Males				
5000	0/5/5	1 day	--	> 5000
Females				
5000	0/5/5	1 day	--	> 5000

[1)] Number of animals which died/number of animals with clinical signs/number of animals used

Table B.6.11-6: Summary of findings of acute oral toxicity study in rats of MON 52276

Mortality:	There were no mortalities during the study.
Clinical signs:	Faecal staining and / or soft stool was noted in all animals after dosing on day 1. A few animals also showed oral and / or nasal discharge, as well as hypoactivity.
Body weight:	Body weight gain was unaffected by the administration of the test substance.
Macroscopic examination:	The gross necropsy conducted at termination of the study revealed no observable abnormalities.

Conclusion
Under the experimental conditions, the oral LD_{50} of MON 52276 is higher than 5000 mg/kg bw in rats. Thus, no classification is required according to the classification criteria of Council Directive 67/548/EEC and subsequent regulations as well as according to Regulation (EC) No. 1272/2008.

B.6.11.4 Acute percutaneous toxicity

Reference: OECD IIIA 7.1.2
Report: [redacted] 1991
Acute Dermal Toxicity Study in Rats.,
BD-91-262,
TOX9552439
Guidelines: US EPA FIFRA guideline 81-2 (1984),
OECD 402 (1987),
EEC directive 84/449/EEC method B.3 (1984)
Deviations: No
GLP: Yes
Acceptability: Yes

Materials and methods

Test material (Lot/Batch No.)	MON 52276 (LLN-9105-3135-F)
Species	Rat, Sprangue-Dawley [CD® - Crl : CD® (SD)BR]
No. of animals (group size)	5 males and 5 females
Dose(s)	5000 mg/kg bw
Exposure	24 hours (dermal, semi-occlusive)
Vehicle/Dilution	None
Post exposure observation period	14 days
Remarks	None

Results and discussions

Table B.6.11-7: Results of acute dermal toxicity study in rats of MON 52276

Dose (mg/kg bw)	Toxicological results [1)]	Duration of signs	Time of death	LD_{50} (mg/kg bw) (14 days)
Males				
5000	0/0/5	--	--	> 5000
Females				
5000	0/0/5	--	--	> 5000

[1)] Number of animals which died/number of animals with clinical signs/number of animals used

Table B.6.11-8: Summary of findings of acute dermal toxicity study in rats of MON 52276

Mortality:	There were no mortalities during the study.
Clinical signs:	There were no dermal effects observed in any of the animals throughout the study period. Two animals showed red ocular discharge and one additional animal had red-stained urine at day 1.
Body weight:	Body weight gain was unaffected by the administration of the test substance.
Macroscopic examination:	The gross necropsy conducted at termination of the study revealed no observable abnormalities.

Conclusion
Under the experimental conditions, the dermal LD_{50} of MON 52276 is higher than 5000 mg/kg bw in rats. Thus, no classification is required according to the classification criteria of Council Directive 67/548/EEC and subsequent regulations as well as according to Regulation (EC) No. 1272/2008.

B.6.11.5 Acute inhalation toxicity

An acute inhalation toxicity study on MON 52276 has not been performed, because the criteria listed in Annex II (7.3.1) of Commission Regulation (EU) 545/2011 are not met (see below):

MON 52276 is not / does not

a) a gas or liquified gas,
The pure active substance, glyphosate acid, is in the form of colourless crystals at ambient temperature, with a melting point of 189.5 °C. The preparation MON 52276 is a soluble liquid (SL) formulation

b) a smoke generating formulation or fumigant,

c) used with fogging/misting equipment,

d) a vapour releasing preparation,
The preparation is not a vapour releasing preparation. It is a soluble liquid (water-based), which is mixed with water for application by hydraulic sprayers

e) an aerosol,

f) a powder or a granule containing a significant proportion of particles of diameter < 50 µm (> 1 % on a weight basis), MON 52276 is a soluble liquid, not a powder

g) to be applied from aircraft in cases where inhalation exposure is relevant,

h) contain an active substance with a vapour pressure > $1x10^{-2}$ Pa and is not to be used in enclosed spaces such as warehouses or glasshouses,
The active ingredient, glyphosate acid, is essentially non-volatile. Its vapour pressure is well below 1×10^{-2} Pa., the threshold for consideration as a volatile substance:
Vapour pressure: 1.31×10^{-5} Pa (25 °C)
Henry's Law Constant: 2.1×10^{-7} Pa × m^3 × mol^{-1}
Based on volatility, the calculated vapour density of glyphosate is less than 1 mg × m^{-3} at 25 °C (equivalent to less than 6×10^{-9} moles × m^3).

In MON 52276, the active ingredient is formulated as the isopropylamine salt of glyphosate. The salt is less volatile than the acid:
Vapour pressure: 2.1×10^{-6} Pa (25 °C)
Henry's Law Constant: 4.6×10^{-10} Pa × m^3 × mol^{-1}

The calculated vapour density of the isopropylamine salt of glyphosate is less than 0.2 mg × m^{-3} at 25 °C (equivalent to less than 1 x 10^{-9} moles × m^{3}).

i) to be applied in a manner which generates a significant proportion (greater than 1 % on a weight basis) of particles or droplets of diameter <50 µm unless the applicant can justify an alternative approach under Directive 1999/45/EC or Regulation (EC) No 1272/2008, where applicable.

The product is recommended for spraying through hydraulic nozzles. Label recommendations propose that the nozzles used to atomise the spray mixture should produce a "medium" to "medium/coarse" spray quality as defined by the International (BCPC) spray classification system. Such nozzles produce a size range of droplets suitable to optimise their deposition on target weeds while reducing the proportion of droplets susceptible to drift.

Droplet spectra were measured for MON 52276 using standard nozzles typical for the type used on field sprayers in a supplementary study ([redacted] 1999; ASB2012-12069). The Spraying Systems 11003 nozzle used in the study is classified as producing a "fine/medium" spray and, therefore, represents a worst case in terms of the proportion of small droplets produced. The results for MON 52276 are comparable to those from studies on other formulations of glyphosate. The droplet size data are measured and reported as % volume, however, the specific gravity of a spray solution is close to 1.00.

Volume median diameter		246 µm	
Number median diameter		55 µm	
% total spray volume	<	50 µm	0.71 %
	<	10 µm	0.00 %

Less than 1 % (w/w) of the droplets have a smaller diameter than 50 µm, the threshold specified in Commission Regulation 545/2011 as a trigger to perform a mandatory acute inhalation toxicity study. Larger nozzles, such as Spraying Systems 11004, or "low drift nozzles", produce fewer small droplets which would even represent a lower risk than those produced by standard nozzles.

B.6.11.6 Skin irritation

Reference:	OECD IIIA 7.1.4
Report:	[redacted] 1991 Primary Dermal Irritation Study in Rabbits., BD-91-263, TOX9552440
Guidelines:	OECD 404 (1992); Commission Directive 92/69/EEC method B.4 (1992), US EPA FIFRA guideline 81-5 (1984)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test material (Lot/Batch No.)	MON 52276 (LLN-9105-3135-F)
Species	Rabbit, New Zealand White
No. of animals (group size)	4 males and 2 females
Initial test using one animal	No
Exposure	0.5 mL (4 hours, semi-occlusive)
Vehicle/Dilution	None
Post exposure observation period	3 days
Remarks	The test substance was applied on two sites of each animal (right and left).

Results and discussions

Table B.6.11-9: Skin irritation of MON 52276

Animal No.			Scores after treatment [1)]				Mean scores (24-72 h)	Reversible [day]
			0.5 h	24 h	48 h	72 h		
1 (male)	Right	Erythema	0	0	0	0	0.0	1
		Oedema	0	0	0	0	0.0	
	Left	Erythema	1	0	0	0	0.0	
		Oedema	0	0	0	0	0.0	
2 (male)	Right	Erythema	1	0	0	0	0.0	1
		Oedema	0	0	0	0	0.0	
	Left	Erythema	2	0	0	0	0.0	
		Oedema	0	0	0	0	0.0	
3 (female)	Right	Erythema	2	1	1	0	0.67	3
		Oedema	0	0	0	0	0.0	
	Left	Erythema	1	1	1	0	0.67	
		Oedema	0	0	0	0 [2)]	0.0	
4 (male)	Right	Erythema	1	0	0	0	0.0	1
		Oedema	0	0	0	0	0.0	
	Left	Erythema	1	0	0	0	0.0	
		Oedema	0	0	0	0	0.0	
5 (male)	Right	Erythema	1	0	0	0	0.0	1
		Oedema	0	0	0	0	0.0	
	Left	Erythema	1	0	0	0	0.0	
		Oedema	0	0	0	0	0.0	
6 (female)	Right	Erythema	1	0	0	0	0.0	1
		Oedema	0	0	0	0	0.0	
	Left	Erythema	1	0	0	0	0.0	
		Oedema	0	0	0	0	0.0	

1) scores in the range of 0 to 4
2) desquamation reported

Clinical signs::No mortality occurred. No clinical signs were reported.

Conclusion
Under the experimental conditions, MON 52276 is not a skin irritant. Thus, no classification is required according to the classification criteria of Council Directive 67/548/EEC and subsequent regulations as well as according to Regulation (EC) No. 1272/2008.

B.6.11.7 Eye irritation

Reference:	OECD IIIA 7.1.5
Report:	[redacted] 1992 Primary Eye Irritation Study in Rabbits, BD-91-60, TOX9552441
Guidelines:	OECD 405 (1987); EC Directive 92/69/EEC method B.5 (1992), US EPA FIFRA guideline 81-4 (1984)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test material (Lot/Batch No.)	MON 52276 (LLN-9102-2794-F)
Species	Rabbit, New Zealand White
No. of animals (group size)	3 males and 3 females
Initial test using one animal	No
Exposure	0.1 mL (single instillation in conjunctival sac)
Irrigation (time point)	No
Vehicle/Dilution	None
Post exposure observation period	7 days
Remarks	None

Results and discussions

Table B.6.11-10: Eye irritation of MON 52276

Animal No.		Scores after treatment [1)]				Mean scores (24-72 h)	Reversible [day]
		1 h	24 h	48 h	72 h		
1 (female)	Corneal opacity	0	0	0	0	0.0	0
	Iritis	0	0	0	0	0.0	0
	Redness conjunctivae	1	1	1	0	0.67	3
	Chemosis conjunctivae	1	0	0	0	0.0	1
2 (male)	Corneal opacity	0	0	0	0	0.0	0
	Iritis	0	0	0	0	0.0	0
	Redness conjunctivae	1	1	1	0	0.67	3
	Chemosis conjunctivae	1	0	0	0	0.0	1
3 (female)	Corneal opacity	0	0	0	0	0.0	0
	Iritis	0	0	0	0	0.0	0
	Redness conjunctivae	2	0	0	0	0.0	1
	Chemosis conjunctivae	1	0	0	0	0.0	1
4 (male)	Corneal opacity	0	0	0	0	0.0	0
	Iritis	0.5	0	0	0	0.0	1
	Redness conjunctivae	2	2	2	1	1.67	7
	Chemosis conjunctivae	1	0	0	0	0.0	1
5 female)	Corneal opacity	0	0	0	0	0.0	0
	Iritis	0	0	0	0	0.0	0
	Redness conjunctivae	1	2	2	1	1.67	7
	Chemosis conjunctivae	1	0	0	0	0.0	0
6 (male)	Corneal opacity	0	0	0	0	0.0	0
	Iritis	0	0	0	0	0.0	0
	Redness conjunctivae	1	2	2	2	2.0	7
	Chemosis conjunctivae	1	0	0	0	0.0	1

[1)] scores in the range of 0 to 4 for cornea opacity and chemosis, 0 to 3 for redness of conjunctivae (1 is not considered positive by the applicants) and 0 to 2 (including 0.5 which is not considered positiv) for iritis

Clinical signs: No mortality occurred. No clinical signs of systemic toxicity were reported.

Conclusion

Under the experimental conditions, MON 52276 is not an eye irritant. Thus, no classification is required according to the classification criteria of Council Directive 67/548/EEC and subsequent regulations as well as according to Regulation (EC) No. 1272/2008.

In addition to the toxicological study on rabbits an open publication was submitted by the applicants (Acquavella, J.F. et al., 1999; TOX2002-699) dealing with human ocular effects from self-reported exposure towards Roundup® herbicides. According to this publication no serious, long-lasting eye injury occurred during a period of 5 years among callers to a single regional poison center.

B.6.11.8 Skin sensitisation

For a summary of the non-GLP compliant study on skin sensitisation (IIIA 7.1.6/01, [redacted] 1992 ; TOX9552442) which was evaluated during the first Annex 1 inclusion procedure of glyphosate and which was not submitted for this renewal it is referred to the DAR.

Reference:	OECD IIIA 7.1.6/02
Report:	[redacted], 2001 Skin Sensitisation Test in Guinea Pigs (Modified Buehler test: 9 Applications), CI-2001-153, TOX2005-1135
Guidelines:	OECD 406 (1992); Commission Directive 96/54/EC B.6 (1996)
Deviations:	No
GLP:	Yes
Acceptability:	Yes Remark: The LLNA, or, if not possible, the M&K-test is clearly preferred to the Buehler-test according to the current state of knowledge and the expected data requirements for plant protection products for authorisation in the EU. According to REACH, the LLNA is the first choice method, too, and a justification for the use of a different test shall be provided. Test Method Guideline B.6 by the European Commission (Reg. (EC) No. 440/2008) or even by its previous version 96/54 also recommends the preferential use of an adjuvant-test (e.g. M&K-test) instead of the Buehler-test without adjuvant unless a justification is given for using the Buehler-method. However, no justification is available. But, since the provided Buehler-test is valid this is to be accepted against the background of animal welfare.

Materials and methods

Test material (Lot/Batch No.)	MON 52276 (A1C1204104)
Species	Guinea pig, Hartley Crl: (HA) BR
No. of animals (group size)	Test substance group: 10 male and 10 female guinea pigs Vehicle control goup: 5 male and 5 femaleguinea pigs
Range finding:	Yes
Exposure (concentration(s), no. of applications)	Topical induction: Undiluted (9 x) Challenge: Undiluted
Vehicle	Purified water
Pretreatment prior to topical application	No

Reliability check	Mercaptobenzothiazole (topical induction: 1st to 4th ind. 20 % w/w, 5th and 6th ind. 10 % w/w, 7th and 8th ind. 5 % w/w and 9th ind. 2.5 % w/w and challenge: 20 %)
Remarks	None

Results and discussions

Table B.6.11-11: Summary of skin responses after challenge exposure towards MON 52276

	24 hours	**48 hours**
	After challenge	
MON 52276	0/20	0/20
Control Group (Vehicle)	0/10	0/10
Positive control	2/10	7/10

1) Number of animals with positive dermal response (scores of 1-3)/number of animals in dose group

Clinical signs::No deaths occurred. No signs of systemic toxicity were reported.

Conclusion
Under the experimental conditions, MON 52276 is not a skin sensitiser. Thus, no classification is required according to the classification criteria of Council Directive 67/548/EEC and subsequent regulations as well as according to Regulation (EC) No. 1272/2008.

B.6.11.9 Supplementary studies for combinations of plant protection products

Combined application of plant protection products is not intended. Therefore, no such studies were performed.

B.6.12 Dermal absorption (Annex IIIA 7.6)

Introduction into this chapter by RMS
In the 2001 EU glyphosate evaluation, dermal absorption of glyphosate was considered to be less than 3% (DAR 1998, ASB2010-10302). This estimate was based on in vivo data in Rhesus monkeys, as well as on in vitro data in human skin, using the original glyphosate formulation Roundup ([redacted] 1983, TOX9552417; [redacted] 1991, TOX9552418). However, these studies do not comply to current standards and should not be taken into consideration anymore even though the 3% assumption was supported by a new study with the active ingredient ([redacted] 2012, ASB2012-11459).

For exposure calculation and risk assessment, it is necessary to determine or estimate dermal absorption of an active ingredient from the formulation under evaluation since co-formulants may have a crucial impact on the absorption rate. Such studies are available for a small number of formulations containing glyphosate. For the representative formulation MON 52276, dermal absorption was assessed in vitro on human skin (isolated epidermis). Valid studies of this type are usually accepted in the EU as "stand alone-information" with their results (after appropriate rounding) being directly and without further adjustment used for exposure calculation and risk assessment (OECD, 2011, ASB2013-2; EFSA, 2012, ASB2012-

6959). Thus, the absence of in vivo data is not considered a data gap. This in vitro study (Ward, 2010, ASB2012-5383) and their results are reported in detail below.

Subsequently, a study is adressed in which dermal absorption of the active ingredient glyphosate itself (i.e., not contained in a commercial formulation) was investigated in vitro on rabbit skin (Hadfield, 2012, ASB2012-11459). Even though rabbit skin is a rather unusual model for studies of this type, the study is reported because it may give an idea of the low dermal absorption of glyphosate that is not formulated to generate a specific plant protection product.

Four more in vitro dermal absorption studies were submitted as part of the GTF dossier (Davies, 2003, ASB2012-11518; Ward, 2010, ASB2012-11515; Ward, 2010, ASB2012-11516; Hadfield, 2012, ASB2012-11517) that all confirmed a low dermal absorption of glyphosate. However, since these studies were performed with products other than the representative formulation, there is no need to take them into consideration for the current re-evaluation of glyphosate. Accordingly, they were not reviewed by the RMS and have been excluded from Volume 3. If necessary, they should be evaluated in future on national or zonal level.

B.6.12.1 Dermal absorption of glyphosate from MON 52276 *in vitro* (Human epidermis)

Reference:	IIIA, 7.6.2/01
Report:	Ward, R.J. 2010 360 g/L Glyphosate SL Formulation (MON 522276) – In vitro absorption of Glyphosate through human epidermis Dermal Technology Laboratory Ltd., Med IC4, Keele University Science and Business Park, Keele, Staffordshire, ST5 5NL, UK Study No.: JV2084, Report No.: JV2084-REG, Date: 2010-02-19, Unpublished ASB2012-5383
Guidelines:	OECD 428 with regard to the OECD guideline, however, some deficiencies are mentioned in the RMS comment below
Deviations:	None
GLP:	Yes
Acceptability:	*See RMS comment*

Dates of experimental work: 2009-06-09 - 2009-08-26

The objective of this study was to evaluate the potential dermal absorption of glyphosate from a 360 g/L SL formulation concentrate, as well as from two representative in-use dilutions prepared as 1:12.5 (v/v) and 1:150 (v/v) aqueous dilutions.
^{14}C-glyphosate was incorporated into the concentrate formulation and dilutions prior to application. The doses were applied to human epidermal membranes at a rate of 10 µL/cm^2 and left unoccluded for an exposure period of 24 hours. The absorption process was followed by taking samples of the receptor fluid (physiological saline) at recorded intervals throughout the exposure period. The distribution of glyphosate within the test system and a 24-hour absorption profile were determined. All samples were analysed by liquid scintillation counting (LSC).

Material and methods

Test materials:

Non radio-labelled test substance:

Identification:	Isopropyamine salt of glyphosate techn. material (glyphosate-IPA)
Description:	Clear, water white to amber viscous liquid (solution in water)
Lot/Batch #:	A8B60170S0
Chemical purity/a.i. content:	Glyphosate-IPA: 63.81 % Glyphosate acid: 47.28 %
Stability of test compound:	Expiry date: 2012-01-25

Analytical reference standard:

Identification:	Glyphosate acid
Description:	White solid
Lot/Batch #:	GLP-0810-19515-A
Chemical purity:	99.8 %
Stability of test compound:	Expiry date: 2011-01-31

Radio-labelled test substance

Identification:	^{14}C-glyphosate (as glyphosate acid)
Lot/Batch #:	53463-3-23
Chemical purity:	99.8 %
Radiochemical purity:	97.8 % (confirmed by analysis)
Specific activity:	47 mCi/mmol; 1739 MBq/mmol; 277.9 µCi/mg; 10.28 MBq/mg
Stability of test compound:	Not reported

Blank formulation

Identification:	Proprietary surfactant blend (MON 8153)
Concentration of a.s.:	0 %
Description:	Not reported
Lot/Batch #:	Not reported
Stability of test compound:	Not reported

Formulated test substance

Identification:	MON 52276 The formulation concentrate used was not supplied as complete formulation, but had to be prepared from the ingredients a) and c) described above, to allow the incorporation of the radiolabel. The test substance concentration in the prepared formulation was confirmed by analysis.

Test skin source:

Species:	Human
Source:	Tissue bank (not further specified)

Study design and methods

Preparation of skin samples: Human skin samples (details regarding the donors not given) were immersed in water at 60 °C for 40-45 seconds and the epidermis was teased away from the dermis. Each membrane was given an identifying number and stored frozen, at approximately -20 ºC, on aluminium foil until required for use.

Test substance preparation

Three test substance concentrations representing the formulation concentrate and two field dilutions were prepared at target concentrations of 360 g/L, 29.6 g/L and 2.51 g/L. The nominal radioactivity contained in the dose preparations was 3.3 MBq.

Radioactive stock solution of ^{14}C-glyphosate

Dry ^{14}C-glyphosate was solubilised in 2 mL of water and mixed thoroughly.

High dose (formulation concentrate, 360 g/L)

A pre-mix was prepared by mixing 3900 mg glyphosate-IPA technical material with an appropriate amount of proprietary surfactant blend. 78 µL (≡ 78 mg) of the radioactive stock solution was mixed with 482 mg of the pre-mix. Water was added to give a total weight of 585 mg. The solution was mixed well. Assuming a density of 1.17 g/mL, the total weight was equivalent of 0.5 mL at a nominal concentration of 369 g glyphosate/L. According to the raw data, only four cells with epidermal samples from three different donors were used

Intermediate dose, (1:12.5 (v/v) aqueous dilution, 29.6 g/L)

A pre-mix was prepared by mixing 305.92 mg glyphosate-IPA technical material with an appropriate amount of proprietary surfactant blend. 78 µL (≡ 78 mg) of the radioactive stock solution was mixed with 38.01 mg of the pre-mix. Water was added to give a total weight of 500 mg. The solution was mixed well. Assuming a density of 1 g/mL, the total weight was equivalent of 0.5 mL at a nominal concentration of 29.6 g glyphosate/L. According to the raw data, six cells with epidermal samples from three different donors were used.

Low dose (1:150 (v/v) aqueous dilution, 2.51 g/L)

A pre-mix was prepared by mixing 76.90 mg glyphosate-IPA technical material with an appropriate amount of proprietary surfactant blend. 78 µL (≡ 78 mg) of the radioactive stock solution was mixed with 2.64 mg of the pre-mix. Water was added to give a total weight of 500 mg. The solution was mixed well. Assuming a density of 1 g/mL, the total weight was equivalent of 0.5 mL at a nominal concentration of 2.51 g glyphosate/L. According to the raw data, agin, only four cells but with epidermal samples from four different donors were used.

Analyses of dose preparations

The radioactivity content of the stock solution was determined by liquid scintillation counting (LSC) analyses of sub-samples of solvent dilutions. The radiochemical purity of the radiolabelled test substance was determined by high performance liquid chromatography (HPLC).

The radioactivity content and homogeneity of the dose preparations were checked by LSC analyses. The radiochemical purity and stability was measured by HPLC analyses.

Preparation of diffusion cells

The skin membranes were placed in static glass diffusion cells providing an exposure area of 2.54 cm^2 of skin. The cells had a receptor chamber volume of approximately 4.5 mL.

An integrity test was performed by measuring the electrical resistance across the skin membranes. Membranes with a resistance of ≥ 10 kΩ were considered having a normal integrity and used for the absorption study.
Physiological saline was chosen as receptor fluid to ensure that the test substance (taking into account its physico-chemical properties) could freely partition into this compartment. However, it was not reported if solubility of the test substance was tested. The skin surface temperature was maintained at 32 ± 1 °C using a water bath.

Test substance application and sampling
Prior to dosing a pre-treatment sample of 500 µL was taken from each diffusion cell, and replaced by an equal amount of fresh receptor fluid.
Each dose formulation was applied to the skin membrane at the rate of 10 µL/cm^2 exposed skin area (25.4 µL dose), corresponding to target concentration of 3693 µg/cm^2, 296 µg/cm^2 and 25.1 µg/cm^2 for the high, intermediate and low dose level, respectively. The applications were left un-occluded for 24 hours.
Receptor fluid samples (500 µL) were taken by an auto-sampler at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours after application. After each sampling the removed amount of receptor fluid was replaced by an equal amount of fresh receptor fluid.

Terminal procedures
After the last sampling 24 hours after application, the remaining receptor fluid was discarded. The receptor chamber was rinsed with receptor fluid that was also discarded.
The donor chamber was carefully removed and the underside wiped with a single natural sponge, pre-wetted with 3 %Teepol L® in water, which was added to the wash sponges. The donor chamber was washed with deionised water and a sample was taken for LSC analysis.
The epidermal surface of the skin was decontaminated by gently swabbing the application site with natural sponges pre-wetted with 3 % Teepol L® in water. Decontamination was shown to be complete following assessment of residual radioactivity levels on the skin surface with a Geiger counter. The skin surface was washed with further sponges pre-wetted with water. All the sponges were combined and digested in Soluene 350® and made up to a recorded volume. A sample was taken for analysis.
The surface of the skin was allowed to dry naturally
Each skin membrane was tape stripped using 3M Scotch 'Magic' tape to a maximum of five strips. The tape strips were soaked individually in 30 % v/v methanol in water to extract any test material. The extracts were sequentially numbered and analysed by LSC. In some cases, it was not possible to take the full five tape strips as the epidermis began to tear, therefore tape stripping was discontinued. The last tape strip for these diffusion cells was digested with the remaining epidermis, so as not to underestimate residues in the remaining epidermis compartment. The remaining epidermis was carefully removed from the receptor chamber and digested in Soluene 350® and the whole digest analysed by LSC.

Analysis of samples
Liquid samples of the receptor fluid, washing solutions, digested wash sponges, tape strip extracts and digested epidermis by LSC using a Packard 3100 TR LSC counter and Goldstar as scintillation fluid.
Results of the analysis of the samples of receptor fluid collected in the study were expressed as amounts of glyphosate in the receptor solution in terms of µg/cm^2. The amounts absorbed, rates of absorption (µg/cm^2/h) and 'percentage of dose absorbed' were calculated. Membranes with absorption profiles that indicate membrane damage during the course of the experiment

have been excluded from calculations. The results of the mass balance and distribution determinations are expressed in terms of amount absorbed and 'percentage of applied dose
The absorbed dose is considered the glyphosate detected in the receptor fluid, while the potentially biologically available proportion of the dose is regarded as the sum of absorbed dose and the amount recovered from the epidermis after tape stripping. The test material removed from the surface of the epidermis by the washing procedure, as well as the glyphosate recovered from the epidermis at the end of the exposure is considered unabsorbed.

Results and discussion

Analysis of unformulated ^{14}C-glyphosate

HPLC analysis of the unformulated sample of ^{14}C-glyphosate confirmed a radiochemical purity of 97.8 %.

Analyses of dose preparations

The achieved concentration of glyphosate in the dose preparations was calculated to be 369.3, 29.6 and 2.52 g glyphosate /L in the formulation concentrate, 1/12.5 v/v dilution and 1/150 v/v dilution, respectively.
LCS analyses confirmed the dose solutions to be homogeneous.

Dermal absorption of glyphosate

The determined distribution of radioactivity for the different dose groups are summarised in Table B.6.12-1 below.

Table B.6.12-1: Summary of results for dermal absorption of ^{14}C-glyphosate from representative SL formulation MON 52278

Dose preparation	**High (concentrate)**		**Intermediate (1:12.5 v/v dilution)**		**Low (1:150 v/v dilution)**	
Nominal concentration [g/L]	360		29.6		2.51	
Actual concentration [g/L]	369.3		29.6		2.52	
Applied dose [µL/cm^2]	10		10		10	
Applied dose [µg/cm^2]	3693		296		25.2	
Number of cells accessed	4*		6		4*	
	Distribution of radioactivity (mean values)					
	µg/cm^2	% of applied dose	µg/cm^2	% of applied dose	µg/cm^2	% of applied dose
Surface compartment						
Stratum corneum (5 tape strips)	2.39	0.065	0.386	0.130	0.081	0.320
Stratum corneum (first 2 tape strips)	1.57	0.043	0.283	0.096	0.065	0.256
Skin wash	3656	99.0	288	97.4	24.8	98.4
Donor chamber	83.4	2.26	6.67	2.26	<LOQ	0.008
Receptor compartment						
Receptor fluid (0-24 h)	0.322	0.009	0.086	0.029	0.023	0.092
Total absorbed	**0.322**	**0.009**	**0.086**	**0.029**	**0.023**	**0.092**
Remaining epidermis (after 5 tape strips)	2.02	0.055	0.310	0.105	0.047	0.185
Remaining epidermis (after 2 tape strips)	2.84	0.077	0.413	0.140	0.063	0.250
Total potentially absorbable** (after 5 tape strips)	2.343	0.063	0.396	0.134	0.070	0.276
Total potentially absorbable (after 2 tape strips)**	**3.162**	**0.086*** **	**0.499**	**0.169**	**0.086**	**0.342*** **
Total recovery	**3744**	**101**	**296**	**100**	**25.0**	**99.0**
Absorption rates [µg/cm^2/h] (0-24h)	0.014		0.003		0.001	

* Some cells for these applications were excluded from calculations as the analytical data indicated that the epidermal membrane may have been damaged during application.
** Total potentially absorbable = total absorbed + remaining epidermis
*** Dermal absorption values used for exposure assessment

Dose preparation	**High (concentrate)**		**Intermediate (1:12.5 v/v dilution)**		**Low (1:150 v/v dilution)**	
Nominal concentration [g/L]	360		29.6		2.51	
Actual concentration [g/L]	369.3		29.6		2.52	
Applied dose [µL/cm^2]	10		10		10	
Applied dose [µg/cm^2]	3693		296		25.2	
Number of cells accessed	4*		6		4*	
	Distribution of radioactivity (mean values with standard deviation)					
	µg/cm^2	% of applied dose, based on the mean	µg/cm^2	% of applied dose, based on the mean	µg/cm^2	% of applied dose, based on the mean
Surface compartment						
Stratum corneum (total)	2.39 ± 0.71	0.065	0.386 ± 0.371	0.130	0.081± 0.082	0.320
Stratum corneum - first tape strip (to be excluded)	1.12 ± 0.81	0.030	0.182 ± 0.209	0.096	0.048 ± 0.051	0.192
Stratum corneum - second tape strip (to be excluded)	0.45 ± 0.26	0.012	0.101 ± 0.106	0.034	0.016 ± 0.019	0.064
Skin wash	3656 ± 181	99.0	288 ± 5.54	97.4	24.8 ± 0.496	98.4
Donor chamber	83.4 ± 167	2.26	6.67 ± 6.12	2.26	<LOQ	n.a.
Receptor compartment						
Receptor fluid (0-24 h)	0.322	0.009	0.086	0.029	0.023	0.092
Receptor fluid (0-12 h)	0.190	0.005	0.045	0.015	0.017	0.067
Total absorbed	**0.322 ± 0.318**	**0.009**	**0.086 ± 0.038**	**0.029**	**0.023 ± 0.017**	**0.092**
Remaining epidermis (after tape stripping)	2.02 ± 0.82	0.055	0.310 ± 0.143	0.105	0.047 ± 0.041	0.185
Remaining epidermis <u>including</u> *Stratum corneum* (after exclusion of 2 tape strips)	2.84	0.077	0.413	0.140	0.063	0.250
Total potentially absorbable (after exclusion of the two upper tapes strips)**	**3.162**	**0.086**	**0.499**	**0.169**	**0.086**	**0.342**
Total recovery	**3744 ± 104**	**101**	**296 ± 4.45**	**100**	**25.0 ± 0.54**	**99.0**
Absorption rates [µg/cm^2/h] (0-24h)	0.014 ± 0.07		0.003 ± 0.001		0.001 ± <0.001	

* Some cells for these applications were excluded from calculations as the analytical data indicated that the epidermal membrane may have been damaged during application.

** Total potentially absorbable = total absorbed + remaining epidermis (including lower layers of the *Stratum corneum*), SD values not provided in the original study

n.a. not applicable

The overall total recovery for the three dose levels was good, with mean values of 99 – 101 % of the applied dose.

Glyphosate absorption from the 360 g/L concentrate formulation was essentially constant over the entire 24 hour exposure period (mean rate = 0.014 µg/cm^2/h). By the end of the exposure period, the mean total amount of absorbed glyphosate was 0.322 µg/cm^2 (0.009 % of applied dose).

From the intermediate and low-dose aqueous dilutions of the formulation, absorption was fastest during the early period of absorption, with 0.010 µg/cm^2/h, (0-1h) and 0.004 µg/cm^2/h, (0-2h), respectively. The rates after this early period until the end of the exposure at 24h were 0.003 µg/cm^2/h and 0.001 µg/cm^2/h for the intermediate and low dose dilutions, respectively.

At the end of the exposure period, the mean total amounts of absorbed glyphosate were 0.086 and 0.023 µg/cm^2 (0.029 % and 0.092 % of applied dose), respectively.
For the formulation concentrate and both aqueous dilutions, the vast majority of the applied glyphosate was removed from the surface of the epidermis during the washing procedure at the end of the 24-hour exposure period (mean 97.4-99.0 %). The mean total amount of glyphosate recovered from the epidermis was 0.120 %, 0.235 % and 0.505 % of the applied dose for the concentrate, intermediate and low dose dilution, respectively.
The amount of potentially biologically available glyphosate (absorbed + epidermis after tape striping) for the concentrate, intermediate and low dose dilutions were 0.064 %, 0.134 % and 0.277 % respectively for 5 tape strips and more conservatively 0.086 %, 0.169 % and 0.342 % respectively for only 2 tape strips.

Conclusions by the Notifier
The results of this *in vitro* dermal absorption study indicate that the absorption of glyphosate through human skin is very limited and very slow. The vast majority of glyphosate was removed from the skin by the washing procedures.
The total absorbed amounts after 24 hour exposure were 0.009 %, 0.029 % and 0.092 % of the applied dose for the formulation concentrate, the 1:12,5 (v/v) and 1:150 (v/v) dilution, respectively.
The corresponding total potentially absorbable amounts, represented by the mean absorbed dose together with the amounts in the remaining skin were 0.063 %, 0.134 % and 0.276 %, respectively for 5 tape strips and more conservatively 0.086 %, 0.169 % and 0.342 % respectively for only 2 tape strips.

Thus, the results predict that the dermal absorption of glyphosate from potential exposure to this 360 g/L glyphosate / L SL formulation would be less than 1 %, irrespective of whether two or five tape strips were considered to contain non-biologically available glyphosate.

Table B.6.12-2: Summary of glyphosate dermal absorption from MON 52276

Study	**% of applied dose***			**Reference**
	Concentrate	Spray dilutions		
SL formulation	360 g/L	29.6 g/L	2.51 g/L	
In vitro (human skin)	0.086	0.169	0.342	IIIA 7.6.2 Ward, 2010a

* The absorption values correspond to total amounts potentially absorbable through human skin (i.e. amounts of radioactivity recovered in the receptor fluid and remaining skin after tape stripping with two strips).

RMS comments
The study is considered acceptable. The mass balance was very good and any adjustment for test material losses not needed. However, a few deficiencies were noted:

- *The intended number of cells (membranes) was six for each application. However, in the low and high dose experiments, 2 membranes had to be excluded because analytical data indicated that epidermal membranes might have been damaged during treatment.*
- *Detailed information on the skin samples (sex and age of donors, body site from which the samples were taken) was not provided.*
- *The number of cells (samples) even at the mid dose level and of skin donors in the high and mid dose experiements was lower than required according to current EFSA guidance (EFSA, 2012, ASB2012-6959). This cannot be considered a deviation that would put the quality and acceptability of the study into question because it was*

commissioned and performed before the guidance document was prepared and came into force but the current guidance must be taken into account for interpretation of the results.

Very low dermal absorption of glyphosate from the representative formulation has been shown. It must be emphasised that the use of isolated epidermis (instead of dermatomed skin) and an exposure period of 24 hours will most probably result in overestimation of the dermal absorption rate.
*The approach taken by the applicant to calculate the dermal absorption is agreed with. In line with current EU practice and also with the new European Guidance document on dermal absorption (EFSA, 2012, ASB2012-6959) the first two (upper) tape strips were excluded. The amount retained in the lower (three) tape strips, in contrast, should be considerd potentially absorbable because ~~raw data have shown that~~ 75 % penetration (expressed as the percentage of radioactivity in the receptor fluid) was not achieved for any of the three applied concentrations after one half of the study duration, i.e., after 12 hours (see Table B.6.12-1). The achieved mean values were rounded according to new rules (EFSA, 2012, ASB2012-6959) giving dermal absorption rates of 0.1, 0.2, and 0.3 % for the high, mid and low concentration ~~that should be used for exposure calculation and risk assessment purposes~~. However, uncertainties with regard to the origin of skin samples and their donors, their actual number in the different experiments and also the occasionally rather high standard deviations should be taken into account. Thus, for exposure calculation and risk assessment purposes, it is proposed to use a (conservative) estimate of **1% dermal absorption** for the formulation concentrate as well as for both dilutions.*

~~*The RMS is aware that the new European Guidance document has come into force long after conduct of the study and also after submission of the glyphosate dossier. Thus, in a strict sense, it might not be applicable. However, it was used here to support the approach taken with regard to inclusion/exclusion of tape strips and for rounding in the hope that it might be helpful for a harmonised evaluation of this study.*~~

B.6.12.2 Dermal absorption of glyphosate active substance *in vitro* (abraded rabbit skin)

Reference:	IIA, 5.3.7/02
Report:	Hadfield, N. 2012a Glyphosate acid - *In Vitro* Absorption through Abraded Rabbit Skin using [^{14}C]-glyphosate Dermal Technology Laboratory Ltd., Med IC4, Keele University Science and Business Park, Keele, Staffordshire, ST5 5NL, UK Study No.: JV2182, Report No.: JV2182-REG Date: 2012-04-18, Unpublished, ASB2012-11459
Guidelines:	OECD 428
Deviations:	None
GLP:	yes
Acceptability:	*See RMS comment*

Dates of experimental work: 2011-12-12 to 2011-12-22

The purpose of this study was to determine the *in vitro* percutaneous absorption of glyphosate acid through abraded rabbit skin following a 6-hour exposure period and subsequent 18 hour monitoring period. This study was designed to assess the potential dermal penetration of test material through rabbit skin and will be of use in estimating the systemic dose achieved in a previous *in vivo* rabbit dermal toxicity study (see IIA 5.3.7, Johnson, 1982, TOX9552366). Therefore, the application rate and exposure conditions used in this study were calculated to be equivalent to 5000 mg/kg bw/day as applied to rabbits in the *in vivo* dermal study (IIA 5.3.7).

^{14}C-glyphosate was incorporated into a wet cake preparation prior to application. The preparations were applied as a paste to abraded rabbit skin membranes at a rate of 79.8 mg/cm^2 (corresponding to 48.3 mg glyphosate acid/cm^2) and left unoccluded for an exposure period of 6 hours, after which the skin surface was washed. The absorption process was followed by taking samples of the receptor fluid (physiological saline) at recorded intervals throughout a total time-period of 24 hours. The distribution of glyphosate within the test system and a 24-hour absorption profile were determined. All samples were analysed by liquid scintillation counting (LSC).

Materials and methods

Test materials:	
Non radio-labelled test substance:	
Identification:	MON 77973 (glyphosate acid)
Description:	White wet cake
Lot/Batch #:	GLP-1103-21149-T
Chemical purity/a.i. content:	85.14 % as glyphosate acid (purity: 95.93 %)
Stability of test compound:	Expiry date: 2012-03-09
Analytical reference standard:	
Identification:	Glyphosate acid
Lot/Batch #:	GLP-0810-1915-A
Chemical purity:	Not reported
Radio-labelled test substance	
Identification:	^{14}C-glyphosate (as glyphosate acid) [phosphonomethylene-^{14}C]-glyphosate
Lot/Batch #:	4675JJN002-1
Radiochemical purity:	96.7 % (confirmed by analysis)
Specific activity:	48 mCi/mmol; 1776 MBq/mmol; 2523µCi/mL; 9.35 MBq/mL
Test skin source:	
Species:	Rabbit
Strain:	New Zealand White Albino
Source:	Harlan
Age:	At least 12 weeks
Type:	Complete pelt

Study design and methods:

Preparation of skin samples

Skin pelts from New Zealand White albino rabbits at least 12 weeks old were obtained from Harlan. The skin samples were transported on cold blocks and were stored on arrival at -20°C, the day after sacrifice. The skin samples arrived clipped and excised and were examined for scars and blemishes. Any extraneous subcutaneous tissue was removed after defrosting and the pelts clipped further if necessary. The pelts were given an identifying number and individually stored frozen, at approximately -20°C, on aluminium foil until required for use.

Test substance preparation

The doses were prepared, to mimic as closely as possible a 5000 mg/kg dose from a previous rabbit *in vivo* study (IIA 5.3.7, [redacted] 1982, TOX9552366). The dose equivalency was calculated on a dose per unit area of skin basis using an average *in vivo* rabbit weight of 2.78 kg. The doses were prepared as close to the time of application as was practicable.

Radioactive stock solution of ^{14}C-glyphosate

The radiolabelled ^{14}C-glyphosate was supplied as a solution in water.

Trial preparation of the radiolabelled glyphosate acid

Glyphosate acid trial preparation was prepared using the method described below, with the exception that different volumes or smaller amounts of radioactivity or unlabelled material were used, where applicable. Three individual vials were prepared as part of the trial preparation, to assess dosing methodology. The paste like composition of the dose preparation was investigated to ensure that it visually provided good skin contact during application to the membranes.

Preparation of radiolabelled glyphosate acid

Firstly 8008 mg of non-labelled glyphosate wet cake was added to a vial, followed by 4162 µL of radiolabelled glyphosate stock solution, providing a nominal 3.85 mg of glyphosate (40 MBq) radioactivity. 5 mL of water was then added and the preparation mixed thoroughly. The preparation was then freeze dried to remove the water added and the water present in the wet cake. When dry, the glyphosate wet cake preparation was then weighed to confirm the removal of all the water. Approximately 521 mg of the dried wet cake preparation was then added to 8 individual vials together with approximately 300 µL of saline to each vial to create a paste. A final weight of each vial was recorded and the preparation was thoroughly mixed with a spatula into a paste before dosing.

Preparation of non-labelled glyphosate acid

To demonstrate that the dose preparations have a close contact during the application procedure, an additional dose preparation without radiolabel was prepared according to the procedure described above.

Analyses of dose preparations

The radioactivity content of the stock solution was determined by liquid scintillation counting (LSC) analyses of sub-samples of solvent dilutions. The radiochemical purity of the radiolabelled stock solution of the test substance was determined by thin layer chromatography (TLC) using unlabelled test substance as reference standard.

The radioactivity content and homogeneity of the dose preparations were checked by LSC analyses. The radiochemical purity and stability was measured by TLC analyses.

Preparation of diffusion cells

The skin membranes were placed in static glass diffusion cells providing an exposure area of 2.54 cm^2 of skin. The cells had a receptor volume of approximately 4.5 mL.

An integrity test was performed by measuring the electrical resistance (ER) across the skin membranes. Non-abraded membranes with a resistance of 1.5 - 5 kΩ were considered having a normal integrity and used for the skin abrasion. Rabbit skin was abraded using a blunt spatula drawn over the skin area approximately six to eight times, in the form of a grid, in order to mimic 'Draize' abrasion as conducted in the in vivo study (IIA 5.3.7, [redacted] 1982, TOX9552366). After the abrasion a further integrity test was performed by measuring the electrical resistance (ER) across the skin membranes. For abraded skin samples membranes with ER values in the range of 0.7 – 1.0 kΩ were selected for the study.

Cells were selected such that the application rate was represented by eight intact skin samples from five different animals. Physiological saline was chosen as receptor fluid. The skin surface temperature was maintained at 32 ± 1 °C using a water bath.

Test substance application and sampling

Prior to dosing a pre-treatment sample of 500 µL was taken from each diffusion cell, and replaced by an equal amount of fresh receptor fluid.

Each dose formulation was applied to the abraded skin membrane as a dried glyphosate acid wet cake paste and spread over the skin surface using a spatula. The weight of each individual preparation and spatula were recorded before and after dosing to allow the applied dose to be calculated.

Each dose was applied at the nominal rate of 79.8 mL/cm^2 exposed skin area (202.8 mg/cell), corresponding to 48.3 mg glyphoate/cm^2. The applications were left un-occluded for 24 hours. Receptor fluid samples (500 µL) were taken by an auto-sampler at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours after application. After each sampling the removed amount of receptor fluid was replaced by an equal amount of fresh receptor fluid.

After the 6-hour sampling, the skin samples were washed by gently swabbing the application site application site with at least three natural sponges pre-wetted with 3 % Teepol L® in water. Decontamination was shown to be complete following assessment of residual radioactivity levels on the skin surface with a Geiger counter. Two further sponges, pre-wetted with water, were used to further swab the surface.

Terminal procedures

After the last sampling, 24 hours after application the remaining receptor fluid was discarded. The receptor chamber was rinsed with receptor fluid that was also discarded.

The donor chamber was carefully removed and the underside wiped with a single natural sponge, pre-wetted with 3 %Teepol L® in water, which was added to the wash sponges. The donor chamber was washed with deionised water and a sample was taken for LSC analysis.

The epidermal surface of the skin was decontaminated by gently swabbing the application site with natural sponges pre-wetted with 3 % Teepol L® in water. Decontamination was shown to be complete following assessment of residual radioactivity levels on the skin surface with a Geiger counter. The skin surface was washed with further sponges pre-wetted with water. All the sponges were combined and digested in Soluene 350® and made up to a recorded volume. A sample was taken for analysis.

Due to the fragility of the abraded skin samples tape stripping could not be performed. Instead a heat separation technique was used to separate the epidermis from the dermis.

The skin was carefully removed from the receptor chamber and the flange area cut away and digested in Soluene 350® and aliquots taken for analysis by LSC.

The remaining skin disc was placed dermis side down, on cling film. A second piece of cling film was then used to cover the epidermis side. A 200g weight was placed in a water bath at 65ºC for an hour prior to use. The weight was placed onto the epidermal surface with moderate pressure for approximately 90 seconds. The epidermis was peeled away from the dermis using forceps. The dermis was digested in Soluene 350® and aliquots taken for analysis by LSC. The epidermis was digested in Soluene 350® and the whole sample analysed by LSC.

Analysis of samples

The radiochemical purity and stability of the ^{14}C-glyphosate preparations was determined by TLC using silica gel plates and methanol : water : acetic acid (6 : 3 : 0.5, v/v/v). Radioactivity on the TLC plates were measured using a Packard Instant Imager (SOP E003). Unlabelled material was visualised under UV light at 254 nm.

For visualising the test material on the TLC plates a 2 % ninhydrine solution in acetone was used.

In addition, for analyses of dose preparations K2 cellulose plates and a revised solvent system (methanol : water : acetic acid (8 : 1.5 : 0.5, v/v/v) was used.

Liquid samples of the receptor fluid, washing solutions, digested wash sponges, and digested dermis and epidermis were measured by LSC using a Packard 3100 TR LSC counter and Goldstar as scintillation fluid.

Results of the analysis of the samples of receptor fluid collected in the study were expressed as amounts of glyphosate in the receptor solution in terms of µg/cm^2. The amounts absorbed, rates of absorption (µg/cm^2/h) and 'percentage of dose absorbed' were calculated. Membranes with absorption profiles that indicate membrane damage during the course of the experiment have been excluded from calculations. The results of the mass balance and distribution determinations are expressed in terms of amount absorbed and 'percentage of applied dose

The absorbed dose is considered the glyphosate detected in the receptor fluid, while the potentially biologically available proportion of the dose is regarded as the sum of absorbed dose and the amount recovered from the dermis. The test material removed from the surface of the epidermis by the washing procedure, as well as the glyphosate recovered from the epidermis at the end of the exposure is considered unabsorbed.

Results and discussion

Analyses of the ^{14}C-glyphosate stock solution

TLC analysis of the ^{14}C-glyphosate stock solution confirmed a radiochemical purity of greater than 95 %.

LSC analysis revealed a radioactivity content of 72.1 MBq, equivalent to a concentration of 0.924 mg/mL. The stock solution was homogeneous with a 1.31 % deviation between the replicates.

Analyses of dose preparations

LSC analyses confirmed the mean application rate to be 48.3 mg glyphosate/cm^2.

The dose preparations had low variability between the replicates analysed (1.66 %-6.26 %) and, considering the physical nature of the preparation, the dose preparations were considered to have acceptable homogeneity.

Membrane integrity check

Based on the ER measurements eight cells with abraded skin samples were selected for the absorption study.

Dermal absorption of glyphosate

Absorption profiles were assessed from eight abraded skin samples. Since one skin sample showed an atypical absorption profile, this was excluded from the calculation of means and SD.

The determined distribution of radioactivity are summarised in Table B.6.12-3 below.

Table B.6.12-3: Summary of results for dermal absorption of ^{14}C-glyphosate (rabbit skin)

Dose preparation				
Applied dose "wet cake" [mg/cm^2]	79.8			
Applied dose glyphosate [mg/cm^2]	48.3			
Number of cells assessed	7			
	Distribution of radioactivity (mean values)			
	µg/cm^2	SD	% of applied dose	SD
Surface compartment				
Dermis (after heat separation)	118	19.4	0.243	0.040
Skin wash at 6 hours	42802	3008	87.9	6.30
Skin wash at 24 hours	1159	1224	2.38	2.51
Donor chamber	59.2	56.9	0.121	0.117
Receptor compartment				
Receptor fluid (0-24 h)	1177	244	2.42	0.503
Total absorbed*	1177	--	2.42	--
Epidermis (after heat separation)	20.1	9.97	0.041	0.020
Flange area	132	68.6	0.270	0.141
Total potentially absorbable**	1295	--	2.663	--
Total recovery	45468	2096	93.3	4.46
Absorption rates [µg/cm^2/h] (0-24h)	53.1	10.2	--	--

SD Standard deviation
* Amount in receptor fluid.
** Total potentially absorbable = total absorbed + remaining dermis (after heat separation)

The total recovery of the individual cells was in the range of 87.3 % to 98.2 %, with an overall mean recovery of 93.3 % of applied dose.

The majority of the applied glyphosate acid (mean 87.9 %) was washed off the skin at 6 hours, with a further 2.38 % washed off at 24 hours. A small proportion (0.041 %) of the dose applied was recovered from the epidermis, with 0.243 % remaining in the dermis.

The mean rate of absorption of glyphosate acid between 0-1 hours was 47.0 µg/cm^2/h, which increased to 166 µg/cm^2/h between 1-4 hours. The mean absorption rate subsequently slowed to 72.3 µg/cm²/h between 4-10 hours and declined further to 13.3 µg/cm²/h for the remainder of the absorption period (10-24 hours). The overall absorption rate (0-24 hours) was 53.1 µg/cm^2/h.

The mean amount of glyphosate acid that penetrated abraded rabbit skin into the receptor fluid over the entire 24-hour experimental period was 1177 µg/cm^2, corresponding to 2.42 % of the applied dose.

Considering that the amount found in the remaining dermis after 24 h is potentially available and could further penetrate through the skin, the total amount of glyphosate potentially available was 2.66 % of the applied dose

Conclusions by the Notifier

The results of this *in vitro* study indicate the dermal absorption of glyphosate through abraded rabbit skin is slow. The vast majority of glyphosate will be washed off during normal washing procedures. The mean total amount absorbed after 24 hours was 2.42 %. The reported total potentially absorbable amount, represented by the mean absorbed dose together with the mean amount in the remaining dermis was 2.66 %.

RMS comments

The study is considered supplementary despite its good quality. Using abraded rabbit skin as an in vitro model, it could be shown that dermal absorption of glyphosate active ingredient was less than 3 %, confirming to some extent a previous assumption (DAR 1998, ASB20110-10302) that was based on data obtained in Rhesus monkeys in vivo ([redacted] 1991, TOX9552418). However, even though the information provided by this study is interesting from a purely scientific point of view, its regulatory value will be quite limited. Dermal absorption of an active ingredient must be always determined or estimated for a certain formulation under evaluation. Exposure to glyphosate in a "wet cake" in this study does neither reflect real exposure conditions of operators, bystanders or workers to liquid concentrates or spray dilutions nor a possible impact of co-formulants.

B.6.12.3 Publications on dermal absorption of glyphosate

Wester *et al.* (2005, ASB2012-12050) compared dermal absorption through human skin *in vitro* after exposure either to a 1 % solution (not further specified) or to cotton sheets that were treated with the same solution on the same day or up to 2 days before to reflect the abundance of glyphosate residues infabric that may occur due to applications of this active ingredient in cotton. The authors could show a lower absorption of glyphosate across the skin from the treated cotton as compared to the liquid solution itself but found also remarkable residues on and in the skin when the cotton sheets had been treated on the same day. Adding of water to the cotton sheets resulted in an increase in absorption rate.

B.6.13 Toxicological data on non active substances (Annex IIIA 7.9 and point 4 of the introduction)

B.6.13.1 Material safety data sheet for each formulant

Copies of the safety data sheets of the formulants are provided in Document J of this dossier.

B.6.13.2 Available toxicological data for each formulant

Please refer to the material safety data sheets provided in Document J of this dossier. There is no toxicological classification of co-formulants which has to be considered for classification and labelling of MON 52276 according to these MSDS.

Remarks on surfactants included into glyphosate-containing plant protection products

All glyphosate-containing plant protection products contain surfactants or - if not present as an integral component – are to be mixed with surfactants as a compulsory additive to produce the ready-to-use dilution. As has already been discussed during the first Annex I inclusion procedure for glyphosate it became apparent that glyphosate-containing products were more toxic than glyphosate alone. This phenomenon was attributed to the presence of particular surfactants predominantly, namely the POE-[redacted]

Some MS may wish to allow for this in the context of the national risk assessment for POE-[redacted] containing glyphosate formulations. Therefore, a toxicological evaluation for POE-[redacted] (including reference values) is provided in a separate paragraph within Vol. 3 (B.6.13.3) of this RAR.

MON 52276 which is the representative formulation here does not contain any POE-[redacted] Instead, a different type of surfactant, i.e. a quarternary ammonium compound, is used for MON 52276.

Since studies on MON 52276 concerning acute toxicity, skin and eye irritation as well as skin sensitisation were performed with the original preparation of MON 52276 the results for these toxicological short-term endpoints also reflect possible effects provoked by the surfactant. No further studies are needed according to the data requirements for plant protection products. Therefore, no toxicological long-term studies were submitted using the formulated product or the surfactant alone. Moreover, up to now no reference values have been considered necessary for the surfactant used, thus, no respective risk assessment was required.

According to the material safety data sheet for the surfactant provided by the applicants this co-formulant was not mutagenic in an Ames-test. No further information on toxicological long-term endpoints was given in this material safety data sheet.

In addition, MON 52276 has been authorised within the EU for many years. There are no medical data which have been collected by occupational physicians or poisoning emergency centres describing long-term adverse health effects for operators provoked by this plant protection product until today.

B.6.13.3 Further toxicological data for other potential co-formulants

The following toxicological evaluation of [redacted] surfactants was prepared by the German Federal Institute for Risk Assessment in 2010 but was not discussed on EU level so far. Even though it is not essential for current risk assessment of glyphosate, i.e., re-evaluation under Regulation (EC) 1107/2009, it might facilitate product assessment and authorisation on zonal or MS level. Therefore, it has been included here as an appendix. Some of the references mentioned in this evaluation have been also submitted in the GTF dossier on glyphosate but had been provided on request to the RMS before or were found as result of a separate literature search. New references were not included and the text of the 2010 evaluation itself was not amended for other than editorial reasons (mainly correction of typos and citation of references). In rare cases, references to other parts of the current RAR were made. The only exception from this approach was dermal absorption that is now estimated by using the default values of 25 and 75 % as recommended in the EU guidance (EFSA, 2012, ASB2012-6959). Enumeration of tables was kept.

B.6.13.3.1 Toxicological evaluation of the POE-[redacted] surfactant (CAS no. 61791-26-2)

Objective of this evaluation:
Many plant protection products (PPP), in particular a number of (but not all) formulations of the widely used herbicidal active ingredient glyphosate, contain a substance with the CAS no. 61791-26-2 as a surfactant. This substance (sum formula, according to U.S.EPA, $C_{48}H_{97}NO_{15}$) is also known as "POE-[redacted] and belongs to the heterogeneous chemical group of polyethoxylated alkylamines (POEA). A huge amount of information from different sources (poisoning incidents in humans; *in vitro* data obtained in different test systems; studies on short-term, reproductive and developmental toxicity of Roundup® formulations or preparations), even though of different quality and reliability, in the whole suggests a higher toxicity of such PPP as compared to the active compound. Glyphosate itself is generally considered to be of low toxicological concern (Williams *et al.*, 2000, ASB2012-12053; EU, 2001, ASB2009-4191; JMPR, 2004, ASB2008-6266). However, in the DAR on glyphosate (DAR, 1998, ASB2010-10302) that was prepared to support first Annex I listing of the active ingredient, it was already mentioned that surfactants could significantly contribute to the toxicity of glyphosate products.

To facilitate a comprehensive risk assessment of products that contain both glyphosate and the POE-[redacted] and to ensure sufficient protection of operators, bystanders, workers, residents and consumers, it was necessary to establish reference values (ADI, AOEL, ARfD) for the surfactant with CAS no. 61791-26-2 and to estimate its dermal absorption rate. These values may be applied in future for risk assessment purposes in addition to those of glyphosate.
Another goal of this evaluation was to check if there was enough evidence to conclude that higher toxicity of certain PPP was in fact due to the surfactant of if there were indications of a synergistic mode of action with glyphosate.

Conclusion:
A systemic AOEL, an ADI and an ARfD in the same magnitude of 0.1 mg/kg bw(/day) are proposed for the POE[redacted] with the CAS no. 61791-26-2 that is contained as a surfactant in many glyphosate-based (and some other) PPP. Furthermore, an inhalative AOEL of 0.0166 mg/kg bw/day was established.

For its dermal absorption rate, in the absence of experimental data, the default values of 25% or 75%, depending on concentration, are proposed.
The substance should be classified and labelled for acute oral toxicity (Xn, R22, corresponding to "Acute tox. 4, H302" according to GHS), for skin and severe eye irritation (Xi, R38-41; corresponding C&L according to the GHS would be "Skin irrit. 2, H315" and "Eye dam. 1, H318) and skin sensitisation (Xi, R43, corresponding to "Skin sens. 1, H~~302~~317"). Most likely, classification for inhalative toxicity will be also needed.

With regard to nearly all toxicological endpoints under investigation, the POE[redacted] was clearly more toxic than glyphosate. Its primary mode of action was a local effect, *i.e.*, strong mucosal irritation. However, occurrence of systemic effects after ingestion or inhalation is also likely. There is some evidence to assume a higher vulnerability of pups.
Eye or mucosal irritation may be produced by both glyphosate and the surfactant and some additivity seems theoretically possible but, with regard to the very different toxic properties of these substances and the apparent differences in the effect doses and severity of findings, higher toxicity of certain PPP as compared to the active ingredient can be allocated to the surfactant alone. The same holds true for poisoning incidents in humans, Therefore, separate reference values of these products are not needed but risk assessment should include a comparison of the expected exposures to the reference values for both glyphosate and POE-[redacted] surfactant.

Justification (detailed evaluation):
The evaluation of and proposed reference values for the POE-[redacted] surfactant with the CAS no. 61791-26-2 are mainly based on toxicological studies with the surfactant formulations MON 0818 and G-3780 that were submitted by the company Monsanto on request of the BfR after they had been identified in a recent evaluation of the U.S. Environmental Protection Agency (EPA). Both formulations contain this [redacted] at an amount of about 70 % and are or were part of various glyphosate-based PPP. The data package consists of subchronic oral studies in rats and dogs and of reproductive and developmental toxicity studies in rats. Furthermore, former evaluations by the EU, the EPA and a number of publications were taken into consideration. With regard to the EPA evaluation (EPA, 2009, ASB2009-9022), it must be emphasised that it was more comprehensive because the whole group of polyethoxylated alkyl amines was addressed with special regard to the need of setting tolerances in crops. The EPA conclusions were drawn under the assumption that the alkyl amine content in herbicidal formulations will not exceed 25 % and will not be higher than 10 % in fungicides and insecticides.

In the following, an overview on the toxicological profile of the POE-[redacted] surfactant with CAS no. 61791-26-2 is given. (For direct comparison to glyphosate, see Table B.6.13-2.) Subsequently, reference values are proposed, the dermal absorption rate is estimated and the possible impact of the surfactant on the toxicity of Roundup® formulations as an example for glyphosate-based herbicides is discussed.

Toxicokinetics and metabolism
No information is available.

Acute toxicity
The acute oral LD_{50} of the surfactant in rats was 864 mg/kg bw when the value of *ca* 1200 mg/kg bw for MON 0818 (EPA, 2009, ASB2009-9022) was corrected for the presumed POE-[redacted] content of 72 %.This result warrants classification and labelling

for acute oral toxicity as "harmful if swallowed" (Xn), *i.e.*, the risk phrase R22, or H302 (Acute toxicity cat. 4, "Warning") according to GHS would be appropriate.

The acute dermal toxicity was tested in rabbits. The LD_{50} was above the highest dose of 907 mg/kg bw when corrected for [redacted] content. Although the amount applied was below the required limit dose of 2000 mg/kg bw, classification and labelling is not considered necessary because no mortality occurred and no clinical signs were reported up to this dose.

Unfortunately, acute inhalation data for the POE-[redacted] under consideration or a surfactant formulation such as MON 0818 is not available. This must be in fact regarded as a data gap because there is evidence coming from an acute inhalation study with a Roundup formulation ([redacted] 1982, TOX2002-693) that inhalative toxicity was higher than with glyphosate alone for which an LC_{50} > 5 mg/L air was determined (see Volume 3, B.6.2.3 of this RAR). In this acute study, an LC_{50} of 3.18 mg/L air for Roundup was obtained resulting in the classification and labelling of the product with Xn, R20 (H332 according to GHS). It is quite likely that this apparent difference was due to the surfactant. MON 0818 was contained in this product at an amount of approximately 18 % w/v, *i.e.*, the POE-[redacted] content was *ca* 14 % w/v. When the strong irritating properties of the POE-[redacted] surfactant (see below) are taken into consideration, one would expect similar effects in the respiratory tract. A higher inhalative toxicity than for glyphosate was also substantiated by a subacute inhalation study ([redacted] 1983, TOX2002-694) with Roundup and by observations in humans after occupational exposure without protective measures ranging from weak symptoms such as a headache to well-documented systemic poisoning with persistent morphological findings ([redacted] 1991, MET9600092; [redacted] 2007, ASB2013-4034).
A high acute inhalative toxicity was experimentally confirmed for other polyethoxylated alkyl amine substances. *Armoblen 557* (CAS no. 68219-26-3) had an LC_{50} of 0.66 mg/L that was established in a study with 4-hour exposure. For Ethomeen C/12 (CAS No. 61791-31-9), an LC_{50} of 0.98 mg/L was calculated on the basis of a one-hour trial (EPA, 2009, ASB2009-9022).
However, acute inhalation data for different products that were submitted to support registration in Germany were partly contradictory although they contain similar amounts of glyphosate (at least 360 g/L) and of the surfactant. Apparently, there is no clear correlation of inhalative toxicity with the surfactant content. In fact, there are PPP with the same surfactant at nearly the same concentration for which classification and labelling for acute inhalation toxicity is not needed. In sum, the available information is not sufficient to conclude on the appropriate classification and labelling of the POE-[redacted] surfactant itself for acute inhalation toxicity although some classification will be probably needed.
If experimental data for a particular formulation containing this surfactant is not available, according to Directive 1999/45/EEC or the CLP regulation, classification and labelling of the PPP for precautionary reasons might be a reasonable option.

The POE-[redacted] surfactant was found irritating to the skin (Xi, R38) and strongly irritating to the eyes. The U.S. EPA (EPA, 2009, ASB2009-9022) labelled the substance for eye effects even as "corrosive" (C) but, according to the EU scheme, R41 seems to be more appropriate. Correct classification according to GHS rules difficult because the studies themselves were not submitted and the assessment is based on the EPA evaluation. However, if Annex VII of the CLP regulation (Translation table from classification under Directive 67/548/EEC to classification and assignment of hazard statements under this regulation) is

applied, Cat.2/H315 for skin irritation and Cat.1/H318 (for eye irritation) would be most appropriate.
Eye irritation is often considered to provide evidence also of mucosal irritation. [redacted] (1987, TOX9552430) studied the irritating effect of the glyphosate isopropylammonium salt, MON 0818 and a Roundup formulation (containing 41% w/v of the IPA salt and 15% of MON 0818) on stomach and small intestine mucosa in dogs. Irritation was more severe with the Roundup formulation than with either the IPA salt or the surfactant alone. The intestine appeared to be more affected than the stomach. The severity of the damage was equivalent to that caused be 0.25 N hydrochloric acid.

Concerning skin sensitisation, the available data is scarce. However, in its 2009 evaluation, U.S. EPA concluded that the POE-[redacted] was sensitising because of a positive Buehler test using three applications (EPA, 2009, ASB2009-9022). Therefore, classification and labelling (Xi, R43 or, according to GHS, Skin sensitisation, Cat.1/H317) is proposed.

For setting of reference values, the acute toxicity data is, of course, not appropriate. However, acute oral toxicity supports a need to establish an ARfD and the evidence of inhalative toxicity suggests that an inhalative AOEL should be established.

Short-term toxicity (subacute and subchronic studies)
Rat, oral
A 90-day feeding study was performed on SD rats ([redacted] 1990, ASB2009-9027). Because current standards were basically met, the study may be considered as valid and reliable. Test substance was the surfactant formulation MON 0818. As stated in the study report, this test item contained 71.9 % of "polyoxyethylen (15) tallow amine" (POEA). Groups of 10 male and 10 female rats were fed MON 0818 at nominal concentrations of 0, 500, 1500, or 4500 ppm. In most groups, however, these nominal concentrations were not achieved. The actual concentrations were in the range from 80 to 90 % of the nominal values.

The highest dose level was clearly toxic. This became apparent mainly by a reduced body weight gain in both sexes that achieved statistical significance and resulted in a lower mean body weight over the whole course of the study. Food consumption was diminished and clinical signs (piloerection, soft faeces) occurred. Furthermore, blood glucose and urea levels were decreased.
Some deviations in organ weights are most probably related to the lower body weight at termination and there were no gross pathological findings at necropsy that could be attributed to treatment. In contrast, histological lesions of the intestinal mucosa were certainly treatment-related. These findings comprised hyperplasia and cell vacuolation in the *Lamina propria* and all animals receiving the high dose were affected.

At the mid dose level of 1500 ppm, 4 out of ten male rats and 5/10 females exhibited the same histopathological changes as described above. In males, there was in addition a statistically significant decrease in food consumption and body weight gain over the first 9 days of treatment.

Feeding of MON 0818 at the low dose level of 500 ppm did not cause any remarkable differences from the control groups and, thus, this dose can be regarded as the NOAEL.
For the nominal dietary concentration of 500 ppm, a mean daily intake of 33.0 mg/kg bw was calculated for the male rats and of 39.9 mg/kg bw for the females. However, to establish the true NOAEL for the POE-[redacted] these values must be corrected for the actually

achieved concentration of 84% of nominal in both the male and female groups and then for the 71.9 % content of the surfactant in MON 0818. These corrections result in intake calculations of 19.9 mg/kg bw/day for males and 24.1 mg/kg bw/day for females. It seems reasonable to round them to 20 mg/kg bw/day that is considered the NOAEL for the surfactant in this study. For the LOAEL that was established at a three times higher dietary level, hence, an achieved intake of 60 mg/kg bw/day is assumed.

The outcome of this study 90-day was at least partly in line with an additional one-month feeding study with ”POEA surfactant” on Sprague-Dawley rats of which the original report was not made available to the BfR. In this experiment, the NOAEL was claimed to be 800 ppm (*ca* 40 mg/kg bw/day). At the next higher dose level of 2000 ppm, body weight gain in male rats was reduced. At the high dose level of 5000 ppm, lower body weight gain and irritation and inflammation of the colon mucosa were observed in both sexes ([redacted] 1989, cited in Williams *et al.*, 2000, ASB2012-12053).

The toxicological findings in these feeding studies in rats point to local irritation in the intestines as the primary mode of action. Because of the known irritating properties of the POE-[redacted] it was first assumed that this local effect was behind the higher toxicity of PPP containing glyphosate and this surfactant as compared to the active ingredient. However, the subchronic study in dogs, the reproduction and developmental studies in rats, comparative mechanistic data and human experience suggest that systemic effects will be most likely due to a second mechanism of POE-tallowamine toxicity.

Dog, oral
Groups of four male and four female Beagle dogs received three times a day a capsule containing the formulation G-3780 for a total of 14 weeks ([redacted] 1973, ASB2009-9026). According to a claim made by Monsanto, G-3780 was very similar to the formulation MON 0818. However, in the study report itself, no information on the composition of the test substance and the content of the POE-[redacted] with the CAS no. 61791-26-2 is given. Therefore, it is assumed that the POE-content, as in MON 0818, was about 72% suggesting a need for correction of the NOAEL/LOAEL.
The total daily doses of G-3780 were 0, 30 (*i.e.*, 3 x 10), 60 (3 x 20), or 90 (3 x 30) mg/kg bw. However, because of a rather tricky dosing scheme, these dose levels were achieved not before the third or fourth week of treatment. This is one of the reasons for a compromised reliability of this study. Transiently, high dose animals even received 120 (3 x 40) mg/kg bw/day but, because of enhanced emesis, diarrhoea and subsequent emaciation, the dose was lowered again after 10 days. It seems that the MTD was exceeded when more than 90 mg/kg bw/day was administered.
At the two upper dose levels, dogs did not gain and sometimes even lost weight. At termination, mean body weight were by more than 10% lower than in the control groups. In addition, vomiting and diarrhoea were more frequently observed than in the controls. Clinical chemistry revealed lower blood calcium and total protein concentrations.
There were no remarkable findings at necropsy and histopathological examination. In particular, local effects on intestinal mucosa, as in the rat study, were not reported. The clinical signs might suggest gastrointestinal irritation but, on the other hand, are common and unspecific signs of general toxicity in dogs.
The lowest dose level of 30 mg/kg bw/day was considered the NOAEL. After correction for presumed POE-[redacted] content, a numeric value of 21 mg/kg bw/day would result that is in the same magnitude as the NOAEL in the subchronic rat study. The LOAEL was 42 mg/kg bw/day.

Despite a wide range of parameters that were investigated, the study design, examination methods and the quality of reporting do not comply with modern standards. Therefore, and because of the uncertainties with regard to the actually applied doses, the study can be considered at best supplementary. However, it can be accepted that the requirement of testing for short-term toxicity in a second species has been fulfilled. The conclusion can be drawn that the sensitivity of rats and dogs in terms of the NOAELs/LOAELs is not that different. In contrast to the studies in rats, however, the findings in dogs suggest rather a systemic effect than local irritation.

Rat, inhalative
In a four-week study on SD rats, 15 male and 15 female animals per group were exposed (whole-body) for 6 hours per day over 5 days per week (total number of treatments 22) to nominal concentrations of 0 (control), 0.37, 0.75, or 2.17 mg/L air of the Roundup formulation MON 2139 ([redacted] 1983, TOX2002-694). However, the analytically determined air concentrations were 0.05 mg/L (low dose), 0.16 mg/L (mid dose), and 0.36 mg/L (high dose). There were no unscheduled deaths in this study. Clinical signs did not occur, and there were no gross pathological changes at necropsy. Body weight and organ weights were not altered but, surprisingly, the lungs had not been weighed.
The only findings that may be attributed to treatment were significantly higher total protein, albumin and globulin serum concentrations in females at the two upper dose levels. Furthermore, the incidence of certain histopathological findings in the lungs (perivascular lymphoid infiltrates or aggregates, interstitial infiltration or pneumonia), in the trachea (mononuclear infiltration and chronic inflammation) and the nasal turbinates (inflammation) was increased in high dose females. Unfortunately, histopathology was performed on control and high dose animals only.
Even though the study author did not mention these findings as adverse or treatment-related, they might be a reaction to Roundup application. Accordingly, the lowest concentration of 0.05 mg/L air is considered the NOAEC.
Under the assumption that the effects were in fact entirely due to the POE-[redacted] an inhalative NOAEL (expressed in mg/kg bw) for this surfactant may be calculated. Since 180 g/L MON 0818 were contained in the test item, a NOAEC of 0.009 mg/L air for this surfactant formulation can be assumed. Taking into consideration the [redacted] content in MON 0818, a respiratory rate of 45 L air/kg bw per hour for the rat and an exposure time of 6 hours per day, the calculation would result in a inhalative NOAEL of *ca* 1.66 mg/kg bw/day.

Mutagenicity
Possible mutagenicity of POE-[redacted](s) was addressed in the past by different regulatory bodies as well as in a review article (Germany, 2000, ASB2013-2748; Williams *et al.*, 2000, ASB2012-12053; EPA, 2009, ASB2009-9022). The overall conclusion was that these substances were not mutagenic but might have caused positive findings in a number of test systems due to cytotoxicity when PPP such as various Roundup formulations were tested. Unfortunately, there are relatively few experiments with formulations in standard test systems available because usually only the active compounds are subject to rigorous testing in a battery of regulatory studies. At least, the Roundup formulation MON 2139 containing the POE-[redacted] with the CAS no. 61791-26-*2* as part of the surfactant formulation MON 0818 proved negative in an Ames test in concentrations of up to 500 µg/plate without and of up to 1500 µg/plate in the presence of metabolic activation by S9 mix ([redacted] 1992, TOX1999-239). In a mouse bone marrow micronucleus assay ([redacted]1992, TOX1999-242), no evidence of a clastogenic potential was found up to the highest tested dose of 555

mg/kg bw/day that was already clearly toxic to mice after single intraperitoneal administration. Roundup proved cytotoxic in the bone marrow.
MON 0818 itself was tested in the Ames test by Stegemann and Li (1990, TOX1999-241) and proved negative. However, due to severe cytotoxicity, it could be tested only at rather low concentrations (up to 300 µg/plate) in the various *Salmonella typhimurium* strains. It proved also negative in a micronucleus assay in mouse bone marrow (Stegemann and Kier, 1998, TOX1999-240) at the dose level of 100 mg/kg bw that was administered by intraperitoneal injection but this latter study was considered supplementary only because no evidence of systemic or bone marrow toxicity was obtained.

There is evidence coming from several studies using the Comet assay or other less validated systems, that products which contain cytotoxic [redacted] (or other) surfactants might produce, mostly in very high concentrations, DNA damage either by direct contact or by enhanced formation of DNA reactive oxygen species (*e.g.*, [redacted] 1997, Z101728; [redacted] 1998, TOX1999-318; [redacted] 2008, ASB2012-11586). A part of these investigations was made in non-mammalian systems that are more relevant to ecotoxicology. Positive findings are nearly always linked to toxicity as recently confirmed by Heydens *et al.* (2008, ASB2012-11845). Unfortunately, no UDS assay in, *e.g.*, rat hepatocytes is available that would be most suitable to investigate a potential for DNA damage also at concentrations below overt toxicity. However, for the time being, weight of evidence suggests that the relevance of possible effects on the DNA to humans under practical exposure conditions is very low.

Chronic toxicity and carcinogenicity
No such data is available. Currently, for co-formulants like surfactants, long-term studies are not required and, accordingly, usually not performed. However, based on the toxicological profile of POE-[redacted] it is not expected that chronic toxicity would be much different from the effects that were noted in the subchronic studies. With regard to carcinogenicity, it should be taken into consideration that, in spite of long-lasting experience and extensive use, there is no convincinig epidemiological evidence in people who had been in frequent occupational contact with glyphosate-based plant protection products (see Vo.lume 3, B.6.5, and Volume 1, 2.6.5 of this RAR). The EPA concluded that the whole group of polyethoxylated alkyl amines was not of concern for carcinogenicity (EPA, 2009, ASB2009-9022).

Reproduction toxicity
Two GLP-compliant studies were performed in rats to investigate possible effects of the surfactant formulation MON 0818 on reproduction ([redacted] 2007, ASB2010-365; [redacted] 2008, ASB2010-364). Furthermore, a rather new published study on reproductive toxicity of a commercial Roundup® formulation ([redacted] 2007, ASB2012-2721) is available. Unfortunately, the design of all three studies was not in line with usual OECD Guideline requirements. The reproductive/developmental screening studies according OCED testing guidelines 421/422 are less sensitive than the full scale study designs according to testing guidelines 414/416.

MON 0818

In a two-generation study (according to OECD 421), Sprague-Dawley rats (20 per sex and dose group) received the test formulation in the F0 generation at dose levels of 0, 100, 300, or 1000 ppm via their diet ([redacted] 2007, ASB2010-365). A broad range of endpoints was examined including hormone measurements, sperm parameters and extensive histopathology. There was no evidence of parental toxicity but the highest dose tested was lower than the LOAEL in the 90-day rat study. In contrast, administration of the high dose resulted in a reduced implantation rate (with 5 of 15 pregnant dams being affected), lower litter size and also an increase in perinatal mortality. Total loss of two litters immediately after birth or at the beginning of the lactation period was noted. However, further development of the surviving pups was not altered.

To produce the F2 generation, three male and female pups from each litter were selected and reared. Between days 21 and 70 after birth, they received the test item at a dietary concentration that was adjusted to the respective actual body weight. The mean daily doses during this period were 0, 7, 21 or 61 mg/kg bw in male rats and 0, 6, 28 or 72 mg/kg bw in females. Two male and female pups per litter were selected for further breeding but only from the control and high dose groups. From the beginning of this third mating period, the selected high dose F1 animals received again 1000 ppm of MON 0818 in their diet. In the F2 generation, no parental toxicity and no impact of this high dose on reproduction were observed.

Histopathological examination of different parts of the intestinal tract (jeunum, ileum, caecum, colon and rectum) did not reveal indications for mucosal irritation in F0 and F1 animals.

Based on the findings in the F1 generation (*i.e.*, lower implantation rate, litter size and pup survival) 300 ppm was considered the NOAEL for reproduction and offspring effects. For the F0 adults, a mean daily intake of 16.6 (males) or 19.5 mg/kg bw/day of MON 0818 was calculated at this dose level that must be corrected for the [redacted] surfactant content. A NOAEL of 12 (males) to 14 (females) mg/kg bw/day for the surfactant would result (EPA, 2009). For parental toxicity, the highest dose of 1000 ppm was the NOAEL. The corrected mean daily surfactant intake was about 38 mg/kg bw (lowest value as calculated for F1 males).

In a second study ([redacted] 2008, ASB2010-364); according to OECD 422), groups of 12 male and female rats of the same strain [Crl:CD(SD)] were fed 1000 ppm of MON 0818 for a total period of 69 – 72 days. This dose was equivalent to a mean daily intake of 66 mg/kg bw by the male animals and 95 mg/kg bw/day by females over the whole course of the study. During the individual study periods (pre-mating, pregnancy, lactation), the intake in females varied between 74 and 126 mg/kg bw/day. A control groups of equal size received untreated diet. From administration day 14 onwards, the animals were mated.

There were two unscheduled deaths among the treated dams. One female rat died showing clinical signs of dystocia. Another dam was did not give birth and was killed 30 days after mating in poor clinical state. At necropsy, uterine rupture was established and two implantation sites were recorded of which one was a dead fetus and the other an early resorption. In contrast to the assessment by the study author, it cannot be excluded that these deaths were treatment-related. However, there were no adverse effects in the remaining 10 dams or in any of the 12 males in the treatment group. Furthermore, reproduction was not affected in the remaining dams. A higher incidence of chronic-progressive nephropathy in treated males as compared to the control group (6/12 vs. 3/12) was not allocated to substance administration because it was only unilateral. Thus, a NOAEL for parental and reproduction

toxicity was not established. Offspring effects could not be fully evaluated because all pups were killed on day 4 *post partum* already. However, if effects on litter size or perinatal mortality as in the first study would have occurred, they might have been noted.

Roundup® herbicide

In a one-generation study, pregnant Wistar rats (15 per group) were administered a Roundup® herbicide that is commercially available in Brazil (containing 36% glyphosate and 18% of the POE-[redacted] surfactant) at dose levels of 50, 150, or 450 mg/kg bw/day by oral gavage from day 1 of presumed gestation through the end of lactation (postnatal day 21). The calculated daily intake of the surfactant was 9, 27, or 81 mg/kg bw. A control group received only the vehicle, *i.e.*, distilled water ([redacted] 2007, ASB2012-2721).

Evaluation of toxicity in the parental generation was based on observations for mortality and clinical signs, body weight measurements and determination of selected organ weights at termination. Reproductive toxicity was assessed by determining litter size, number of living and dead pups, viability and sex ratio. Possible effects on offspring development, in particular with regard to sexual maturation, were studied in one male pup and one female pup per litter which were killed at an age of 65 days and in one more pup per sex and litter which were sacrificed on postnatal day 140. Organ weights were also determined in these animals.

Up to the highest dose level, there was no evidence of maternal toxicity. Likewise, litter size, mean number of live and dead pups and sex ratio among the pups were not affected at any dose level. Thus, the NOAEL for parental and reproductive toxicity was 450 mg Roundup/kg bw/day, corresponding to a dose of 81 mg/kg bw/day for the surfactant.

However, sexual development in offspring was affected. In female pups, delayed vaginal opening suggested a slower sexual development in all treated groups. Since this finding was associated with a markedly lower body weight in the low dose group, a possible treatment-related effect was assumed only for the two upper dose levels. In male pups, functional disturbances were confined to the highest dose level of 450 mg Roundup/kg bw/day and were partly contradictory. On one hand, preputial separation was noted to occur a bit earlier than in the control group. On the other hand, at an age of 65 days, testosterone serum concentration was significantly lower (but not at 140 days) and, when measured at 140 days, daily sperm production and total number of sperm in the epididymis tail were diminished suggesting rather a delay in sexual development. Interpretation of this data is difficult since a similar decrease in both parameters was observed at the low dose level, too, whereas the mean values in the mid dose group were similar to the controls. At all three dose levels, histopathological examination revealed degenerative changes in the testes with the absence of tubular lumen being the most outstanding finding. However, all these observations were flawed by a low number of animals on which the findings are based, *e.g.*, histopathology of the testes was confined to only five male pups per dose level.

Thus, a NOAEL for offspring effects cannot be established.

A direct comparison of the results by [redacted] (2007, ASB2012-2721) with the two studies of [redacted] (2007, ASB2010-365 and 2008, ASB2010-364) is not possible because the test material was different and the same holds true for the study design and the range of parameters under investigation. Unfortunately, it is also not feasible to compare these studies and their results with the Guideline-compliant reproduction studies with glyphosate. Thus, no definite conclusion can be drawn if the effects of treatment with Roundup can be allocated to the surfactant. Nonetheless, the published findings suggest that offspring development was in fact a particularly sensitive target of Roundup and the POE-[redacted] The findings in young male rats might indicate impairment of spermatogenesis.

Developmental toxicity

The surfactant formulation MON 0818 and a Roundup® herbicide that is commercially available in Brazil were tested for developmental toxicity and teratogenicity in rats. A study in a second species was not submitted. However, it is unclear whether the data requirements for pesticides or drugs can be applied to co-formulants. Furthermore, the rabbit as the usual second species is known to be very sensitive to gut irritation. Thus, severe maternal toxicity at low doses due to the well known irritation potential of the [redacted] surfactant must be expected in a rabbit study that might prevent meaningful evaluation of fetal effects at sufficiently high dose levels.

<u>MON 0818</u>

A preliminary (range-finding) and a main study were performed under GLP conditions in which MON 0818 was administered to pregnant rats (Charles River Crl:CD Br). Although the studies themselves can be considered acceptable, it is not clear how much POE-[redacted] was contained because its amount even in different batches of MON 0818 can vary. However, based on the information given in the study report of [redacted] (1990, ASB2009-9027) and in line with the EPA evaluation (EPA, 2009, ASB2009-9022), it is assumed that the amount of this surfactant in the tested formulation had accounted for 71.9 %, too. The NOAELs/LOAELs will be corrected accordingly.

Preliminary study

MON 0818 was administered by oral gavage to groups of 8 pregnant rats from day 6 through day 15 post mating at dose levels of 0, 25, 50, 100, 200, or 400 mg/kg bw/day. On pregnancy day 20, the dams were killed and fetuses developed by caesarian section. The uteri were dissected and examined. Foetuses were counted and inspected for external anomalies.

Mortality, clinical signs and body weight losses were clear indications of severe maternal toxicity at a dose of 100 mg/kg bw/day and above. The NOAEL for maternal toxicity was 50 mg MON 0818/kg bw/day, corresponding to 36 mg/kg bw/day for the POE-[redacted] Obvious developmental toxicity, in contrast, was confined to the highest dose level of 400 mg/kg bw/day at which post implantation losses were increased. However, due to mortality among the dams, only 3 litters were available at this dose for evaluation preventing meaningful evaluation of teratogenicity ([redacted] 1989, ASB2009-9028). Based on these results, the dose selection for the subsequent main study appears acceptable.

Main study

25 pregnant rats per groups received MON 0818 from days 6 through 15 post mating by oral gavage at dose levels of 0, 15, 100 and 300 mg/kg bw/day. Following sacrifice of the dams and caesarean section, uteri were inspected and fetuses examined for external, visceral and skeletal anomalies by appropriate methods ([redacted] 1990, ASB2009-9029).

Severe maternal toxicity became apparent at the top dose level by the death of 6 dams between treatment days 8 and 13, clinical signs, initial body weight losses and a diminished body weight gain thereafter. Furthermore, food consumption was decreased. After cessation of treatment, body weight gain and food consumption showed a trend towards normalisation from study day 16 onwards. Soft and mucous faeces might suggest mucosal damage. A lower mean liver weight was probably a reflection of the lower body weight.

At the mid dose level of 100 mg/kg bw/day, mean food consumption was significantly reduced during the first three days of treatment and five out of 25 dams lost weight although the mean body weight and body weight gain were not different from the control group. Clinical signs were only rarely seen. Based on these minor findings, and in accordance to the study author, this dose is considered the LOAEL. For this assessment, it was also taken into

account that clear maternal toxicity occurred at the same dose level in the range-finding study. After adjustment for a [redacted] content of 71.9 %, the LOAEL for the surfactant was calculated to be 72 mg/kg bw/day. The low dose of 15 mg MON 0818/kg bw/day was the NOAEL in this study corresponding to 10.8 mg [redacted]/kg bw/day.
(In the 2009 EPA evaluation (ASB2009-9022), the findings at 100 mg MON 0818/kg bw/day were disregarded and, accordingly, 72 mg/kg bw/day was considered the NOAEL for the [redacted] This dose was also used as starting point to derive the ARfD.)

In the study report ([redacted] 1990, ASB2009-9029) as well as in the EPA evaluation (EPA, 2009, ASB2009-9022), it is stated that no developmental toxicity was observed up to the highest dose level of 300 mg/kg bw/day. However, the total number of visceral and skeletal anomalies at this dose was increased. Due to maternal mortality, only 15 litters were available for evaluation but the incidence of exencephalia and stenosis of the right carotid were already above the historical control range. It may be expected that, with a higher number of litters, the frequency of these anomalies would be even higher. Furthermore, malformations such as *Situs inversus* and absent bladder were noted only in this high dose group for which no historical control data was provided. Therefore, the NOAEL for developmental toxicity of MON 0818 was the mid dose level of 100 mg/kg bw/day corresponding to 72 mg/kg bw/day for the [redacted] The highest dose of 300 mg/kg bw/day (216 mg/kg bw/day) was considered the LOAEL for this endpoint.

<u>Roundup® herbicide</u>
Pregnant Wistar rats (14 - 16 per group) were administered a Roundup® formulation that is commercially distributed in Brazil and was reported to contain 36% glyphosate and 18% of the POE-[redacted] surfactant ([redacted] 2003, ASB2012-11600). The test material was applied in distilled water once a day by oral gavage from day 6 through 15 of gestation at dose levels of 500, 750, or 1000 mg/kg bw whereas the control group received only the vehicle. The respective doses of the [redacted] accounted for approximately 91, 135, and 180 mg/kg bw/day. On gestation day 21, dams were anaesthetised and the uteri with contents were removed by caesarean section and weighed. Afterwards, the dams were sacrificed, necropsied and organ weights of heart, lung, liver, kidneys, and spleen determined. Uteri were inspected for live and dead fetuses and number of implantation sites. The fetuses were weighed, sexed and examined for external and skeletal but not for visceral anomalies.

Maternal toxicity was severe with 50% of the dams (7 out of 14 in that group) dying between gestation days 7 and 14 but was confined to the highest dose level of 1000 mg Roundup/kg bw/day. In the surviving dams, no remarkable findings were reported and relative organ weights did not show statistically significant differences although relative liver weight at the top dose level tended to be increased.
Developmental toxicity was observed in all dose groups and was characterised by a developmental delay of the skeleton and an increase in certain skeletal anomalies. Whereas only 15.4% of the fetuses in the control group exhibited skeletal findings of any kind, the total frequency was higher in a dose-related manner in the treated groups (33.1%, 42.0%, and 57.3%). Incomplete ossification of the skull was noted in all three dose groups and was dose-related as well as a reduced number of caudal vertebrae at the two upper dose levels. In contrast, for other findings, a clear relation to dose was absent. Thus, the incidence of the malformation ”fused zygomatic bone” was higher only at the lowest dose level and, accordingly, cannot be attributed to treatment.

The NOAEL for maternal toxicity of the POE-[redacted] in a Roundup formulation in this study was 135 mg/kg bw/day. In contrast, a NOAEL for fetotoxicity/teratogenicity could not be established.

Similar maternal or developmental effects in rats were not reported for the active ingredient glyphosate (see Volume 3, B.6.6 and Volume, 2.6.6 of this RAR). It may be concluded that the higher maternal and developmental toxicity of Roundup® was due to co-formulants in the herbicidal formulation. It is quite likely that they result from the rather high amount of the POE-[redacted] surfactant in the product but a definitive proof is lacking. It must be emphasised that the selected dose levels in the more recent Brazilian study were clearly above the LOAELs in the 90-day study ([redacted] 1990, ASB2009-9027) and the developmental studies ([redacted] 1989, ASB2009-9028; [redacted] 1990, ASB2009-9029) with the surfactant in the formulation MON 0818. However, these previous studies were apparently not known to the authors because the reports were unpublished. When the different studies are compared, it is surprising that maternal toxicity in the study by [redacted] (2003, ASB2012-11600) occurred only at the highest dose level. It may be doubted if the investigations in the dams were sufficiently extensive to reveal adverse findings at the lower dose levels. In contrast, developmental (skeletal) effects were obviously more pronounced as in the more comprehensive study by [redacted] (1990, ASB2009-9029).

Neurotoxicity
No data available. Based on chemical structure, a specific neurotoxic potential is not expected. The available studies do not suggest neurotoxicity of the [redacted] surfactant.

Mechanistic studies
A systemic effect of a [redacted] surfactant was demonstrated by [redacted]. (1990, TOX9552419) who studied the haemodynamic effects of continuous i.v. application of either glyphosate IPA salt, the formulation Roundup or the surfactant in dogs. The impact on cardiovascular functions was studied in groups of five anesthetised and artificially ventilated female Beagle dogs. Duration of i.v. exposure was 60 minutes. A total of 8.2 g glyphosate (IPA salt administration) or 2.8 g glyphosate (Roundup) was injected. These amounts would correspond to doses of about 550 - 820 mg/kg bw or 180 - 280 mg/kg bw, respectively, since the body weight of the dogs was 10 to 15 kg.
The surfactant alone and Roundup significantly reduced the blood pressure, cardiac output and left ventricular stroke work index suggesting a marked effect on circulation. It could be shown that the cardiac depression observed with Roundup was likely due to the surfactant since, in contrast, arterial blood pressure was even increased when glyphosate isopropylamine salt had been injected. Similarly, the IPA salt did not cause changes in heart rate or cardiac output. A decrease in blood pH observed in this group could be either due to a direct effect of administration or to metabolic acidosis. In any case, it was not strong enough to affect the circulatory system.

[redacted] (1990, Z44833) reported a high toxicity of a [redacted] following intratracheal application to dogs, due to severe lung irritation. A similar effect after oral administration was assumed to result from aspiration because vomiting occurred.

Human data (poisoning incidents)
Despite the low acute toxicity of glyphosate technical, a number of poisoning incidents in humans sometimes even resulting in death were reported in particular from Asian countries (DAR, 1998, ASB2010-10302). Severe intoxication was mainly characterized by a decrease

in blood pressure and further cardiovascular symptoms followed by pulmonary dysfunction and renal failure and by signs of irritation in the gastointestinal tract. Pathophysiology of poisoning by the oral route is assumed to include irritation or corrosion of the intestinal mucosa as a first step resulting in electrolyte imbalances, shock and disturbances in the cardiovascular system. The respiratory signs, as well as renal symptoms, are considered secondary to this mechanism being caused either by pulmonary edema related to disturbed circulation or by aspiration pneumonia following emesis. It is generally assumed that all these effects were mainly due to the ([redacted] surfactants ([redacted] 2004, ASB2012-11576; [redacted]n, 2004, ASB2012-12038), as well as disturbances of lung function and circulation and histopathological lung lesions after acute inhalation ([redacted], 2007, ASB2013-4034). [redacted] (1987, Z35531) reported two cases of human poisonings with surfactants causing clinical signs resembling very much those observed after ingestion of large amounts of Roundup.

Reference doses

Table B.6.13-1 provides an overview on the toxicological studies with the surfactant formulations MON 0818 or G-3780 that might be used for setting reference values for the POE-[redacted] with the CAS no. 61791-26-2. For correction of the NOAELs/LOAELs, a [redacted] content of 72% is assumed. Although acute studies might point to, e.g., a need for setting an ARfD, they are usually not considered an appropriate basis to derive any of the reference doses. Likewise, mechanistic studies are often performed under very artificial conditions and unrealistic high doses are employed. Long-term studies that are mostly used to derive the ADI are not available. Therefore, only short-term toxicity, reproduction and developmental toxicity studies can be taken into consideration for this purpose.

Table B.6.13-1: NOAELs and effect doses for the POE-[redacted] with CAS no. 61791-26-2 in relevant toxicological studies

Study type / Formulation	NOAEL	LOAEL	Effects at LOAEL	Reference
90-days (feeding), rat / MON 0818	20 mg/kg bw/d	60 mg/kg bw/d	Histopathological lesions of intestinal mucosa, bw gain and food consumption ↓	[redacted] 1990 ASB2009-9027
14-weeks (capsules), dog / G-3780	21 mg/kg bw/d	42 mg/kg bw/d	Clinical signs (vomiting, diarrhea); bw (gain) ↓	[redacted] 1973 ASB2009-9026
4-weeks (inhalation), rat / MON 2139 (Roundup)	1.6 mg/kg bw/d (calculated from NOAEC)	5 mg/kg bw/d (calculated from LOAEC)	Clinical chemistry findings, histopathological lesions (lung, trachea, nasal turbinates) at higher concentration	[redacted] 1983 TOX2002-694
2-generation (feeding), rat / MON 0818	12 mg/kg bw/d	38 mg/kg bw/d	Implantation rate, litter size, pup survival ↓	[redacted] 2007 ASB2010-365
One-generation (feeding), rat / MON 0818	Not established	74 mg/kg bw/d	Equivocal evidence of maternal toxicity	[redacted] 2008 ASB2010-364
One-generation (feeding), rat / Roundup	81 mg/kg bw/d (reproductive and parental toxicity), not established for offspring effects	> 81 mg/kg bw/d (reproduction and parental toxicity); 9 mg/kg bw/d (offspring effects)	Sexual development mainly of male pups affected	[redacted] 2007 ASB2012-2721
Developmental toxicity (gavage), rat (range-finding study) / MON 0818	36 mg/kg bw/d	72 mg/kg bw/d	Clinical signs and bw losses in pregnant dams	[redacted] 1989 ASB2009-9028
Developmental toxicity (gavage), rat (main study) / MON 0818	10.8 mg/kg bw/d (maternal toxicity); 72 mg/kg bw/d (developmental effects)	72 mg/kg bw/d (maternal toxicity); 216 mg/kg bw/d (developmental effects)	Food consumption ↓ and bw losses in dams; visceral and skeletal anomalies in fetuses ↑	[redacted] 1990 ASB2009-9029
Developmental toxicity (gavage), rat / Roundup	135 mg/kg bw/d (maternal toxicity); not established for developmental effects	180 mg/kg bw/d (maternal toxicity); 91 mg/kg bw/d (developmental effects)	Mortality in pregnant dams; skeletal anomalies in fetuses ↑, delay in ossification	[redacted] 2003 ASB2012-11600

Studies with MON 0818 are of greater value for deriving the reference values because it cannot be excluded that the active ingredient or co-formulants other than the [redacted] to a certain degree may have contributed to the toxicity of Roundup. In the studies with oral administration of MON 0818, the lowest NOAELs were obtained in the Two-generation study by [redacted] (2007, ASB2010-365) and, with regard to maternal toxicity, in the developmental toxicity study by [redacted] (1990, ASB2009-9029). Numerically, both NOAELs were in the same magnitude of 10 – 12 mg/kg bw/day. The LOAEL of 9 mg/kg bw/day as calculated from the reproduction study with Roundup is in the same range. It is proposed to derive both the ADI and the AOEL on this basis. Since postnatal effects on pup survival in the reproduction study could be an acute effect and because a lower food consumption and body weight losses in pregnant dams in the developmental study at the

LOAEL were observed during the first days of treatment, a NOAEL of 10 – 12 mg/kg bw/day is also considered suitable for deriving the ARfD.

The toxic effects of the POE-[redacted] can be partly attributed to its irritating potential. However, systemic effects were also noted and, therefore, reduction of the usual safety factor is not feasible. When a safety factor of 100 is applied, a numeric value of 0.1 mg/kg bw(/day) for all three reference values (ADI, systemic AOEL, ARfD) will result.

In its recent evaluation, U.S. EPA has set a "chronic RfD" (ADI) and a reference value for "incidental oral (short-term and intermediate-term) exposure" (corresponding to the AOEL) of 0.15 mg/kg bw/day each (EPA, 2009, ASB2009-9022). However, these values are intended to be applied for the whole group of ethoxylated alkyl amines and are based on the NOAEL of 15 mg/kg bw/day as obtained in a 90-day rat study with ATMER®163. In this study, mortality occurred at the next higher dose level of 30 mg/kg bw/day ([redacted] 1991, ASB2009-10488). ATMER®163 is an alkylamine formulation (CAS no. 70955-14-5) that is not contained as a co-formulant in plant protection products which are authorised in Germany. Therefore, this study is of no relevance for this evaluation.

The POE-[redacted] with CAS no. 61791-26-2 proved more toxic by the inhalation route than by oral intake. Therefore, setting of an inhalative AOEL as an additional reference value is needed. In the absence of an appropriate inhalative study with the surfactant itself, a calculation must be performed on the basis of a 4-week study with Roundup ([redacted] 1983, TOX2002-694) under the (conservative) assumption that the effects were entirely due to the [redacted] Based on the calculated NOAEL of 1.66 mg/kg bw/day for the [redacted] in this study (see section on short-term toxicity), an inhalative AOEL for this surfactant of 0.0166 mg/kg bw/day can be set when the safety factor of 100 is applied.

Dermal absorption

The POE-[redacted] with CAS no. 61791-26-2 is a surfactant that is used to enhance the uptake of the herbicidal compound glyphosate (or other herbicides) into the leaves or other part of the weeds which are intended to be controlled. Accordingly, it is a surface-active substance and a certain ability to penetrate through biological membranes can be assumed. However, estimation of dermal absorption for substances with strong irritating properties is difficult because dermal penetration can be either inhibited or facilitated.

Experimental data on dermal absorption is not available. Furthermore, there is no reliable data available that would allow "read-across". Thus, the default values as proposed in the EFSA guidance document (EFSA, 2012, ASB2012-6959) should be used, depending on the concentration of the surfactant in the plan protection product.

Also if physico-chemical properties are taken into consideration, a high molecular weight of 928.31 D (EPA, 2009, ASB2009-9022) suggests poor dermal penetration. However, according to the EU guidance document on dermal absorption, the $\log P_{OW}$ of 3[redacted]15 does not allow reduction of the default values to 10 %.

Impact of POE-[redacted] on the toxicity of Roundup formulations

With regard to nearly all endpoints investigated, the POE-[redacted] (CAS no. 61791-26-2) was clearly more toxic than glyphosate. A direct comparison is shown in Table B.6.13-2. Data for the active substance have been taken from a recent evaluation (EU, 2001, ASB2009-4191).

Table B.6.13-2: Comparison of toxicity data for glyphosate and the POE-[redacted] surfactant with CAS no. 61791-26-2

End point	**Glyphosate**		**POE-[redacted] surfactant**	
Acute oral (rat)	LD_{50} >5000 mg/kg bw		LD_{50}: 864 mg/kg bw	
Acute dermal (rabbit)	LD_{50} >2000 mg/kg bw		LD_{50} >907 mg/kg bw	
Skin irritation	Not irritant		Irritant	
Eye irritation	Moderately to severely irritant		Severely irritant	
Skin sensitization	Negative		Sensitising	
Mutagenicity (gene mutations)	Negative		Negative	
Mutagenicity (chromosome aberrations)	Negative		Negative	
DNA damage	Negative		Equivocal (some evidence at high and clearly toxic doses)	
	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)
Short-term toxicity (rat, oral, 90 d)	150	300	20	60
Short-term toxicity (dog, oral, ca 3 mo)	300	1000	21	42
Reproduction toxicity (rat)	700 (parental) 2000 (repro) 700 (offspring)	2000 (parental) >2000 (repro) 2000 (offspring)	38 (parental) 12 (repro) 12 (offspring)	74 (parental) 38 (repro) 38 (offspring)
Developmental studies (rat), maternal toxicity	300	1000	10.8	72
Developmental studies (rat), fetal effects	300	1000	72	216

The higher toxicity of the surfactant might explain that also Roundup formulations when tested for different endpoints were more toxic than glyphosate ([redacted] 1982, TOX2002-693 and [redacted] 1983, TOX2002-694; [redacted] 2003, ASB2012-11600 and [redacted]., 2007, ASB2012-2721). This is also the most likely explanation for poisoning incidents in humans by the oral or the inhalation route. A possible potentiation of toxicity of glyphosate IPA salt and the [redacted] in animals was suspected by [redacted] (1991, Z80636) who tested the acute oral toxicity of Roundup formulations in rats. However, taking into consideration the toxicological profile of glyphosate, synergism is not very likely. Most effects of both substances are different by nature. Even if the surfactant would enhance the oral absorption of glyhosate (usually about 20~~30~~ % only), adverse effects are not expected because they occur only at exaggerated doses. The only effect for which dose additivity could be theoretically assumed is eye (and perhaps mucosal) irritation. However, the low acute oral toxicity and the high NOAELs of glyphosate in short-term oral studies (see Table B.6.13-2) suggest that the irritating potential of this active ingredient is not relevant after oral intake. Therefore, it is not very likely that glyphosate itself contributed that much to the toxicity of Roundup products in poisoning incidents in humans.

In sum, the available data is sufficient to support the assumption that critical effects of glyhosate-based PPP that were not seen with the active ingredient were due to toxicity of the POE-[redacted] surfactant alone.

B.6.14 Exposure data (Annex IIIA 7.3 to 7.5)

The review report for glyphosate 6511/VI/99-final – 21 January 2002 (ASB2009-4191) is considered to provide relevant basic information on risk assessment for glyphosate. But, new studies on the active substance glyphosate have been performed since then (see IIA 5 of the dossier for the active substance and chapter IIIA 7.6). Based on all available data, more appropriate values for the AOEL and dermal absorption have been derived (see Table B.6.14-1). The assessment presented below is based on these data.

Table B.6.14-1: Product information and toxicological reference values used for exposure assessment

Product	**MON 52276**
Formulation type	SL
Category	Herbicide
Container sizes, short description	1 L, 5 L, 10 L HDPE-containers with 63 mm openings, 20 L HDPE-containers with 61 mm openings and 60 – 1000 L drums
Active substances (incl. content)	**Glyphosate (as its isopropylamine salt)** 360 g/L (salt techn.: 486 g/L)
AOEL systemic	0.1 mg/kg bw/d
Inhalative absorption	100 %
Oral absorption	20 %
Dermal absorption	Concentrate: ~~0.~~1 % Dilution: ~~0.3~~ 1 % (Dilution rate: ~ 1:150, i.e. concentration of glyphosate: 2.5 g/L)* (SL-formulation containing 360 g/L glyphosate, MON 52276)

* Although the lowest concentration of glyphosate in the ready-to-use spray dilution of intended uses, i.e. 0.9 g a.s./L (360 g a.s./400 L, cf Table B.6.14-2) is not fully covered by the concentration of the dermal absorption study (see above) no correction of the value is considered necessary since there is no significant dose dependance of dermal absorption based on the available *in vitro* study using human skin.

MON 52276 is used as a herbicide against annual, perennial and biennial weeds. It is applied pre- and post-planting, pre-emergence of crops or pre-harvest as well as post-emergence of weeds. Spray treatments are performed using tractor-mounted ground-boom sprayers and knapsack sprayers. A summary of the representative uses for MON 52276 is presented in Table B.6.14-2 below.

Table B.6.14-2: Summary of supported uses of MON 52276

Crops	F	Application rate per treatment		Spray volume	Maximum in-use concentration	Number of treatments	Application technique	Acceptability of exposure assessment				
		[L product /ha]	[kg a.s./ha]	[L/ha]	[kg a.s./hL]	min - max		Operator GM	Operator UK POEM	Worker	Bystander	Residents
All crops (pre-planting) [1), 6)]	F	1 – 6*	0.36 – 2.16	100 - 400	2.16	1 – 2*	Tractor-mounted ground boom sprayer with hydraulic nozzles					
All crops (pre-planting) [1), 7)]	F	1 – 6*	0.36 – 2.16	100 - 400	2.16	1 – 2*						
All crops (post-planting/pre-emergence of crops)	F	1 - 3	0.36 – 1.08	100 - 400	1.08	1						
Cereals, oil seeds [1)] (both pre-harvest)	F	2 - 6	0.72 – 2.16	100 - 400	2.16	1						
Orchard crops, vines, incl. citrus & tree nuts [2), 3), 4), 5), 6)] (post emergence of weeds)	F	2 – 8*	0.72 – 2.88	100 - 400	2.88	1 – 3*	Knapsack sprayer					
Orchard crops, vines, incl. citrus & tree nuts [2), 3), 4), 5), 6)] (post emergence of weeds; spot treatment)	F	2 – 8*	0.72 – 2.88	100 - 400	2.88	1 – 3*						

F = field use
GM = German model
* Maximum dose per season not to exceed 4.32 kg a.s./ha
1) critical use for operators in low crops
2) critical use for operators during applications under high crops
3) critical use for bystanders
4) critical use for residents
5) critical use for workers
Since it could not be figured out unequivocally what is meant by 'all crops' by the applicant two different 'worst case'scenarios for residents are presented:
6) Applications on lawn, pasture or meadow not included in 'all crops'
7) Applications on lawn, pasture or meadow included in 'all crops'

	Exposure acceptable without PPE / risk mitigation measures
	Exposure acceptable with PPE or risk mitigation measures required
	Exposure not acceptable/ Evaluation not possible
	No critical use

The results of exposure estimations for operators, bystanders, residents and workers are summarised in Table B.6.14-4, Table B.6.14-6, Table B.6.14-8 and Table B.6.14-10 below. Detailed estimations are provided in A.1.1, 1.2 and 1.3.

B.6.14.1 Operator exposure (IIIA 7.3.1 and 7.3.2)

B.6.14.1.1 Estimation of operator exposure

A summary of the exposure models used for estimation of operator exposure towards the active substances during application of MON 52276 according to intended uses is presented in Table B.6.14-3. Outcome of the estimation is presented in Table B.6.14-4. Detailed calculations are given in 0.

Table B.6.14-3: Exposure models used for operator exposure estimations

Critical use	Cereals, oil seed rape, etc. (max. 6 L product/ha); Orchard crops, vines, incl. citrus & tree nuts (max. 8 L product/ha)
Model	German model [Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products (Uniform Principles for Operator Protection), Mitteilungen aus der Biologischen Bundesanstalt für Land-und Forstwirtschaft, Berlin-Dahlem, Heft 277, 1992]
Critical use	Cereals, oil seed rape, etc. (max. 6 L product/ha); Orchard crops, vines, incl. citrus & tree nuts (max. 8 L product/ha)
Model	Revised UK-POEM [Estimation of Exposure and Absorption of Pesticides by Spray Operators, Scientific subcommittee on Pesticides and British Agrochemical Association Joint Medical Panel Report (UK MAFF), 1986 and the Predictive Operator Exposure Model (POEM) V 1.0, (UK MAFF), 1992]

Table B.6.14-4: Estimated operator exposure towards glyphosate from the use of MON 52276

Model data	Level of PPE	Total absorbed dose (mg/kg/day)	% of systemic AOEL
Tractor mounted boom spray applications outdoors to low crops Application rate: 2.16 kg a.s./ha			
German Model	no PPE [1]	~~0.006245~~0.028389	~~6.2~~28.4
Body weight: 70 kg	+ Gloves mixing/loading	~~0.004779~~0.013725	~~4.8~~13.7
UK POEM	no PPE [2]	~~0.075~~0.2612	~~75.5~~261.2
Application volume: 100 L/ha Container: 10 litres 63 mm closure Body weight: 60 kg	+ Gloves mixing/loading and application	~~0.067~~0.0493	~~66.9~~49.3
Hand-held spray applications outdoors to high crops [3] Application rate: 2.88 kg a.s./ha			
German Model	no PPE [1]	~~0.0278~~0.115365	~~27.8~~115.4
Body weight: 70 kg	+ Gloves mixing/loading	~~0.0195~~0.031865	~~19.5~~31.9
Hand-held spray applications (15 L tank) outdoors to low crops Application rate: 2.88 kg a.s./ha			
UK POEM	no PPE [2]	~~0.207~~0.5682	~~206.6~~568.2
Application volume: 100 L/ha Container: 10 litres 63 mm closure Body weight: 60 kg	+ Gloves mixing/ loading and application and impermeable coverall during application	~~0.085~~0.1487	~~84.7~~148.7

[1] no PPE: Operator wearing T-shirt and shorts
[2] no PPE: Operator wearing long sleeved shirt, long trousers ("permeable") but no gloves
[3] Since there are no data for outdoor applications of herbicides under high crops in the German model, this represents 'worst case'.

In conclusion, MON 52276 can be applied safely by operators using tractor-mounted and hand-held application techniques based on exposure calculations. Only in the case of hand-held spray applications using the UK POEM allocation of PPE to operators is necessary.

B.6.14.1.2 Measurement of operator exposure (mixer/loader/applicator) (IIIA 7.3.3)

Since the operator exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) will not be exceeded under conditions of intended uses according to the German model (with~~out~~ PPE for hand-held applications), a study to provide measurements of operator exposure ~~was not necessary and~~ was therefore not performed.
~~or if PPE is worn a~~According to the UK POEM the acceptable operator exposure level (AOEL) will not be exceeded (with PPE) in the case of applications using tractor mounted boom spray equipment, but will be exceeded for hand-held applications even if PPE is worn.

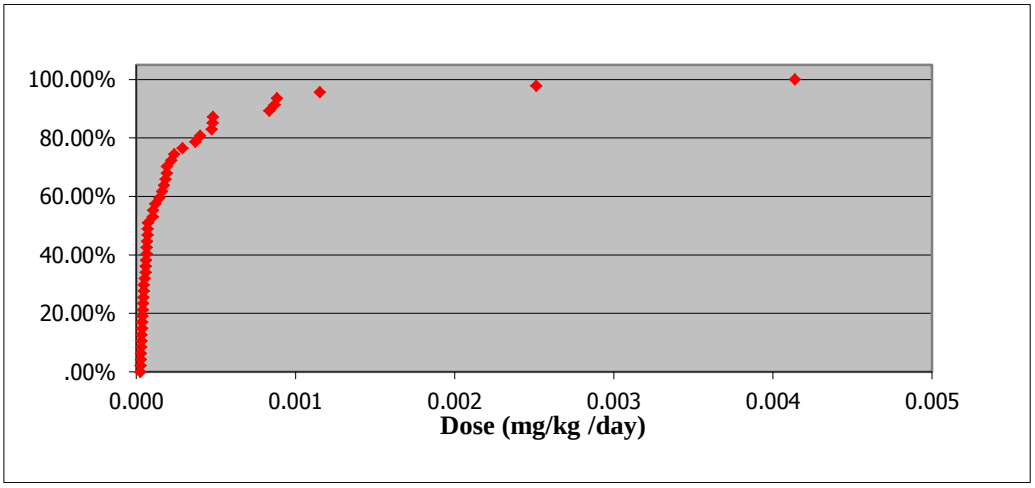
~~Nevertheless,~~ Additionally, the applicants submitted respective biomonitoring data from the Farm Family Exposure Study for farmers and their families published in the open literature ([redacted] 2004; ASB2012-11528). This study was conducted in a southern (South Carolina) and northern (Minnesota) agricultural production area of the U.S.. The purpose of the study was to quantify actual internal pesticide exposure immediately before, during and after pesticide application.

For forty-eight farmer families, including 79 children, urine samples were collected for a 24-hour period prior to application, on the day of application and for three consecutive 24-hour periods thereafter. Urine specimens from 24-hour periods were mixed in such a way that the proportion of the individual volumes of the specimens remained unchanged in the composite mixture. Subsequently glyphosate concentrations were determined in the samples.

Farmers were not instructed or coached by the study investigators on how to apply the products. All farmers used tractor-mounted boom-sprayers and applied Roundup® Ultra (Monsanto Company) over glyphosate tolerant crops early in the growing season. About one-third of the farmers made applications on between 4 and 18 hectares, another third on 18–50 ha, and another third on 50–178 ha. Application rates were according to label recommendations. Sixty percent of applications were carried out using closed-cabs. 71 % of the farmers wore rubber gloves during application. 27 % of the farmers repaired their equipment during the application.

Glyphosate concentrations in the farmers' urine ranged from less than the limit of detection (LOD = 1 ppb) to a maximum of 233 ppb. Overall, only 60 % of the farmers showed detectable levels on the day of application, which further declined afterwards. Figure B.6.14-1 shows the cumulative frequency distribution of systemic doses obtained by the farmers.

Figure B.6.14-1: Systemic dose distribution for farmers applying glyphosate (Acquavella et al., 2004; ASB2012-11528)



The maximum estimated systemic dose for operators was 0.004 mg/kg/day, whereas the geometric mean was 0.0001 mg/kg bw/day.

B.6.14.2 Bystander and resident exposure (IIIA 7.4)

B.6.14.2.1 Estimation of bystander and resident exposure

Table B.6.14-5 shows the exposure model used for estimation of bystander and resident exposure towards glyphosate. Estimations are presented for adults as well as for children. In the case applications on lawn, on meadows and on pasture can be excluded, outcome of the 'worst case' estimations for applications in orchards etc. is presented in Table B.6.14-6. Detailed calculations are given in A.1.2.

If applications on lawn etc. have to be considered this scenario will represent 'worst case' conditions for residents. In this case it is referred to Table B.6.14-7 and Table B.6.14-8 and calculations in 0, too.

Table B.6.14-5: Exposure model used for bystander and resident exposure estimations

Critical use	Orchard crops, vines, incl. citrus & tree nuts (max. 3 x 8 L product/ha *)
Model	Martin, S. et al. (2008, ASB2009-450) [Guidance for Exposure and Risk Evaluation for Bystanders and Residents Exposed to Plant Protection Products During and After Application; J. Verbr. Lebensm. 3 (2008): 272-281 Birkhäuser Verlag Basel] and Bundesanzeiger (BAnz), 06 January 2012, Issue No. 4, pp. 75-76

* Maximum dose per season not to exceed 4.32 kg as/ha

Table B.6.14-6: Estimated bystander and resident exposure towards glyphosate from the use of MON 52276

	Glyphosate	
Model data	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**
Hand-held spray application outdoors under high crops ('worst case') Application rate: 2.88 kg a.s./ha (bystander) or 4.32 kg a.s./ha (resident) [1)]		
Bystanders (adult) Drift rate: 8.02 % [~~1~~2)] (3 m) Body weight: 60 kg	~~0.00135~~0.00405	~~1.35~~4.05
Bystanders (children) Drift rate: 8.02 % [~~1~~2)] (3 m) Body weight: 16.15 kg	~~0.00133~~0.00343	~~1.33~~3.43
Residents (adult) Drift rate: 7.23 % [~~2~~3)] (3 m) Body weight: 60 kg	~~0.00039~~0.00066	~~0.39~~0.66
Residents (children) Drift rate: 7.23 % [~~2~~3)] (3 m) Body weight: 16.15 kg	~~0.00163~~0.00198	~~1.63~~1.98

[1)] although up to 3 x 2.88 kg a.s./ha are intended under high crops the maximum dose per season may not exceed 4.32 kg a.s./ha
[~~1~~2)] drift rate for ornamentals > 50 cm used as a default for herbicidal applications under high crops
[~~2~~3)] 82th percentile for two applications

In the case applications on lawn are included in the term 'all crops' given by the applicant an alternative calculation for resident exposure is presented below.

Table B.6.14-7: Exposure model used for resident exposure estimations for applications on lawn

Critical use	Lawn, meadows, pasture (max. 2 x 6 L product/ha)
Model	Martin, S. et al. (2008, ASB2009-450) [Guidance for Exposure and Risk Evaluation for Bystanders and Residents Exposed to Plant Protection Products During and After Application; J. Verbr. Lebensm. 3 (2008): 272-281 Birkhäuser Verlag Basel] and Bundesanzeiger (BAnz), 06 January 2012, Issue No. 4, pp. 75-76

Table B.6.14-8: Estimated resident exposure towards glyphosate from the use of MON 52276 on lawn

	Glyphosate	
Model data	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**
Tractor mounted spray application on lawn, pasture, meadow Application rate: 2 x 2.16 kg a.s./ha		
Bystanders (adult) Deposit: 100 % Body weight: 60 kg	~~0.00185~~0.00553	~~1.85~~5.53
Bystanders (children) Deposit: 100 % Body weight: 16.15 kg	~~0.01598~~0.02084	~~16.0~~20.84

B.6.14.2.2 Measurement of bystander or resident exposure

Since the bystander and resident exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) will not be exceeded under conditions of intended uses, a study to provide measurements of bystander or resident exposure was not necessary and was therefore not performed.

B.6.14.3 Worker exposure

For the intended uses of MON 52276 there are no foreseen re-entry activities. The only reasonable re-entry scenario is inspection of the crops. Furthermore, for spray treatments pre- and post-planting, and pre-emergence of the crops, as well as post-emergence of weeds in orchards, crop inspection activities normally require no dermal contact to the foliage, but rather consist of a visual inspection. Nevertheless, dermal contact with residues after application is considered as a 'worst case' scenario.

B.6.14.3.1 Estimation of worker exposure

Due to the low vapour pressure of glyphosate (1.31×10^{-5} Pa (25 °C)) respiratory exposure is considered irrelevant for re-entry tasks, so that exposure during these tasks occurs via skin contact with treated surfaces predominantly.

Table B.6.14-9 shows the exposure model used for estimation of dermal worker exposure after entry into a previously treated area or handling a crop treated with MON 52276 according to the critical use. Outcome of the estimation is presented in Table B.6.14-10. Detailed calculations are in A.1.3.

Table B.6.14-9: Exposure model for intended uses

Critical use	Orchard crops, vines, incl. citrus & tree nuts (max. 3 x 8 L product/ha *)
Model	German re-entry model, Krebs et al. (2000, TOX2004-1971) [Uniform Principles for Safeguarding the Health of Workers Re-entering Crop Growing Areas after Application of Plant Protection Products, Nachrichtenbl. Deut. Pflanzenschutzdienst., 52(1), p. 5-9]

* Maximum dose per season not to exceed 4.32 kg as/ha

Table B.6.14-10: Estimated worker exposure

		Glyphosate	
Model data	Level of PPE	Total absorbed dose (mg/kg/day)	% of systemic AOEL
Number of applications and application rate: 4.32 kg a.s./ha [1)]			
8 hours/day [2)], TC: 5000 cm^2/person/h [3)] DFR 1 µg/cm²/kg a.s. [4)] Body weight: 60 kg	no PPE [5)]	~~0.00864~~0.0288	~~8.6~~28.8
	+ Gloves and protective suit	~~0.00043~~0.0014	~~0.4~~1.4

1) Maximum dose per season; 'worst case' (no degradation between splitted dose applications taken into account)
2) 8 h/day for professional applications; 'worst case', since normally there is no classical re-entry scenario for herbicidal applications under high crops
3) Ornamentals, EUROPOEM II, 2002, Post-Application Exposure of Workers to Pesticides in Agriculture; default used for herbicidal applications under high crops
4) no harmonised default is available, therefore default value acc. to the above mentioned model (even if a more conservative default value of 3 µg/cm²/kg a.s. is used, worker exposure is < 100 % of AOEL (86.4~~26~~ % of AOEL))
5) no PPE: Worker wearing long sleeved shirt, long trousers ("permeable") but no gloves

B.6.14.3.2 Measurement of worker exposure

Since the worker exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) will not be exceeded under conditions of intended uses, a study to provide measurements of worker exposure was not necessary and was therefore not performed.

Appendix 1 Exposure calculations

Operator exposure calculations (IIIA 7.3)

Table A 1: Input parameters considered for the estimation of operator exposure according to the German model (FCTM)

Formulation type:	**SL**		**Application technique:**	**Field Crop Tractor Mounted (FCTM)**	
Application rate (AR):	2.16	kg a.s./ha			
Area treated per day (A):	20	ha	Dermal hands m/l ($D_{M(H)}$):	2.4	mg/person/kg a.s.
Dermal absorption (DA):	~~0.~~1.0	% (concentr.)	Dermal hands appl. ($D_{A(H)}$):	0.38	mg/person/kg a.s.
	~~0.3~~1.0	% (dilution)	Dermal body appl. ($D_{A(B)}$):	1.6	mg/person/kg a.s.
Inhalation absorption (IA):	100	%	Dermal head appl. ($D_{A(C)}$):	0.06	mg/person/kg a.s.
Body weight (BW):	70	kg/person	Inhalation m/l (I_M):	0.0006	mg/person/kg a.s.
AOEL	0.1	mg/kg bw/d	Inhalation appl. (I_A):	0.001	mg/person/kg a.s.

Table A 2: Estimation of operator exposure towards glyphosate using the German model

Without PPE			**With PPE**		
Operators: Systemic dermal exposure after application in cereals, oil seeds, pre-harvest; all crops, pre-planting					
Dermal exposure during mixing/loading					
Hands			Hands		
$SDE_{OM(H)} = (D_{M(H)}$ x AR x A x DA) / BW			$SDE_{OM(H)} = (D_{M(H)}$ x AR x A x PPE [1)] x DA) / BW		
(2.4 x 2.16 x 20 x ~~0.~~1.0 %) / 70			(2.4 x 2.16 x 20 x 0.01 x ~~0.~~1.0 %) / 70		
External dermal exposure	103.68	mg/person	External dermal exposure	1.0368	mg/person
External dermal exposure	1.481143	mg/kg bw/d	External dermal exposure	0.014811	mg/kg bw/d
Systemic dermal exposure	0.~~0~~01481	mg/kg bw/d	Systemic dermal exposure	~~0.000015~~0.000148	mg/kg bw/d
Dermal exposure during application					
Hands			Hands		
$SDE_{OA(H)} = (D_{A(H)}$ x AR x A x DA) / BW			$SDE_{OA(H)} = (D_{A(H)}$ x AR x A x PPE x DA) / BW		
(0.38 x 2.16 x 20 x ~~0.3~~1.0 %) / 70			(0.38 x 2.16 x 20 x 1 x ~~0.3~~1.0%) / 70		
External dermal exposure	16.416	mg/person	External dermal exposure	16.416	mg/person
External dermal exposure	0.234514	mg/kg bw/d	External dermal exposure	0.234514	mg/kg bw/d
Systemic dermal exposure	~~0.000704~~0.002345	mg/kg bw/d	Systemic dermal exposure	~~0.000704~~0.002345	mg/kg bw/d
Body			Body		
$SDE_{OA(B)} = (D_{A(B)}$ x AR x A x DA) / BW			$SDE_{OA(B)} = (D_{A(B)}$ x AR x A x PPE x DA) / BW		
(1.6 x 2.16 x 20 x ~~0.3~~1.0 %) / 70			(1.6 x 2.16 x 20 x 1 x ~~0.3~~1.0 %) / 70		
External dermal exposure	69.12	mg/person	External dermal exposure	69.12	mg/person
External dermal exposure	0.987429	mg/kg bw/d	External dermal exposure	0.987429	mg/kg bw/d
Systemic dermal	~~0.002962~~0.009874	mg/kg bw/d	Systemic dermal	~~0.002962~~0.009874	mg/kg bw/d

Without PPE			**With PPE**		
exposure			exposure		
Head			Head		
$SDE_{OA(C)} = (D_{A(C)}$ x AR x A x DA) / BW			$SDE_{OA(C)} = (D_{A(C)}$ x AR x A x PPE x DA) / BW		
(0.06 x 2.16 x 20 x ~~0.3~~1.0 %) / 70			(0.06 x 2.16 x 20 x 1 x ~~0.3~~1.0 %) / 70		
External dermal exposure	2.592	mg/person	External dermal exposure	2.592	mg/person
External dermal exposure	0.037029	mg/kg bw/d	External dermal exposure	0.037029	mg/kg bw/d
Systemic dermal exposure	~~0.0001110~~.000370	mg/kg bw/d	Systemic dermal exposure	~~0.0001110~~.000370	mg/kg bw/d
Total systemic dermal exposure: $SDE_O = SDE_{OM(H)} + SDE_{OA(H)} + SDE_{OA(B)} + SDE_{OA(C)}$			Total systemic dermal exposure: $SDE_O = SDE_{OM(H)} + SDE_{OA(H)} + SDE_{OA(B)} + SDE_{OA(C)}$		
Total external dermal exposure	191.808	mg/person	Total external dermal exposure	89.1648	mg/person
Total external dermal exposure	2.740114	mg/kg bw/d	Total external dermal exposure	1.273783	mg/kg bw/d
Total systemic dermal exposure	~~0.0052580~~.027401	mg/kg bw/d	Total systemic dermal exposure	~~0.0037920~~.012748	mg/kg bw/d
Operators: Systemic inhalation exposure after application in Cereals, oil seeds, pre-harvest; all crops, pre-planting					
Inhalation exposure during mixing/loading					
$SIE_{OM} = (I_M$ x AR x A x IA) / BW			$SIE_{OM} = (I_M$ x AR x A x PPE x IA) / BW		
(0.0006 x 2.16 x 20 x 100 %) / 70			(0.0006 x 2.16 x 20 x 1 x 100 %) / 70		
External inhalation exposure	0.02592	mg/person	External inhalation exposure	0.02592	mg/person
External inhalation exposure	0.00037	mg/kg bw/d	External inhalation exposure	0.00037	mg/kg bw/d
Systemic inhalation exposure	0.00037	mg/kg bw/d	Systemic inhalation exposure	0.00037	mg/kg bw/d
Inhalation exposure during application					
$SIE_{OA} = (I_A$ x AR x A x IA) / BW			$SIE_{OA} = (I_A$ x AR x A x PPE x IA) / BW		
(0.001 x 2.16 x 20 x 100 %) / 70			(0.001 x 2.16 x 20 x 1 x 100 %) / 70		
External inhalation exposure	0.0432	mg/person	External inhalation exposure	0.0432	mg/person
External inhalation exposure	0.000617	mg/kg bw/d	External inhalation exposure	0.000617	mg/kg bw/d
Systemic inhalation exposure	0.000617	mg/kg bw/d	Systemic inhalation exposure	0.000617	mg/kg bw/d
Total systemic inhalation exposure: $SIE_O = SIE_{OM} + SIE_{OA}$			Total systemic inhalation exposure: $SIE_O = SIE_{OM} + SIE_{OA}$		
Total external inhalation exposure	0.06912	mg/person	Total external inhalation exposure	0.06912	mg/person
Total external inhalation exposure	0.000987	mg/kg bw/d	Total external inhalation exposure	0.000987	mg/kg bw/d
Total systemic inhalation exposure	0.000987	mg/kg bw/d	Total systemic inhalation exposure	0.000987	mg/kg bw/d
Total systemic exposure: $SE_O = SDE_O + SIE_O$			Total systemic exposure: $SE_O = SDE_O + SIE_O$		
Total systemic exposure	~~0.4371841~~.987200	mg/person	Total systemic exposure	~~0.3345410~~.960768	mg/person

Without PPE			**With PPE**		
Total systemic exposure	~~0.006245~~0.028389	mg/kg bw/d	Total systemic exposure	~~0.004779~~0.013725	mg/kg bw/d
% of AOEL	~~6.2~~28.4	%	% of AOEL	~~4.8~~13.7	%
[1)] reduction factor for gloves is 0.01 (professional appl.)					

Table A 3: Input parameters considered for the estimation of operator exposure according to the German model (HCHH)

Formulation type:	**SL**		**Application technique:**	**High Crop Hand Held (HCHH)**	
Application rate (AR):	2.88	kg a.s./ha			
Area treated per day (A):	1	ha	Dermal hands m/l ($D_{M(H)}$):	205	mg/person/kg a.s.
Dermal absorption (DA):	~~0.~~1.0	% (concentr.)	Dermal hands appl. ($D_{A(H)}$):	10.6	mg/person/kg a.s.
	~~0.3~~1.0	% (dilution)	Dermal body appl. ($D_{A(B)}$):	25	mg/person/kg a.s.
Inhalation absorption (IA):	100	%	Dermal head appl. ($D_{A(C)}$):	4.8	mg/person/kg a.s.
Body weight (BW):	70	kg/person	Inhalation m/l (I_M):	0.05	mg/person/kg a.s.
AOEL	0.1	mg/kg bw/d	Inhalation appl. (I_A):	0.3	mg/person/kg a.s.

Table A 4: Estimation of operator exposure towards glyphosate using the German model (HCHH)

Without PPE			**With PPE**		
Operators: Systemic dermal exposure after application in grapevine, orchard crops, tree nuts					
Dermal exposure during mixing/loading					
Hands			Hands		
$SDE_{OM(H)} = (D_{M(H)}$ x AR x A x DA) / BW			$SDE_{OM(H)} = (D_{M(H)}$ x AR x A x PPE [1)] x DA) / BW		
(205 x 2.88 x 1 x ~~0.~~1.0 %) / 70			(205 x 2.88 x 1 x 0.01 x ~~0.~~1.0 %) / 70		
External dermal exposure	590.4	mg/person	External dermal exposure	5.904	mg/person
External dermal exposure	8.434286	mg/kg bw/d	External dermal exposure	0.084343	mg/kg bw/d
Systemic dermal exposure	0.~~0~~08434	mg/kg bw/d	Systemic dermal exposure	~~0.000084~~ 0.000843	mg/kg bw/d
Dermal exposure during application					
Hands			Hands		
$SDE_{OA(H)} = (D_{A(H)}$ x AR x A x DA) / BW			$SDE_{OA(H)} = (D_{A(H)}$ x AR x A x PPE x DA) / BW		
(10.6 x 2.88 x 1 x ~~0.3~~1.0 %) / 70			(10.6 x 2.88 x 1 x 1 x ~~0.3~~1.0 %) / 70		
External dermal exposure	30.528	mg/person	External dermal exposure	30.528	mg/person
External dermal exposure	0.436114	mg/kg bw/d	External dermal exposure	0.436114	mg/kg bw/d
Systemic dermal exposure	~~0.001308~~ 0.004361	mg/kg bw/d	Systemic dermal exposure	~~0.001308~~ 0.004361	mg/kg bw/d
Body			Body		
$SDE_{OA(B)} = (D_{A(B)}$ x AR x A x DA) / BW			$SDE_{OA(B)} = (D_{A(B)}$ x AR x A x PPE x DA) / BW		
(25 x 2.88 x 1 x ~~0.3~~1.0 %) / 70			(25 x 2.88 x 1 x 1 x ~~0.3~~1.0 %) / 70		
External dermal exposure	72	mg/person	External dermal exposure	72	mg/person
External dermal	1.028571	mg/kg bw/d	External dermal exposure	1.028571	mg/kg bw/d

Without PPE			**With PPE**		
exposure					
Systemic dermal exposure	~~0.003086~~ 0.010286	mg/kg bw/d	Systemic dermal exposure	~~0.003086~~ 0.010286	mg/kg bw/d
Head			Head		
$SDE_{OA(C)} = (D_{A(C)}$ x AR x A x DA) / BW			$SDE_{OA(C)} = (D_{A(C)}$ x AR x A x PPE x DA) / BW		
(4.8 x 2.88 x 1 x ~~0.3~~1.0 %) / 70			(4.8 x 2.88 x 1 x 1 x ~~0.3~~1.0 %) / 70		
External dermal exposure	13.824	mg/person	External dermal exposure	13.824	mg/person
External dermal exposure	0.197486	mg/kg bw/d	External dermal exposure	0.197486	mg/kg bw/d
Systemic dermal exposure	~~0.000592~~ 0.001975	mg/kg bw/d	Systemic dermal exposure	~~0.000592~~ 0.001975	mg/kg bw/d
Total systemic dermal exposure: $SDE_O = SDE_{OM(H)} + SDE_{OA(H)} + SDE_{OA(B)} + SDE_{OA(C)}$			Total systemic dermal exposure: $SDE_O = SDE_{OM(H)} + SDE_{OA(H)} + SDE_{OA(B)} + SDE_{OA(C)}$		
Total external dermal exposure	706.752	mg/person	Total external dermal exposure	122.256	mg/person
Total external dermal exposure	10.096457	mg/kg bw/d	Total external dermal exposure	1.746514	mg/kg bw/d
Total systemic dermal exposure	~~0.013421~~ 0.100965	mg/kg bw/d	Total systemic dermal exposure	~~0.005071~~ 0.017465	mg/kg bw/d
Operators: Systemic inhalation exposure after application in Grapevine, orchard crops, tree nuts					
Inhalation exposure during mixing/loading					
$SIE_{OM} = (I_M$ x AR x A x IA) / BW			$SIE_{OM} = (I_M$ x AR x A x PPE x IA) / BW		
(0.05 x 2.88 x 1 x 100 %) / 70			(0.05 x 2.88 x 1 x 1 x 100 %) / 70		
External inhalation exposure	0.144	mg/person	External inhalation exposure	0.144	mg/person
External inhalation exposure	0.002057	mg/kg bw/d	External inhalation exposure	0.002057	mg/kg bw/d
Systemic inhalation exposure	0.002057	mg/kg bw/d	Systemic inhalation exposure	0.002057	mg/kg bw/d
Inhalation exposure during application					
$SIE_{OA} = (I_A$ x AR x A x IA) / BW			$SIE_{OA} = (I_A$ x AR x A x PPE x IA) / BW		
(0.3 x 2.88 x 1 x 100 %) / 70			(0.3 x 2.88 x 1 x 1 x 100 %) / 70		
External inhalation exposure	0.864	mg/person	External inhalation exposure	0.864	mg/person
External inhalation exposure	0.012343	mg/kg bw/d	External inhalation exposure	0.012343	mg/kg bw/d
Systemic inhalation exposure	0.012343	mg/kg bw/d	Systemic inhalation exposure	0.012343	mg/kg bw/d
Total systemic inhalation exposure: $SIE_O = SIE_{OM} + SIE_{OA}$			Total systemic inhalation exposure: $SIE_O = SIE_{OM} + SIE_{OA}$		
Total external inhalation exposure	1.008	mg/person	Total external inhalation exposure	1.008	mg/person
Total external inhalation exposure	0.0144	mg/kg bw/d	Total external inhalation exposure	0.0144	mg/kg bw/d
Total systemic inhalation exposure	0.0144	mg/kg bw/d	Total systemic inhalation exposure	0.0144	mg/kg bw/d
Total systemic exposure: $SE_O = SDE_O + SIE_O$			Total systemic exposure: $SE_O = SDE_O + SIE_O$		
Total systemic exposure	~~1.947456~~ 8.075520	mg/person	Total systemic exposure	~~1.36296~~ 2.230560	mg/person
Total systemic exposure	~~0.027821~~ 0.115365	mg/kg bw/d	Total systemic exposure	~~0.019471~~ 0.031865	mg/kg bw/d
% of AOEL	~~27.8~~115.4	%	% of AOEL	~~19.5~~31.9	%
[1)] reduction factor for gloves is 0.01 (professional appl.)					

Table A 5: Estimation of operator exposure towards glyphosate using the UK-POEM (FCTM)

Without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)				
Active substance	Glyphosate			
Product	MON 52276			
Formulation type	water-based			
Concentration of a.s.	360	mg/mL		
Dose	6	L preparation/ha	(2.16 kg a.s./ha)	
Application volume	100	L/ha		
Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Container	10 litres 63 mm closure			
Work rate/day	50	ha		
Duration of spraying	6	h		
PPE during mix./loading	None			
PPE during application	None			
Dermal absorption from product	~~0.~~1.0	%		
Dermal absorption from spray	~~0.3~~1.0	%		
EXPOSURE DURING MIXING AND LOADING				
Container size	10	Litres		
Hand contamination/operation	0,05	mL		
Application dose	6	Litres product/ha		
Work rate	50	ha/day		
Number of operations	30	/day		
Hand contamination	1.5	mL/day		
Protective clothing	None			
Transmission to skin	100	%		
Dermal exposure to formulation	1.5	mL/day		
DERMAL EXPOSURE DURING SPRAY APPLICATION				
Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	100	spray/ha		
Volume of surface contamination	10	mL/h		
Distribution	Hands	Trunk	Legs	
	65 %	10 %	25 %	
Clothing	None	Permeable	Permeable	
Penetration	100 %	5 %	15 %	
Dermal exposure	6.5	0.05	0.375	mL/h
Duration of exposure	6	h		
Total dermal exposure to spray	41.55	mL/day		
ABSORBED DERMAL DOSE				
	Mix/load		Application	
Dermal exposure	1.5	mL/day	41.55	mL/day
Concen. of a.s. product or spray	360	mg/mL	21.6	mg/mL
Dermal exposure to a.s.	540	mg/day	897.48	mg/day
Percent absorbed	~~0.~~1.0	%	~~0.3~~1.0	%
Absorbed dose	~~0.~~5.40	mg/day	~~2.692~~8.97	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.01	mL/h		
Duration of exposure	6	h		
Concentration of a.s. in spray	21.6	mg/mL		
Inhalation exposure to a.s.	1.296	mg/day		
Percent absorbed	100	%		
Absorbed dose	1.296	mg/day		
PREDICTED EXPOSURE				
Total absorbed dose	~~4.528~~15.671	mg/day		
Operator body weight	60	kg		

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)				
Operator exposure	~~0.075~~0.2612	mg/kg bw/day		
Amount of AOEL	~~75.5~~261.2	%		

With PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)				
Active substance	Glyphosate			
Product	MON 52276			
Formulation type	water-based			
Concentration of a.s.	360	mg/mL		
Dose	6	L preparation/ha	(2.16 kg a.s./ha)	
Application volume	100	L/ha		
Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Container	10 litres 63 mm closure			
Work rate/day	50	ha		
Duration of spraying	6	h		
PPE during mix./loading	Gloves			
PPE during application	~~None~~Gloves			
Dermal absorption from product	~~0.~~1.0	%		
Dermal absorption from spray	~~0.3~~1.0	%		
EXPOSURE DURING MIXING AND LOADING				
Container size	10	Litres		
Hand contamination/operation	0,05	mL		
Application dose	6	Litres product/ha		
Work rate	50	ha/day		
Number of operations	30	/day		
Hand contamination	1.5	mL/day		
Protective clothing	Gloves			
Transmission to skin	5	%		
Dermal exposure to formulation	0.075	mL/day		
DERMAL EXPOSURE DURING SPRAY APPLICATION				
Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	100	spray/ha		
Volume of surface contamination	10	mL/h		
Distribution	Hands	Trunk	Legs	
	65 %	10 %	25 %	
Clothing	~~None~~Gloves	Permeable	Permeable	
Penetration	10~~0~~ %	5 %	15 %	
Dermal exposure	0.65	0.05	0.375	mL/h
Duration of exposure	6	h		
Total dermal exposure to spray	~~41.55~~6.45	mL/day		
ABSORBED DERMAL DOSE				
	Mix/load		Application	
Dermal exposure	0.075	mL/day	~~41.55~~6.45	mL/day
Concen. of a.s. product or spray	360	mg/mL	21.6	mg/mL
Dermal exposure to a.s.	27	mg/day	~~897.48~~139.32	mg/day
Percent absorbed	~~0.~~1.0	%	~~0.3~~1.0	%
Absorbed dose	0.~~0~~2700	mg/day	~~2.692~~1.3932	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.01	mL/h		
Duration of exposure	6	h		
Concentration of a.s. in spray	21.6	mg/mL		
Inhalation exposure to a.s.	1.296	mg/day		
Percent absorbed	100	%		
Absorbed dose	1.296	mg/day		
PREDICTED EXPOSURE				
Total absorbed dose	~~4.015~~2.9592	mg/day		

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)				
Operator body weight	60	kg		
Operator exposure	~~0.067~~0.0493	mg/kg bw/day		
Amount of AOEL	~~66.9~~49.3	%		

Table A 6: Estimation of operator exposure towards glyphosate using the UK-POEM (Hand-held, 15 L tank, downwards)

Without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)				
Active substance	Glyphosate			
Product	MON 52276			
Formulation type	water-based			
Concentration of a.s.	360	mg/mL		
Dose	8	L preparation/ha	(2.88 kg a.s./ha)	
Application volume	100	L/ha		
Application method	Hand-held sprayer (15 L tank): hydraulic nozzles. Outdoor, low level target			
Container	10 litres 63 mm closure			
Work rate/day	1	ha		
Duration of spraying	6	h		
PPE during mix./loading	None			
PPE during application	None			
Dermal absorption from product	~~0.~~1.0	%		
Dermal absorption from spray	~~0.3~~1.0	%		
EXPOSURE DURING MIXING AND LOADING				
Container size	10	Litres		
Hand contamination/operation	0,05	mL		
Application dose	8	Litres product/ha		
Work rate	1	ha/day		
Number of operations	7	/day		
Hand contamination	0.35	mL/day		
Protective clothing	None			
Transmission to skin	100	%		
Dermal exposure to formulation	0.35	mL/day		
DERMAL EXPOSURE DURING SPRAY APPLICATION				
Application technique	Hand-held sprayer (15 L tank): hydraulic nozzles. Outdoor, low level target			
Application volume	100	spray/ha		
Volume of surface contamination	50	mL/h		
Distribution	Hands	Trunk	Legs	
	25 %	25 %	50 %	
Clothing	None	Permeable	Permeable	
Penetration	100 %	20 %	18 %	
Dermal exposure	10	2.5	4.5	mL/h
Duration of exposure	6	h		
Total dermal exposure to spray	102	mL/day		
ABSORBED DERMAL DOSE				
	Mix/load		Application	
Dermal exposure	0.35	mL/day	102	mL/day
Concen. of a.s. product or spray	360	mg/mL	28.8	mg/mL
Dermal exposure to a.s.	126	mg/day	2937.6	mg/day
Percent absorbed	~~0.~~1.0	%	~~0.3~~1.0	%
Absorbed dose	~~0.~~1.260	mg/day	~~8.813~~29.376	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.02	mL/h		

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)				
Duration of exposure	6	h		
Concentration of a.s. in spray	28.8	mg/mL		
Inhalation exposure to a.s.	3.456	mg/day		
Percent absorbed	100	%		
Absorbed dose	3.456	mg/day		
PREDICTED EXPOSURE				
Total absorbed dose	~~12.395~~34.0920	mg/day		
Operator body weight	60	kg		
Operator exposure	~~0.207~~0.5682	mg/kg bw/day		
Amount of AOEL	~~206.6~~568.2	%		

With PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)				
Active substance	Glyphosate			
Product	MON 52276			
Formulation type	water-based			
Concentration of a.s.	360	mg/mL		
Dose	8	L preparation/ha	(2.88 kg a.s./ha)	
Application volume	100	L/ha		
Application method	Hand-held sprayer (15 L tank): hydraulic nozzles. Outdoor, low level target			
Container	10 litres 63 mm closure			
Work rate/day	1	ha		
Duration of spraying	6	h		
PPE during mix./loading	Gloves			
PPE during application	Gloves and impermeable coveralls			
Dermal absorption from product	~~0.~~1.0	%		
Dermal absorption from spray	~~0.3~~1.0	%		
EXPOSURE DURING MIXING AND LOADING				
Container size	10	Litres		
Hand contamination/operation	0,05	mL		
Application dose	8	Litres product/ha		
Work rate	1	ha/day		
Number of operations	7	/day		
Hand contamination	0.35	mL/day		
Protective clothing	Gloves			
Transmission to skin	5	%		
Dermal exposure to formulation	0.01~~8~~75	mL/day		
DERMAL EXPOSURE DURING SPRAY APPLICATION				
Application technique	Hand-held sprayer (15 L tank): hydraulic nozzles. Outdoor, low level target			
Application volume	100	spray/ha		
Volume of surface contamination	50	mL/h		
Distribution	Hands	Trunk	Legs	
	25 %	25 %	50 %	
Clothing	Gloves	Impermeable	Impermeable	
Penetration	10 %	5 %	5 %	
Dermal exposure	1.25	0.625	1.25	mL/h
Duration of exposure	6	h		
Total dermal exposure to spray	18.75	mL/day		
ABSORBED DERMAL DOSE				
	Mix/load		Application	
Dermal exposure	0.01~~8~~75	mL/day	18.75	mL/day
Concen. of a.s. product or spray	360	mg/mL	28.8	mg/mL
Dermal exposure to a.s.	6.3	mg/day	540	mg/day
Percent absorbed	~~0.~~1.0	%	~~0.3~~1.0	%

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)				
Absorbed dose	0.~~0~~063	mg/day	~~1.62~~5.4	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.02	mL/h		
Duration of exposure	6	h		
Concentration of a.s. in spray	28.8	mg/mL		
Inhalation exposure to a.s.	3.456	mg/day		
Percent absorbed	100	%		
Absorbed dose	3.456	mg/day		
PREDICTED EXPOSURE				
Total absorbed dose	~~5.082~~8.9190	mg/day		
Operator body weight	60	kg		
Operator exposure	~~0.085~~0.1487	mg/kg bw/day		
Amount of AOEL	~~84.7~~148.7	%		

Bystander and resident exposure calculations (IIIA 7.4)

Table A 7: Input parameters considered for the estimation of bystander exposure

Intended use::	**All high crops intended**		**Drift (D) [1]:**	**8.02**	**% (HC, 3 m)**
Application rate (AR):	2.88	kg a.s./ha	Exposed body surface area (BSA):	1	m² (adults)
	288	mg/m^2		0.21	m² (children)
Body weight (BW):	60	kg/person (adults)	Specific Inhalation Exposure (I^*_A):	0.3	mg/kg a.s. (6 hours, adults)
	16.15	kg/person (children)		0.172414	mg/kg a.s. (6 hours, children)
Dermal absorption (DA):	~~0.3~~1.0	% ('worst case')	Area Treated (A):	1	ha/d (based on HCHH)
Inhalation absorption (IA):	100	%			
AOEL:	0.1	mg/kg bw/d	Exposure duration (T):	5	min

[1] drift rate for ornamentals > 50 cm used as a default for herbicidal applications under high crops

Table A 8: Estimation of bystander exposure towards glyphosate

Adults			**Children**		
Bystander: Systemic dermal exposure during/after application under high crops (via spray drift)					
SDE_B = (AR x D x BSA x DA) / BW			SDE_B = (AR x D x BSA x DA) / BW		
(288 x 8.02 % x 1 x ~~0.3~~1.0 %) / 60			(288 x 8.02 % x 0.21 x ~~0.3~~1.0 %) / 16.15		
External dermal exposure	23.0976	mg/person	External dermal exposure	4.850496	mg/person
External dermal exposure	0.38496	mg/kg bw/d	External dermal exposure	0.30034	mg/kg bw/d
Systemic dermal exposure	~~0.001155~~0.00385	mg/kg bw/d	Systemic dermal exposure	~~0.000901~~0.00300	mg/kg bw/d
Bystander: Systemic inhalation exposure during/after application under high crops (via spray drift)					
SIE_B = (I^*_A x AR x A x T x IA) / BW			SIE_B = (I^*_A x AR x A x T x IA) / BW		
(0.3 / 360 x 2.88 x 1 x 5 x 100 %) / 60			(0.172414 / 360 x 2.88 x 1 x 5 x 100 %) / 16.15		
External inhalation exposure	0.012	mg/person	External inhalation exposure	0.006897	mg/person
External inhalation exposure	0.0002	mg/kg bw/d	External inhalation exposure	0.000427	mg/kg bw/d
Systemic inhalation exposure	0.0002	mg/kg bw/d	Systemic inhalation exposure	0.000427	mg/kg bw/d
Total systemic exposure: $SE_B = SDE_B + SIE_B$			Total systemic exposure: $SE_B = SDE_B + SIE_B$		
Total systemic exposure	~~0.081293~~0.242976	mg/person	Total systemic exposure	~~0.021448~~0.055401	mg/person
Total systemic exposure	~~0.001355~~0.004050	mg/kg bw/d	Total systemic exposure	~~0.001328~~0.0034304	mg/kg bw/d
% of AOEL	~~1.35~~4.05	%	% of AOEL	~~1.33~~3.43	%

Table A 9: Input parameters considered for the estimation of resident exposure ('worst case' if no applications on lawn, pasture and meadow are intended)

Intended uses:	**All high crops intended**		**Drift (D) [2]:**	**7.23**	**% (HC, 3 m)**
Application rate (AR): [1]	4.32	kg a.s./ha	Transfer coefficient (TC):	7300	cm^2/h (adults)
	0.0432	mg/cm^2		2600	cm^2/h (children)
Number of applications (NA):	2		Turf Transferable Residues (TTR):	5	%
Body weight (BW):	60	kg/person (adults)	Exposure Duration (H):	2	h
	16.15	kg/person (children)	Airborne Concentration of Vapour (ACV):	0.001	mg/m^3
Dermal absorption (DA):	~~0.3~~1.0	% ('worst case')	Inhalation Rate (IR):	16.57	m^3/d (adults)
Inhalation absorption (IA):	100	%		8.31	m^3/d (children)
Oral absorption (OA):	20	%	Saliva Extraction Factor (SE):	50	%
AOEL:	0.1	mg/kg bw/d	Surface Area of Hands (SA):	20	cm^2
			Frequency of Hand to Mouth (Freq):	20	events/h
			Dislodgeable foliar residues (DFR):	20	%
			Ingestion Rate for Mouthing of Grass/Day (IgR):	25	cm^2/d

[1] maximum dose per season

[2] drift rate for ornamentals > 50 cm used as a default for herbicidal applications under high crops, 82 th percentile for 2 applications

Table A 10: Estimation of resident exposure towards glyphosate ('worst case' if no applications on lawn, pasture and meadow are intended)

Adults			**Children**		
Residents: Systemic dermal exposure after application under high crops (via deposits caused by spray drift)					
SDE_R = (AR x NA x D x TTR x TC x H x DA) / BW			SDE_R = (AR x NA x D x TTR x TC x H x DA) / BW		
(0.0432 x 7.23 % x 5 % x 7300 x 2 x ~~0.3~~1.0 %) / 60			(0.0432 x 1 x 7.23 % x 5 % x 2600 x 2 x ~~0.3~~1.0 %) / 16.15		
External dermal exposure	2.280053	mg/person	External dermal exposure	0.812074	mg/person
External dermal exposure	0.038001	mg/kg bw/d	External dermal exposure	0.050283	mg/kg bw/d
Systemic dermal exposure	~~0.000114~~ 0.000380	mg/kg bw/d	Systemic dermal exposure	~~0.000151~~ 0.000503	mg/kg bw/d
Residents: Systemic inhalation exposure after application under high crops (via vapour)					
SIE_R = (AC_V x IR x IA) / BW			SIE_R = (AC_V x IR x IA) / BW		
(0.001 x 16.57 x 100 %) / 60			(0.001 x 8.31 x 100 %) / 16.15		
External inhalation exposure	0.01657	mg/person	External inhalation exposure	0.00831	mg/person
External inhalation exposure	0.000276	mg/kg bw/d	External inhalation exposure	0.000515	mg/kg bw/d
Systemic inhalation exposure	0.000276	mg/kg bw/d	Systemic inhalation exposure	0.000515	mg/kg bw/d
			Residents: Systemic oral exposure (hand-to-mouth transfer)		
			$SOE_{R(H)}$ = (AR x NA x D x TTR x SE x SA x Freq x H x OA) / BW		
			(0.0432 x 1 x % x 5 % x 50 % x 20 x 20 x 2 x 20 %) / 16.15		
			External oral exposure	0.062467	mg/person
			External oral exposure	0.003868	mg/kg bw/d
			Systemic oral exposure	0.000774	mg/kg bw/d
			Residents: Systemic oral exposure (object-to-mouth transfer)		
			$SOE_{R(O)}$ = (AR x NA x D x DFR x IgR x OA) / BW		
			(0.0432 x 1 x % x 20 % x 25 x 20 %) / 16.15		
			External oral exposure	0.015617	mg/person
			External oral exposure	0.000967	mg/kg bw/d
			Systemic oral exposure	0.000193	mg/kg bw/d
Total systemic exposure: SE_R = SDE_R + SIE_R			Total systemic exposure: SE_R = SDE_R + SIE_R + $SOE_{R(H)}$ + $SOE_{R(O)}$		
Total systemic exposure	~~0.02341~~ 0.039372	mg/person	Total systemic exposure	~~0.026363~~ 0.032048	mg/person
Total systemic exposure	~~0.00039~~ 0.000656	mg/kg bw/d	Total systemic exposure	~~0.001632~~ 0.001984	mg/kg bw/d
% of AOEL	~~0.39~~0.66	%	% of AOEL	~~1.63~~1.98	%

Table A 11: Input parameters considered for the estimation of resident exposure ('worst case' if applications on lawn, pasture and meadow are intended)

Intended uses:	**Pastures, lawn, meadow**		**Deposit (D):**	**100**	
Application rate (AR):	2.16	kg a.s./ha	Transfer coefficient (TC):	7300	cm^2/h (adults)
	0.0216	mg/cm^2		2600	cm^2/h (children)
Number of applications (NA):	2		Turf Transferable Residues (TTR):	5	%
Body weight (BW):	60	kg/person (adults)	Exposure Duration (H):	2	h
	16.15	kg/person (children)	Airborne Concentration of Vapour (ACV):	0.001	mg/m^3
Dermal absorption (DA):	~~0.3~~1.0	% ('worst case')	Inhalation Rate (IR):	16.57	m^3/d (adults)
Inhalation absorption (IA):	100	%		8.31	m^3/d (children)
Oral absorption (OA):	20	%	Saliva Extraction Factor (SE):	50	%
AOEL:	0.1	mg/kg bw/d	Surface Area of Hands (SA):	20	cm^2
			Frequency of Hand to Mouth (Freq):	20	events/h
			Dislodgeable foliar residues (DFR):	20	%
			Ingestion Rate for Mouthing of Grass/Day (IgR):	25	cm^2/d

Table A 12: Estimation of resident exposure towards glyphosate ('worst case' if applications on lawn, pasture and meadow are intended)

Adults			**Children**		
Residents: Systemic dermal exposure after application on pastures, lawn, meadow (via deposits caused by spray drift)					
SDE_R = (AR x NA x D x TTR x TC x H x DA) / BW			SDE_R = (AR x NA x D x TTR x TC x H x DA) / BW		
(0.0216 x 2 x 100% x 5% x 7300 x 2 x ~~0.3~~1.0%) / 60			(0.0216 x 2 x 100% x 5% x 2600 x 2 x ~~0.3~~1.0%) / 16.15		
External dermal exposure	31.536	mg/person	External dermal exposure	11.232	mg/person
External dermal exposure	0.52560	mg/kg bw/d	External dermal exposure	0.69548	mg/kg bw/d
Systemic dermal exposure	~~0.001577~~ 0.0052560	mg/kg bw/d	Systemic dermal exposure	~~0.002086~~ 0.0069548	mg/kg bw/d
Residents: Systemic inhalation exposure after application on pastures, lawn, meadow (via vapour)					
SIE_R = (AC_V x IR x IA) / BW			SIE_R = (AC_V x IR x IA) / BW		
(0.001 x 16.57 x 100%) / 60			(0.001 x 8.31 x 100%) / 16.15		
External inhalation exposure	0.01657	mg/person	External inhalation exposure	0.00831	mg/person
External inhalation exposure	0.000276	mg/kg bw/d	External inhalation exposure	0.000515	mg/kg bw/d
Systemic inhalation exposure	0.000276	mg/kg bw/d	Systemic inhalation exposure	0.000515	mg/kg bw/d
			Residents: Systemic oral exposure (hand-to-mouth transfer)		
			$SOE_{R(H)}$ = (AR x NA x D x TTR x SE x SA x Freq x H x OA) / BW		
			(0.0216 x 2 x % x 5% x 50% x 20 x 20 x 2 x 20%) / 16.15		
			External oral exposure	0.864	mg/person
			External oral exposure	0.053498	mg/kg bw/d
			Systemic oral exposure	0.0107	mg/kg bw/d
			Residents: Systemic oral exposure (object-to-mouth transfer)		
			$SOE_{R(O)}$ = (AR x NA x D x DFR x IgR x OA) / BW		
			(0.0216 x 2 x % x 20% x 25 x 20%) / 16.15		
			External oral exposure	0.216	mg/person
			External oral exposure	0.013375	mg/kg bw/d
			Systemic oral exposure	0.002675	mg/kg bw/d
Total systemic exposure: SE_R = SDE_R + SIE_R			Total systemic exposure: SE_R = SDE_R + SIE_R + $SOE_{R(H)}$ + $SOE_{R(O)}$		
Total systemic exposure	~~0.111178~~ 0.331932	mg/person	Total systemic exposure	~~0.258006~~ 0.336631	mg/person
Total systemic exposure	~~0.001853~~ 0.005532	mg/kg bw/d	Total systemic exposure	~~0.015976~~ 0.020844	mg/kg bw/d
% of AOEL	~~1.85~~5.53	%	% of AOEL	~~15.98~~ 20.84	%

Worker exposure calculations (IIIA 7.5)

Table A 13: Input parameters considered for the estimation of worker exposure

Intended use(s):	**All high crops intended**		**Dislodgeable foliar residues (DFR):**	**1**	**µg/cm²/kg a.s.**
Application rate (AR):	4.32 [1)]	kg a.s./ha	Transfer coefficient (TC):	5000	cm^2/person/h
Number of applications (NA):			Work rate per day (WR):	8	h/d
Body weight (BW):	60	kg/person	PPE	5	%
Dermal absorption (DA):	~~0.3~~1.0	% ('worst case')			
AOEL	0.1	mg/kg bw/d			

[1)] Maximum dose per season, therefore no number of applications considered; no degradation between splitted dose applications taken into account

Table A 14: Estimation of worker exposure towards glyphosate using the German re-entry model

Without PPE [1)]			**With PPE [2)]**		
Worker (re-entry): Systemic dermal exposure after application under high crops					
SDE_W = (DFR x TC x WR x AR x DA) / BW			SDE_W = (DFR x TC x WR x AR x PPE x DA) / BW		
(1 x 5000 x 8 x 4.32 x x ~~0.3~~1.0 %) / 60			(1 x 5000 x 8 x 4.32 x x 5 % x ~~0.3~~1.0 %) / 60		
External dermal exposure	172.8	mg/person	External dermal exposure	8.64	mg/person
External dermal exposure	2.88	mg/kg bw/d	External dermal exposure	0.144	mg/kg bw/d
Total systemic exposure	~~0.5184~~ 1.7280	mg/person	Total systemic exposure	~~0.02592~~ 0.0864	mg/person
Total systemic exposure	~~0.00864~~ 0.02880	mg/kg bw/d	Total systemic exposure	~~0.000432~~ 0.00144	mg/kg bw/d
% of AOEL	~~8.6~~28.8	%	% of AOEL	~~0.4~~1.4	%

[1)] acceptable without PPE: Treated areas/crops may not be entered until the spray coating has dried
[2)] acceptable only with PPE: see 'instructions for use'

B.6.15 References relied on

Studies marked in yellow are not part of the dossier for renewal.

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[5]
KIIA 5 KIIIA1 7 (OECD)	EFSA	2009	Reasoned opinion: Modification of the residue definition of Glyphosate in genetically modified maize grain and soybeans, and in products of animal origin EFSA Journal 2009; 7(9):1310 ! EFSA-Q-2009-00372 ASB2012-3480	N	---
KIIA 5 KIIIA1 7 (OECD)	EFSA	2012	Final review of the Séralini et al. (2012a) publication on a 2-year rodent feeding study with glyphosate formulations and GM maize NK603 as published online on 19 September 2012 in Food and Chemical Toxicology *EFSA Journal 2012;10(11):2986* ASB2012-15513	N	---
KIIA 5 KIIIA1 7 (OECD)	European Commission	2002	Review report for the active substance glyphosate. Finalised in the Standing Committee on Plant Health at its meeting on 29 June 2001in view of the inclusion of glyphosate in Annex I of Directive 91/414/EEC. Glyphosat 6511/VI/99-final ASB2009-4191	N	---
KIIA 5 KIIIA1 7 (OECD)	Germany	1998	Glyphosate (Monograph) ASB2010-10302	N	---
KIIA 5 KIIIA1 7 (OECD)	Germany	1998	Glyphosate-trimesium (Monograph), ASB2010-10493	N	---
KIIA 5 KIIIA1 7 (OECD)	Germany	2000	Glyphosate (Monograph): Addendum B.6, ASB2013-2748	N	---
KIIA 5 KIIIA1 7 (OECD)	OECD	2002	OECD; Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies ENV/JM/MONO(2002)19 ASB2013-3754	N	---

[5] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[5]
KIIA 5 KIIIA1 7 (OECD)	Anonymous	2006	Backgrounder Response to "Glyphosate Toxic & Roundup Worse". Monsanto statement. http://www.monsanto.com/products/Documents/glyphosate-background-materials/Response_ISIS_apr_06.pdf ASB2013-5455	N	---
KIIA 5.1 K IIA 5.6 (OECD	Antoniou M, Habib MEEM, Howard CV, Jennings RC, Leifert C, No-dari RO, C Robinson, Fa-gan J.	2011	Roundup and birth defects: Is the public being kept in the dark? Earth Open Source report. Available from: http://www.earthopensource.org/files/pdfs/Roundup-and-birth-defects/RoundupandBirthDefectsv5.pdf ASB2011-7202	N	---
KIIA 5.1 KIIA 5.10 (OECD)	Carr, K.H., Bleeke, M.S.	2012	Process Description for Identification, Review, and Categorization of Scientific Literature Concerning Glyphosate and AMPA Side-Effects on Health, the Environment, and Non-Target Species k.A. GLP: N, published: Y 2309656 / ASB2012-11583	N	LIT
KIIA 5.1 KIIA 5.10 (OECD)	Klimisch, H.J., Andreae, M., Tillmann, U.	1997	A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data Regulatory Toxicology and Pharmacology 25, 1-5 GLP: N, published: Y 2309856 / ASB2010-14388	N	LIT
KIIA 5.1.1 KIIA 5.5.3 KIIA 5.9 KIIA 5.10 KIIIA1 7.6.4 (OECD)	Acquavella, J.F., Alexander, B.H., Mandel, J.S., Gustin, C., Baker, B., Chapman, P., Bleeke, M.	2004	Glyphosate biomonitoring for farmers and their families: Results from the farm family exposure study Environmental Health Perspectives 112, 321-326 GLP: N, published: Y 2309536 / ASB2012-11528	N	LIT
KIIA 5.1.1 KIIA 5.4.4 KIIA 5.7.4 KIIA 5.10 (OECD)	Anadon, A., Martinez-Larranaga, M.R., Martinez, M.A., Castellano, V.J., Martinez, M., Martin, M.T., Nozal, M.J., Bernal, J.L.	2009	Toxicokinetics of glyphosate and its metabolite aminomethyl phosphonic acid in rats Toxicol Lett 190, 91-95 GLP: N, published: Y 2309568 / ASB2012-11542	N	LIT

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KIIA 5.1.1 (OECD)	[redacted]	1995	Glyphosate: ADME-study in rats - Final report A&M 038/94, TOX9552251	N	---
KIIA 5.1.1 KIIA 5.4.4 (OECD)	[redacted]	1991	Metabolism of glyphosate in Sprague-Dawley rats: Tissue distribution, identification, and quantitation of glyphosate-derived materials following a single oral dose, Fundamental and Applied Toxicology 17(1991): 43-51 TOX9551791	N	---
KIIA 5.1.1 KIIA 5.3.2 KIIA 5.4 KIIA 5.5 KIIA 5.10 (OECD)	[redacted]	1992	NTP technical report on toxicity studies of Glyphosate administered in dosed feed to F344/N rats and B6C3F1 mice, National Institutes of Health 16(1992) 1-57 TOX9551954	N	---
KIIA 5.1.1 (OECD)	[redacted]	1973	Final report on CP 67573 residue and metabolism. Part 9: The gross distribution of n-phosphonomethylglycine-^{14}C in the rabbit TOX9552353	N	---
KIIA 5.1.1 (OECD)	[redacted]	1973	CP 67573 residue and metabolism. Part 13: The dynamics of accumulation and depletion of orally ingested N-phosphonomethylglycine-^{14}C TOX9552355	N	---
KIIA 5.1.1 (OECD)	[redacted]	1996	Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat CTL/4940 SYN GLP: Y, published: N 2309074 / TOX2000-1977	N	SYN
KIIA 5.1.1 (OECD)	[redacted]	1996	Glyphosate acid: Excretion and tissue retention of a single oral dose (1000 mg/kg) in the rat CTL/4942 SYN GLP: Y, published: N 2309076 / TOX2000-1978	N	SYN
KIIA 5.1.1 KIIA 5.1.3 (OECD)	[redacted]	1996	Glyphosate acid: Excretion and Tissue Retention of a Single Oral Dose (10 mg/kg) in the Rat Following Repeat Dosing CTL/P/4944 SYN GLP: Y, published: N 2309078 / TOX2000-1979	N	SYN
KIIA 5.1.1 KIIA 5.1.3 (OECD)	[redacted]	1996	Glyphosate acid: Whole body autoradiography in the rat (10 mg/kg) CTL/P/4943 SYN GLP: Y, published: N 2309080 / TOX2000-1980	N	SYN

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[5]
KIIA 5.1.1 KIIA 5.9 KIIA 5.10 (OECD)	Hoppe, H.-W.	2013	Glyphosate and AMPA: Determination of glyphosate residues in human urine samples from 18 European countries Medical Laboratory Bremen, MLHB-2013-06-06 ASB2013-8037	N	---
KIIA 5.1.1 (OECD)	[redacted]	1988	The metabolism of glyphosate in Sprague/Dawley rats. Part II. Identification, characterization, and quantitation of glyphosate and its metabolites after intravenous and oral administration, MSL-7206 ! 206300, TOX9552357	N	---
KIIA 5.1.1 (OECD)	[redacted]	1996	[^{14}C]-glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat 1413/2-1011 NUF GLP: Y, published: N 2309072 / ASB2012-11380	Y	NUF
KIIA 5.1.1 (OECD)	[redacted]	1995	Metabolism study of ^{14}C-labelled glyphosate after single oral and intravenous administration to Sprague-Dawley rats, 9202/95 TOX9650071	N	---
KIIA 5.1.1 KIIA 5.1.3 (OECD)	[redacted]	1996	Glyphosate acid: Biotransformation in the rat CTL/P/5058 SYN GLP: Y, published: N 2309082 / TOX2000-1981	N	SYN
KIIA 5.1.1 KIIA 5.10 (OECD)	Mage, D.T.	2006	Suggested corrections to the Farm Family Exposure Study Environmental Health Perspectives 114, A633-A634 GLP: N, published: Y 2309900 / ASB2012-11888	N	LIT
KIIA 5.1.1 (OECD)	[redacted]	1995	HR-001: Metabolism in the rat SNY 332/951256 HLS GLP: Y, published: N 2309070 / ASB2012-11379	N	ALS
KIIA 5.1.1 (OECD)	[redacted]	1992	(^{14}C)-glyphosate: Absorption and distribution in the rat - preliminary study TOX9552358	N	---
KIIA 5.1.1 (OECD)	[redacted]	1992	(^{14}C)-glyphosate: Absorption, distribution, metabolism and excretion in the rat, TOX9300343	N	---

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KIIA 5.1.1 (OECD)	[redacted]	1988	The metabolism of glyphosate in Sprague/Dawley rats. I. Excretion and tissue distribution of Glyphosate and its metabolites following intravenous and oral administration MSL-7215 ! EHL 86139 ! ML-86-438 TOX9552356	N	---
KIIA 5.2.1 (OECD)	[redacted]	2007	Glyphosate technical material: Acute oral toxicity study in rats (Up and Down procedure) B02755; T007035-05 SYN GLP: Y, published: N 2309111 / ASB2012-11391	Y	SYN
KIIA 5.2.1 (OECD)	[redacted]	1981	Acute oral toxicity of MON 0139 to rats 800257 ! ML-80-261 TOX9552321	N	---
KIIA 5.2.1 (OECD)	[redacted]	1990	Acute oral toxicity in the rat: Glyphosate technical R231 ! AGC-900823B ! AGC-101 TOX9500261	N	---
KIIA 5.2.1 (OECD)	[redacted]	1995	Glyphosate technical 95 %: Acute oral toxicity (LD_{50}) test in rat 10670 ! IRI 556073 TOX9500377	N	---
KIIA 5.2.1 (OECD)	[redacted]	1989	Glyphosate technical: Acute oral toxicity (limit) test in rats 5883 ! IRI 243268 TOX9552319	N	---
KIIA 5.2.1 (OECD)	[redacted]	1991	Assessment of acute oral toxicity of "Glyphosate technical" to mice - incl. Addendum 12321 TOX9552320	N	---
KIIA 5.2.1 (OECD)	[redacted]	2008	Acute Oral Toxicity Study in Wistar Hannover Rats for Glyphosate Technical RF -3996.305.475.07 HAG GLP: Y, published: N 2309100 / ASB2012-11389	Y	HAG
KIIA 5.2.1 (OECD)	[redacted]	1996	Glyphosate Acid: Acute Oral Toxicity Study in Rats CTL/P/4660 SYN GLP: Y, published: N 2309109 / TOX2000-1982	N	SYN
KIIA 5.2.1 (OECD)	[redacted]	1994	Glyphosate premix: Acute oral toxicity (limit test) in the rat 545/37 TOX9552322	N	---

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KIIA 5.2.1 (OECD)	[redacted].	1995	Acute Toxicity Study of MON 0139 By Oral Administration in Mice XX-95-205 MON GLP: Y, published: N 2309115 / ASB2012-11393	Y	MON
KIIA 5.2.1 (OECD)	[redacted]	2009	Acute Oral Toxicity Study of Glyphosate TC in Rats 23910 HAG GLP: Y, published: N 2309092 / ASB2012-11385	Y	HAG
KIIA 5.2.1 (OECD)	[redacted]	2010	Acute Oral Toxicity Study of Glyphosate TC in Rats 24874 HAG GLP: Y, published: N 2309094 / ASB2012-11386	Y	HAG
KIIA 5.2.1 (OECD)	[redacted]	2010	Acute Oral Toxicity Study of Glyphosate TC in Rats 24602 HAG GLP: Y, published: N 2309096 / ASB2012-11387	Y	HAG
KIIA 5.2.1 (OECD)	[redacted]	1979	Acute Oral Toxicity Study in Rats. BD-77-428 MON GLP: N, published: N 2309107 / Z35541	N	MON
KIIA 5.2.1 (OECD)	[redacted]	1995	HR-001: Acute Oral Toxicity Study In Rats IET 94-0134 ALS GLP: Y, published: N 2309086 / ASB2012-11382	Y	ALS
KIIA 5.2.1 (OECD)	[redacted]	1995	HR-001: Acute Oral Toxicity Study In Mice IET 94-0133 ALS GLP: Y, published: N 2309088 / ASB2012-11383	Y	ALS
KIIA 5.2.1 (OECD)	[redacted]	2005	Glyphosate Acid Technical - Acute Oral Toxicity Up and Down Procedure in Rats PSL 15274 HAG GLP: Y, published: N 2309098 / ASB2012-11388	Y	HAG
KIIA 5.2.1 (OECD)	[redacted]	1999	NUP5a99 62 % glyphosate MUP: Acute oral toxicity study in rats - Limit test 7907 NUF GLP: Y, published: N 2309117 / ASB2012-11394	Y	NUF

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[5]
KIIA 5.2.1 (OECD)	[redacted]	2014	Glyphosate: Acute oral toxicity in the rat - fixed dose method Report No.: 41401853, Harlan Laboratories Ltd., Derbyshire, DE72 2GD, UK Date: 2014-00-01, not published ASB2014-9147	Y	
KIIA 5.2.1 (OECD)	[redacted]	1987	Acute oral LD_{50} study of MON-8750 in Sprague-Dawley rats FDRL 9308A TOX9552323	N	---
KIIA 5.2.1 (OECD)	[redacted]	1987	Acute oral toxicity of MON 8750 in Sprague-Dawley rats FD-86-431/9308A Z85869	N	---
KIIA 5.2.1 (OECD)	[redacted]	1988	Acute Oral Toxicity Study of Glyphosate Batch/lot/nbr no. XLI-55 in Sprague/Dawley rats FD-88-29 (FDRL 88.20 MON 88.2053.007) GLP: Y, published: N 2309105 / Z35389	N	MON
KIIA 5.2.1 (OECD)	[redacted]	1995	Final report for oral and dermal LD_{50} tests with Sanachem glyphosate acid technical in rats, limit test 00917 TOX9650909	N	---
KIIA 5.2.1 KIIA 5.2.2 (OECD)	[redacted]	1995	Final report for oral and dermal LD_{50} tests with Sanachem glyphosate 62 % IPA in rats, limit test 00926 TOX9650910	N	---
KIIA 5.2.1 (OECD)	[redacted]	2009	Glyphosate Technical: Acute oral Toxicity Study in Rat, C22864, C22864 EXC GLP: Y, published: N 2309090 / ASB2012-11384	Y	EXC
KIIA 5.2.1 (OECD)	[redacted]	1994	Glyphosate: Acute oral toxicity (limit test) in the rat 710/14 TOX9500245	N	---
KIIA 5.2.1 (OECD)	[redacted]	1991	Acute oral toxicity study with glyphosate technical (FSG 03090 H/05 march 90) in Wistar rats ES.874.AOR ! ES-GPT-AOR ! TOXI-874/1990 TOX9551088	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[5]
KIIA 5.2.1 (OECD)	[redacted]	1991	Acute oral toxicity study with glyphosate technical (FSG 03090 H/05 march 90) in swiss albino mice ES.875.AOM ! ES-GPT-AOM ! TOXI-875/1990 TOX9551089	N	---
KIIA 5.2.1 (OECD)	[redacted]	2007	GLYPHOSATE TECHNICAL (NUP05068) : Acute oral toxicity study in rats BO2272 NUF GLP: Y, published: N 2309103 / ASB2012-11390	Y	NUF
KIIA 5.2.1 (OECD)	[redacted]	2011	Glyphosate technical - Acute Oral Toxicity Study in the Rat (Up and Down Procedure) 10/218-001P SYN GLP: Y, published: N 2309113 / ASB2012-11392	Y	SYN
KIIA 5.2.1 (OECD)	[redacted]	1994	Glyphosate (Alkaloida, Tiszavasvari): Acute oral toxicity in rats GHA-94-401/R TOX9650142	N	---
KIIA 5.2.1 (OECD)	[redacted]	1994	Glyphosate technical: Acute oral toxicity study in mice 940020 ! PRO629 TOX9551624	N	---
KIIA 5.2.1 (OECD)	[redacted]	1989	Acute oral toxicity study with glyphosate technical (isopropylamine salt 62 % in water equivalent to 46 % of N-phosphonomethylglycine acid) in rats 238050 ! PRO439 TOX9551623	N	---
KIIA 5.2.1 (OECD)	[redacted]	1992	Glyphosate technical: Acute oral toxicity (limit test) in the rat 134/37 TOX9551810	N	---
KIIA 5.2.1 (OECD)	[redacted]	1987	Acute oral toxicity of 41 % SN750721 solution in mice - Test report entrusted by Shinung Corporation TX58AO2 TOX9500376	N	---
KIIA 5.2.1 (OECD)	[redacted]	1987	Acute oral toxicity of 64 % SN750721 technical liquid in mice Test report entrusted by Shinung Corporation TX58AO1 TOX9500375	N	---

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KIIA 5.2.1 (OECD)	[redacted]	2009	Glyphosate: Acute Oral Toxicity Study (UDP) In Rats 12170-08 HEL GLP: Y, published: N 2309084 / ASB2012-11381	Y	HAG
KIIA 5.2.2 (OECD)	[redacted]	2007	Glyphosate technical material: Acute dermal toxicity study in rats B02766 (T007036-05) SYN GLP: Y, published: N 2309141 / ASB2012-11404	Y	SYN
KIIA 5.2.2 (OECD)	[redacted]	1981	Acute dermal toxicity of MON 0139 to rabbits 800258 ! ML-80-261 TOX9552326	N	---
KIIA 5.2.2 (OECD)	[redacted]	1990	Acute dermal toxicity study in the rat: Glyphosate technical AGC-900823A ! AGC-301 ! R232 TOX9551793	N	---
KIIA 5.2.2 (OECD)	[redacted]	1987	Acute dermal toxicity study of Mon 8750 in New Zealand white rabbits FDRL 9308A ! FD-86-431 TOX9552327	N	---
KIIA 5.2.2 (OECD)	[redacted]	1987	Acute dermal toxicity study of Mon 8722 in New Zealand white rabbits FDRL 9307A ! FD-86-430 TOX9552328	N	---
KIIA 5.2.2 (OECD)	[redacted]	1989	Glyphosate Technical Acute Dermal Toxicity (Limit) Test in Rats 5884 CHE GLP: Y, published: N 2309119 / TOX9300328	N	CHE
KIIA 5.2.2 (OECD)	[redacted]	2008	Acute Dermal Toxicity in Wistar Hannover Rats for Glyphosate Technical RF-3996.310.456.07 HAG GLP: Y, published: N 2309135 / ASB2012-11402	Y	HAG
KIIA 5.2.2 (OECD)	[redacted]	1996	Glyphosate Acid: Acute Dermal Toxicity in the Rat CTL/P/4664 SYN GLP: Y, published: N 2309139 / TOX2000-1983	Y	SYN
KIIA 5.2.2 (OECD)	[redacted]	2009	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats LPT 23912 HAG GLP: Y, published: N 2309127 / ASB2012-11398	Y	HAG

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[5]
KIIA 5.2.2 (OECD)	[redacted]	2010	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats LPT 24876 HAG GLP: Y, published: N 2309129 / ASB2012-11399	Y	HAG
KIIA 5.2.2 (OECD)	[redacted]	2010	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats LPT 24604 HAG GLP: Y, published: N 2309131 / ASB2012-11400	Y	HAG
KIIA 5.2.2 (OECD)	[redacted]	1995	HR-001: Acute dermal toxicity study in rats IET 94-0154 ALS GLP: Y, published: N 2309123 / ASB2012-11396	N	ALS
KIIA 5.2.2 (OECD)	[redacted]	2005	Glyphosate Acid Technical: Acute Dermal Toxicity Study in Rats - Limit Test PSL 15275 HAG GLP: Y, published: N 2309133 / ASB2012-11401	Y	HAG
KIIA 5.2.2 (OECD)	[redacted]	1994	Acute dermal toxicity of glyphosate technical in the rat T1586.3.A TOX9500378	N	---
KIIA 5.2.2 (OECD)	[redacted]	1988	Acute dermal toxicity of glyphosate Batch/lot/nbr no. XLI-55 in new zealand white rabbits 88.2053.008 ! FD-88-29 TOX9552325	N	---
KIIA 5.2.2 (OECD)	[redacted]	2009	Glyphosate Technical: Acute Dermal Toxicity Study in Rat C22875 EXC GLP: Y, published: N 2309125 / ASB2012-11397	Y	EXC
KIIA 5.2.2 (OECD)	[redacted]	1994	Glyphosate: Acute dermal toxicity (limit test) in the rat 710/15 TOX9500246	N	---
KIIA 5.2.2 (OECD)	[redacted]	1991	Acute dermal toxicity study with glyphosate technical (FSG 03090 H/05 march 90) in Wistar rats ES.876.ADR ! ES-GPT-ARD ! TOXI-876/1990 TOX9551090	N	---

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KIIA 5.2.2 (OECD)	[redacted]	2007	GLYPHOSATE TECHNICAL (NUP05068): Acute dermal toxicity study in rats B02283 NUF GLP: Y, published: N 2309137 / ASB2012-11403	Y	NUF
KIIA 5.2.2 (OECD)	[redacted]	1994	Glyphosate (Alkaloida, Tiszavasvari): Acute dermal toxicity in rats GHA-94-402/R TOX9650143	N	---
KIIA 5.2.2 (OECD)	[redacted]	1989	Acute dermal toxicity study with glyphosate technical (isopropylamine salt 62 % in water equivalent to 46 % of N-phosphonomethylglycine acid) in rats 238061 ! PRO425 TOX9551625	N	---
KIIA 5.2.2 (OECD)	[redacted]	1992	Glyphosate technical: Acute dermal toxicity (limit test) in the rat 134/38 TOX9551813	N	---
KIIA 5.2.2 (OECD)	[redacted]	2009	Glyphosate - Acute Dermal Toxicity Study in Rats 12171-08 HAG GLP: Y, published: N 2309121 / ASB2012-11395	Y	HAG
KIIA 5.2.2 (OECD)	[redacted]	2011	Glyphosate Technical - Acute Dermal Toxicity Study in Rats - Final Report Amendmend 1 10/218-002P SYN GLP: Y, published: N 2309143 / ASB2012-11405	Y	SYN
KIIA 5.2.3 (OECD)	[redacted]	1988	Acute inhalation study of MON 8750 technical EHL 87147 ! ML-87-228 TOX9552332	N	---
KIIA 5.2.3 (OECD)	[redacted]	1994	Glyphosate premix: Acute inhalation toxicity study four-hour exposure (nose only) in the rat 523-001 ! 545/39 TOX9552331	N	---
KIIA 5.2.3 (OECD)	[redacted]	1995	Glyphosate: Acute inhalation toxicity study four-hour exposure (nose only) in the rat 710/16 TOX9500247	N	---
KIIA 5.2.3 (OECD)	[redacted]	2004	An acute nose-only inhalation toxicity study in rats with MON 78623 SB-2003-116 MON GLP: Y, published: N 2309169 / ASB2012-11417	Y	MON

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KIIA 5.2.3 (OECD)	[redacted]	2009	Glyphosate - Acute Inhalation Toxicity Study in Rats 12107-08 HAG GLP: Y, published: N 2309155 / ASB2012-11411	Y	HAG
KIIA 5.2.3 (OECD)	[redacted]	2007	Glyphosate technical (NUP05068) : 4-Hour acute inhalation toxicity study in rats B02327 NUF GLP: Y, published: N 2309161 / ASB2012-11414	Y	NUF
KIIA 5.2.3 (OECD)	[redacted]	1987	Acute toxicity of Rodeo herbicide adminis-tered by inhalation to male and female Spra-gue-Dawley rats EHL 86105 ! ML-86-281 ! MSL 6582 TOX9552330	N	---
KIIA 5.2.3 (OECD)	[redacted]	2009	Glyphosate Tech: Acute Inhalation Toxicity (Nose only) Study in the Rat 2743/0001 EXC GLP: Y, published: N 2309149 / ASB2012-11408	Y	EXC
KIIA 5.2.3 (OECD)	[redacted]	2009	Acute Inhalation Toxicity Study of Glyphosate TC in Rats LPT 23911 HAG GLP: Y, published: N 2309151 / ASB2012-11409	Y	HAG
KIIA 5.2.3 (OECD)	[redacted]	2010	Acute Inhalation Toxicity Study of Glyphosate TC In Rats 24603 HEL GLP: Y, published: N 2309145 / ASB2012-11406	Y	HAG
KIIA 5.2.3 (OECD)	[redacted]	2010	Acute Inhalation Toxicity Study of Glyphosate TC in Rats LPT 24875 HAG GLP: Y, published: N 2309153 / ASB2012-11410	Y	HAG
KIIA 5.2.3 (OECD)	[redacted]	1995	HR-001: Acute inhalation toxicity study in rats IET 94-0155 ALS GLP: Y, published: N 2309147 / ASB2012-11407	Y	ALS
KIIA 5.2.3 (OECD)	[redacted]	1989	Glyphosate technical: Acute inhalation toxicity study in rats (limit test) 5993 ! IRI 642062 TOX9552329	N	---

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KIIA 5.2.3 (OECD)	[redacted]	2005	Glyphosate Acid Technical: Acute Inhalation Toxicity Study in Rats - Limit Test PSL 15276 HAG GLP: Y, published: N 2309157 / ASB2012-11412	Y	HAG
KIIA 5.2.3 (OECD)	[redacted]	2011	Glyphosate Technical - Acute inhalation Toxicity Study (Nose-only) in the Rat 11/054-004P SYN GLP: Y, published: N 2309165 / ASB2012-11415	Y	SYN
KIIA 5.2.3 (OECD)	[redacted]	1996	Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study in the Rat CTL/P/4882 SYN GLP: Y, published: N 2309163 / TOX2000-1984	Y	SYN
KIIA 5.2.3 (OECD)	[redacted]	1989	4-hour, acute inhalation toxicity study with glyphosate technical in rats 238105 ! PRO426 TOX9551626	N	---
KIIA 5.2.3 (OECD)	[redacted]	1994	Glyphosate (Alkaloida, Tiszavasvari): Acute inhalation toxicity in rats GHA-94-403/R TOX9650144	N	---
KIIA 5.2.3 (OECD)	[redacted]	1999	NUP5a99 62 % glyphosate MUP: Acute inhalation toxicity study in rats - Limit test 7909 NUF GLP: Y, published: N 2309167 / ASB2012-11416	Y	NUF
KIIA 5.2.4 (OECD)	[redacted]	2007	Glyphosate technical material: Primary skin irritation study in rabbits (4-hour semi-occlusive application) B02777 (T007037-05) SYN GLP: Y, published: N 2309193 / ASB2012-11426	Y	SYN
KIIA 5.2.4 (OECD)	[redacted]	1990	Acute dermal irritation/corrosion of glyphosate technical in the rabbit (intact and abraded skin) AGC-900822A ! AGC-001 ! R233 TOX9551794	N	---
KIIA 5.2.4 (OECD)	[redacted]	1987	Primary dermal irritation study of Mon-8750 in New Zealand white rabbits FDRL 9308A ! FD-86-431 TOX9552336	N	---

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KIIA 5.2.4 (OECD)	[redacted]	2008	Acute Dermal Irritation/Corrosion Study in Rabbits with Glyphosate Technical RF-3996.311.476.07 HAG GLP: Y, published: N 2309185 / ASB2012-11425	Y	HAG
KIIA 5.2.4 (OECD)	[redacted]	1989	Glyphosate technical: Primary skin irritation test in rabbits 5885 ! IRI 243268 TOX9552333	N	---
KIIA 5.2.4 (OECD)	[redacted]	1996	Glyphosate Acid: Skin Irritation To The Rabbit CTL/P/4695 SYN GLP: Y, published: N 2309191 / TOX2000-1985	Y	SYN
KIIA 5.2.4 (OECD)	[redacted]	1994	Glyphosate premix: Acute dermal irritation test in the rabbit 565-003 ! 545/40 TOX9552335	N	---
KIIA 5.2.4 (OECD)	[redacted]	1995	HR-001: Primary Dermal irritation study in rabbits IET 95-0035 ALS GLP: Y, published: N 2309175 / ASB2012-11420	Y	ALS
KIIA 5.2.4 (OECD)	[redacted]	2009	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC In Rabbits 24877 HEL GLP: Y, published: N 2309173 / ASB2012-11419	Y	HAG
KIIA 5.2.4 (OECD)	[redacted]	2009	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits LPT 23913 HAG GLP: Y, published: N 2309177 / ASB2012-11421	Y	HAG
KIIA 5.2.4 (OECD)	[redacted]	2010	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits LPT 24605 HAG GLP: Y, published: N 2309179 / ASB2012-11422	Y	HAG
KIIA 5.2.4 (OECD)	[redacted]	2005	Glyphosate Acid Technical - Primary Skin Irritation Study in Rabbits PSL 15278 HAG GLP: Y, published: N 2309183 / ASB2012-11424	Y	HAG

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KIIA 5.2.4 (OECD)	[redacted]	1988	Primary Dermal Irritation Study of Glyphosate Batch/lot/nbr no. XLI-55 in New Zealand White Rabbits FD-88-29 (FDRL 88.20 MON GLP: Y, published: N 2309187 / Z35394	N	MON
KIIA 5.2.4 (OECD)	[redacted]	1994	Glyphosate 360g/L: Acute dermal irritation test in the rabbit 710/29 TOX9500248	N	---
KIIA 5.2.4 (OECD)	[redacted]	1991	Primary skin irritation study with glyphosate technical (FSG 03090 H/05 march 90) in New Zealand white rabbits ES.878.SKIN ! TOXI-878/1990 ! ES-GPT-SKIN TOX9551092	N	---
KIIA 5.2.4 (OECD)	[redacted]	2007	Glyphosate Technical (NUP 05068): Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application) B02294 NUF GLP: Y, published: N 2309171 / ASB2012-11418	Y	NUF
KIIA 5.2.4 (OECD)	[redacted]	1994	Glyphosate (Alkaloida, Tiszavasvari): Primary dermal irritation study in rabbits GHA-93-404/N TOX9650145	N	---
KIIA 5.2.4 (OECD)	[redacted]	1991	Acute dermal irritation study in New Zealand White rabbits treated with the test article glyphosate tecnico 98 % 910259 ! PRO495 TOX9551627	N	---
KIIA 5.2.4 (OECD)	[redacted]	1989	Primary skin irritation study with glyphosate technical (isopropylamine salt 62 % in water equivalent to 46 % of N-phosphonomethylglycine acid) in rabbits (4-hour semi-occlusive application on intact and abraded skin) 238072 ! PRO438 TOX9551628	N	---
KIIA 5.2.4 (OECD)	[redacted]	2009	Glyphosate - Acute Dermal Irritation Study in Rabbits 12173-08 HAG GLP: Y, published: N 2309181 / ASB2012-11423	Y	HAG

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KIIA 5.2.4 (OECD)	[redacted]	2011	Glyphosate technical - Primary skin irritation study in rabbits - Final report Amendment 1 10/218-006N SYN GLP: Y, published: N 2309195 / ASB2012-11427	Y	SYN
KIIA 5.2.5 (OECD)	[redacted]	2007	Glyphosate technical material: Primary eye irritation study in rabbits B02788 (T007038-05) SYN GLP: Y, published: N 2309219 / ASB2012-11437	Y	SYN
KIIA 5.2.5 (OECD)	[redacted]	1990	Acute eye irritation/corrosion of glyphosate technical in the rabbit AGC-900822 ! AGC-002 ! R234 TOX9500264	N	---
KIIA 5.2.5 (OECD)	[redacted]	1987	Primary eye irritation of Mon 8722 in New Zealand white rabbits FDRL 9307A ! FD-86-430 TOX9552342	N	---
KIIA 5.2.5 (OECD)	[redacted]	2008	Acute Eye Irritation/Corrosion Study in Rabbits with Glyphosate Technical RF-3996.312.599.07 HAG GLP: Y, published: N 2309213 / ASB2012-11436	Y	HAG
KIIA 5.2.5 (OECD)	[redacted]	1989	Glyphosate technical: Primary eye irritation test in rabbits 5886 ! IRI 243268 TOX9552338	N	---
KIIA 5.2.5 (OECD)	[redacted]	1994	Glyphosate premix: Acute eye irritation test in the rabbit 566-003 ! 545/41 TOX9552340	N	---
KIIA 5.2.5 (OECD)	[redacted]	1995	HR-001: Primary Eye Irritation study in rabbits IET 95-0034 ALS GLP: Y, published: N 2309201 / ASB2012-11430	Y	ALS
KIIA 5.2.5 (OECD)	[redacted]	1997	Glyphosate Acid: Eye Irritation to the Rabbit CTL/P/5138 SYN GLP: Y, published: N 2309217 / TOX2000-1986	Y	SYN
KIIA 5.2.5 (OECD)	[redacted]	1996	CHA 440: Primary eye irritation study in rab-bits 2981-96 ! S9-FF81-4.C41 TOX1999-881	N	---

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KIIA 5.2.5 (OECD)	[redacted]	2009	Acute Eye Irritation/Corrosion Test Of Glyphosate TC In Rabbits 24878 HEL GLP: Y, published: N 2309199 / ASB2012-11429	Y	HAG
KIIA 5.2.5 (OECD)	[redacted]	2009	Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits LPT 23914 HAG GLP: Y, published: N 2309205 / ASB2012-11432	Y	HAG
KIIA 5.2.5 (OECD)	[redacted]	2010	Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits LPT 24606 HAG GLP: Y, published: N 2309207 / ASB2012-11433	Y	HAG
KIIA 5.2.5 (OECD)	[redacted]	2005	Eye Irritation/Corrosion Effects in Rabbits (*Oryctolagus cuniculus*) of Glyphosate 95 TC PSL 15277 HAG GLP: Y, published: N 2309211 / ASB2012-11435	Y	HAG
KIIA 5.2.5 (OECD)	[redacted]	1988	Primary Eye Irritation Study of Glyphosate FD-88-29 MON GLP: N, published: N 2309215 / Z35395	N	MON
KIIA 5.2.5 (OECD)	[redacted]	2009	Expert Statement Expert Statement Expert Statement Glyphosate technical: Primary eye irritation study in rat C22897 EXC GLP: Y, published: N 2309203 / ASB2012-11431	Y	EXC
KIIA 5.2.5 (OECD)	[redacted]	1994	Glyphosate: Acute eye irritation test in the rabbit 710/18 TOX9500249	N	---
KIIA 5.2.5 (OECD)	[redacted]	1991	Primary eye irritation study with glyphosate technical (FSG 03090 H/05 march 90) in New Zealand white rabbits ES.879.EYE ! TOXI-879/1990 ! ES-GPT-EYE TOX9551093	N	---
KIIA 5.2.5 (OECD)	[redacted]	2007	Glyphosate Technical (NUP 05068): Primary Eye Irritation Study In Rabbits B02305 NUF GLP: Y, published: N 2309197 / ASB2012-11428	Y	NUF

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KIIA 5.2.5 (OECD)	[redacted]	2011	Glyphosate Technical - Acute Eye Irritation Study in Rabbits 10/218-005N NUF GLP: Y, published: N 2309221 / ASB2012-11438	Y	SYN
KIIA 5.2.5 (OECD)	[redacted]	1994	Glyphosate (Alkaloida, Tiszavasvari): Primary eye irritation study in rabbits GHA-93-405/N TOX9650146	N	---
KIIA 5.2.5 (OECD)	[redacted]	1991	Acute eye irritation study in New Zealand White rabbits treated with the test article glyphosate tecnico 98 % 910260 ! PRO496 Z101610	N	---
KIIA 5.2.5 (OECD)	[redacted]	1989	Primary eye irritation with glyphosate technical (isopropylamine salt 62 % in water equivalent to 46 % of N-phosphonomethylglycine acid) in the rabbit (rinsed / unrinsed eyes) 238083 ! PRO423 TOX9551629	N	---
KIIA 5.2.5 (OECD)	[redacted]	2009	Glyphosate - Acute Eye Irritation Study in Rabbits 12172-08 HAG GLP: Y, published: N 2309209 / ASB2012-11434	Y	HAG
KIIA 5.2.6 (OECD)	[redacted]	1983	A dermal sensitization study in guinea pigs with Glyphosate BD-83-008 ! B/d 4235-82 Z35238	N	---
KIIA 5.2.6 (OECD)	[redacted]	2007	Glyphosate Technical Material - Skin Sensitisation (Local Lymph Node Assay in the Mouse) GM8048-REG SYN GLP: Y, published: N 2309245 / ASB2012-11449	Y	SYN
KIIA 5.2.6 (OECD)	[redacted]	1989	Glyphosate technical: Magnusson-Kligman maximisation test in guinea pigs 5887 ! IRI 243268 TOX9552343	N	---
KIIA 5.2.6 (OECD)	[redacted]	1996	Glyphosate Acid: Skin Sensitisation to the Guinea Pig CTL/P/4699 SYN GLP: Y, published: N 2309243 / TOX2000-1987	Y	SYN

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KIIA 5.2.6 (OECD)	[redacted]	1994	Glyphosate premix: Magnusson & Kligman maximisation study in the guinea pig 567-003 ! 545/42 TOX9552345	N	---
KIIA 5.2.6 (OECD)	[redacted]	2009	Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) LPT 23915 HAG GLP: Y, published: N 2309231 / ASB2012-11443	Y	HAG
KIIA 5.2.6 (OECD)	[redacted]	2010	Examination Of Glyphosate TC In The Skin Sensitisation Test In Guinea Pigs According To Magnusson And Kligman (Maximisation Test) 24879 HEL GLP: Y, published: N 2309225 / ASB2012-11440	Y	HAG
KIIA 5.2.6 (OECD)	[redacted]	2010	Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) LPT 24607 HAG GLP: Y, published: N 2309233 / ASB2012-11444	Y	HAG
KIIA 5.2.6 (OECD)	[redacted]	1995	HR-001: Dermal sensitisation study in guinea pigs IET 95-0036 ALS GLP: Y, published: N 2309227 / ASB2012-11441	Y	ALS
KIIA 5.2.6 (OECD)	[redacted].	2008	Skin Sensitisation Test for Glyphosate Technical in Guinea Pigs. Buehler Test RF-3996.318.431.07 HAG GLP: Y, published: N 2309239 / ASB2012-11447	Y	HAG
KIIA 5.2.6 (OECD)	[redacted]	2005	Glyphosate acid technical - Dermal Sensitisation in Guinea Pigs (Buehler Method) PSL 15279 HAG GLP: Y, published: N 2309237 / ASB2012-11446	Y	HAG
KIIA 5.2.6 (OECD)	[redacted]	1993	Skin sensitisation test in guinea-pigs with glyphosate technical 95 % min of Excel Industries Ltd., Bombay. IIT 1230 TOX9650652	N	---

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KIIA 5.2.6 (OECD)	[redacted]	2006	Glyphosate Technical: Skin Sensitisation in the Guinea Pig - Magnusson and Kligman Maximisation method 2060/009 (SMK-PH-05- NUF GLP: Y, published: N 2309241 / ASB2012-11448	Y	NUF
KIIA 5.2.6 (OECD)	[redacted]	2009	Glyphosate Technical: Contact Hypersensitivity in albino guinea pigs - Maximisation-Test C22908 EXC GLP: Y, published: N 2309229 / ASB2012-11442	Y	EXC
KIIA 5.2.6 (OECD)	[redacted]	1994	Glyphosate: Magnusson & Kligman maximisation study in the guinea pig 710/19 TOX9500250	N	---
KIIA 5.2.6 (OECD)	[redacted]	2007	Glyphosate Technical (NUP 05068): Contact Hypersensitivity in Albino Guinea Pigs, Maximisation Test B02316 NUF GLP: Y, published: N 2309223 / ASB2012-11439	Y	NUF
KIIA 5.2.6 (OECD)	[redacted], [redacted]	2011	Glyphosate technical - Local lymph node assay in the mouse - Final report amendment 2 10/218-037E SYN GLP: Y, published: N 2309247 / ASB2012-11450	Y	SYN
KIIA 5.2.6 (OECD)	[redacted]	1991	Luxan glyphosate techn.: Magnusson & Kligman maximisation study in the guinea pig 349/11 TOX9551796	N	---
KIIA 5.2.6 (OECD)	[redacted]	2009	Glyphosate - Skin Sensitisation Study in Guinea Pigs. Buehler Test 12174-08 HAG GLP: Y, published: N 2309235 / ASB2012-11445	Y	HAG
KIIA 5.3.1 (OECD)	[redacted]	1989	Glyphosate: 4 week dietary toxicity study in rats 5626 ! IRI 437462 TOX9552351	N	---
KIIA 5.3.1 (OECD)	[redacted]O.	1989	Glyphosate: Oral maximum tolerated dose study in dogs 5660 ! IRI 640683 TOX9552352	N	---

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KIIA 5.3.1 KIIIA1 7.6.2 (OECD)	[redacted]	2012	Glyphosate acid - *In Vitro* absorption through abraded rabbit skin using [^{14}C]-glyphosate JV2182-REG GTF GLP: Y, published: N 2309282 / ASB2012-11459	Y	EGT
KIIA 5.3.1 (OECD)	[redacted]	1993	Glyphosate: 3 week toxicity study in rats with dermal administration 7839 ! IRI 450881 TOX9552367	N	---
KIIA 5.3.1 KIIA 5.3.7 KIIIA1 7.6.2 (OECD)	[redacted]	1982	21-Day dermal toxicity study in rabbits IR-81-195 MON GLP: N, published: N 2309280 / TOX9552366	N	MON
KIIA 5.3.1 (OECD)	[redacted]	1982	Range finding study of MON 0139 and iso-propylamine administered orally to dogs ML-81-032/810036 ! MSL-2155 TOX9552349	N	---
KIIA 5.3.1 (OECD)	[redacted]	1996	Glyphosate acid: 21-day dermal toxicity study in rats CTL/P/4985 SYN GLP: Y, published: N 2309288 / ASB2012-11461	Y	SYN
KIIA 5.3.1 (OECD)	[redacted]	1991	28-day dietary study in rats on glyphosate technical ES.881.28 DDR ! TOXI-881/1991 ! ES-GPT-28 DDR TOX9551095	N	---
KIIA 5.3.1 (OECD)	[redacted]	1994	28-day dietary study in rats on glyphosate technical –Amendment ES.881.28 DDR ! TOXI-881/1991 ! ES-GPT-28 DDR Z102035	N	---
KIIA 5.3.1 (OECD)	[redacted]	1994	28-day dietary study in rats on glyphosate technical - Second Amendment ES.881.28 DDR ! TOXI-881/1991 ! ES-GPT-28 DDR Z102043	N	---
KIIA 5.3.1 KIIA 5.3.7 (OECD)	[redacted]	1994	GGlyphosate technical (Alkaloida, Tiszavasvári): Repeated dose twenty-eight-Day dermal toxicity study in rabbits MÜF 214/94 MON GLP: Y, published: N 2309284 / TOX9650151	N	MON

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KIIA 5.3.1 KIIIA1 7.1.3 (OECD)	[redacted]	1983	Four-week study of 33-1/3 % use-dilution of Roundup in water administered to male and female Sprague-Dawley rats by inhalation 830025 ! ML-83-015 TOX2002-694	N	---
KIIA 5.3.2 (OECD)	[redacted]	1981	Glyphosate: Subchronic toxicological study 90-day rats TOX9650152	N	---
KIIA 5.3.2 (OECD)	[redacted]	1996	First Revision to Glyphosate Acid: 90 Day Oral Feeding Study in Rats CTL/P/1599 SYN GLP: Y, published: N 2309249 / TOX2000-1990	Y	SYN
KIIA 5.3.2 (OECD)	[redacted]	1990	Glyphosate technical: 90 day oral toxicity study in the rat AGC-900914 ! AGC-401 ! R230 TOX9500266	N	---
KIIA 5.3.2 (OECD)	[redacted]	1996	Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study In The Rat 434/016 NUF GLP: Y, published: N 2309256 / ASB2012-11451	Y	NUF
KIIA 5.3.2 (OECD)	[redacted]	1989	Glyphosate technical: 90 day oral toxicity study in the rats - incl. Amendment to Protocol BY-401 BY-891002 ! BY-401 TOX9551821	N	---
KIIA 5.3.2 (OECD)	[redacted]	1995	HR-001: 13-week Subchronic Oral Toxicity Study in Rats IET 94-0138 ALS GLP: Y, published: N 2309258 / ASB2012-11452	Y	ALS
KIIA 5.3.2 (OECD)	[redacted]	1995	HR-001: 13-week Oral Subchronic Toxicity Study in Mice IET 94-0136 ALS GLP: Y, published: N 2309260 / ASB2012-11453	N	ALS
KIIA 5.3.2 (OECD)	[redacted]	1993	90 day range finding study of glyphosate in rats 011-0001 ALK GLP: Y, published: N 2309252 / TOX9650149	N	ALK

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KIIA 5.3.2 (OECD)	[redacted]	1991	Glyphosate: 13-week dietary toxicity study in rats 7136 ! IRI 437876 TOX9552364	N	---
KIIA 5.3.2 (OECD)	[redacted]	1991	Glyphosate: 13-week dietary toxicity study in mice 7024 ! IRI 437918 TOX9552363	N	---
KIIA 5.3.2 (OECD)	[redacted] W.	1987	90-day study of glyphosate administered in feed to Sprague-Dawley rats MSL 7375 ! ML-86-351 ! EHL 86128 TOX9552362	N	---
KIIA 5.3.2 (OECD)	[redacted]	1992	Glyphosat techn. (FSG 03090 H/05 March 1990): 90 day oral toxicity study in wistar rats TOXI-882/1991 ! ES-GPT-90 OR ! ES-882 90 OR TOX9551096	N	---
KIIA 5.3.3 (OECD)	[redacted]	2007	Glyphosate Technical: 13-Week Toxicity Study By Oral Route (Capsule) In Beagle Dogs 29646 TCC NUF GLP: Y, published: N 2309262 / ASB2012-11454	Y	NUF
KIIA 5.3.3 (OECD)	[redacted]	1996	First Revision to Glyphosate Acid: 90-Day Oral Toxicity Study in Dogs CTL/P/1802 SYN GLP: Y, published: N 2309271 / TOX2000-1991	Y	SYN
KIIA 5.3.3 (OECD)	[redacted]	1999	Subchronic (90 Day) Oral Toxicity Study With Glyphosate Technical In Beagle Dogs AND Test compound stability in experimental diet (dog feed) 1816 / 1817-R.FST FSG GLP: Y, published: N 2309264 / ASB2012-11455	Y	ADM
KIIA 5.3.3 (OECD)	[redacted]	1985	Twelve month study of glyphosate adminis-tered by gelatin capsule to beagle dogs MSL-5069 ! 636 Z35385	N	---
KIIA 5.3.3 (OECD)	[redacted]	1983	Six month study of MON 0139 administered by gelatin capsule to beagle dogs 810166 ! ML-81-368 TOX9552361	N	---

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KIIA 5.3.3 (OECD)	[redacted]	1996	HR-001: 13-week Oral Subchronic Toxicity Study in Dogs IET 94-0158 ALS GLP: Y, published: N 2309269 / ASB2012-11456	Y	ALS
KIIA 5.3.4 (OECD)	[redacted]	1996	Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs CTL/P/5079 SYN GLP: Y, published: N 2309278 / TOX2000-1992	Y	SYN
KIIA 5.3.4 (OECD)	[redacted]	1990	Glyphosate: 52-week oral toxicity study in dogs 7502 ! IRI 642675 TOX9552384	N	---
KIIA 5.3.4 (OECD)	[redacted]	2007	Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule)in Beagle Dogs 29647 TCC NUF GLP: Y, published: N 2309274 / ASB2012-11457	Y	NUF
KIIA 5.3.4 (OECD)	[redacted]	1997	HR-001: 12-Month Oral Chronic Toxicity Study in Dogs IET 94-0157 ALS GLP: Y, published: N 2309276 / ASB2012-11458	Y	ALS
KIIA 5.4.1 (OECD)	[redacted]	1995	HR-001: Reverse Mutation Test IET 94-0142 ALS GLP: Y, published: N 2309291 / ASB2012-11462	Y	ALS
KIIA 5.4.1 (OECD)	Callander, R.D.	1996	Glyphosate acid: An evaluation of mutagenic potential using *S. typhimurium* and *E. coli* CTL/P/4874 SYN GLP: Y, published: N 2309313 / ASB2012-11473	Y	SYN
KIIA 5.4.1 (OECD)	Flügge, C.	2009	Mutagenicity Study of Glyphosate TC in the *Salmonella typhimurium* Reverse Mutation Assay (*in vitro*) LPT 23916 HAG GLP: Y, published: N 2309303 / ASB2012-11468	Y	HAG
KIIA 5.4.1 (OECD)	Flügge, C.	2010	Mutagenicity Study of Glyphosate TC in the *Salmonella typhimurium* Reverse Mutation Assay (*in vitro*) LPT 24880 HAG GLP: Y, published: N 2309305 / ASB2012-11469	Y	HAG

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KIIA 5.4.1 (OECD)	Jensen, J. C.	1991	Mutagenicity test: Ames salmonella assay with glyphosate, batch 206-JaK-25-1 Report: 12323, TOX9552371	Y	---
KIIA 5.4.1 KIIIA1 7.6.3 (OECD)	Kier, L. D.; Stegeman, S. D.; Costello, J. G.; Schermes, S.	1992	Ames/salmonella mutagenicity assay of MON 2139 (Roundup herbicide formulation) EHL 91183 ! ML-91-440 ! MSL-11729 TOX1999-239	N	---
KIIA 5.4.1 (OECD)	Kier, L. D.; Stegeman, S. D.; Costello, J. G.; Schermes, S.	1992	Ames/salmonella mutagenicity assay of MON 14445 (DIRECT Herbicide formulation) MSL-11731 ! EHL 91185/ML-91-442 TOX1999-320	N	---
KIIA 5.4.1 KIIA 5.4.4 (OECD)	Kier, L. D.; Stegeman, S. D.; Costello, J. G.; Schermes, S.	1992	Ames/Salmonella mutagenicity assay of Rodeo MSL-11730 ! EHL 91184/ML-91-441 TOX9552373	N	---
KIIA 5.4.1 (OECD)	Li, A. P.; Long, T. J.	1988	An evaluation of the genotoxic potential of glyphosate, Fundamental and Applied Toxicology 10 (1988)537 – 546 published: Y, TOX9500253	N	---
KIIA 5.4.1 KIIA 5.4.4 (OECD)	Rank, J.; Jensen, A. G.; Skov, B.	1993	Genotoxicity testing of the herbicide roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrow micronucleus test, Salmonella mutagenicity test, and Allium anaphase-telephase test Z82234	Y	---
KIIA 5.4.1 KIIA 5.4.4 (OECD)	Rasmussen, E. S.	1997	Genotoxicity of Roundup/Glyphosate, Danish Environmental Protection Agency, AA036753, 7042-0110 ASB2013-9671	N	---
KIIA 5.4.1 (OECD)	Riberri do Val, R.	2007	Bacterial reverse mutation test (Ames Test) for Glifosato Técnico Helm 3393/2007-2.0AM-B HAG GLP: Y, published: N 2309299 / ASB2012-11466	Y	HAG
KIIA 5.4.1 (OECD)	Schreib, G.	2012	Reverse mutation assay using Bacteria (Salmonella typhimurium) with Glyphosate tech. 126159 ASB2014-9133		

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KIIA 5.4.1 (OECD)	Shirasu, Y.; Moriya, M.; Ota, T.; Ohta, T.	1978	Glyphosate: The report of mutagenic study with bacteria for CP 67573 - Microbial mutagenicity testing on CP67573 Report: ET-78-241, TOX9552368	N	---
KIIA 5.4.1 (OECD)	Sokolowski, A.	2007	*Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with glyphosate technical (NUP-05068) 1061401 NUF GLP: Y, published: N 2309293 / ASB2012-11463	Y	NUF
KIIA 5.4.1 (OECD)	Sokolowski, A.	2007	*Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with glyphosate technical (NUP-05070) 1061402 NUF GLP: Y, published: N 2309295 / ASB2012-11464	Y	NUF
KIIA 5.4.1 (OECD)	Sokolowski, A.	2007	*Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with glyphosate technical (NUP-05067) 1061403 NUF GLP: Y, published: N 2309297 / ASB2012-11465	Y	NUF
KIIA 5.4.1 (OECD)	Sokolowski, A.	2009	Glyphosate technical - *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay 1264500 SYN GLP: Y, published: N 2309315 / ASB2012-11474	Y	SYN
KIIA 5.4.1 (OECD)	Sokolowski, A.	2010	*Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with Solution of Glyphosate TC spiked with Glyphosine 1332300 HAG GLP: Y, published: N 2309307 / ASB2012-11470	Y	HAG
KIIA 5.4.1 (OECD)	Thompson, P.W.	1996	Technical glyphosate: Reverse mutation assay "Ames test" using *Salmonella typhimurium* and *Escherichia coli* 434/014 NUF GLP: Y, published: N 2309311 / ASB2012-11472	Y	NUF
KIIA 5.4.1 (OECD)	Thompson, P.	2014	Glyphosate: Reverse mutation assay 'Ames test' using Salmonella typhimurium and Escherichia coli 41401854 ASB2014-9148	Y	

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KIIA 5.4.1 (OECD)	Vargas, A. A. T.; Bonetti, R.	1996	The *Salmonella typhimurium* reverse mutation by Glifos G.1.1 - 050/96 TOX1999-884	N	---
KIIA 5.4.1 (OECD)	Wallner, B.	2010	Reverse Mutation Assay using Bacteria (*Salmonella typhimurium*) with Glyphosate TC BSL 101268 HAG GLP: Y, published: N 2309309 / ASB2012-11471	Y	HAG
KIIA 5.4.2 (OECD)	Fox, V.	1998	Glyphosate acid: *In vitro* cytogenetic assay in human lymphocytes CTL/P/6050 SYN GLP: Y, published: N 2309321 / TOX2000-1995	Y	SYN
KIIA 5.4.2 (OECD)	Jensen, J. C.	1991	Mutagenicity test: *In vitro* mammalian cell gene mutation test with glyphosate, batch 206-JaK-25-1, Report: 12325, published: N, TOX9552372	N	---
KIIA 5.4.2 (OECD)	Kyomu, M.	1995	HR-001: *In vitro* cytogenetics test IET 94-0143 ALS GLP: Y, published: N 2309317 / ASB2012-11475	Y	ALS
KIIA 5.4.2 KIIA 5.4.3 KIIA 4.4.4 (OECD)	Li, A. P.	1983	CHO/HGPRT gene mutation assay with glyphosate, Report ML-83-155 ! 830079, published: N, TOX9552369	N	---
KIIA 5.4.2 (OECD)	Rossberger, S.	1994	Glyphosate: DNA repair test with primary rat hepatocytes, Report: 931564 ! 94-03-28 ro, published: N, TOX9400697/ TOX9551099	N	ADM
KIIA 5.4.2 (OECD)	van de Waart, E. J.	1995	Evaluation of the ability of glyphosate to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat) Report: 141918, published: N, TOX9651525	N	---
KIIA 5.4.2 (OECD)	Wright, N.P.	1996	Technical glyphosate: Chromosome aberration test in CHL cells *in vitro* 434/015 NUF GLP: Y, published: N 2309319 / ASB2012-11476	Y	NUF
KIIA 5.4.3 (OECD)	Akanuma, M.	1995	HR-001: DNA Repair Test (Rec-Assay) IET 94-0141 ALS GLP: Y, published: N 2309325 / ASB2012-11477	N	ALS

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KIIA 5.4.3 (OECD)	Clay, P.	1996	Glyphosate acid: L5178 TK+/- mouse lymphoma gene mutation assay CTL/P/4991 SYN GLP: Y, published: N 2309323 / TOX2000-1994	Y	SYN
KIIA 5.4.4 KIIA 5.10 (OECD)	Alvarez-Moya, C., Silva, M.R., Arambula, A.R.V., Sandoval, A.I., Vasquez, H.C., Montes, R.M.G.	2011	Evaluation of genetic damage induced by glyphosate isopropylamine salt using Tradescantia bioassays Genetics and Molecular Biology 34 (1):127-130 34, 127-130 GLP: N, published: Y 2309560 / ASB2012-11538	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Amer, S.M., Aly, F.A.E., Farghaly, A.A., Ibrahim, A.A.E.	2006	*In vitro* and *in vivo* evaluation of the genotoxicity of the herbicide glyphosate in mice Bulletin of the National Research Centre (Egypt) 31, 427-446 GLP: N, published: Y 2309562 / ASB2012-11539	N	MOD
KIIA 5.4.4 KIIA 5.10 (OECD)	Andre, V., Goff, J.L., Pottier, D., Lebailly, P., Peluso, M., Munnia, A., Gauduchon, P.	2007	Evaluation of bulky DNA adduct levels after pesticide use: Comparison between open-field farmers and fruit growers Toxicological & Environmental Chemistry 89, 125-139 GLP: N, published: Y 2309570 / ASB2012-11543	N	LIT
KIIA 5.4.4 KIIA 5.5.3 KIIA 5.6 KIIA 5..7.2 KIIA 5.10 KIIIA1 7.6.3 (OECD)	Anonym.	2004	WORLD HEALTH ORGANIZATION and FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, Rome: Pesticide residues in food – 2004; Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues Rome, Italy, 20–29 September 2004, ASB2008-6266	N	---
KIIA 5.4.4 KIIA 5.10 (OECD)	Benachour, N., Seralini, G.E.	2009	Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells Chem Res Toxicol 22, 97-105 GLP: N, published: Y 2309606 / ASB2012-11561	N	LIT

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KIIA 5.4.4 KIIA 5.10 (OECD)	Bolognesi, C., Bonatti, S., Degan, P., Gallerani, E., Peluso, M., Rabboni, R., Roggieri, P., Abbondandolo, A.	1997	Genotoxic activity of glyphosate and its technical formulation roundup Journal of Agricultural and Food Chemistry 45, 1957-1962 GLP: N, published: Y 2309628 / Z59299	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Bolognesi, C., Carrasquilla, G., Volpi, S., Solomon, K.R., Marshall, E.J.	2009	Biomonitoring of genotoxic risk in agricultural workers from five colombian regions: association to occupational exposure to glyphosate J Toxicol Environ Health A 72, 986-997 GLP: N, published: Y 2309630 / ASB2012-11570	N	JCC
KIIA 5.4.4 KIIA 5.10 (OECD)	Bolognesi, C., Landini, E., Perrone, E., Roggieri, P.	2004	Cytogenetic biomonitoring of a floriculturist population in Italy: micronucleus analysis by fluorescence in situ hybridization (FISH) with an all-chromosome centromeric probe Mutation Research-Genetic Toxicology and Environmental Mutagenesis 557, 109-117 GLP: N, published: Y 2309634 / ASB2012-11572	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Bolognesi, C., Perrone, E., Landini, E.	2002	Micronucleus monitoring of a floriculturist population from western Liguria, Italy Mutagenesis 175, 391-397 GLP: N, published: Y 2309636 / ASB2012-11573	N	LIT
KIIA 5.4.4 (OECD)	[redacted]	1999	A micronucleus study in mice for glifosate técnico Nufarm RF-G12.79/99 NUF GLP: Y, published: N 2309335 / ASB2012-11482	Y	NUF
KIIA 5.4.4 KIIA 5.10 KIIIA1 7.6.3 (OECD)	Cavalcante, D.G.S.M., Martinez, C.B.R., Sofia, S.H.	2008	Genotoxic effects of Roundup (R) on the fish *Prochilodus lineatus* Mutation Research-Genetic Toxicology and Environmental Mutagenesis 655, 41-46 GLP: N, published: Y 2309662 / ASB2012-11586	N	LIT

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KIIA 5.4.4 KIIA 5.10 (OECD)	Cavas, T., Konen, S.	2007	Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (*Carassius auratus*) exposed to a glyphosate formulation using the micronucleus test and the comet assay Mutagenesis 22, 263-268 GLP: N, published: Y 2309664 / ASB2012-11587	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Chruscielska, K.; Brzezinski, J.; Grafstein, B.	2000	Glyphosate: Evaluation of chronic activity and possible far -reaching effects - Part 2. Studies on mutagenic activity Pestycydy, 2000, (3-4), 21-25 ASB2013-9830	N	---
KIIA 5.4.4 KIIA 5.10 KIIIA1 7.6.3 (OECD)	Clements, C.; Ralph, S.; Petras, M.	1997	Glyphosate: Genotoxicity of select herbicides in *Rana catesbeiana* tadpoles using the alkaline single-cell gel DNA electrophoresis (comet) assay Environ. Molec. Mutagen., 29, 277-288 Z101728	N	---
KIIA 5.4.4 KIIA 5.10 (OECD)	[redacted]	2000	Comparative analysis between micronuclei tests in mice and in peripheral erythrocytes of *Oreochromis niloticus* in evaluation ofmutagenic potential ofthe agrotoxins deltamethrin, dicofol, glyphosate, and lmazapyr ASB2013-11477	N	---
KIIA 5.4.4 (OECD)	[redacted]	2010	Amendment No. 1 to report: Evaluation of the mutagenic potential of Glyphosate technical by micronucleus assay in mice 3996.402.395.07 ASB2014-9284		
KIIA 5.4.4 (OECD)	[redacted]	2008	Evaluation of the mutagenic potential of Glyphosate Technical Micronucleus assay in mice Bioagri Laboratories Ltda., Brazil Data owner: HAG (original sponsor: Jingma Chemicals, Longyou Zhejian, China) Report No.: RF - 3996.402.395.07 Date: 2008-09-29 Unpublished; ASB2012-11481		
KIIA 5.4.4 KIIA 5.5.3 KIIA 5.10 (OECD)	[redacted]	2000	Glyphosate: Evaluation of chronic activity and possible far - reaching effects - Part 1. Studies on chronic toxicity Pestycydy, 2000, (3 -4), 11-20 ASB2013-9829	N	---

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KIIA 5.4.4 KIIA 5.10 (OECD)	[redacted]	2000	Glyphosate: Evaluation of chronic activity and possible far - reaching effects - Part 3. Prenatal toxicity Pestycydy, 2000, (3-4), 27-31 ASB2013-9831	N	---
KIIA 5.4.4 KIIA 5.10 (OECD)	Dimitrov, B.D., Gadeva, P.G., Benova, D.K., Bineva, M.V.	2006	Comparative genotoxicity of the herbicides Roundup, Stomp and Reglone in plant and mammalian test systems Mutagenesis 21, 375-382 GLP: N, published: Y 2309708 / ASB2012-11607	N	LIT
KIIA 5.4.4 (OECD)	[redacted]	2006	Glyphosate Technical: Micronucleus Test In The Mouse 2060/014 NUF GLP: Y, published: N 2309327 / ASB2012-11478	Y	NUF
KIIA 5.4.4 (OECD)	Flowers, L. J.	1981	Ames/salmonella mutagenicity assay of MON 8080 MSL 1538 ! ML-80-294/800281 TOX1999-319	N	---
KIIA 5.4.4 (OECD)	[redacted]	2009	Micronucleus Test of Glyphosate TC in Bone Marrow Cells of the CD Rat by oral administration LPT 23917 HAG GLP: Y, published: N 2309329 / ASB2012-11479	Y	HAG
KIIA 5.4.4 (OECD)	[redacted]	1996	Glyphosate acid: mouse bone marrow micronucleus test CTL/P/4954 SYN GLP: Y, published: N 2309337 / TOX2000-1996	N	SYN
KIIA 5.4.4 KIIA 5.10 (OECD)	[redacted]	2002	A comparison between mouse and fish micronucleus test using cyclophosphamide, mitomycin C and various pesticides Mutation Research-Genetic Toxicology and Environmental Mutagenesis 518, 145-150 GLP: N, published: Y 2309776 / ASB2012-11834	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Guilherme, S., Gaivão, I., Santos, M.A., Pacheco, M.	2010	European eel (*Anguilla anguilla*) genotoxic and pro-oxidant responses following short-term exposure to Roundup®a glyphosate-based herbicide Mutagenesis 25, 523-530 GLP: N, published: Y 2309780 / ASB2012-11836	N	LIT

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KIIA 5.4.4 KIIA 5.10 (OECD)	Helal, A.D., Moussa, H.M.	2005	Chromosomal aberrations induced by glyphosate isopropylamine herbicide and trials for diminuting its toxicity using some chemical inactivators and antioxidant Veterinary Medical Journal Giza 53, 169-187 GLP: N, published: Y 2309794 / ASB2012-11841	N	LIT
KIIA 5.4.4 KIIA 5.10 KIIIA1 7.6.3 (OECD)	Heydens, W.F., Healy, C.E., Hotz, K.J., Kier, L.D., Martens, M.A., Wilson, A.G.E, Farmer, D.R.	2008	Genotoxic potential of glyphosate formulations: Mode-of-action investigations Journal of Agricultural and Food Chemistry 56, 1517-1523 GLP: N, published: Y 2309802 / ASB2012-11845	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Holeckova, B.	2006	Evaluation of the *in vitro* effect of glyphosate-based herbicide on bovine lymphocytes using chromosome painting Bulletin of the Veterinary Research Institute in Pulawy 50, 533-536 GLP: N, published: Y 2309806 / ASB2012-11847	N	LIT
KIIA 5.4.4 (OECD)	[redacted]	2008	Glyphosate Technical - Micronucleus Assay in Bone Marrow Cells of the Mouse 1158500 SYN GLP: Y, published: N 2309339 / ASB2012-11483	Y	SYN
KIIA 5.4.4 (OECD)	[redacted]	1991	Mutagenicity test: Micronucleus test with glyphosate, batch 206-JaK-25-1, Report: 12324, published: N, TOX9552374	N	---
KIIA 5.4.4 KIIA 5.10 (OECD)	Kale, P.G., Petty, B.T., Walker, S., Ford, J.B., Dehkordi, N., Tarasia, S., Tasie, B.O., Kale, R., Sohni, Y.R.	1995	Mutagenicity testing of 9 herbicides and pesticides currently used in agriculture Environmental and Molecular Mutagenesis 25, 148-153 GLP: N, published: Y 2309834 / Z73986, ASB2012-11860	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Kaya, B.: Creus, A.; Yanikoglu, A.; et al.;	2000	Use of the Drosophila wing spot test in the genotoxicity testing of different herbicides ASB2013-9832	N	---
KIIA 5.4.4 (OECD)	[redacted]	1992	Mouse micronucleus study of Rodeo herbicide formulation MSL-11772 ! EHL 91201/91205/ML-91-438 TOX9552376	N	---

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KIIA 5.4.4 KIIIA1 7.6.3 (OECD)	[redacted]	1992	Mouse micronucleus study of Roundup herbicide formulation MSL-11771 ! EHL 91200/91204 ! ML-91-434/ML-91-437 TOX1999-242	N	---
KIIA 5.4 .4 (OECD)	[redacted]	1992	Glyphosate: Mouse micronucleus study of DIRECT Herbicide formulation MSL-11773 ! EHL 91202/91206 ! ML-91-436/ML-91-439 TOX1999-322	N	---
KIIA 5.4.4 KIIA 5.10 (OECD)	Knopper, L.D., Lean, D.R.S.	2004	Carcinogenic and genotoxic potential of turf pesticides commonly used on golf courses Journal of Toxicology and Environmental Health-Part B-Critical Reviews 7, 267-279 GLP: N, published: Y 2309864 / ASB2012-11871	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Lebailly, P., Devaux, A., Pottier, D., De Meo, M., Andre, V., Baldi, I., Severin, F., Bernaud, J., Durand, B., Henry-Amar, M., Gauduchon, P.	2003	Urine mutagenicity and lymphocyte DNA damage in fruit growers occupationally exposed to the fungicide captan Occupational & Environmental Medicine 60, 910-917 GLP: N, published: Y 2309878 / ASB2012-11878	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Levine, S.L., Han, Z., Liu, J., Farmer, D.R., Papadopoulos, V.	2007	Disrupting mitochondrial function with surfactants inhibits MA-10 Leydig cell steroidogenesis Cell Biol Toxicol 23, 385-400 GLP: N, published: Y 2309890 / ASB2009-9030	N	LIT
KIIA 5.4.4 (OECD)	[redacted]	1983	*In vivo* bone marrow cytogenetics study of glyphosate in Sprague-Dawley rats, Report: ML-83-236 ! 830083, published: N, TOX9552375	N	---
KIIA 5.4.4 KIIA 5.10 (OECD)	[redacted]	1998	Genotoxicity and oxidative stress induced by pesticide exposure in bovine lymphocyte cultures *in vitro* Mutation Research 403 (1998) 13-20 ASB2013-9836	N	---

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KIIA 5.4.4 KIIA 5.10 (OECD)	[redacted]	1998	Cytogenetic damage and induction of pro-oxidant state in human lymphocytes exposed *in vitro* to glyphosate, vinclozolin,aAtrazine and DPX-E9636 Environmental and Molecular Mutagenesis 32: 39-46 (1998) ASB2013-9837	N	---
KIIA 5.4.4 KIIA 5.10 (OECD)	Manas, F., Peralta, L., Raviolo, J., Ovandoa, H.G., Weyers, A., Ugnia, L., Cid, M.G., Larripa, I., Gorla, N.	2009	Genotoxicity of glyphosate assessed by the comet assay and cytogenetic tests Environmental Toxicology and Pharmacology 28, 37-41 GLP: N, published: Y 2309908 / ASB2012-11892	N	LIT
KIIA 5.4.4 KIIA 5.10 KIIIA1 7.6.3 (OECD	[redacted]	1991	Glyphosate: Oral and pulmonary toxicology of the surfactant used in Roundup herbicide Z80636	N	---
KIIA 5.4.4 KIIA 5.11 (OECD)	[redacted]	1994	Environmental health criteria 159, Glyphosate TOX9500301	N	---
KIIA 5.4.4 KIIA 5.10 (OECD)	Mladinic, M., Berend, S., Vrdoljak, A.L., Kopjar, N., Radic, B., Zeljezic, D.	2009	Evaluation of genome damage and its relation to oxidative stress induced by glyphosate in human lymphocytes in vitro Environmental and Molecular Mutagenesis 50, 800-807 GLP: N, published: Y 2309942 / ASB2012-11906	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Mladinic, M., Perkovic, P., Zeljezic, D.	2009	Characterization of chromatin instabilities induced by glyphosate, terbuthylazine and carbofuran using cytome FISH assay Toxicol Lett 189, 130-137 GLP: N, published: Y 2309944 / ASB2012-11907	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Monroy, C., Cortes, A., Sicard, D., de Restrepo, H.	2005	Cytotoxicity and genotoxicity of human cells exposed *in vitro* to glyphosate Biomedica 25, 335-345 GLP: N, published: Y 2309950 / ASB2012-11910	N	LIT

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KIIA 5.4.4 KIIA 5.10 (OECD)	Pastor, S., Creus, A., Parron, T., Cebulska-Wasilewska, A., Siffel, C., Piperakis, S., Marcos, R.	2003	Biomonitoring of four European populations occupationally exposed to pesticides: use of micronuclei as biomarkers Mutagenesis 18, 249-258 GLP: N, published: Y 2310004 / ASB2012-11991	N	LIT
KIIA 5.4.4 (OECD)	Patel, N. N.	2012	Micronucleus test of Glyphosate TGAI in mice 120709 ! 485-1-06-4696 ! DR-0112-6927-003 ! 10001701-27-1 ASB2014-9277		
KIIA 5.4.4 KIIA 5.10 (OECD)	Paz-Y-Mino, C., Sanchez, M.E., Arevalo, M., Munoz, M.J., Witte, T., De-La-Carrera, G.O., Leone, P.E.	2007	Evaluation of DNA damage in an Ecuadorian population exposed to glyphosate Genetics and Molecular Biology 30, 456-460 GLP: N, published: Y 2310006 / ASB2012-11992	N	LIT
KIIA 5.4.4 KIIA 5.10 KIIIA1 7.6.3 (OECD)	Peluso, M., Munnia, A., Bolognesi, C., Parodi, S.	1998	32P-postlabeling detection of DNA adducts in mice treated with the herbicide Roundup Environmental and Molecular Mutagenesis 31, 55-59 GLP: N, published: Y 2310014 / TOX1999-318	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Piesova, E.	2004	The Influence Of Different Treatment Length On the Induction Of Micronuclei In Bovine Lymphocytes After Exposure To Glyphosate Folia Veterinaria 48, 130-134 GLP: N, published: Y 2310026 / ASB2012-12001	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Piesova, E.	2005	The effect of glyphosate on the frequency of micronuclei in bovine lymphocytes *in vitro* Acta Veterinaria-Beograd 55, 101-109 GLP: N, published: Y 2310024 / ASB2012-12000	N	MOD
KIIA 5.4.4 KIIA 5.10 (OECD)	Poletta, G.L., Larriera, A., Kleinsorge, E., Mudry, M.D.	2009	Genotoxicity of the herbicide formulation Roundup (R) (glyphosate) in broad-snouted caiman (*Caiman latirostris*) evidenced by the Comet assay and the Micronucleus test Mutation Research-Genetic Toxicology and Environmental Mutagenesis 672, 95-102 GLP: N, published: Y 2310028 / ASB2012-12002	N	LIT

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KIIA 5.4.4 KIIA 5.10 (OECD)	Prasad, S., Srivastava, S., Singh, M., Shukla, Y.	2009	Clastogenic effects of glyphosate in bone marrow cells of swiss albino mice J Toxicol GLP: N, published: Y 2310034 / ASB2012-12005	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Raipulis, J., Toma, M., Balode, M.	2009	Toxicity and genotoxicity testing of Roundup Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences. 63, 29-32 GLP: N, published: Y 2310040 / ASB2012-12008	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Rodrigues, H.G., Penha-Silva, N., Araujo, M.F.P, Nishijo, H., Aversi-Ferreira, T.A.	2011	Effects of Roundup Pesticide on the Stability of Human Erythrocyte Membranes and Micronuclei Frequency in Bone Marrow Cells of Swiss Mice Open Biology Journal 54-59 GLP: N, published: Y 2310046 / ASB2012-12010	N	LIT
KIIA 5.4.4 (OECD)	Roth, M.		Glyphosate technical - Micronucleus assay in bone marrow cells of the mouse 1479200 ! TK0112981 ASB2014-9333		
KIIA 5.4.4 KIIA 5.10 (OECD)	Salvagni, J., Ternus, R., Fuentefria, A.	2011	Assessment of the genotoxic impact of pesticides on farming communities in the countryside of Santa Catarina State, Brazil Genetics and Molecular Biology 34, 122-126 GLP: N, published: Y 2310060 / ASB2012-12017	N	LIT
KIIA 5.4.4 KIIA 5.9 KIIIA1 7.6.3 (OECD)	Sawada, Y., Nagai, Y.	1987	Roundup® poisoning - its clinical observation possible involvement - englische Version Journal of Clinical and Experimental Medicine (paper) 143, 25-27 GLP: N, published: Y 2309502 / Z35531	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Shaham, J., Kaufman, Z., Gurvich, R., Levi, Z.	2001	Frequency of sister-chromatid exchange among greenhouse farmers exposed to pesticides Mutat Res 491-, 71-80 GLP: N, published: Y 2310076 / ASB2012-12025	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Sivikova, K., Dianovsky, J.	2006	Cytogenetic effect of technical glyphosate on cultivated bovine peripheral lymphocytes Int J Hyg Environ Health 209, 15-20 GLP: N, published: Y 2310084 / ASB2012-12029	N	LIT

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KIIA 5.4.4 KIIA 5.8.1 (OECD)	[redacted]	1992	Dodigen 4022: Chromosome aberrations in vitro in V79 chinese hamster cells 92.1024 ! 92.0337 TOX1999-325	N	---
KIIA 5.4.4 KIIA 5.8.1 (OECD)	Stammberger, I.; Mayer, D.	1992	Dodigen 4022: Study of the mutagenic potential in strains of *Salmonella typhimurium* (ames test) and *Escherichia coli* 92.0487 ! 92.0336 TOX1999-324	N	---
KIIA 5.4.4 KIIA 5.10 KIIIA1 7.6.3 (OECD)	[redacted]	1998	Mouse micronucleus screening assay of MON 0818 ML-89-463 ! EHL 89182 TOX1999-240	N	---
KIIA 5.4.4 KIIIA1 7.6.3 (OECD)	Stegeman, S. D.; Li, A. P.	1990	Ames/salmonella mutagenicity assay of MON 0818 EHL 89178 ! ML-89-461 ! MSL-10625 TOX1999-241	N	---
KIIA 5.4.4 (OECD)	[redacted]	1993	Glyphosate technical (FSG 03090 H/05 March 1990): Mutagenicity-micronucleus test in swiss albino mice, Report: 889-MUT.MN ! TOXI-889/1993 ! ES-GPT-MUT-MN, published: N, TOX9551100	N	---
KIIA 5.4.4 (OECD)	[redacted]	1994	Glyphosate technical (FSG 03090 H/05 March 1990): Genetic toxicology - *In vivo* mammalian bone marrow cytogenetic test, Report: 890-MUT-CH.AB ! TOXI-890/1993 ! ES-GPT-MUT-CH.AB, published: N, TOX9400323 / TOX9551101	N	---
KIIA 5.4.4 KIIA 5.10 (OECD)	Vigfusson, N.V., Vyse, E.R.	1980	The effect of the pesticides Dexon, Captan and Roundup on sister chromatid exchanges in human lymphocytes *in vitro* Mutation Research 79, 53-57 GLP: N, published: Y 2310114 / TOX970056 / ASB2012-12044	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Vlastos, D., Stivaktakis, P., Matthopoulos, D.P.	2006	Pesticide exposure and genotoxicity correlations within a Greek farmers' group International Journal of Environmental Analytical Chemistry 86, 215-223 GLP: N, published: Y 2310116 / ASB2012-12045	N	LIT
KIIA 5.4.4 KIIA 5.5.3 KIIA 5.10 KIIIA1 7.6.3 (OECD)	Williams, G.M., Kroes, R., Munro, I.C.	2000	Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans Regulatory Toxicology and Pharmacology 31, 117-165 GLP: N, published: Y 2310132 / ASB2012-12053	N	LIT

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KIIA 5.4.4 (OECD)	[redacted]	1996	A micronucleus study in mice for the product GILFOS G1206096 ! G.1.2 - 60/96 TOX1999-253	N	---
KIIA 5.4.4 (OECD)	[redacted]	2007	Mammalian Erythrocyte Micronucleus Test for Glifosato Técnico Helm 3393/2007-3.0MN-B ASB2012-11480		
KIIA 5.4.6 (OECD)	[redacted]	1992	Glyphosate technical (FSG 03090 H/05, March 1990): Dominant lethal test in wistar rats Report: 888-DLT ! TOXI-888/1992 ! ES-GPT-DLT, published: N, TOX9551102	N	---
KIIA 5.4.6 (OECD)	[redacted] C.	1980	Dominant lethal mutagenicity assay with technical Glyphosate in mice, Report: 401-064 ! IR-79-014, published: N, TOX9552377	N	---
KIIA 5.5 (OECD)	Anon.	2015	Lesion-related incidence data. RITA database ASB2015-2532		
KIIA 5.5 (OECD)	Eaton, G.; Johnson, F. N.; Custer, R. P.; Crane, A. R.;	1980	The Icr:Ha(ICR) mouse: a current account of breeding, mutations, diseases and mortality Lab. Animals 14(1980)17-24 ASB2015-2537		LIT
KIIA 5.5 (OECD)	Giknis, M. L. A.; Clifford, C. B.;	2010	Spontaneous neoplastic lesions in the Crl:CD1 (ICR) mouse in control groups from 18 month to 2 year studies Selected pages ASB2015-2529		
KIIA 5.5 (OECD)	Greim, H.; Saltmiras, D.; Mostert, V.; Strupp, C.;	2015	Evaluation of carcinogenic potential of the herbicide glyphosate, drawing on tumor incidence data from fourteen chronic/carcinogenicity rodent studies Crit Rev Toxicol, 2015; 45(3): 185–208 ASB2015-2287		LIT
KIIA 5.5 (OECD)	Roe, F. J. C.; Tucker, M. J.;	1974	Recent developments in the design of carcinogenicity tests on laboratory animals Proc. Europ. Soc. Stud. Drug Tox., 15:171-177 (1974) ASB2015-2534		LIT
KIIA 5.5 (OECD)	Sher, S. P.	1974	Review article - Tumors in control mice: Literature tabulation Toxicol. Appl. Pharmacol. 30(1974)337-359 Z22020		LIT
KIIA 5.5 (OECD)	Son, W.-C.; Gopinath, C.;	2004	Early occurrence of spontaneous tumors in CD-1 mice and Sprague–Dawley rats Toxicologic Pathology, 32:371–374, 2004 ASB2015-2533		LIT

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KIIA 5.5 (OECD)	Taddesse-Heath, L.; Chattopadhyay, S. K.; Dillehay, D. L.; et al.;	2000	Lymphomas and high-level expression of murine leukemia viruses in CFW mice J. Virol. 74(2000)15:6832-6837 ASB2015-2535		LIT
KIIA 5.5 (OECD)	Toth, B.; Rappaport, H.; Shubik, P.;	1963	Influence of dose and age on the induction of malignant lymphomas and other tumors by 7,12-Dimethylbenz(α)anthracene in Swiss mice J. Nat. Cancer Institute, 30(1963)4:723-732 ASB2015-2536		LIT
KIIA 5.5 (OECD)	Tucker, M. J.	1979	The effect of long-term food restriction on tumours in rodents Int. J. Cancer: 23, 803-807 (1979) Z83266		LIT
KIIA 5.5 (OECD)	Wood, E.;	2010	Historical Incidence of Malignant lymphoma in CD-1 Mouse ASB2015-2531		
KIIA 5.5.1 KIIA 5.10 (OECD)	[redacted]	1996	Glyphosate Acid: One Year Dietary Toxicity Study in Rats CTL/P/5143 SYN GLP: Y, published: N 2309341 / TOX2000-1998	N	SYN
KIIA 5.5.2 (OECD)	[redacted]	1993	Glyphosate - 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks) 7867 CHE GLP: Y, published: N 2309374 / TOX9750499	N	CHE
KIIA 5.5.2 KIIA 5.10 (OECD)	[redacted]	2001	Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats CTL/PR1111 SYN GLP: Y, published: N 2309368 / ASB2012-11488	N	SYN
KIIA 5.5.2 (OECD)	[redacted]	1974	2-year chronic oral toxicity study with CP 67573 in albino rats B564 ! BTL-71-32 Z35230	N	---
KIIA 5.5.2 (OECD)	[redacted]	1997	HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats, Vol. 1 (Seite 1-500) IET 94-0150 Vol.1 ALS GLP: Y, published: N 2309360 / ASB2012-11484	N	ALS

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KIIA 5.5.2 (OECD)	[redacted]	1997	HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats, Vol. 2 (Seite 501-1000) IET 94-0150 Vol. 2 ALS GLP: Y, published: N 2309362 / ASB2012-11485	N	ALS
KIIA 5.5.2 (OECD)	[redacted]	1997	HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats, Vol.3 (Seite 1001-1500) IET 94-0150 Vol. 3 ALS GLP: Y, published: N 2309364 / ASB2012-11486	N	ALS
KIIA 5.5.2 (OECD)	[redacted]	1997	HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats, Vol. 4 (Seite 1501-2051) IET 94-0150 Vol. 4 ALS GLP: Y, published: N 2309366 / ASB2012-11487	N	ALS
KIIA 5.5.2 (OECD)	[redacted]	1990	Chronic study of glyphosate administered in feed to Albino rats MSL-10495 MON GLP: Y, published: N 2309384 / TOX9300244	N	MON
KIIA 5.5.2 (OECD)	[redacted]	1996	Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats TOXI:886.C.C-R FSG GLP: Y, published: N 2309343 / TOX9651587 / TOX9600015	N	ADM
KIIA 5.5.2 (OECD)	[redacted]	2009	Glyphosate Technical: Dietary combined chronic toxicity / carcinogenicity study in the rat SPL2060-0012 NUF GLP: Y, published: N 2309391 / ASB2012-11490	Y	NUF
KIIA 5.5.3 KIIA 5.10 (OECD)	Acquavella, J.F., Gustin, C., Alexander, B.H., Mandel, J.S.	2005	Implications for epidemiologic research on variation by pesticide in studies of farmers and their families Scandinavian Journal of Work Environment & Health 31, 105-109 GLP: N, published: Y 2309540 / ASB2012-11530	N	LIT

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KIIA 5.5.3 KIIA 5.10 (OECD)	Alavanja, M.C., Samanic, C., Dosemeci, M., Lubin, J., Tarone, R., Lynch, C.F., Knott, C., Thomas, K., Hoppin, J.A., Barker, J., Coble, J., Sandler, D.P., Blair, A.	2003	Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort Am J Epidemiol 157, 800-814 GLP: N, published: Y 2309554 / ASB2012-11535	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Andreotti, G., Freeman, L.E.B., Hou, L., Coble, J., Rusiecki, J., Hoppin, J.A., Silverman, D.T., Alavanja, M.C.R.	2009	Agricultural pesticide use and pancreatic cancer risk in the Agricultural Health Study Cohort International Journal of Cancer 124, 2495-2500 GLP: N, published: Y 2309572 / ASB2012-11544	N	LIT
KIIA 5.5.3 (OECD)	[redacted]	1993	Glyphosate: 104-week dietary carcinogenicity study in mice 7793 ! IRI 438618 TOX9552382	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Band, P.R., Abanto, Z., Bert, J., Lang, B., Fang, R., Gallagher, R.P., Le, N.D.	2011	Prostate Cancer Risk and Exposure to Pesticides in British Columbia Farmers Prostate 71, 168-183 GLP: N, published: Y 2309594 / ASB2012-11555	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Barale-Thomas, E.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 473–474 ASB2013-10998	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Berry, C.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 445–446 ASB2013-10988	N	--
KIIA 5.5.3 KIIA 5.10 (OECD)	Blair, A., Freeman, L.B.	2009	Epidemiologic Studies in Agricultural Populations: Observations and Future Directions Journal of Agromedicine 14, 125-131 GLP: N, published: Y 2309618 / ASB2012-11566	N	LIT

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KIIA 5.5.3 KIIA 5.10 (OECD)	Carreon, T., Butler, M.A., Ruder, A.M., Waters, M.A., Davis-King, K.E., Calvert, G.M., Schulte, P.A., Connally, B., Ward, E.M., Sanderson, W.T., Heinemann, E.F., Mandel, J.S., Morten, R.F., Reding, D.J., Rosenmann, K.D., Talaska, G.	2005	Gliomas and farm pesticide exposure in women: The Upper Midwest Health Study Environmental Health Perspectives 113, 546-551 GLP: N, published: Y 2309660 / ASB2012-11585	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	McDuffie, H.H., Pahwa, P., McLaughlin, J.R., Spinelli, J.J., Fincham, S., Dosman, J.A., Robson, D., Skinnider, L.F., Choi, N.W.	2001	Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health Cancer Epidemiol Biomarkers Prev 10, 1155-1163 GLP: N, published: Y 2309924 / ASB2011-364	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Engel, L.S., Hill, D.A., Hoppin, J.A., Lubin, J.H., Lynch, C.F., Pierce, J., Samanic, C., Sandler, D.P., Blair, A., Alavanja, M.C.	2005	Pesticide use and breast cancer risk among farmers' wives in the agricultural health study American Journal of Epidemiology 161, 121-135 GLP: N, published: Y 2309720 / ASB2012-11613	N	MOD
KIIA 5.5.3 KIIA 5.10 (OECD)	Eriksson, M., Hardell, L., Carlberg, M., Akerman, M.	2008	Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis Int J Cancer 123, 1657-1663 GLP: N, published: Y 2309722 / ASB2012-11614	N	LIT

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KIIA 5.5.3 KIIA 5.10 (OECD)	Farmer, D.R., Lash, T.L., Acquavella, J.F.	2005	Glyphosate Results Revisited Environmental Health Perspectives 113, A365-A366 GLP: N, published: Y 2309726 / ASB2012-11616	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Flower, K.B., Hoppin, J.A., Lynch, C.F., Blair, A., Knott, C., Shore, D.L., Sandler, D.P.	2004	Cancer risk and parental pesticide application in children of agricultural health study participants Environmental Health Perspectives 112, 361-635 GLP: N, published: Y 2309734 / ASB2012-11620	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Freeman,L.B.	2009	Evaluation of agricultural exposures: the agricultural health study and the agricultural cohort consortium Reviews on Environmental Health 24, 311-318 GLP: N, published: Y 2309740 / ASB2012-11623	N	MOD
KIIA 5.5.3 KIIA 5.10 (OECD)	Fritschi, L., Benke, G., Hughes, A.M., Kricker, A..,Turner, J., Vajdic, C.M., Grulich, A., Milliken, S., Kaldor, J., Armstrong, B.K.	2005	Occupational exposure to pesticides and risk of non-Hodgkin's lymphoma American Journal of Epidemiology 162, 849-857 GLP: N, published: Y 2309746 / ASB2012-11624	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	George, J., Prasad, S., Mahmood, Z., Shukla, Y.	2010	Studies on glyphosate-induced carcinogenicity in mouse skin: a proteomic approach J Proteomics 73, 951-964 GLP: N, published: Y 2309766 / ASB2012-11829	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Grunewald, W.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 447–448 ASB2013-11001	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Hammond, B.; Goldstein, D. A.; Saltmiras, D.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 459–464 ASB2013-10995	N	---

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KIIA 5.5.3 KIIA 5.10 (OECD)	Hardell, L., Eriksson, M.	1999	A case-control study of non-Hodgkin lymphoma and exposure to pesticides Cancer 85, 1353-1360 GLP: N, published: Y 2309788 / ASB2012-11838	N	MOD
KIIA 5.5.3 KIIA 5.10 (OECD)	Hardell, L., Eriksson, M., Nordstrom, M.	2002	Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: Pooled analysis of two Swedish case-control studies Leukemia & Lymphoma 43, 1043-1049 GLP: N, published: Y 2309790 / ASB2012-11839	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Heinemann, J. A.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 442 ASB2013-10987	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Karunanayake, C.P., Spinelli, J.J., McLaughlin, J.R., Dosman, J.A., Pahwa, P., McDuffie, H.H.	2011	Hodgkin Lymphoma and Pesticides Exposure in Men: A Canadian Case-Control Study Journal of Agromedicine 17, 30-39 GLP: N, published: Y 2309844 / ASB2012-11865	N	LIT
KIIA 5.5.3 (OECD)	[redacted]	1983	A chronic feeding study of glyphosate (Roundup technical) in mice 77-2061 ! (BDN-77-420) TOX9552381	N	---
KIIA 5.5.3 (OECD)	[redacted]	2001	Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice TOXI: 1559.CARCI-M FSG GLP: Y, published: N 2309396 / ASB2012-11491	Y	ADM
KIIA 5.5.3 KIIA 5.10 (OECD)	Landgren, O., Kyle, R.A., Hoppin, J.A., Freeman, L.E.B., Cerhan, J.R., Katzmann, J.A., Rajkumar, S.V., Alavanja, M.C.	2009	Pesticide exposure and risk of monoclonal gammopathy of undetermined significance in the Agricultural Health Study Blood 113, 6386-6391 GLP: N, published: Y 2309874 / ASB2012-11875	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Langridge, P.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 441 ASB2013-10986	N	---

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KIIA 5.5.3 KIIA 5.10 (OECD)	Lash, T.L.	2007	Bias analysis applied to Agricultural Health Study publications to estimate non-random sources of uncertainty J Occup Med Toxicol 2, 1-9 GLP: N, published: Y 2309876 / ASB2012-11877	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Lee, W.J., Lijinsky, W., Heineman, E.F., Markin, R.S., Weisenburger, D.D., Ward, M.H.	2004	Agricultural pesticide use and adenocarcinomas of the stomach and oesophagus Occupational and Environmental Medicine 61 (9):743-749 61, 743-749 GLP: N, published: Y 2309888 / ASB2012-11883	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Lee, W.J., Colt, J.S., Heineman, E.F., McComb, R., Weisenburger, D.D., Lijinsky, W., Ward, M.H.	2005	Agricultural pesticide use and risk of glioma in Nebraska, United States Occupational and Environmental Medicine 62, 786-792 GLP: N, published: Y 2309886 / ASB2012-11882	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Monge, P., Wesseling, C., Guardado, J., Lundberg, I., Ahlbom, A., Cantor, K.P., Weideroass, E., Partanen, T.	2007	Parental occupational exposure to pesticides and the risk of childhood leukemia in Costa Rica Scandinavian Journal of Work Environment & Health 33, 293-303 GLP: N, published: Y 2309948 / ASB2012-11909	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Multigner, L., Ndong, J.R., Oliva, A., Blanchet, P.	2008	Environmental pollutants and prostate cancer: epidemiological data Gynecol Obstet Fertil 36, 848-856 GLP: N, published: Y 2309964 / ASB2012-11917	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Ndong, J.R., Blanchet, P., Multigner, L.	2009	Pesticides and prostate cancer: epidemiological data Bulletin Du Cancer 96, 171-180 GLP: N, published: Y 2309974 / ASB2012-11922	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Nordström, M.; Hardell, L.; Magnuson, A.; Hagberg, H.; Rask-Andersen, A.	1998	Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukae-mia evaluated in a case-control study TOX1999-687	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Ollivier, L.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 458 ASB2013-11000	N	---

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KIIA 5.5.3 KIIA 5.10 (OECD)	Pahwa, P., Karunanayake, C.P., Dosman, J.A., Spinelli, J.J., McDuffie, H.H., McLaughlin, J.R.	2011	Multiple Myeloma and Exposure to Pesticides: A Canadian Case-Control Study Journal of Agromedicine 17, 40-50 GLP: N, published: Y 2309996 / ASB2012-11987	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Panchin, A. Y.;	2013	Toxicity of roundup-tolerant genetically modified maize is not supported by statistical tests Food and Chemical Toxicology 53 (2013) 475 ASB2013-10937	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Pilu, R.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 454 ASB2013-10992	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	De Roos, A.J., Blair, A., Rusiecki, J.A., Hoppin, J.A., Svec, M., Dosemeci, M., Sandler, D.P., Alavanja, M.C.	2005	Cancer incidence among glyphosate-exposed pesticide applicators in the agricultural health study Environmental Health Perspectives 113, 49-54 GLP: N, published: Y 2309704 / ASB2012-11605	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	De Roos, A.J., Zahm, S.H., Cantor, K.P., Weisenburger, D.D., Holmes, F.F., Burmeister, L.F., Blair, A.	2003	Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men Occupational and Environmental Medicine 60 GLP: N, published: Y 2309706 / ASB2012-11606	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Schorsch, F.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 465–466 ASB2013-10996	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Séralini, G.-E.; Clair, E.; Mesnage, R.; Gress, S.; Defarge, N.; Malatesta, M.; Hennequin, D.; Spiroux de Vendômois, J.;	2012	Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Food and Chem Toxicol., in Press, ASB2012-15514	N	---

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KIIA 5.5.3 KIIA 5.10 (OECD)	Séralini, G. E.; Mesnage, R.; Defarge, N.; Gress, S.; Hennequin, D.; Clair, E.; Malatesta, M.; Spiroux de Vendômois, J.;	2013	Answers to critics: Why there is a long term toxicity due to a Rounduptolerant genetically modified maize and to a Roundup herbicide Food and Chemical Toxicology 53 (2013) 476–483 ASB2013-10985	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	de Souza, L.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 440 ASB2013-10999	N	---
KIIA 5.5.3 (OECD)	[redacted]	1997	HR-001: 18-Month Oral Oncogenicity Study in Mice IET 940151 ALS GLP: Y, published: N 2309415 / ASB2012-11493	Y	ALS
KIIA 5.5.3 KIIA 5.10 (OECD)	Tester, M.;	2012	Letter to the Editor Food and Chemical Toxicology 53 (2013) 457 ASB2013-10994	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Tien, D. L.; Huy, H. L.;	2012	Comments on ‘‘Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize’’ Food and Chemical Toxicology 53 (2013) 443–444 ASB2013-10984	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Trewavas, A.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 449 ASB2013-10989	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Tribe, D.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 467–472 ASB2013-10997	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Wager, R.;	2013	Letter to the editor Food and Chemical Toxicology 53 (2013) 455–456 ASB2013-10993	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Weichenthal, S., Moase, C., Chan, P.	2010	A review of pesticide exposure and cancer incidence in the Agricultural Health Study cohort Environ Health Perspect 118, 1117-1125 GLP: N, published: Y 2310122 / ASB2012-12048	N	LIT

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KIIA 5.5.3 (OECD)	[redacted]	2009	Glyphosate Technical: Dietary carcinogenicity study in the mouse SPL 2060-0011 NUF GLP: Y, published: N 2309412 / ASB2012-11492	Y	NUF
KIIA 5.6.1 (OECD)	[redacted]	1985	Three-generation reproduction study in rats with the oral administration of glyphosate TOX9650161	N	---
KIIA 5.6.1 (OECD)	[redacted]	1988	Report on effect of glyphosate technical of Excel Industries Ltd., Bombay, on fertility and general reproductive performance (Segment I) TOX9551832	N	---
KIIA 5.6.1 (OECD)	[redacted]	1988	Report on effect of pesticides on reproductive process - Segment IV - three generation reproduction study with albino rats using glyphosate technical of Excel Industries Ltd., Bombay TOX9551965	N	---
KIIA 5.6.1 (OECD)	[redacted]	1991	Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring CHV 42/90619 TOX9552388	N	---
KIIA 5.6.1 (OECD)	[redacted]	1992	The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat CHV 47/911129 CHE GLP: Y, published: N 2309436 / TOX9552389	N	CHE
KIIA 5.6.1 (OECD)	[redacted]	2007	Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat 2060/0013 NUF GLP: Y, published: N 2309418 / ASB2012-11494	Y	NUF
KIIA 5.6.1 (OECD)	[redacted]	2000	Glyphosate acid: Multigeneration reproduction toxicity study in rats CTL/P/6332 SYN / MON GLP: Y, published: N 2309423 / TOX2000-2000	Y	SYN
KIIA 5.6.1 (OECD)	[redacted]	1990	Two Generation Reproduction Feeding Study with Glyphosate in Sprague-Dawley Rats MSL-10387 MON GLP: Y, published: N 2309439 / ASB2012-11496 / TOX9552387	N	MON

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KIIA 5.6.1 (OECD)	[redacted]	1993	Two Generation Reproduction Study in Wistar Rats TOXI: 885-RP-G2 GLP: Y, published: N 2309427 / TOX9300009 / TOX9551104	N	ADM
KIIA 5.6.1 (OECD)	[redacted]	1997	HR-001: A two-generation reproduction study in rats IET 96-0031 ALS GLP: Y, published: N 2309425 / ASB2012-11495	N	ALS
KIIA 5.6.10 (OECD	Antoniou, M.; Habib, M.E.M; Howard, C.V.; Jennings, R.C.; Leifert, C.; Nodari, R.O.; Robinson, C.J.; Fagan, J.	2012	Teratogenic Effects of Glyphosate-Based Herbicides: Divergence of Regulatory Decisions from Scientific Evidence J Environ Anal Toxicol 2012, S:4, ASB2012-15927	N	---
KIIA 5.6.10 (OECD)	[redacted]	1991	The effect of glyphosate on pregnancy of the rat (incorporates preliminary investigation) CHV 43 u. 41/90716 TOX9552393	N	---
KIIA 5.6.10 (OECD)	[redacted]	1995	HR-001: Teratogenicity Study in Rats IET 94-0152 ALS GLP: Y, published: N 2309444 / ASB2012-11497	Y	ALS
KIIA 5.6.10 (OECD)	[redacted]	2012	Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity Toxicology, in Press ASB2012-13917	N	---
KIIA 5.6.10 (OECD)	[redacted]	2002	Amendment 001 to glyphosate acid: Developmental toxicity study in the rat CTL/P/4819 ! RR0690 ASB2012-10080	N	---
KIIA 5.6.10 (OECD)	[redacted]	1991	Glyphosate techn. (FSG 03090 H/05 March 1990): Teratogenicity study in Wistar rats ES.883.TER-R ! TOXI-883/1991 ! ES-GPT-TER-R TOX9551105	N	---
KIIA 5.6.10 (OECD)	[redacted]	1980	Glyphosate: Teratology study in rats 401-054 ! IR-79-016 TOX9552392	N	---

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KIIA 5.6.11 (OECD)	[redacted]	2013	No evidence of endocrine disruption by glyphosate in male and female pubertal assays. Abstract ASB2013-3464	N	---
KIIA 5.6.11 (OECD)	[redacted]	1989	Rabbit Teratology Study with Glyphosate Technical IIT Project No. 1086 EXC GLP: Y, published: N 2309462 / TOX9551960	N	EXC
KIIA 5.6.11 (OECD)	[redacted]	1991	The Effect of Glyphosate on Pregnancy of the Rabbit (Incorporates Preliminary Investigations) CHV 45 & 39 & 40/901 CHE GLP: Y, published: N 2309454 / TOX9552391	N	CHE
KIIA 5.6.11 (OECD)	[redacted]	1996	Glyphosate technical: Oral gavage teratology study in the rabbit 434/020 NUF GLP: Y, published: N 2309448 / ASB2012-11499	Y	NUF
KIIA 5.6.11 (OECD)	[redacted]	1995	HR-001: A Teratogenicity Study in Rabbits IET 94-0153 ALS GLP: Y, published: N 2309446 / ASB2012-11498	N	ALS
KIIA 5.6.11 (OECD)	Kimmel, G.L.; Kimmel, C.A.; Williams, A.L.; DeSesso, J.M.;	2013	Evaluation of developmental toxicity studies of glyphosate with attention to cardiovascular development Critical Reviews in Toxicology 43(2013)2: 79-95, ASB2013-3462	N	---
KIIA 5.6.11 (OECD)	[redacted]	1996	Glyphosate acid: Developmental toxicity study in the rabbit CTL/P/5009 SYN GLP: Y, published: N 2309450 / TOX2000-2002	N	SYN
KIIA 5.6.11 (OECD)	[redacted]	1993	Teratogenicity study in rabbits - Tets compound: Glyphosate technical TOXI: 884-TER-RB GLP: Y, published: N 2309457 / TOX9551106	N	ADM
KIIA 5.6.11 (OECD)	[redacted]	1980	Technical Glyphosate: Teratology study in rabbits IR-79-018 MON GLP: N, published: N 2309452 / TOX9552390	N	MON

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KIIA 5.6.2 (OECD)	[redacted]	1996	Glyphosate acid: Developmental toxicity study in the rat 29.03.1996 CTL/P/4819 ! RR 0690 TOX2000-2001	N	---
KIIA 5.6.2 (OECD)	[redacted]	2011	Glyphosate Technical: Dietary carcinogenicity study in the mouse – Amendment SPL 2060-0011 ASB2014-9149		
KIIA 5.6.2 (OECD)	[redacted]	2011	Assessment and further discussion on relevance of perceived elevation in testicular atrophy for SafePharm project number 2060/0011 (Glyphosate technical: mouse oncogenicity study) SPL 2060-0011 ASB2014-9150		
KIIA 5.7. (OECD	[redacted]	1996	Glyphosat Acid: Acute delayed neurotoxicity study with in the domestic hen CTL/C/3122 ! C2.8/01 ! ISN 361 ASB2013-9828	N	---
KIIA 5.7.1 (OECD)	[redacted]	1996	Glyphosate acid: Acute neurotoxicity study in rats CTL/P/4866 SYN GLP: Y, published: N 2309464 / ASB2012-11500	N	SYN
KIIA 5.7.4 KIIA 5.10 (OECD)	Astiz, M., de Alaniz, M.J., Marra, C.A.	2009	Effect of pesticides on cell survival in liver and brain rat tissues Ecotoxicol Environ Saf 72, 2025-2032 GLP: N, published: Y 2309582 / ASB2012-11549	N	LIT
KIIA 5.7.4 KIIA 5.9 KIIA 5.10 (OECD)	Barbosa, E.R., da Costa, M.D.L., Bacheschi, L.A., Scaff, M., Leite, C.C.	2001	Parkinsonism after glycine-derivate exposure Movement Disorders 16, 565-568 GLP: N, published: Y 2309598 / ASB2012-11557	N	LIT
KIIA 5.7.4 KIIA 5.10 (OECD)	Cole, R.D., Anderson, G.L., Williams, P.L.	2004	The nematode Caenorhabditis elegans as a model of organophosphate-induced mammalian neurotoxicity Toxicology and Applied Pharmacology 194, 248-256 GLP: N, published: Y 2309680 / ASB2012-11594	N	LIT

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KIIA 5.7.4 KIIA 5.10 (OECD)	da Costa, M.D.L., Goncalves, L.R., Barbosa, E.R., Bacheschi, L.A.	2003	Neuroimaging abnormalities in parkinsonism: study of five cases Arquivos De Neuro-Psiquiatria 61, 381-386 GLP: N, published: Y 2309688 / ASB2012-11598	N	LIT
KIIIA 5.7.4 KIIA 5.10 (OECD)	Engel, L.S., Checkoway, H., Keifer, M.C., Seixas, N.S., Longstreth, W.T., Jr., Scott, K.C., Hudnell, K., Anger, W.K., Camicioli, R.	2001	Parkinsonism and occupational exposure to pesticides Occup Environ Med 28, 582-589 GLP: N, published: Y 2309718 / ASB2012-11612	N	LIT
KIIA 5.7.4 KIIA 5.10 (OECD)	Gui, Y.-x., Fan, X.-n., Wang, H.-m., Wang, G., Chen, S.-d.	2012	Glyphosate induced cell death through apoptotic and autophagic mechanisms Neurotoxicology and Teratology GLP: N, published: Y 2309778 / ASB2012-11835	N	LIT
KIIA 5.7.4 KIIA 5.10 (OECD)	Heu, C., Elie-Caille, C., Mougey, V., Launay, S., Nicod, L.	2012	A step further toward glyphosate-induced epidermal cell death: Involvement of mitochondrial and oxidative mechanisms Environmental Toxicology and Pharmacology 34, 144-153 GLP: N, published: Y 2309800 / ASB2012-11844	N	LIT
KIIA 5.7.4 (OECD)	[redacted]	1996	Glyphosate Acid: Subchronic Neurotoxicity Study In Rats CTL/P/4867 SYN GLP: Y, published: N 2309466 / ASB2012-11501	N	SYN
KIIA 5.7.4 KIIA 5.10 (OECD)	Kamel, F., Tanner, C.M., Umbach, D.M., Hoppin, J.A., Alavanja, M.C.R., Blair, A., Comyns, K., Goldman, S.M., Korell, M., Langston, J.W., Ross G.W., Sandler, D.P.	2007	Pesticide exposure and self-reported Parkinson's disease in the agricultural health study American Journal of Epidemiology 165, 364-374 GLP: N, published: Y 2309838 / ASB2012-11862	N	LIT

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KIIA 5.7.4 KIIA 5.10 (OECD)	Mink, P.J., Mandel, J.S., Lundin, J.I., Sceurman, B.K.	2011	Epidemiologic studies of glyphosate and non-cancer health outcomes: A review Regulatory Toxicology and Pharmacology 61, 172-184 GLP: N, published: Y 2309938 / ASB2012-11904	N	LIT
KIIA 5.7.4 KIIA 5.10 (OECD)	Negga, R., Rudd, D.A., Davis, N.S., Justice, A.N., Hatfield, H.E., Valente, A.L., Fields, A.S., Fitsanakis, V.A.	2011	Exposure to Mn/Zn ethylene-bis-dithiocarbamate and glyphosate pesticides leads to neurodegeneration in Caenorhabditis elegans NeuroToxicology 32, 331-341 GLP: N, published: Y 2309976 / ASB2012-11923	N	LIT
KIIA 5.7.4 KIIA 5.9 KIIA 5.10 (OECD)	Wang, G., Fan, X.N., Tan, Y.Y., Cheng, Q., Chen, S.D.	2011	Parkinsonism after chronic occupational exposure to glyphosate Parkinsonism & Related Disorders 17, 486-487 GLP: N, published: Y 2310120 / ASB2012-12047	N	LIT
KIIA 5.8 (OECD)	[redacted]	1996	AMPA, Reverse Mutation Test IET 96-0076 ALS GLP: Y, published: N 2309478 / ASB2012-11507	Y	ALS
KIIA 5.9 (OECD)	Bakke, J. P.	1991	Evaluation of the potential of AMPA to induce unscheduled DNA synthesis in the in vitro hepatocyte DNA repair assay using the male F-344 rats 2495-V01-91 ! SR-91-234 TOX9552409	N	---
KIIA 5.8 (OECD)	Callander, R.D.	1988	Aminomethyl Phosphonic Acid: An Evaluation of Mutagenic Potential Using *S. typhimurium* and *E. coli* CTL/P/2206 SYN GLP: Y, published: N 2309476 / TOX9500043	N	SYN
KIIA 5.8 (OECD)	[redacted]	1993	AMPA: Acute oral toxicity (limit) test in rats 8763 ! IRI 552409 TOX9552395	N	---
KIIA 5.8 (OECD)	[redacted]	1993	AMPA: Acute dermal toxicity (limit) test in rats 8764 ! IRI 552409 TOX9552396	N	---
KIIA 5.8 (OECD)	[redacted]	1993	AMPA: Magnusson-Kligman maximisation test in guinea pigs 8765 ! IRI 552409 TOX9300374	N	---

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KIIA 5.8 (OECD)	[redacted]	1979	CP 50435: 90-day subacute rat toxicity study 401-050 ! IRD-78-174 TOX9552401	N	---
KIIA 5.8 (OECD)	[redacted]	1992	AMPA: Teratogenicity study in rats 7891 ! IRI 490421 TOX9300348	N	---
KIIA 5.8 (OECD)	[redacted]	1993	AMPA: 4 week dose range finding study in rats with administration by gavage 7803 ! IRI 450860 TOX9300349	N	---
KIIA 5.8 (OECD)	[redacted]	1991	A developmental toxicity study of AMPA in rats WIL-50159 ! WI-90-266 TOX9552414	N	---
KIIA 5.8 (OECD)	[redacted]	1991	Assessment of acute oral toxicity of (N-methyl-N-phosphonomethyl)glycine to rats 12837 TOX9552398	N	---
KIIA 5.8 (OECD)	[redacted]	1993	Mutagenicity test: Ames salmonella test with AMPA, batch 286-JRJ-73-4, 13269 TOX9300378	N	---
KIIA 5.8 (OECD)	[redacted]	1993	AMPA, batch 286-JRJ-73-4: Mutagenicity test: *In vitro* mammalian cell gene mutation test performed with mouse lymphoma cells (L5178Y) 13270 TOX9300380	N	---
KIIA 5.8 (OECD)	[redacted]	1993	Mutagenicity test: Micronucleus test with AMPA, batch 286-JRJ-73-4 13268 TOX9300379	N	---
KIIA 5.8 (OECD)	[redacted]	1993	Mouse micronucleus study of AMPA EHL-90170/ML-90-404 ! MSL 13243 TOX9552413	N	---
KIIA 5.8 (OECD)	[redacted]	1996	AMPA: Acute Oral Toxicity Study In Mice IET 96-0075 ALS GLP: Y, published: N 2309468 / ASB2012-11502	Y	ALS
KIIA 5.8 (OECD)	[redacted]	1988	Aminomethyl Phosphonic Acid: Acute Oral Toxicity to the Rat CTL/P/2266 SYN GLP: Y, published: N 2309470 / TOX9500044	N	SYN

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KIIA 5.8 (OECD)	[redacted]	2002	Acute Toxicity Study of AMPA (Aminomethyl Phosphonic Acid) in CD Rats by Dermal Administration - LIMIT TEST 16168/02 GLP: Y, published: N 2309472 / ASB2012-11503	Y	ADM
KIIA 5.8 (OECD)	[redacted]	2002	Examination of AMPA (Aminomethyl Phosphonic Acid) in the Skin Sensitisation Test in Guinea Pigs according to Magnusson And Kligman (Maximisation Test) 16169/02 GLP: Y, published: N 2309474 / ASB2012-11506	Y	ADM
KIIA 5.8 (OECD)	[redacted]	2002	Measurement of unscheduled DNA synthesis (UDS) in rat hepatocytes in vitro procedure with AMPA (Amino methyl phosphonic acid) IPL-R 020625 ALS GLP: Y, published: N 2309480 / ASB2012-11508	Y	ALS
KIIA 5.8 (OECD)	[redacted]	1993	AMPA: 13-week toxicity study in rats with administration by gavage 7866 ! IRI 450876 TOX9300377	N	---
KIIA 5.8 (OECD)	[redacted]	1991	90-day oral (capsule) toxicity study in dogs with AMPA WIL-50173 ! WI-90-354 TOX9552406	N	---
KIIA 5.9 (OECD)	Acquavella, J.F., Weber, J.A., Cullen, M.R., Cruz, O.A., Martens, M.A., Holden, L.R., Riordan, S., Thompsen, M., Farmer, D.	1999	Human ocular effects from self-reported exposures to Round-up herbicides Human & Experimental Toxicology (paper) 18, 479-486 GLP: N, published: Y 2309482 / TOX2002-699	N	LIT
KIIA 5.9 (OECD)	Bando, H., Murao, Y., Aoyagi, U., Hirakawa, A., Iwase, M., Nakatani, T.	2010	[Extreme hyperkalemia in a patient with a new glyphosate potassium herbicide poisoning: report of a case] Chudoku Kenkyu 23, 246-249 GLP: N, published: Y 2309596 / ASB2012-11556	N	LIT
KIIA 5.9 (OECD)	BfR	2011	Frauenmilch: Dioxingehalte sinken kontinuierlich. Information Nr. 011/2011 des BfR vom 23.03.2011 ASB2014-8171	N	---

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KIIA 5.9 KIIIA1 7.6.3 (OECD)	Bradberry, S.M., Proutfoot, A.T., Vale, J.A.	2004	Glyphosate poisoning Toxicological reviews (paper), 23, 159-167 GLP: N, published: Y 2309484 / ASB2012-11509	N	LIT
KIIA 5.9 (OECD)	Bradberry, S.M., Proudfoot, A.T., Vale, J.A.	2004	Glyphosate poisoning Toxicol Rev 23, 159-167 GLP: N, published: Y 2309642 / ASB2012-11576	N	LIT
KIIA 5.9 (OECD)	Burger, R.; Begemann, K.; Meyer, H.; Hahn, A.;	2009	Severe dyspnoea after spraying of a pesticide containing glyphosate. Lung damage histologi-cally confirmed Clinical Toxicology (2009) 47, 506 ASB2013-11831	N	---
KIIA 5.9 (OECD)	Chang, C.-J., Peng, Y.-C., Hung, W.-H., Yang, D.-Y., Lin, T.-J.	1999	Clinical impact of upper gastrointestinal tract injuries in glyphosate-surfactant oral intoxication Human & Experimental Toxicology (paper), 18, 475-478 GLP: N, published: Y 2309486 / ASB2012-11510	N	LIT
KIIA 5.9 (OECD)	Fromme, H.; Gruber, L.; Seckin, E.; et al.;	2011	Phthalates and their metabolites in breast milk — Results from the Bavarian Monitoring of Breast Milk (BAMBI) Environment International 37 (2011) 715–722 ASB2014-8169	N	LIT
KIIA 5.9 (OECD)	Fürst, P.	2006	Dioxins, polychlorinated biphenyls and other organohalogen compounds in human milk Mol. Nutr. Food Res. 2006, 50, 922 – 933 ASB2014-8168	N	LIT
KIIA 5.9 (OECD)	Goldstein, D.A., Johnson, G., Farmer, D.R., Martens, M.A.	1999	Pneumonitis and herbicide exposure Chest (paper), 16, 1139-1140 GLP: N, published: Y 2309490 / ASB2012-11511	N	LIT
KIIA 5.9 (OECD)	Goldstein, D.A., Acquavella, J.F., Mannion, R.M., Farmer, D.R.	2002	An analysis of glyphosate data from the California Environmental Protection Agency Pesticide Illness Surveillance Program Journal of Toxicology-Clinical Toxicology 40, 885-892 GLP: N, published: Y 2309770 / ASB2012-11831	N	LIT
KIIA 5.9 (OECD)	Kamijo, Y., Mekari, M., Yoshimura, K., Kano, T., Soma, K.	2012	Glyphosate-surfactant herbicide products containing glyphosate potassium salt can cause fatal hyperkalemia if ingested in massive amounts Clinical Toxicology 50, 159 GLP: N, published: Y 2309840 / ASB2012-11863	N	LIT

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KIIA 5.9 (OECD)	Lee, H-L., Chen, K.-W., Chi, C.-H., Huang, J.-J., Tsai, L.-M.	2000	Clinical presentations and prognostic factors of a glyphosate-surfactant herbicide intoxication: a review of 131 cases Academic Emergency Medicine (paper) 7, 906-910 GLP: N, published: Y 2309492 / ASB2012-11512	N	LIT
KIIA 5.9 (OECD)	Lee, C.H., Shih, C.P., Hsu, K.H., Hung, D.Z., Lin, C.C.	2008	The early prognostic factors of glyphosate-surfactant intoxication Am J Emerg Med 26, 275-281 GLP: N, published: Y 2309880 / ASB2012-11879	N	LIT
KIIA 5.9 KIIA 5.10 KIIIA1 7.6.3 (OECD)	[redacted]	1987	Irritating effect of glyphosate, surfactant and roundup on stomach and small intestine in dogs MON GLP: N, published: N 2309496 / TOX9552430	N	MON
KIIA 5.9 (OECD)	Paumgartten, F.J.R.	2012	ANVISA - Glyphosate Intoxications 2010 to 2012 in Brasil; ASB2013-13413	N	---
KIIA 5.9 (OECD)	Pushnoy, L.A., Avnon, L.S., Carel, R.S.	1998	Herbicide (Roundup) Pneumonitis Chest (paper), 114, 1769-1771 GLP: N, published: Y 2309498 / ASB2012-11513	N	LIT
KIIA 5.9 (OECD)	Raab, U.; Alb-recht, M.; Preiss, U.; et al.;	2013	Organochlorine compounds, nitro musks and perfluorinated substances in breast milk – Results from Bavarian Monitoring of Breast Milk 2007/8 Chemosphere 93 (2013) 461–467 ASB2014-8170	N	LIT
KIIA 5.9 (OECD)	Sawada, Y., Nagai, Y., Ueyama, M., Yamamoto, I.	1988	Probable toxicity of surface-active agent in commercial herbicide containing glyphosate The Lancet (paper) 1, 299-301 GLP: N, published: Y 2309504 / Z35532	N	LIT
KIIA 5.9 (OECD)	Tominack, R., Conner, P., Yamashita, M.	1989	Clinical Management of Roundup® herbicide exposure The Japanese Journal of Toxicology (paper) 2, 187-192 GLP: N, published: Y 2309506 / TOX9552426	N	LIT

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KIIA 5.9 (OECD)	UBA	2008	Aktualisierung der Referenzwerte für HCB, β-HCH, DDT und PCB in Frauenmilch. Stellungnahme der Kommission Human-Biomonitoring des Umweltbundesamtes *BfR Stillkommission* Bundesgesundheitsbl - Gesundheitsforsch - Gesundheitsschutz 2008 · 51:1239–1242 ASB2014-8167	N	---
KIIA 5.9 (OECD)	Verdugo-Raab, U.;	2012	Ergebnisse der Muttermilchuntersuchungen 1984–2010 Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit ASB2014-8173	N	---
KIIA 5.10 (OECD)	AFSSA	2009	Avis de l'Agence française de sécurité sanitaire des aliments relatif au glyphosate et aux préparations phytopharmaceutiques à base de cette substance active GLP: N, published: Y 2309546 / ASB2012-11532	N	LIT
KIIA 5.10 (OECD)	[redacted]	1996	Glyphosate Acid: Comparison of Salivary Gland Effects in Three Strains of Rat CTL/P/5160 SYN GLP: Y, published: N 2309518 / ASB2012-11520	N	SYN
KIIA 5.10 (OECD)	[redacted]	1996	Glyphosate Acid: Comparison of Salivary Gland Effects in Three Strains of Rat CTL/P/5160 SYN GLP: Y, published: N 2309558 / ASB2012-11537	Y	MOD
KIIA 5.10 (OECD)	Alavanja, M. C. R.; Ross, M. K.; Bonner, M. R.	2013	Increased cancer burden among pesticide applicators and others due to pesticide exposure CA: A Cancer Journal for Clinicians, 2013;63:120–142 ASB2014-9174	N	LIT
KIIA 5.10 (OECD)	Alavanja, M. C. R.; Bonner, M. R.	2012	Occupational pesticide exposures and cancer risk: a review Journal of Toxicology and Environmental Health, Part B, 15:238–263, 2012 ASB2014-9173	N	LIT
KIIA 5.10 (OECD)	Alvarez-Moya, C.; Reynoso Silva, M.; Valdez Ramírez, C.; et al.;	2014	Comparison of the in vivo and in vitro genotoxicity of Glyphosate Isopropylamine salt in three different organisms Genetics and Molecular Biology, 37, 1, 105-110 (2014) ASB2014-6902	N	LIT

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KIIA 5.10 (OECD)	Altenburger, R.; Scholz, S.; Schmitt-Jansen, M.; Busch, W.; Escher B. I.	2012	Mixture toxicity revisited from a toxicogenomic perspective Environ. Sci. Technol. 2012, 46, 2508−2522 ASB2014-9176	N	LIT
KIIA 5.10 (OECD)	Andreotti, G.; Koutros, S.; Berndt, S. I. et al.	2012	The Interaction between pesticide use and genetic variants involved in lipid metabolism on prostate cancer risk Journal of Cancer Epidemiology Volume 2012, Article ID 358076, ASB2014-9198	N	LIT
KIIA 5.10 (OECD)	Anonymous	2009	Final List of Initial Pesticide Active Ingredients and Pesticide Inert Ingredients to be Screened Under the Federal Food, Drug, and Cosmetic Act Federal Register /Vol. 74, No. 71 /Wednesday, April 15, 2009 /Notices, 17579-17585 74, 17579-17585 GLP: N, published: Y 2310108 / ASB2012-12041	N	LIT
KIIA 5.10 (OECD)	Anonymous	2012	GM Soy linked to health damage in pigs -- a Danish Dossier ASB2013-11007	N	---
KIIA 5.10 (OECD)	Anonymous	2013	Effects on ruminants and other herbivores (livestock and wild life) ASB2013-11007	N	---
KIIA 5.10 (OECD)	Antoniou, M.; Brack, P.; Carrasco, A. et al.	2010	GV-SOYA - Nachhaltig? - Verantwortungsbewusst? - Zusammenfassung der wichtigsten Ergebnisse ASB2012-803	N	---
KIIA 5.10 (OECD)	Arbuckle, T.E., Lin, Z.Q., Mery, L.S.	2001	An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population Environmental Health Perspectives 109, 851-857 GLP: N, published: Y 2309574 / ASB2012-11545	N	LIT
KIIA 5.10 (OECD)	Aris, A., Leblanc, S.	2011	Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada Reproductive Toxicology 31, 528-533 GLP: N, published: Y 2309578 / ASB2012-11547	N	LIT

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KIIA 5.10 (OECD)	Astiz, M.; de Catalfo, G.; Garcia, M.	2013	Pesticide-induced decrease in rat testicular steroidogenesisis differentially prevented by lipoate and tocopherol Ecotoxicology and Environmental Safety 91 (2013) 129–138 ASB2014-7493	N	LIT
KIIA 5.10 (OECD)	Astiz, M.; de Alaniz, M. J. T.; Marra, C. A.	2012	The oxidative damage and inflammation caused by pesticides are reverted by lipoic acid in rat brain Neurochemistry International 61 (2012) 1231–1241 ASB2014-9201	N	LIT
KIIA 5.10 (OECD)	Axelrad, J.C., Howard, C.V., McLean, W.G.	2003	The effects of acute pesticide exposure on neuroblastoma cells chronically exposed to diazinon Toxicology 185, 67-78 GLP: N, published: Y 2309590 / ASB2012-11553	N	LIT
KIIA 5.10 (OECD)	Bailey, J.; Hauswirth, J.; Stump, D.;	2013	No evidence of endocrine disruption by glyphosate in male and female pubertal assays. Abstract SOT 2013 Annual Meeting, PS 1937: p 412 ASB2013-3464	N	---
KIIA 5.10 (OECD)	Basrur, P. K.;	2006	Disrupted sex differentiation and feminization of man and domestic animals Environmental Research 100 (2006) 18–38 ASB2014-7492	N	LIT
KIIA 5.10 (OECD)	Bates, N.; Edwards, N.	2013	Letter to the editor: Glyphosate toxicity in animals Clinical Toxicology (2013), 51, 1243 ASB2014-9249	N	LIT
KIIA 5.10 (OECD)	Bell, E.M., Hertz-Picciotto, I., Beaumont, J.J.	2001	A case-control study of pesticides and fetal death due to congenital anomalies Epidemiology 12, 148-156 GLP: N, published: Y 2309602 / ASB2012-11559	N	LIT
KIIA 5.10 (OECD)	Belle, R., Le Bouffant, R., Morales, J., Cosson, B., Cormier, P., Mulner-Lorillon, O.	2007	Sea urchin embryo, DNA-damaged cell cycle checkpoint and the mechanisms initiating cancer development J Soc Biol 201, 317-327 GLP: N, published: Y 2309604 / ASB2012-11560	N	LIT

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KIIA 5.10 (OECD)	Bellé, R.; Morales, J.; Cormier, P.; Mulner-Lorillon, O.	2012	Letter to the editor: Toxicity of Roundup and Glyphosate Journal of Toxicology and Environmental Health, Part B, 15:233–237, 2012 ASB2014-9251	N	LIT
KIIA 5.10 (OECD)	Benachour, N., Sipahutar, H., Moslerni, S., Gasnier, C., Travert, C., Seralini, G.E.	2007	Time- and dose-dependent effects of roundup on human embryonic and placental cells Archives of Environmental Contamination and Toxicology 53, 126-133 GLP: N, published: Y 2309608 / ASB2009-9018	N	LIT
KIIA 5.10 (OECD)	Benedetti, A.L., Vituri, C.D., Trentin, A.G, Domingues, M.A.C., Alvarez-Silva, M.	2004	The effects of sub-chronic exposure of Wistar rats to the herbicide Glyphosate-Biocarb (R) Toxicology Letters 153, 227-232 GLP: N, published: Y 2309610 / ASB2012-11562	N	LIT
KIIA 5.10 (OECD)	Benitez-Leite, S., Macchi, M., Acosta, M.	2009	Malformaciones congénitas asociadas a agrotóxicos Archives of Pediatrics 80 (3):377-378. 80, 377-378 GLP: N, published: Y 2309612 / ASB2012-11563	N	LIT
KIIA 5.10 (OECD)	Barry, K.; Koutros, S.; Berndt, S. et al.	2011	Genetic variation in base excision repair pathway genes, pesticide exposure, and prostate cancer risk Environmental Health Perspectives, 119(2011)12 ASB2014-9247	N	LIT
KIIA 5.10 (OECD)	Benedettia, D.; Nunesa, E.; Sarmentoa, M. et al.	2013	Genetic damage in soybean workers exposed to pesticides: Evaluation with the comet and buccal micronucleus cytome assays Mutation Research 752 (2013) 28– 33 ASB2014-9279	N	LIT
KIIA 5.10 (OECD)	Beuret, C.J., Zirulnik, F., Gimenez, M.S.	2005	Effect of the herbicide glyphosate on liver lipoperoxidation in pregnant rats and their fetuses Reproductive Toxicology 19, 501-504 GLP: N, published: Y 2309614 / ASB2012-11564	N	LIT
KIIA 5.10 (OECD)	Beswick, E.; Millo, J.	2011	Fatal poisoning with Glyphosate - surfactant herbicide JICS Volume 12, Number 1, January 2011 ASB2014-9283	N	LIT

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KIIA 5.10 (OECD)	BfR	2009	BfR-Bewertung der Studie "Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines" vom 06.08.2009 GLP: N, published: Y 2309616 / ASB2012-11565	N	LIT
KIIA 5.10 (OECD)	[redacted]	1987	Synergism and potentiation in rats of Glyphosate (tech.) of Excel Industries Ltd., Bombay TOX9551964	N	---
KIIA 5.10 (OECD)	Blair, A., Zahm, S.H.	1993	Patterns of pesticide use among farmers: implications for epidemiologic research Epidemiology 4, 55-62 GLP: N, published: Y 2309620 / ASB2012-11567	N	LIT
KIIA 5.10 (OECD)	Bleeke, M.S., Kurtzweil, M.L., Saltmiras, D.A.	2010	Dietary Exposure Assessment of Polyoxyethylenealkylamines (POEA) Surfactants MON GLP: N, published: Y 2309622 / ASB2010-6123	N	LIT
KIIA 5.10 (OECD)	Borgert, C. J.; Baker, S. P.; Matthews, J. C.	2013	Potency matters: Thresholds govern endocrine activity Regulatory Toxicology and Pharmacology 67 (2013) 83–88 ASB2014-9292	N	LIT
KIIA 5.10 (OECD)	Brändli, D.; Reinacher, S.	2011	Herbizide im Urin Ithaka Journal 1 | 2012:1–4 ASB2012-804	N	LIT
KIIA 5.10 (OECD)	BVL	2010	Glyphosate - Comments from Germany on the paper by Paganelli, A. et al. (2010): "Glyphosate-based Herbicides Produce Teratogenic Effects on Vertebrates by Impairing Retinoic Acid Signaling" GLP: N, published: Y 2309648 / ASB2012-11579	N	LIT
KIIA 5.10 (OECD)	Caglar, S., Kolankaya, D.	2008	The effect of sub-acute and sub-chronic exposure of rats to the glyphosate-based herbicide Roundup Environmental Toxicology and Pharmacology 25 (2008) 57–62 ASB2012-11580	N	LIT
KIIA 5.10 (OECD)	Campaña, H.; Pawluk, M. S.; López Camelo, J. S.; Grupo de Estudio del ECLAMC	2010	Prevalencia al nacimiento de 27 anomalías congénitas seleccionadas, en 7 regiones geográficas de la Argentina. Births prevalence of 27 selected congenital anomalies in 7 geographic regions of Argentina Arch Argent Pediatr 2010;108(5):409-417 ASB2013-10559	N	---

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KIIA 5.10 (OECD)	Campo, N. B.-C.; Zarate, D. H. V.; Hernandez, E. D. R.	2009	Toxicity of the main pesticides used in Popayán Valley with Bacillus subtillis Facultad de Ciencias Agropecuarias 16 Vol 7 No. 1 Enero - Junio 2009 ASB2014-9281	N	---
KIIA 5.10 (OECD)	Carmichael, S. L.; Yang, W.; Roberts, E. M. et al.	2013	Hypospadias and residential proximity to pesticide applications Pediatrics 2013;132(5)e1216–e1226 ASB2014-9307	N	LIT
KIIA 5.10 (OECD)	Carroll, R.; Metcalfe, C.; Gunnell, D. et al.	2012	Diurnal variation in probability of death following self-poisoning in Sri Lanka—evidence for chronotoxicity in humans International Journal of Epidemiology 2012;41:1821–182 ASB2014-9308	N	LIT
KIIA 5.10 (OECD)	Cassault-Meyer, E.; Gress, S.; Seralini, G. E.; Galeraud-Denis, I.;	2014	An acute exposure to glyphosate-based herbicide alters aromatase levels in testis and sperm nuclear quality Environ Toxicol. Pharmacol 38 (2014) 131–140 ASB2014-5615	N	LIT
KIIA 5.10 (OECD)	Cattani, D.; de Liz Oliveira Cavalli, V. L.; Heinz Rieg, C. E. et al.	2014	Mechanisms underlying the neurotoxicity induced by Glyphosate-based herbicide in immature rat hippocampus: Involvement of glutamate excitotoxicity Toxicology 320 (2014) 34–45 ASB2014-3919	N	LIT
KIIA 5.10 (OECD)	Chaufan, G.; Coalova, I.; Molina, M.	2014	Glyphosate commercial formulation causes cytotoxicity, oxidative effects, and apoptosis on human cells: Differences with its active ingredient International Journal of Toxicology 2014, Vol. 33(1) 29-38 ASB2014-9314 / ASB2014-7616	N	LIT
KIIA 5.10 (OECD)	Chen, Y. J.; Wu, M.-L.; Deng, J.-F.; Yang, C.-C.	2009	The epidemiology of Glyphosate-surfactant herbicide poisoning in Taiwan, 1986–2007: a poison center study Clinical Toxicology (2009) 47, 670–677 ASB2014-9318	N	LIT
KIIA 5.10 (OECD)	Chen, H.-H.; Lin, J.-L.; Huang, W-H. et al.	2013	Spectrum of corrosive esophageal injury after intentional paraquat or Glyphosate-surfactant herbicide ingestion International Journal of General Medicine 2013:6 677–683 ASB2014-9321	N	LIT

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KIIA 5.10 (OECD)	Chien, W.-C.; Chung, C.-H.; Jaakkola, J. J. K. et al.	2012	Risk and prognostic factors of inpatient mortality associated with unintentional insecticide and herbicide poisonings: A retrospective cohort study PLOS one September 2012 Volume 7 Issue 9 e45627 ASB2014-9326	N	LIT
KIIA 5.10 (OECD)	Chorfa, A.; Bétemps, D.; Morignat, E. et al.	2013	Specific pesticide-dependent increases in alpha-synuclein levels in human neuroblastoma (SH-SY5Y) and melanoma (SK-MEL-2) cell lines Toxicological Sciences 133(2), 289–297 2013 ASB2014-9328	N	LIT
KIIA 5.10 (OECD)	Clair, E., Linn, L., Travert, C., Amiel, C., Seralini, G.-E., Panoff, J.-M.	2012	Effects of Roundup and Glyphosate on Three Food Microorganisms: Geotrichum candidum, Lactococcus lactis subsp. cremoris and Lactobacillus delbrueckii subsp. bulgaricus Current Microbiology 64, 486-491 GLP: N, published: Y 2309674 / ASB2012-11592	N	LIT
KIIA 5.10 (OECD)	Clair, E., Mesnage, R., Travert, C., Séralini, G.-E.	2012	A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells *in vitro*, and testosterone decrease at lower levels Toxicology *in Vitro* 26, 269-279 GLP: N, published: Y 2309678 / ASB2012-1628	N	LIT
KIIA 5.10 (OECD)	Coalova, I.; Ríos de Molina, M. C.; Chaufan, G.;	2014	Influence of the spray adjuvant on the toxicity effects of a Glyphosate formulation Toxicology in Vitro 28 (2014) 1306–1311 ASB2014-7615	N	LIT
KIIA 5.10 (OECD)	Cocco, P.; Satta, G.;	2014	Lymphoma risk and occupational exposure to pesticides: results of the Epilymph study Occup Environ Med 2012;0:1–7 ASB2014-7523	N	LIT
KIIA 5.10 (OECD)	Corsini, E.; Sokooti, M.; Galli, C. L.; Moretto, A.; Colosio, C.	2012	Pesticide induced immunotoxicity in humans: A comprehensive review of the existing evidence Toxicology (2012) ASB2014-9352	N	LIT
KIIA 5.10 (OECD)	Culbreth, M. E.; Harrill, J. A.; Freudenrich, T. M. et al.	2012	Comparison of chemical-induced changes in proliferation and apoptosis in human and mouse neuroprogenitor cells NeuroToxicology 33 (2012) 1499–1510 ASB2014-9355	N	LIT

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KIIA 5.10 (OECD)	Curwin, B.D., Hein, M.J., Sanderson, W.T., Striley, C., Heederik, D., Kromhout, H., Reynolds, S.J., Alavanja, M.C.	2006	Urinary pesticide concentrations among children, mothers and fathers living in farm and non-farm households in iowa Ann. Occup. Hyg. 2006, 1-33 ASB2012-11597	N	LIT
KIIA 5.10 KIIIA1 7.6.3 (OECD)	Dallegrave, E., Mantese, F.D., Coelho, R.S., Pereira, J.D., Dalsenter, P.R., Langeloh, A.	2003	The teratogenic potential of the herbicide glyphosate-Roundup (R) in Wistar rats Toxicology Letters 142, 45-52 GLP: N, published: Y 2309692 / ASB2012-11600	N	LIT
KIIA 5.10 KIIIA1 7.6.3 (OECD)	Dallegrave, E., Mantese, F.D., Oliveira, R.T., Andrade, A.J.M., Dalsenter, P.R., Langeloh, A.	2007	Pre- and postnatal toxicity of the commercial glyphosate formulation in Wistar rats Archives of Toxicology 81, 665-673 GLP: N, published: Y 2309694 / ASB2012-2721	N	LIT
KIIA 5.10 (OECD)	de Liz Oliveira Cavalli, V. L.; Cattani, D.; Rieg, C. E. H. et al.	2013	Roundup disrupts male reproductive functions by triggering calcium-mediated cell death in rat testis and Sertoli cells FreeRadicalBiologyandMedicine65(2013)335–346 ASB2014-7495	N	LIT
KIIA 5.10 (OECD)	Daruich, J., Zirulnik, F., Gimenez, M.S.	2001	Effect of the herbicide glyphosate on enzymatic activity in pregnant rats and their fetuses Environmental Research 85, 226-231 GLP: N, published: Y 2309696 / ASB2012-11601	N	LIT
KIIA 5.10 (OECD)	Da Silva, F. R.; Kvitko, K.; Rohr, P. et al.	2014	Genotoxic assessment in tobacco farmers at different crop times Science of the Total Environment 490 (2014) 334–341 ASB2014-9358	N	LIT
KIIA 5.10 (OECD)	DeSesso, J. M.; Williams, A.	2012	Comment on ‘‘Glyphosate impairs male off-spring reproductive development by disrupting gonadotropin expression" by Romano et al. 2012 Arch Toxicol (2012) 86:1791–1793 ASB2014-9369	N	LIT

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KIIA 5.10 (OECD)	De Souza Filho, J.; Neves Sousa, C. C.; Da Silva, C. C. et al.	2013	Mutagenicity and genotoxicity in gill erythro-cyte cells of Poecilia reticulata exposed to a Glyphosate formulation Bull Environ Contam Toxicol (2013) 91:583–587 ASB2014-7617	N	LIT
KIIA 5.10 (OECD)	El-Shenawy, N.S.	2009	Oxidative stress responses of rats exposed to Roundup and its active ingredient glyphosate Environmental Toxicology and Pharmacology 28 (2009) 379–385 ASB2012-11611	N	LIT
KIIA 5.10 (OECD)	El-Zaemey, S.; Heyworth, J.	2013	Noticing pesticide spray drift from agricultural pesticide application areas and breast cancer: a case-control study Aust NZ J Public Health. 2013; Online ASB2014-9473	N	LIT
KIIA 5.10 (OECD)	European Commission	2011	Standing Committee on the Food Chain and Animal Health, Section Phytopharmaceuticals - Plant Protection Products - Legislation - 22-23 November 2010 GLP: N, published: Y 2309724 / ASB2012-11615	N	LIT
KIIA 5.10 (OECD)	Faria, N. M. X.; Fassa, A. G.; Meucci, R. D. et al.	2014	Occupational exposure to pesticides, nicotine and minor psychiatric disorders among tobacco farmers in southern Brazil NeuroToxicology (2014) in Press ASB2014-9477	N	---
KIIA 5.10 (OECD)	Folta, K.	2014	Letter to the editor Food and Chemical Toxicology: 65 (2014) 392 ASB2014-9478	N	LIT
KIIA 5.10 (OECD)	Forgacs, A.L., Ding, Q., Jaremba, R.G., Huhtaniemi, I.T., Rahman, N.A., Zacharewski, T.R.	2012	BLTK1 Murine Leydig Cells: A Novel Steroidogenic Model for Evaluating the Effects of Reproductive and Developmental Toxicants Toxicological Sciences GLP: N, published: Y 2309736 / ASB2012-11621	N	LIT
KIIA 5.10 (OECD)	Freire, C.; Koifman, S.	2012	Pesticide exposure and Parkinson's disease: Epidemiological evidence of association NeuroToxicology 33 (2012) 947–971 ASB2014-9479	N	LIT

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KIIA 5.10 (OECD)	French Committee for the Study of Toxicity	2005	Enquiry into the referral of the Committee for the Study of Toxicity by the DGAL regarding the article "Differential effects of glyphosate and Roundup on human placental cells and aromatase." Richard S., Moslemi S., Sipahutar H., Benachour GLP: N, published: Y 2309742 / ASB2009-9025	N	LIT
KIIA 5.10 (OECD)	Garlich, F. M.; Goldman, M.; Pepe, J. et al.	2014	Hemodialysis clearance of glyphosate following a life-threatening ingestion of glyphosate-surfactant herbicide Clinical Toxicology (2014), 52, 66–71 ASB2014-9480	N	LIT
KIIA 5.10 (OECD)	Garry, V.F., Harkins, M.E., Erickson, L.L., Long-Simpson, L.K., Holland, S.E., Burroughs, B.L.	2002	Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA Environmental Health Perspectives 110:441-449 110, 441-449 GLP: N, published: Y 2309750 / ASB2012-11626	N	LIT
KIIA 5.10 (OECD)	Garry, V.F., Holland, S.E., Erickson, L.L., Burroughs, B.L.	2003	Male reproductive hormones and thyroid function in pesticide applicators in the Red River Valley of Minnesota Journal of Toxicology and Environmental Health-Part A 66, 965-986 GLP: N, published: Y 2309752 / ASB2012-11627	N	LIT
KIIA 5.10 (OECD)	Gasnier, C., Benachour, N., Clair, E., Travert, C., Langlois, F., Laurant, C., Decroix-Laporte, C., Seralini, G.E.	2010	Dig1 protects against cell death provoked by glyphosate-based herbicides in human liver cell lines J Occup Med Toxicol 5 GLP: N, published: Y 2309754 / ASB2012-11628	N	LIT
KIIA 5.10 (OECD)	Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M.C., Seralini, G.E.	2009	Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines Toxicology 262, 184-191 GLP: N, published: Y 2309756 / ASB2012-11629	N	LIT

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KIIA 5.10 (OECD)	Gasnier, C., Laurant, C., Decroix-Laporte, C., Mesnage, R., Clair, E., Travert, C., Seralini, G.E.	2011	Defined plant extracts can protect human cells against combined xenobiotic effects J Occup Med Toxicol 6, 3 GLP: N, published: Y 2309758 / ASB2012-11630	N	LIT
KIIA 5.10 (OECD)	Gencer, N.; Ergün, A.; Demir, D.	2012	In vitro effects of some herbicides and fungicides on human erythrocyte carbonic anhydrase activity Fresenius Environmental Bulletin, Volume 21 - No 3. 2012 ASB2014-9481	N	LIT
KIIA 5.10 (OECD)	Gentile, N.; Manas, F.; Bosch, B.; Peralta, L.; Gorla, N.; Aiassa, D.	2012	Micronucleus assay as a biomarker of genotoxicity in the occupational exposure to agrochemicals in rural workers Bull Environ Contam Toxicol (2012) 88:816–822 ASB2014-9482	N	LIT
KIIA 5.10 (OECD)	George, J.; Shukla, Y.;	2013	Emptying of intracellular calcium pool and oxidative stress imbalance are associated with the Glyphosate-induced proliferation in human skin keratinocytes HaCaT cells ISRN Dermatology, Volume 2013, Article ID 825180 ASB2014-8034	N	LIT
KIIA 5.10 (OECD)	Gil, H. W.; Park, J. S.; Hong; S. Y.	2013	Effect of intravenous lipid emulsion in patients with acute Glyphosate intoxication Clinical Toxicology (2013), 51, 767–771 ASB2014-9488	N	LIT
KIIA 5.10 (OECD)	Glyphosat task force	2014	Response to EFSA non-confidential comment 48 (EFSA non-confidential letter page 9, in reference to public comment 2(78 ASB2014-9624	N	LIT
KIIA 5.10 (OECD)	Goldner, W. S.; Sandler, D. P.; Yu, F. et al.	2013	Hypothyroidism and pesticide use among male private pesticide applicators in the agricultural health study JOEM Volume 55, Number 10, October 2013 ASB2014-9492	N	LIT
KIIA 5.10 (OECD)	Goldstein, D. A.; Saltmiras, D. A	2014	Neurodevelopmental toxicity: still more questions than answers neurology Vol 13 July 2014 ASB2014-9493	N	LIT

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KIIA 5.10 (OECD)	Grandjean, P.; Landrigan, P. J.	2014	Neurobehavioural effects of developmental toxicity Lancet Neurol 2014; 13: 330–38 ASB2014-9494	N	LIT
KIIA 5.10 (OECD)	Greenpeace	2011	Herbicide tolerance and GM cropsfedd - Why the world should be ready to round up Glyphosate ASB2012-810	N	---
KIIA 5.10 (OECD)	Gress, S.; Lemoine, S.; Puddu, P.-E.; Seralini, G.-E.; Rouet, R.;	2014	Cardiotoxic electrophysiological effects of the herbicide Roundup in rat and rabbit ventricular myocardium in vitro Cardiovasc Toxicol ASB2014-12161	N	LIT
KIIA 5.10 (OECD)	Guilherme, S.; Santos, M. A.; Barroso, C.; et al.;	2012	Differential genotoxicity of Roundupformulation and its constituents in blood cells of fish (Anguilla anguilla): considerations on chemical interactions and DNA damaging mechanisms Ecotoxicology (2012) 21:1381–1390 ASB2014-7619	N	LIT
KIIA 5.10 (OECD)	[redacted]	2010	An 8-Week Oral (Diet and Gavage) Toxicity Study of Citric Acid in Male Rats WIL-50361 GTF GLP: Y, published: N 2309782 / ASB2012-11519	Y	MOD
KIIA 5.10 (OECD)	[redacted]	2012	Glyphosate - A 28-Day Oral (Dietary) Immunotoxicity Study in Female B6C3F1 Mice WIL-50393 MON GLP: Y, published: N 2309522 / ASB2012-11521	Y	EGT
KIIA 5.10 (OECD)	Harrill, J. A.; Freudenich, T. M.; Robinette, B. L.; Mundy, W. R.	2011	Comparative sensitivity of human and rat neural cultures to chemical-induced inhibition of neurite outgrowth Toxicology and Applied Pharmacology 256 (2011) 268–280 ASB2014-9558	N	LIT
KIIA 5.10 (OECD)	Hayes; A. W.	2014	Reply to letter to the editor Food and Chemical Toxicology: 65 (2014) 394–395 ASB2014-9559	N	LIT

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KIIA 5.10 (OECD)	Hecker, M., Hollert, H., Cooper, R., Vinggaard, A.M., Akahori, Y., Murphy, M., Nellemann, C., Higley, E., Newsted, J., Laskey, J., Buckalew, A.	2011	The OECD validation program of the H295R steroidogenesis assay: Phase 3. Final inter-laboratory validation study Environmental Science and Pollution Research 18, 503-515 GLP: N, published: Y 2309792 / ASB2012-11840	N	LIT
KIIA 5.10 (OECD)	Hedberg, D.; Wallin, M.;	2010	Effects of Roundup and glyphosate formulations on intracellular transport, microtubules and actin filaments in Xenopus laevis melanophores Toxicology in Vitro 24 (2010) 795–802 ASB2014-7494	N	LIT
KIIA 5.10 (OECD)	Heu, C., Berquand, A., Elie-Caille, C., Nicod, L.	2012	Glyphosate-induced stiffening of HaCaT keratinocytes, a Peak Force Tapping study on living cells Journal of structural biology 178, 1-7 GLP: N, published: Y 2309798 / ASB2012-11843	N	LIT
KIIA 5.10 (OECD)	Hinojosa, R.; Baud, F.; Marque, S.; Barreteau, H.	2013	Severe poisonings in intensive care unit: Study of announced substances in 2011 Annales Pharmaceutiques Françaises (2013) 71, 174—185 ASB2014-9566	N	LIT
KIIA 5.10 (OECD)	Hoare, A.	2014	QSAR assessment on the toxicological properties of Glyphosate and its impurities Report EE/14/002, Battelle UK Limited ASB2014-9157		
KIIA 5.10 (OECD)	Hokanson, R., Fudge, R., Chowdhary, R., Busbee, D.	2007	Alteration of estrogen-regulated gene expression in human cells induced by the agricultural and horticultural herbicide glyphosate Hum Exp Toxicol 26, 747-752 GLP: N, published: Y 2309804 / ASB2012-11846	N	LIT
KIIA 5.10 (OECD)	Honeycutt, Z.; Rowlands, H.;	2014	Glyphosate Testing Report: Findings in American Mothers' Breast Milk, Urine and Water. "Moms Across America" and "Sustainable Pulse" ASB2014-6793	N	---

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KIIA 5.10 (OECD)	Horiuchi, N.; Oguchi, S.; Nagami, H. et al.	2007	Pesticide-related dermatitis in Saku District, Japan, 1975-2000 Int. J Occup. Environ Health 2007;14:25-34 ASB2014-9570	N	LIT
KIIA 5.10 (OECD)	Hour, B. T.; Belen, C.; Zar, T. et al.	2012	Herbicide Roundup intoxication: Successful treatment with continuous renal replacement therapy The American Journal of Medicine, Vol 125, No 8, August 2012 ASB2014-9571	N	LIT
KIIA 5.10 (OECD)	Jamkhande, P. G.; Chintawar, K. D.; Chandak, P. G.	2014	Teratogenicity: A mechanism based short review on common teratogenic agents Asian Pac J Trop Dis 2014; 4(6): 421-432 ASB2014-9573	N	LIT
KIIA 5.10 (OECD)	Jany, K.-D.	2013	Die Langzeitfütterungsstudie von Seralini et al. (2012) - eine kritische Replik Ernährungs Umschau 8/2013 ASB2014-9580	N	LIT
KIIA 5.10 (OECD)	Jasper, R.; Locatelli, G. O.; Pilati, C. et al.	2012	Evaluation of biochemical, hematological and oxidative parameters in mice exposed to the herbicide Glyphosate-Roundup Interdiscip Toxicol. 2012; Vol. 5(3): 133–140. ASB2014-9583	N	LIT
KIIA 5.10 (OECD)	Jayasumana, C.; Gunatilake, S.; Senanayake, P.;	2014	Glyphosate, hard water and nephrotoxic metals: Are they the culprits behind the epidemic of chronic kidney disease of unknown etiology in Sri Lanka? *Int. J. Environ. Res. Public Health* **2014**, *11*, 2125-2147 ASB2014-3085	N	LIT
KIIA 5.10 (OECD)	John, B.	2014	Letter to the editor Food and Chemical Toxicology:65 (2014) 391 ASB2014-9584	N	LIT
KIIA 5.10 (OECD)	Kachuri, L.; Demers, P. A.; Blair, A. et al.	2013	Multiple pesticide exposures and the risk of multiple myeloma in Canadian men Int. J. Cancer: 133, 1846–1858 (2013) ASB2014-8030	N	LIT
KIIA 5.10 (OECD)	Kamel, F.; Umbach, D. M.; Bedlack, R. S. et al.	2012	Pesticide exposure and amyotrophic lateral sclerosis NeuroToxicology 33 (2012) 457–462 ASB2014-9586	N	LIT

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KIIA 5.10 (OECD)	Kelce, W.R., Lamb, J.C., DeSesso, J.M.	2010	A Critique of prepubertal exposur eto commercial formulation of the herbicide glyphosate GLP: N, published: Y 2309848 / ASB2012-11867	N	LIT
KIIA 5.10 (OECD)	Kier, L. D.; Kirkland, D. J.	2013	Review of genotoxicity studies of Glyphosate and Glyphosate-based formulations Crit Rev Toxicol, 2013; 43(4): 283–315 ASB2014-9587	N	LIT
KIIA 5.10 (OECD)	Kilinc, N.; Isgör, M. M.; Sengül, B. et al.	2013	Influence of pesticide exposure on carbonic anhydrase II from sheep stomach Toxicology and Industrial Health 1–8 ASB2014-9588	N	LIT
KIIA 5.10 (OECD)	Kim, J.; Ko, W.; Lee, W. J.	2013	Depressive symptoms and severity of acute occupational pesticide poisoning among male farmers Occup Environ Med 2013;0:1–7 ASB2014-9592	N	LIT
KIIA 5.10 (OECD)	Kim, Y.; Hong, J.; Gil, H. et al.	2013	Mixtures of Glyphosate and surfactant TN20 accelerate cell death via mitochondrial damage-induced apoptosis and necrosis Toxicology in Vitro 27 (2013) 191–197 ASB2014-9591	N	LIT
KIIA 5.10 (OECD)	Kitazawa, T.		IET historical control data on milignant lymphoma incidence in control ICR (Crj:CD-1) mice HR-001: Carcinogenicity study in mice (IET 94-0151) 13-C015 ASB2014-9146		
KIIA 5.10 (OECD)	Knezevic, V.; Bozic, D.; Budosan, I. et al.	2012	Early continuous dialysis in acute Glyphosate-surfactant poisoning Srp Arh Celok Lek. 2012 Sep-Oct;140(9-10):648-652 ASB2014-9593	N	LIT
KIIA 5.10 (OECD)	Koller, V. J.; Fürhacker, M.; Nersesyan, A. et al.	2012	Cytotoxic and DNA-damaging properties of Glyphosate and Roundup in human-derived buccal epithelial cells Arch Toxicol (2012) 86:805–813 ASB2014-7618	N	LIT
KIIA 5.10 (OECD)	Koureas, M.; Tsezou, A.; Tsakalof, A. et al.	2014	Increased levels of oxidxative DNA damage in pesticide sprayers in Thessaly Region (Greece). Implications of pesticide exposure Science of the Total Environment 496 (2014) 358–364 ASB2014-9724	N	LIT

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KIIA 5.10 (OECD)	Koutros, S.; Andreotti, G.; Berndt, S. I. et al.	2011	Xenobiotic-metabolizing gene variants, pesticide use, and the risk of prostate cancer Pharmacogenetics and Genomics 2011, Vol 21 No 10 ASB2014-9594	N	LIT
KIIA 5.10 (OECD)	Krüger, M.; Schrödl, W.; Neuhaus, J.; Shehata, A. A.;	2013	Field investigations of glyphosate in urine of Danish dairy cows J Environ Anal Toxicol, 3:5 ASB2013-11599	N	---
KIIA 5.10 (OECD)	Krüger, M.; Shehata, A. A.; Schrödl, W.; Rodloff, A.;	2013	Glyphosate suppresses the antagonistic effect of *Enterococcus* spp. on *Clostridium botulinum* in Press, Anaerobe 20: 74-78 (2013) ASB2013-8527	N	---
KIIA 5.10 (OECD)	Krüger, M.; Schledorn, P.; Schrödl, W.; Hoppe, H. W.; Lutz, W.; Shehata, A. A.;	2014	Detection of Glyphosate residues in animals and humans J Environ Anal Toxicol 2014, 4:2 ASB2014-5024	N	LIT
KIIA 5.10 (OECD)	Krüger, M.;Große-Herrenthey, A.; Schrödl, W.; Gerlach, A.; Rodloff, A.;	2012	Visceral botulism at dairy farms in Schleswig Holstein, Germany - Prevalence of *Clostridium botulinum* in feces of cows, in animal feeds, in feces of the farmers, and in house dust Anaerobe 18 (2012) 221-223 ASB2013-13312	N	---
KIIA 5.10 (OECD)	Krüger, M.; Schrödl, W.; Pedersen, I; Shehata, A. A.	2014	Detection of Glyphosate in malformed piglets J Environ Anal Toxicol 2014, 4:5 ASB2014-8935	N	LIT
KIIA 5.10 (OECD)	Kumar, S.	2011	Occupational, environmental and lifestyle factors associated with spontaneous abortion Reproductive Sciences 18(10) 915-930 ASB2014-9725	N	LIT
KIIA 5.10 (OECD)	Kwiatkowska, M.; Huras, B.; Bukowska, B.	2014	The effect of metabolites and impurities of Glyphosate on human erythrocytes (in vitro) Pesticide Biochemistry and Physiology 109 (2014) 34–43 ASB2014-9603	N	LIT
KIIA 5.10 (OECD)	Kwiatkowska, M.; Nowacka-Krukowska, H.; Bukowska, B.	2014	The effect of Glyphosate, its metabolites and impurities on erythrocyte acetylcholinesterase activity Environ. Toxicol. Pharmacol. 37 (2014) 1101–1108 ASB2014-8085	N	LIT

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KIIA 5.10 (OECD)	Labite, H.; Cummins, E.	2012	A quantitative approach for ranking human health risks from pesticides in Irish groundwater Human and Ecological Risk Assessment, 18: 1156–1185, 2012 ASB2014-9604	N	LIT
KIIA 5.10 (OECD)	Lamb, J. C.; Bofetta, P.; Foster, W. G. et al.	2014	Critical comments on the WHO-UNEP state of the science of endocrine disrupting chemicals – 2012 Regulatory Toxicology and Pharmacology 69 (2014) 22–40 ASB2014-9605	N	LIT
KIIA 5.10 (OECD)	Larsen, K.; Najle, R.; Lifschitz, A.; Virkel, G.	2012	Effects of sub-lethal exposure of rats to the herbicide Glyphosate in drinking water: Glutathione transferase enzyme activities, levels of reduced Glutathione and lipid peroxidation in liver, kidneys and small intestine Environ. Toxicol. Pharmacol. 34(2012)811-818 ASB2014-6905	N	LIT
KIIA 5.10 (OECD)	Larsen, K.; Najle, R.; Lifschitz, A. et al.	2014	Effects of sublethal exposure to a Glyphosate-based herbicide formulation on metabolic activities of different xenobiotic-metabolizing enzymes in rats International Journal of Toxicology 2014, Vol. 33(4) 307-318 ASB2014-9606	N	LIT
KIIA 5.10 (OECD)	Lee, B. K.; Lee, H. K.; Ryu, H. H. et al.	2012	Continuous renal replacement therapy in a patient with cardiac arrest after Glyphosate-surfactant herbicide poisoning Hong Kong Journal of Emergency Medicine ASB2014-9607	N	LIT
KIIA 5.10 (OECD)	LeFew, W. R.; McConnell, E. R.; Crooks, J. L. et al.	2013	Evaluation of microelectrode array data using Bayesian modeling as an approach to screening and prioritization for neurotoxicity testing NeuroToxicology 36 (2013) 34–41 ASB2014-9608	N	LIT
KIIA 5.10 (OECD)	Lesmes-Fabian, C.; García-Santos, G.; Leuenberger, F. et al.	2012	Dermal exposure assessment of pesticide use: The case of sprayers in potato farms in the Colombian highlands Science of the Total Environment 430 (2012) 202–208 ASB2014-9726	N	LIT

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KIIA 5.10 (OECD)	Levine, S.	2012	EDSP assays and regulatory safety studies provide a weight of evidence that Glyphosate is not an endocrine disruptor SETAC North America 33rd Annual Meeting ASB2014-9609	N	LIT
KIIA 5.10 (OECD)	Lopez, S. L.; Aiassa, D.; Benitez-Leite, S.; Lajmano-vich, R.; Manas, F.; Poletta, G.; Sanchez, N.; Simoniello, M. F.; Carrasco, A. E.;	2012	Pesticides used in South American GMO-based agriculture: A review of their effects on humans and animal models Advances in Molecular Toxicology, Volume 6, 41-75 doi.org/10.1016/B978-0-444-59389-4.00002-1 ASB2013-10534	N	---
KIIA 5.10 (OECD)	Malhotra, R.C., Ghia, D.K., Cordato, D.J., Beran, R.G.	2010	Glyphosate-surfactant herbicide-induced re-versible encephalopathy Case Reports / Journal of Clinical Neurosci-ence 17 (2010) 1472–1473 ASB2012-11890	N	LIT
KIIA 5.10 (OECD)	Manas, F., Peralta, L., Raviolo, J., Ovando, H.G., Weyers, A., Ugnia, L., Cid, M.G., Larripa, I., Gorla, N.	2009	Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests Ecotoxicology and Environmental Safety 72, 834-837 GLP: N, published: Y 2309906 / ASB2012-11891	N	LIT
KIIA 5.10 (OECD)	Mandel, J.S., Alexander, B.H., Baker, B.A., Acquavella, J.F., Chapman, P., Honeycutt, R.	2005	Biomonitoring for farm families in the farm family exposure study Scand J Work Environ Health 31, 98-104 GLP: N, published: Y 2309910 / ASB2012-11893	N	LIT
KIIA 5.10 (OECD)	Mañas, F.; Pe-ralta, L.; Ugnia, L. et al.	2013	Oxidative stress and comet assay in tissues of mice administered Glyphosate and Ampa in drinking water for 14 days Journal of Basic & Applied Genetics | 2013 | Vol-ume 24 | Issue 2 | Article 7 ASB2014-6909		
KIIA 5.10 (OECD)	Manfo, F. P. T.; Moundipa, P. F.; Déchaud, H. et al.	2010	Effect of agropesticides use on male reproduc-tive function: A study on farmers in Djutitsa (Cameroon) Environmental Toxicology ASB2014-9611		

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KIIA 5.10 (OECD)	Markard, C.;	2014	Umweltprobenbank des Bundes. Ergebnisse der Vorstudie "HBM von Glyphosat" II 1.2-93404/21 ASB2014-2057		
KIIA 5.10 (OECD)	Marc, J.; Mulner-Lorillon, O.; Boulben, S. et al.	2002	Pesticide Roundup provokes cell division dysfunction at the level of CDK1/Cyclin B activation Chemical Research in Toxicology 15:326-331 (2002) ASB2013-9838 / ASB2009-9012	N	---
KIIA 5.10 (OECD)	Marc, J., Belle, R., Morales, J., Cormier, P., Mulner-Lorillon, O.	2004	Formulated glyphosate activates the DNA-response checkpoint of the cell cycle leading to the prevention of G2/M transition Toxicological Sciences 82, 436-442 GLP: N, published: Y 2309912 / ASB2012-11894	N	LIT
KIIA 5.10 (OECD)	Marc, J., Mulner-Lorillon, O., Belle, R.	2004	Glyphosate-based pesticides affect cell cycle regulation Biology of the Cell 96, 245-249 GLP: N, published: Y 2309914 / ASB2009-9014	N	LIT
KIIA 5.10 (OECD)	Marc, J., Mulner-Lorillon, O., Durand, G., Belle, R.	2003	Embryonic cell cycle for risk assessment of pesticides at the molecular level Environmental Chemistry Letters 1, 8-12 GLP: N, published: Y 2309916 / ASB2009-9013	N	LIT
KIIA 5.10 (OECD)	Mariager, T. P.; Madsen, P. V.; Ebbehoj, N. E. et al.	2013	Severe adverse effects related to dermal exposure to a Glyphosate-surfactant herbicide Clinical Toxicology (2013), 51, 111–113 ASB2014-9612		
KIIA 5.10 (OECD)	Martini, C. M.; Gabrielli, M.	2012	A commercial formulation of Glyphosate inhibits proliferation and differentiation to adipocytes and induces apoptosis in 3T3-L1 fibroblasts Toxicology in Vitro 26 (2012) 1007–1013 ASB2014-9613		
KIIA 5.10 (OECD)	McConnell, E. R.; McClain, M. A.; Ross, J. et al.	2012	Evaluation of multi-well microelectrode arrays for neurotoxicity screening using a chemical training set NeuroToxicology 33 (2012) 1048–1057 ASB2014-9615		

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KIIA 5.10 (OECD)	McQueen, H., Callan, A.C., Hinwood, A.L.	2012	Estimating maternal and prenatal exposure to glyphosate in the community setting. International Journal of Hygiene and Environmental Health GLP: N, published: Y 2309926 / ASB2012-11898	N	LIT
KIIA 5.10 (OECD)	Mesnage, R., Clair, E., Gress, S., Then, C., Székács, A., Séralini, G.E.	2012	Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide Journal of Applied Toxicology:n/a-n/a GLP: N, published: Y 2309930 / ASB2012-11900	N	LIT
KIIA 5.10 (OECD)	Mesnage, R.; Moesch, C.; Le Grand, R. et al.	2012	Glyphosate exposure in a farmer's family Journal of Environmental Protection, 2012, 3, 1001-1003 ASB2014-3846		
KIIA 5.10 (OECD)	Mesnage R.; Defarge N.; Spiroux de Vendômois et al.	2014	Major pesticides are more toxic to human cells than their declared active principles Hindawi Publishing Corporation, BioMed Reserch International Vol 2014 ASB2014-1755		
KIIA 5.10 (OECD)	Mesnage, R.; Séralini, G.-E.	2014	The need for a closer look at pesticide toxicity during GMO assessment Practical Food Safety: Contemporary Issues and Future Directions, First Edition. Edited by Rajeev Bhat and Vicente M. Gómez-López. ASB2014-9616		
KIIA 5.10 (OECD)	Mink, P. J.; Mandel, J. S.; Sceurman, B. K. et al.	2012	Epidemiologic studies of Gyphosate and cancer: A review Regulatory Toxicology and Pharmacology 63 (2012) 440–452 ASB2014-9617		
KIIA 5.10 (OECD)	Modesto, K. A.; Martinez, C. B. R.	2010	Effects of roundup transorb on fish: Hematology, antioxidant defenses and acetylcholinesterase activity Chemosphere 81 (2010) 781–787 ASB2012-811		
KIIA 5.10 (OECD)	Moreno, N. C.; Sofia, S. H.; Martinez, C. B. R.;	2014	Genotoxic effects of the herbicide Round-upTransorb®and its active ingredient glyphosate onthe fish Prochilodus lineatus Environ. Toxicol. Pharmacol. 37 (2014) 448–454 ASB2014-7522		

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KIIA 5.10 (OECD)	Mose, T., Kjaerstad, M.B., Mathiesen, L., Nielsen, J.B., Edelfors, Sn., Knudsen L.E.	2008	Placental passage of benzoic acid, caffeine, and glyphosate in an ex vivo human perfusion system Journal of Toxicology and Environmental Health-Part a-Current Issues 71, 984-991 GLP: N, published: Y 2309958 / ASB2012-11914	N	LIT
KIIA 5.10 (OECD)	Mostafalou, S.; Abdollahi, M.	2013	Pesticides and human chronic diseases: Evidences, mechanisms, and perspectives Toxicology and Applied Pharmacology 268 (2013) 157–177 ASB2014-9618		
KIIA 5.10 (OECD)	Mulet, J.M.	2011	Letter to the Editor Regarding the Article by Paganelli et al. Chemical Research in Toxicology 24, 609 GLP: N, published: Y 2309962 / ASB2012-11916	N	MOD
KIIA 5.10 (OECD)	NABU	2011	Glyphosat & Ag rogentechnik - Risiken des Anbaus herbizidresistenter Pflanzen für Mensch und Umwelt Ausgabe: 04/2011 ASB2012-8016		
KIIA 5.10 (OECD)	Narayan, S.; Liew, Z.; Paul, K. et al.	2013	Household organophosphorus pesticide use and Parkinson's disease International Journal of Epidemiology 2013;1–10 ASB2014-9620		
KIIA 5.10 (OECD)	Niemann, L.; Sieke, C.; Pfeil, R.; Solecki, R.;	2014	A critical review of glyphosate findings in human urine samples and comparison with the exposure of operators and consumers ~~*in Press*~~ *J. Verbr. Lebensm., (2015) 10:3-12* ASB2014-11029		
KIIA 5.10 (OECD)	Omran, N. E.; Salama, W. M.;	2013	The endocrine disrupter effect of atrazine and glyphosate on Biomphalaria alexandrina snails Toxicology and Industrial Health 1–10 ASB2014-7614		
KIIA 5.10 (OECD)	Paumgartten, F.J.R. Cremonese, C. Freiere, C. Meyer, A. Koiman, S.	2012	Pesticide exposure and poor pregnancy outcomes: weaknesses of the evidence // Exposição a agrotóxicos e resultados adversos da gravidez: a fragilidade da evidência Cad. Saúde Pública, Rio de Janeiro, 28(10):2009-2012, out, 2012 ASB2013-10538	N	---

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KIIA 5.10 (OECD)	Paganelli, A., Gnazzo, V., Acosta, H., Lopez, S.L., Carrasco, A.E.	2010	Glyphosate-Based Herbicides Produce Teratogenic Effects on Vertebrates by Impairing Retinoic Acid Signaling Chem Res Toxicol 23, 1586-1595 GLP: N, published: Y 2309994 / ASB2012-11986, ASB2010-11410	N	LIT
KIIA 5.10 (OECD)	Pahwa, P. P.; Karunanayak, C. P.; Dosman, J. A. et al.	2011	Soft-tissue sarcoma and pesticides exposure in men results of a canadian case-control study JOEM _ Volume 53, Number 11, November 2011 ASB2014-9625		
KIIA 5.10 (OECD)	Palma, G.	2011	Letter to the Editor Regarding the Article by Paganelli et al. Chemical Research in Toxicology 24, 775-776 GLP: N, published: Y 2310000 / ASB2012-11989	N	LIT
KIIA 5.10 (OECD)	PANAP	2009	Glyphosate - Summary ASB2012-8017		
KIIA 5.10 (OECD)	Perry, L.; Adams, R. D.; Benett, A. R. et al.	2014	National toxicovigilance for pesticide exposures resulting in health care contact – An example from the UK's National poisons information service Clinical Toxicology (2014), 52, 549–555 ASB2014-9626		
KIIA 5.10 (OECD)	Quassinti, L., Maccari, E., Murri, O., Bramucci, M.	2009	Effects of paraquat and glyphosate on steroidogenesis in gonads of the frog Rana esculenta *in vitro* Pesticide Biochemistry and Physiology 93, 91-95 GLP: N, published: Y 2310038 / ASB2012-12007	N	MOD
KIIA 5.10 (OECD)	Razi, M.; Najaft, G.; Feyzi, S. et al.	2012	Histological and histochemical effects of Glyphosate on testicular tissue and function Iran J Reprod Med Vol. 10. No. 3. pp: 181-192, May 2012 ASB2014-9390		
KIIA 5.10 (OECD)	Relyea, A.R.	2005	The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities Ecological Applications, 15(2), 2005, pp. 618–627 ASB2012-204		

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KIIA 5.10 (OECD)	Relyea, A.R.	2012	New effects of Roundup on amphibians: Predators reduce herbicide mortality; herbicides induce antipredator morphology Ecological Applications, 22(2), 2012, pp. 634–647 ASB2012-2791		
KIIA 5.10 (OECD)	Rhomberg, L. R.; Goodman, J. E.	2012	Low-dose effects and nonmonotonic dose–responses of endocrine disrupting chemicals: Has the case been made? Regulatory Toxicology and Pharmacology 64 (2012) 130–133 ASB2014-9391		
KIIA 5.10 (OECD)	Richard, S., Moslemi, S., Sipahutar, H., Benachour, N., Seralini, G.E.	2005	Differential effects of glyphosate and roundup on human placental cells and aromatase Environmental Health Perspectives 113, 716-720 GLP: N, published: Y 2310042 / ASB2009-9024 / TOX2005-1743	N	LIT
KIIA 5.10 (OECD)	Riede, S.; Schafft, H.; Lahrssen-Wiederholt, M.; Breves, G.;	2014	Effects of a glyphosate-based herbicide on in vitro ruminal fermentation and microbial community with special attention to clostridia. Proc. Soc. Nutr. Physiol. ; VOL 23 2014; 23; 34 Gesellschaft fur Ernahrungsphysiologie Conference; 68th, Gesellschaft fur Ernahrungsphysiologie ASB2013-14684		
KIIA 5.10 (OECD)	Roberts, J. R.; Karr, C. J.	2012	Pesticide exposure in children PEDIATRICS Volume 130, Number 6, December 2012 ASB2014-9394		
KIIA 5.10 (OECD)	Rodloff, A. C.; Krüger, M.;	2012	Chronic *Clostridium botulinum* infections in farmers Anaerobe 18 (2012) 226-228 ASB2013-13311	N	---
KIIA 5.10 (OECD)	Romano, M., Romano, R., Santos, L., Wisniewski, P., Campos, D., de Souza, P., Viau, P., Bernardi, M., Nunes, M., de Oliveira, C.	2012	Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression Archives of Toxicology 86, 663-673 GLP: N, published: Y 2310048 / ASB2012-12011	N	LIT

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KIIA 5.10 (OECD)	Romano, R.M., Romano, M.A., Bernardi, M.M., Furtado, P.V., Oliveira, C.A.	2010	Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology Arch Toxicol 84, 309317 GLP: N, published: Y 2310050 / ASB2012-12012	N	LIT
KIIA 5.10 (OECD)	Romano, M. A.; Romano, R. M.	2012	Reply to comment of John M. DeSesso and Amy L. Williams regarding "Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression" by Romano et al. 2012 Arch Toxicol (2012) 86:1795–1797 ASB2014-9396		
KIIA 5.10 (OECD)	Rosanoff, A.	2014	Letter to the editor Food and Chemical Toxicology: 65 (2014) 389 ASB2014-9397		
KIIA 5.10 (OECD)	Roberfroid, M.	2014	Letter to the editor Food and Chemical Toxicology: 65 (2014) 390 ASB2014-9393		
KIIA 5.10 (OECD)	Roberfroid, M.	2014	Letter to the editor Food and Chemical Toxicology: 66 (2014) 385 ASB2014-9392		
KIIA 5.10 (OECD)	Roustan, A.; Aye, M.; De Meo, M.; Di Giorgio, C.;	2014	Genotoxicity of mixtures of glyphosate and atrazine and their environmental transformation products before and after photoactivation Chemosphere 108 (2014) 93–100 ASB2014-8086		
KIIA 5.10 (OECD)	[redacted]	1987	The subacute oral toxicity of the isopropylamine salt of glyphosate (MON 0139) in female cattle 82002 ! VT-82-003 TOX9552424	N	---
KIIA 5.10 (OECD)	[redacted]	1987	The subacute toxicity of Roundup herbicide (MON-2139) in female cattle 82001 ! VT-82-002 ASB2010-8131	N	---

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KIIA 5.10 (OECD)	[redacted]	1987	The acute toxicity of glyphosate in female goats 80006 ! VT-80-450 TOX9552422	N	---
KIIA 5.10 (OECD)	[redacted]	1987	The acute oral toxicity of the isopropylamine salt of glyphosate (MON 0139) in female goats 80007 ! VT-80-451 TOX9552423	N	---
KIIA 5.10 (OECD)	Saltmiras, D., Bus, J.S., Spanogle, T., Hauswirth, J., Tobia, A., Hill, S.	2011	Letter to the Editor Regarding the Article by Paganelli et al. Chemical Research in Toxicology 24, 607-608 GLP: N, published: Y 2310056 / ASB2012-12015	N	LIT
KIIA 5.10 (OECD)	Saltmiras, D.A., Tobia, A.	2012	No Evlidence of Endocrine Disruption by Glyphosate in Hershberger and Uterotrophic Assays. Abstract PS 2198 The Toxicologist (supplement to Toxicological Sciences) 126, 474 GLP: N, published: Y 2310058 / ASB2012-12016	N	LIT
KIIA 5.10 (OECD)	Samsel, A.; Seneff, S.	2013	Glyphosate's suppression of Cytochrome P450 enzymes and amino acid biosynthesis by the Gut Microbiome: Pathways to modern diseases Entropy 2013, 15, 1416-1463 ASB2013-8535		
KIIA 5.10 (OECD)	Savitz, D.A., Arbuckle, T., Kaczor, D., Curtis, K.M.	1997	Male pesticide exposure and pregnancy outcome American Journal of Epidemiology 146, 1025-1036 GLP: N, published: Y 2310070 / ASB2012-12022	N	LIT
KIIA 5.10 (OECD)	Schinasi, L.; Leon, M. E.;	2014	Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: A systematic review and meta-analysis Int. J. Environ. Res. Public Health 2014, 11, 4449-4527 ASB2014-4819		

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KIIA 5.10 (OECD)	Seneff, S.; Lauritzen, A.; Davidson, R. M. et al.	2013	Is encephalopathy a mechanism to renew sulfate in autism? Entropy 2013, 15, 372-406 ASB2014-9729		
KIIA 5.10 (OECD)	Sengupta, P.; Banerjee, R.	2013	Environmental toxins: Alarming impacts of pesticides on male fertility Human and Experimental Toxicology 1-23 ASB2014-9730		
KIIA 5.10 (OECD)	Séralini, G. E.	2014	Conclusiveness of toxicity data and double standards Food and Chemical Toxicology 69 (2014) 357–359 ASB2014-9632		
KIIA 5.10 (OECD)	Seyboldt, C.; Hoedemaker, M.;	2014	Bedeutung von Clostridium botulinum bei chronischem Krankheitsgeschehen und Teilprojekt: Mikrobiologisches Risikopotenzial von Biogasanlagen unter besonderer Berücksichtigung von Hühnertrockenkot als Gärsubstrat. Abschlussbericht Projekt: 2810HS005 Tierärztliche Hochschule Hannover ASB2014-10736		
KIIA 5.10 (OECD)	Shehata, A. A.; Schrödl, W.; Aldin, A. A.; Hafez, H. M.; Krüger, M.;	2012	The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro Curr Microbiol, published online 09.12.2012 ASB2012-16301	N	---
KIIA 5.10 (OECD)	Shehata, A.; Schrödl, W.; Neuhaus, J.; Krüger, M.;	2012	Antagonistic effect of different bacteria on *Clostridium botulinum* types A, B, D and E *in vitro* Downloaded from veterinaryrecord.bmj.com on December 19, 2012 ASB2013-8529	N	---
KIIA 5.10 (OECD)	Sirinathsinghji, E.;	2014	Sri Lanka Partially Bans Glyphosate for Deadly Kidney Disease Epidemic ISIS Report 09/04/14 ASB2014-10742		
KIIA 5.10 (OECD)	Sørensen, M. T.; Damgaard Poulsen, H.; Højberg, O.	2014	Memorandum on "The feeding of genetically modified Glyphosate resistant soy products to livestock" ASB2014-5761		

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KIIA 5.10 (OECD)	Song, H. Y.; Kim, J. H.; Seok, S. J.; Gil, H. W.; Hong, S, Y.;	2012	*In vitro* cytotoxic effect of glyphosate mixture containing surfactants J Korean Med Sci 2012; 27: 711-715 ASB2013-10531	N	---
KIIA 5.10 (OECD)	Sribanditmong-kol, P.; Jutavijit-tum, P.; Pon-graveevongsa, P.; Wunnapuk, K.; Durongka-dech, P.	2012	Pathological and toxicological findings in Glyphosate-surfactant herbicide fatality Am J Forensic Med Pathol & Volume 33, Number 3, September 2012 ASB2014-9731		
KIIA 5.10 (OECD)	Sugeng, A. J.; Beamer, P. I.; Lutz, E. A. et al.	2013	Hazard-ranking of agricultural pesticides for chronic health effects in Yuma County, Arizo-na Science of the Total Environment 463-464 (2013) 35–41 ASB2014-9733		
KIIA 5.10 (OECD)	Takahashi, H.; Kakinuma, Y.	1992	Ammonium salt of glyphosate (MON 8750) general pharmacology study IET 90-0149/ET-92-15 TOX9552421	N	---
KIIA 5.10 (OECD)	Thongprakai-sang, S.; Thi-antanawat, A.; Rangkadilok, N.; Suriyo, T.; Satayavivad, J.;	2013	Glyphosate induces human breast cancer cells growth via estrogen receptors Food and Chemical Toxicology, 59(2013)129–136 ASB2013-11991	N	---
KIIA 5.10 (OECD)	Tizhe, E. V.; Ibrahim, N. D. G.; Fatihu, M. Y. et al.	2013	Haematogical changes induced by subchronic Glyphosate exposure: Ameliorative effect of zinc in Wistar rats Sokoto Journal of Veterinary Sciences, Vol-ume 11 (Number 2). December, 2013 ASB2014-6963		
KIIA 5.10 (OECD)	Tizhe, E. V.; Ibrahim, N. D. G.; Fatihu, M. Y. et al.	2013	Influence of zinc supplementation on histo-pathological changes in the stomach, liver, kidney, brain, pancreas and spleen during sub-chronic exposure of Wistar rats to Glyphosate Comp Clin Pathol (2013) ASB2014-6965		
KIIA 5.10 (OECD)	Tizhe, E. V.; Ibrahim, N. D. G.; Fatihu, M. Y. et al.	2013	Serum biochemical assessment of hepatic and renal functions of rats during oral exposure to Glyphosate with zinc Comp Clin Pathol (2014) 23:1043–1050 ASB2014-6964		

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KIIA 5.10 (OECD)	Vandenberg, L. N.; Colborn, T.; Hayer, T. B. et al.	2012	Hormones and endocrine-disrupting chemicals: Low-dose effects and nonmonotonic dose responses Endocrine Reviews, June 2012, 33(3):378–455 ASB2014-9635		
KIIA 5.10 (OECD)	Walsh, L.P., McCormick, C., Martin, C., Stocco, D.M.	2000	Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression Environmental Health Perspectives 108, 769-776 GLP: N, published: Y 2310118 / ASB2012-12046	N	LIT
KIIA 5.10 (OECD)	Wigle, D. T.; Arbuckle, T. E.; Turner, M. C.; Bérube, A.; Yang, Q.; Liu, S.; Krewski, D.	2008	Epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants Journal of Toxicology and Environmental Health, Part B, 11:373–517, 2008 ASB2014-9637		
KIIA 5.10 (OECD)	Williams, A.L., Watson, R.E., DeSesso, J.M.	2012	Developmental and Reproductive Outcomes in Humans and Animals After Glyphosate Exposure: A Critical Analysis Journal of Toxicology and Environmental Health, Part B 15, 39-96 GLP: N, published: Y 2310130 / ASB2012-12052	N	LIT
KIIA 5.10 (OECD)	[redacted]	1996	Glyphosate Technical: Pharmacology Screening Study in the Rat 434/021 NUF GLP: Y, published: N 2310134 / ASB2012-12054	Y	NUF
KIIA 5.10 (OECD)	Wunnapuk, K.; Gobe, G.; Endre Z. et al.	2013	Use of a glyphosate-based herbicide-induced nephrotoxicity model toinvestigate a panel of kidney injury biomarkers Toxicology Letters 225 (2014) 192– 200 ASB2014-9638		
KIIA 5.10 (OECD)	Xia, S.; Zhao, Y.-B.; Yang, M.-Q. et al.	2013	Induction of vitellogenin gene expression in medaka exposed to Glyphosate and potential molecular mechanism China Environmental Science 2013, 33(9): 1656-1663 ASB2014-9642		

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KIIA 5.10 (OECD)	Yang, W.; Carmichael, S. L.; Roberts, E. M. et al.	2013	Residential agricultural pesticide exposures and risk of neural tube defects and orofacial clefts among offspring in the San Joaquin Valley of California American Journal of Epidemiology ASB2014-9644		
KIIA 5.10 (OECD)	Yousef, M.I., Salem, M.H., Ibrahim, H.Z., Helmi, S., Seehy, M.A., Bertheussen, K.	1995	Toxic effects of carbofuran and glyphosate on semen characteristics in rabbits Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes 30, 513-534 GLP: N, published: Y 2310142 / ASB2012-12058	N	LIT
KIIA 5.10 (OECD)	Zhang, Z.-L.; Yang, Z.-F.	2013	Research Progress on Rep roductive and Developmental Toxicity of Glyphosate J Environ o.:.:up Med. reb.2013 Vol.30 "",,.2 ASB2014-9643		
KIIA 5.10 (OECD)	Zhao, W.; Yu, H.; Zhang, J. et al.	2013	Effects of Glyphosate on apoptosis and expressions of androgen-binding protein and vimentin mRNA in mouse Sertoli cells J South Med Univ, 2013, 33(11): 1709-1712 ASB2014-9645		
KIIA 5.10 (OECD)	Zouaoui, K.; Dulaurent, S.; Gaulier, J. M. et al.	2012	Determination of Glyphosate and AMPA in blood and urine from humans: About 13 cases of acute intoxication Forensic Science International (2012) ASB2014-9734		
KIIIA1 7.1.1 (OECD)	[redacted]	1991	Acute Oral Toxicity Study In Rats BD-91-261 MON GLP: Y, published: N 2315976 / TOX9552438	N	MOD
KIIIA1 7.1.2 (OECD)	[redacted]	1991	Acute Dermal Toxicity Study In Rats BD-91-262 MON GLP: Y, published: N 2315978 / TOX9552439	N	MOD
KIIIA1 7.1.3 (OECD)	Polveche, V . Rombaut, M. Bonicelli, B.	1999	Measurements of granulometry and distribution of a spray nozzle - Comparison of different glyphosate formulations 106/Pulv MON GLP: N, published: N 2315980 / ASB2012-12069	N	MOD
KIIIA1 7.1.3 (OECD)	[redacted]	1982	Acute inhalation toxicity of Roundup formulation to male and female Sprague-Dawley rats - incl. Amendment No. 1, Date: 15.12.1982 810093 ! ML-81-201 TOX2002-693	N	---

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KIIIA1 7.1.4 (OECD)	[redacted]	1991	Primary dermal irritation study in rabbits BD-91-263 MON GLP: Y, published: N 2315983 / TOX9552440	N	MOD
KIIIA1 7.1.5 (OECD)	[redacted]	1991	Primary eye irritation study in rabbits BD-91-60 MON GLP: Y, published: N 2315985 / TOX9552441	N	MOD
KIIIA1 7.1.6 (OECD)	[redacted]	1987	Genamin T-200 BM: A closed-patch repeated insult dermal sensitization study in guinea pigs - (Buehler method) 6816-86 ! BD-86-290 ASB2010-366	N	---
KIIIA1 7.1.6 (OECD)	Griffon, B.	2001	Skin sensitization test in guinea pigs (Modified Buehler test: 9 applications) CI-2001-153 MON GLP: Y, published: N 2315987 / TOX2005-1135	N	MOD
KIIIA1 7.4 (OECD)	Martin, S.; Westphal, D.; Erdtmann-Vourliotis, M.; Dechet, F.; Schulze-Rosario, C.; Stauber, F.; Wicke, H.; Chester, G.	2008	Guidance for exposure and risk evaluation for bystanders and residents exposed to plant protection products during and after application 2008/1070089 ! 1661-5751/00/000001-10 ! DOI 10.1007/s00003-008-0361-5 ASB2009-450	N	---
KIIIA1 7.5 (OECD)	Krebs. B.; Maasfeld, W.; Schrader, J.; Wolf, R.; Hoernicke, E.; Nolting, H. G.; Backhaus, G. F.; Westphal, D.	2000	Uniform Principles for safeguarding the health of workers re-entering crop-growing areas after application of plant protection product TOX2004-1971	N	---
KIIIA1 7.6.2 (OECD)	Davies, D.J.	2003	Glyphosate SL (360 g/L) Formulation (A12798Q): in vitro absorption through human epidermis CTL JV1732 SYN GLP: Y, published: N 2309514 / ASB2012-11518	Y	SYN

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KIIIA1 7.6.2 (OECD)	Hadfield, N.	2011	Glyphosate 360 IPA Salt (CA2273): *In Vitro* Absorption through Human Epidermis using [^{14}C]-glyphosate JV2147-REG NUF GLP: Y, published: N 2309512 / ASB2012-11517	Y	NUF
KIIIA1 7.6.2 (OECD)	EFSA	2012	Panel on Plant Protection Products and their Residues (PPR). Guidance on Dermal Absorption. EFSA Journal (2012), 10(4), 2665-2695, ASB2012-6959	N	---
KIIIA1 7.6.2 (OECD)	Franz, T.J.	1983	Evaluation of the percutaneous absorption of Roundup formulations in man using an *in-vitro* technique MON GLP: N, published: N 2309488 / TOX9552417	N	MON
KIIIA1 7.6.2 (OECD)	OECD	2011	Guidance notes on dermal absorption. Adopted 18 August 2011. Series on Testing and Assessment, No. 156. ENV/JM/MONO(2011)36, JT03305971. ASB2013-2 http://www.oecd.org/dataoecd/63/12/48532204.pdf.	N	---
KIIIA1 7.6.2 (OECD)	Ward, R.J.	2010	360 g/L Glyphosate SL Formulation (MON 52276) - *In vitro* absorption of glyphosate through human epidermis JV2084-REG MON GLP: Y, published: N 2315989 / ASB2012-5383	Y	MOD
KIIIA1 7.6.2 (OECD)	Ward	2010	450 g/L Glyphosate SL Formulation (MON 79545) - *In vitro* absorption of glyphosate through human epidermis JV2083-REG MON GLP: Y, published: N 2309508 / ASB2012-11515	Y	MON
KIIIA1 7.6.2 (OECD)	Ward	2010	480 g/L Glyphosate SL Formulation (MON 79351) - *In vitro* absorption of glyphosate through human epidermis JV2085-REG MON GLP: Y, published: N 2309510 / ASB2012-11516	Y	MON
KIIIA1 7.6.2 (OECD)	Wester, R. C.; Melendres, J.; Sarason, R.; McMaster, J.; Maibach, H. I.	1991	Glyphosate skin binding, absorption, residual tissue distribution and skin decontamination TOX9552418	N	---

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KIIIA1 7.6.2 (OECD)	Wester, R.C., Quan, D., Maibach, H.I., Wester, R.M.	2005	Percutaneous Absorption of Hazardous Chemicals from Fabric into and Through Human Skin. In Percutaneous Absorption: Drugs, Cosmetics, Mechanisms, Methods Boca Raton, FL. Taylor and Francis Group, LLC. 22, 303-310 GLP: N, published: Y 2310126 / ASB2012-12050	N	LIT
KIIIA1 7.6.3 (OECD)	EPA	2009	Alkyl Amine Polyalkoxylates; Exemption from the requirement of a tolerance Fed. Reg. 74(2009)115:28616 ASB2009-9022	N	---
KIIIA1 7.6.3 (OECD)	[redacted]	1973	G-3780: 14-week oral subacute study in dogs 33372 ! MRD-165 ! XX-95-336 ! MON 0818 ASB2009-9026	N	---
KIIIA1 7.6.3 (OECD)	Hahn, A.; Be-gemann, K.; Burger, R. Hillebrand, J, Meyer, H. Preußner, K. Gessener, M	2007	Ärztliche Mitteilungen bei Vergiftungen 2007, BfR ASB2013-4034	N	---
KIIIA1 7.6.3 (OECD)	Holson, J.F.	1989	A dose range-finding developmental toxicity study of MON 0818 in rats. WIL Research Labs., Ashland, Ohio, USA, on behalf of Monsanto. Project no. WIL-50042, Sponsor no. WI-88-304, unpublished; ASB2009-9028	N	---
KIIIA1 7.6.3 (OECD)	[redacted]	1990	A develomental toxicity study of MON 0818 in rats, Final report: WI-89-388 GLP: N, published: Y 2309808 / ASB2009-9029	N	LIT
KIIIA1 7.6.3 (OECD)	Jauhiainen, A.; Räsänen, K.; Sarantila, R.; Nuutinen, J.; Kangas, J.	1991	Occupational exposure of forest workers to glyphosate during brush saw spraying work American Industrial Hygiene Association Journal, 52(1991)2:61-64 MET9600092	N	---
KIIIA1 7.6.3 (OECD)	[redacted]	2007	A ReproductionDevelopmental Toxicity Screening Study of MON 0818 in Rats WIL-50282 GLP: Y, published: N 2309858 / ASB2010-365	Y	MOD

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[5]
KIIIA1 7.6.3 (OECD)	[redacted]	2008	A Combined 28-Day Repeated Dose Oral (Dietary) Toxicity Study with the Reproduction/Developmental Toxicity Screening Test of MON 8109 and MON 0818 in Rats WIL-50337 MON GLP: N, published: N 2309861 / ASB2010-364	N	MOD
KIIIA1 7.6.3 (OECD)	Martinez, T. T.; Long, W. C.; Hiller, R.	1990	Comparison of the toxicology of the herbicide Roundup by oral and pulmonary routes of exposure Z44833	N	---
KIIIA1 7.6.3 (OECD)	Stella, J., Ryan, M.	2004	Glyphosate herbicide formulation: a potentially lethal ingestion Emerg Med Australas 16, 235-239 GLP: N, published: Y 2310102 / ASB2012-12038	N	LIT
KIIIA1 7.6.3 (OECD)	[redacted]	1990	Ninety-day study of MON 0818 administered in feed to albino rats MSL-10468 ! ML-89-359/EHL 89161 ASB2009-9027	N	---
KIIIA1 7.6.3 (OECD)	Tai, T.; Yamashita, M.; Wakimori, H.	1990	Hemodynamic effects of Roundup, glyphosate and surfactant in dogs The Japanese Journal of Toxicology, 3, 63-68. TOX9552419	N	---
KIIIA1 7.6.3 (OECD)	[redacted]	1991	Subchronic toxicity study in rats with Atmer 163. Hazleton Washington, Inc., Vienna, Virginia, USA, on behalf of ICI Americas, submitted by Monsanto. HWA 564-162 ASB2009-10488	N	---
KIIIA1 7.9 (OECD)	BfR; Hahn, A.; Begemann, K.; Burger, R. et al.	2007	Ärztliche Mitteilungen bei Vergiftungen ISBN 3-938163-40-2 ! ISSN 1435-4047 ASB2014-9290		

Codes of owner

ALK	Alkaloida Europe
ALS	Alschu-Chemie GmbH
CHE	Cheminova A/S
EGT	European Glyphosate Task Force AIR 2
EXC	ExxonMobile Chemical Belgium
ADM	ADAMA Agan Ltd
HAG	Handelsgesellschaft für Baustoffe mbH & Co. KG
HEL	Helm AG
JCC	Jiangsu Changlong

LIT	Published literature
MAH	Makhteshim-AGAN Group
MOD	Monsanto Europe S.A./N.V.
MON	Montedison (Deutschland) Chemie Handels GmbH
NUF	Nufarm GmbH & Co. KG
SYN	Syntana Handelsgesellschaft

Studies marked in yellow are not part of the dossier for renewal.

Renewal Assessment Report

18 December 2013

Revised
29 January 2015
31 March 2015

Glyphosate

Volume 3

Annex B.7

Residue data

RMS: Germany **Co-RMS: Slovakia**

Version history (Volume 3, chapter B.7)

Date	**Reason for revision**
19 December 2013	Initial Renewal Assessment Report
29 January 2015	Updated following RAR peer review, taking into account the reporting table, the evaluation table and additional data
31 March 2015	Updated following Pestidices Peer Review 127 expert meeting (3-5 March 2015)

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B.7 Residue data

B.7.1 Metabolism, distribution and expression of residues in plants (Annex IIA 6.2.1; Annex IIIA 8.2)

The metabolism of glyphosate was investigated in a large variety of crops, including non-tolerant and tolerant crops (GOX, CP4 EPSPS and NAG). Metabolism studies involving ^{14}C-glyphosate were normally conducted using the phosphonomethyl-labell, unless specifically noted. Further labels used in some studies used ^{14}C-glycine- or ^{14}C-methane-glyphosate.

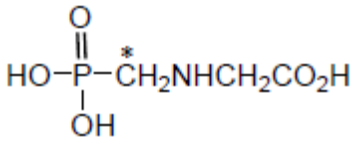

Figure B.7.1-1: ^{14}C-phosphonomethyl-labelled glyphosate

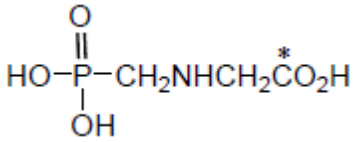

Figure B.7.1-2: ^{14}C-1-glycine-labelled glyphosate

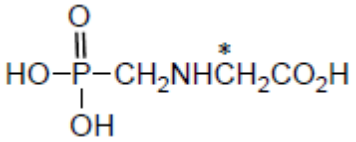

Figure B.7.1-3: ^{14}C-2-glycine-labelled glyphosate

B.7.1.1 Non-tolerant crops

B.7.1.1.1 Fruit crops

Reference: OECD KIIA 6.2.1

Report: Sutherland, M. L.
The metabolism of CP 67573 by citrus - February 1973 - October 1974
June 1975, Report No. 328, RIP9501194

Guidelines: none

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be acceptable.

Material and Methods:

In this study the uptake and translocation of ^{14}C-glyphosate and ^{14}C-AMPA in citrus was investigated. The active substances were applied to the soil of plant pots containing mandarins (calamondin) at rates equivalent to 2.24 kg as/ha. Alternatively the same amount of ^{14}C-glyphosate was applied to selected leaves simulating foliar treatment. After each month leaf samples were collected. At 4 months the study was terminated and soil, roots, stems, leaves, immature and mature fruits were collected.

A transpiration experiment was conducted to investigate the uptake of radioactivity from a hydroponic solution at 10 ppm (Glyphosate: 4.4 x 10^8 dmp or AMPA: 8.0 x 10^8 dpm) and the formation of $^{14}CO_2$. In addition the radioactivity in leaves, stem, roots and the rest of the plant was investigated after 1 or two weeks.

In a third experiment 4 mg ^{14}C-glyphosate was applied to leaves. After 1, 2, 3, 4, 6 and 8 weeks samples of treated leaves, untreated leaves, stems and fruits were collected.

All plant samples were extracted with water or extracted under reflux with hexane, diethylether and acetone. Total radioactive residues were analysed using LSC methods. The quantification and identification following aqueous extraction was performed by GC/MS following derivatisation to N-trifluoroacetyltrimethyl-esters. ion exchange chromatography or high voltage electrophoresis.

Findings:

In the following table the uptake of radioactivity following soil or foliar treatment equivalent to 2.24 kg as/ha was summarised.

Table B.7.1-1: Recovered radioactivity following foliar or soil treatment of citrus trees with ^{14}C-glyphosate or ^{14}C-AMPA at rates equivalent to 2.24 kg as/ha

Treatment	% AR										
	1 month leaves	2 months leaves	3 months leaves	4 months							
				leaves	soil	roots	stems	treated leaves	immature fruit	mature fruit	total
^{14}C-glyphosate (soil)	0.08	0.09	0.09	0.09	63.82	0.41	0.08	-	0.06	0.05	64.51
^{14}C-glyphosate (foliar)	0.27	1.01	0.29	0.76	10.52	0.29	0.41	11.92	0.26	1.3	25.46
^{14}C-AMPA (soil)	0.08	0.06	0.06	0.07	64.67	0.34	0.09	-	0.04	0.04	65.25

For the transpiration experiment recovered radioactivity was:

Table 7.1-2: Uptake and distribution of radioactivity following hydroponic application of ^{14}C-glyphosate or ^{14}C-AMPA at 10 ppm over two weeks

Fraction	**% AR**			
	Chamber 1 (1 week) Glyphosate (10 ppm) 4.4 x 10^8 dpm	Chamber 2 (2 weeks) Glyphosate (10 ppm): 4.4 x 10^8 dpm	Chamber 3 (1 week) AMPA (10 ppm): 8.0 x 10^8 dpm	Chamber 4 (2 weeks) AMPA (10 ppm): 8.0 x 10^8 dpm
Leaves				
1 week	1.3	-	1.8	-
2 weeks	-	1.2	-	0.8
Stems				
1 week	0.3	-	0.3	-
2 weeks	-	0.4	-	0.3
Roots				
1 week	4.2	-	5.5	-
2 weeks	-	2.6	-	2.5
Plant CO_2				
1 week	2.1	2.9	1.4	0.8
2 weeks	-	0.3	-	0.2
Nutrient Solution				
1 week	85.3	82.5	82.7	85.3
2 weeks	-	2.3[a]	-	3.3[a]
Total	95.2	92.2	91.7	93.2

a: after one week the nutrient solution was replaced by fresh, unlabelled nutrient solution

The long-term behaviour of radioactive residues following foliar treatment to leaves showed a strong loss in the accountability. No information on the total balance of radioactivity was provided in the study.

Table B.7.1-3: Recovered radioactivity following foliar application of 4 mg ^{14}C-glyphosate per citrus plants

Interval (weeks)	**% AR**				
	treated leaves	non-treated leaves	stems	fruit	accountability
1	76.6	1.8	1.3	<0.1	79.7
2	23.0	0.8	1.6	1.0	26.4
3	26.8	1.8	1.9	0.3	30.8
4	24.5	1.6	2.2	-	28.3
6	10.3	2.6	1.7	1.4	16.0
8	-	2.2	1.5	9.8	13.5

Conclusions:

In this study the uptake of glyphosate and AMPA via the roots was minimal. In addition application of glyphosate to the leaves also gave very low rates of translocation into untreated plant parts. The composition of the extracted radioactivity was stated to mainly consist of the unchanged substances applied, however no information on the results of the identification were presented in the study report.

Reference: OECD 6.2.1

Report: Nadeau, R. G.; Cozad, S. J.
Absorption, translocation and metabolism of Roundup herbicide in walnut, almond and pecan trees
April 1976, Report No. 403, RIP9501196

Guidelines: none

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study the uptake and metabolism of ^{14}C-glyphosate following soil treatment or foliar application to tree nut seedlings (almond, pecan and walnut) was investigated.
In the soil experiment 12.9 mg ^{14}C-glyphosate were applied to the surface of pots (18 cm diameter for pecan and walnut, 26 cm diameter for almonds). For the foliar treatment an amount of 100 µg ^{14}C-glyphosate was applied to the surface of two trees per variety. Samples were collected after 16 weeks for the soil treatment and after 2 or 5 week for the foliar treatment.

Total radioactive residues were analysed using LSC methods. The quantification and identification following aqueous extraction was performed by GC/MS following derivatisation to N-trifluoroacetyltrimethyl-esters. ion exchange chromatography or high voltage electrophoresis.

Findings:
For the soil application the uptake via the roots was limited, not resulting in residues sufficient for identification.

Table B.7.1-4: Total radioactive residues in tree nut plants following soil treatment 16 weeks before sampling

Sample	% AR		% extracted
	Tree 1	Tree 2	
Walnut, tops (treated)	0.13	0.14	11.5
Walnut, roots (treated)	0.28	0.19	19.8
Walnut, tops (control)	0.09	0.05	11.7
Walnut, roots (control)	0.16	0.07	12.3
Almond, tops (treated)	0.23	0.29	10.6
Almond, roots (treated)	0.14	0.08	29.8
Almond, tops (control)	0.29	0.29	9.1
Almond, roots (control)	0.14	0.14	14.2
Pecan, tops (treated)	0.36	0.19	11.4
Pecan, roots (treated)	0.25	0.27	17.4
Pecan, tops (control)	0.07	0.07	25.4
Pecan, roots (control)	0.04	0.10	15.8

Following foliar application the recovered radioactivity and the identified composition of the residues were:

Table B.7.1-5: Total recovered radioactivity, extractability and composition of ^{14}C-glyphosate in tree nut trees following foliar treatment

Sample	% AR		%	Composition (% extracted) following electrophoresis					
	Tree 1	Tree 2	extracted	Non-retard-ed	Neu-tral	Gly	AMPA	other	Total
Walnut, treated leaves (35 DAT)	64.5	47.7	82	2.0	-	77.1	8.0	9.0	96.1
Walnut, other tops (35 DAT)	5.8	8.2	26	26.1	-	70.3	4.6	-	101.0
Walnnut, roots (35 DAT)	27.4	14.8	48	4.2	-	87.0	4.0	-	95.2
Almond, treated leaves (35 DAT)	71.3	61.1	54	1.1	-	77.0	8.0	10.1	97.1
Almond, other tops (35 DAT)	14.5	12.6	26	37.8	4.8	52.7	9.5	-	104.8
Almond, roots (35 DAT)	6.6	8.2	79	11.7	-	79.3	5.9	-	96.9
Pecan, treated leaves (35 DAT)	54.0	53.1	72	1.0	-	86.2	6.0	6.3	99.5
Pecan, other tops (35 DAT)	10.0	4.2	70	9.1	-	88.2	2.0	-	99.3
Pecan, roots (35 DAT)	16.6	3.3	63	1.6	-	95.0	-	-	96.6
Walnut, treated leaves[a] (14 DAT)	60.5	45.0	103	1	-	92	3	5	101
Walnut, other tops[a] (14 DAT)	7.3	6.2	85	2	-	96	2	1	101
Walnnut, roots[a] (14 DAT)	10.4	23.5	88	1	-	97	2	1	101

a: repeated experiment

Conclusions:
For tree nut plants the uptake of glyphosate from the soil was very low, not resulting in TRR levels sufficient for further identification. After foliar treatment of single plants most of the radioactivity applied was located on the treated compartiments. The translocation into untreated plant parts was minor. Identification of the residue revealed mainly unchanged parent and minor levels of AMPA, being present <10% of the extracted radioactivity.

Reference: OECD KIIA 6.2.1

Report: Rueppel, M. L.; Moran, S. J.
CP 67573 residue and metabolism Part 23: The metabolism of CP 67573 in apple trees
Dec. 1974, Report No. 342, RIP9501190

Guidelines: none

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study the uptake and translocation of ^{14}C-radiolabelled glyphosate and ^{14}C-AMPA were investigated following foliar, trunk or soil treatment of dwarft apple trees (Golden Delicious variety) grown in 15 L buckets.

For the soil treatment stocking solutions were sprayed at rates equivalent to 3.4 kg glyphosate per ha or 1.7 kg AMPA per ha. After 6 and 12 weeks leaves, stems, branches and the trunk were sampled.
For the uptake via the trunk an amount of 92.4 µg/tree was applied and covered to prevent splashing of the area during watering. After 8 and 42 days samples were collected (leaves and stems, treated trunk, untreated trunk and branches and roots).
The behaviour of glyphosate following foliar application was investigated on trees five weeks after end of dormancy. On each tree shoots with at least 4-5 leaves were selected and treated with 5.356 µg ^{14}C-glyphosate "as evenly as possible with a syringe". After 4, 7 and 10 weeks samples of treated leaves, new growth above treatment, other new growth, branches, trunk and roots were collected.

All samples collected were homogenised and extracted with water or 0.5N NH_4OH. Total radioactive residues were determined using LSC. For the identification of metabolites GC-MS, TLC and NMR were used and the results compared with reference substances.

Findings:
In the following tables the recovered radioactivity in the various matrices is summarised.

Table B.7.1-6: Recovery of applied radioactivity following soil treatment to apple trees at rates equivalent to 3.4 kg ^{14}C-glyphosate or 1.7 kg ^{14}C-AMPA per ha

Applied compound	Interval	Leaves and stems (% applied)	Branches and trunk (% applied)	Total uptake (% applied)
Control[a]	6 weeks	0.0095	0.0043	0.0138
	12 weeks	0.02	0.009	0.029
^{14}C-glyphosate, 3.4 kg as/ha	6 weeks	0.0013	0.002	0.0033
	12 weeks	0.093	0.041	0.134
^{14}C-AMPA, 1.7 kg as/ha	6 weeks	0.0016	0.0018	0.0034
	12 weeks	0.059	0.011	0.07

a: control plants grown in same greenhouse as treated plants

Table B.7.1-7: Recovery of applied radioactivity following trunk treatment to apple trees with 92.4 µg ^{14}C-glyphosate per tree

Applied dose in DPM	Recovered dose in DPM (% applied)				
	Stem and leaves	Untreated trunk	Roots	Treated trunk	Total recovered
9,514,880	7,550 (0.08)	8,240 (0.08)	10,080 (0.1)	6,872,760 (72.05)	6,897,630 (72.31)

Table B.7.1-8: Recovery of applied radioactivity following foliar treatment to four apple leaves with 5.356 µg ^{14}C-glyphosate

Interval	% applied					
	treated leaves	new growth above treatment	other new growth	branches and trunk	roots	total recovered
7 days	64.86	3.24	0.33	0.89	0.94	70.26
21 days	75.06	2.32	3.87	1.78	0.53	83.56
28 days	45.06	3.56	1.68	1.68	3.17	55.25
49 days	60.03	4.13	2.84	2.31	2.19	71.61
70 days	60.51	5.03	1.47	2.70	2.47	72.18

For samples collected after foliar treatment the residue present was extracted and analysed for its composition.

Table B.7.1-9: Extractability and composition of radioactivity in samples following foliar treatment to four apple leaves with 5.356 µg ^{14}C-glyphosate

Interval	Sample	% applied activity extracted	% of extract identified as	
			glyphosate	sum of AMPA and N-methy-AMPA
7 days	new growth above treatment	87.94	97.69	0.54
	treated leaves	90.49	98.06	0.76
21 days	new growth above treatment	102.59	98.75	0.65
	other new growth	88.98	98.11	0.78
	treated leaves	82.35	98.71	0.78
28 days	new growth above treatment[1]	97.13	97.77	0.22
	other new growth[1]	71.91	92.92	1.43
	treated leaves[1]	101.43	94.74	3.99
	roots	71.51	92.84	-
	trunk and branches	79.47	81.08	-
	new growth above treatment[2]	97.13	92.44	2.28
	other new growth[2]	91.45	92.20	0.91
	treated leaves[2]	96.86	95.69	2.84
49 days	new growth above treatment	93.86	93.14	2.62
	other new growth	100.4	91.83	2.88
	treated leaves	98.15	92.31	5.70
70 days	new growth above treatment	94.65	92.07	5.32
	other new growth	99.91	95.17	2.72
	treated leaves	91.39	91.75	7.06

1: data based on 2 trees
2: data based on 24 trees

Conclusions:
In this study the uptake of glyphosate and AMPA via the roots was minimal. In addition application of glyphosate to the trunk or to leaves also gave very low rates of translocation into untreated plant parts. The composition of the extracted radioactivity mainly consisted of unchanged glyphosate, in treated leaves as well as in untreated compartiments.

Reference: OECD KIIA 6.2.1, RIP9500012

Report: Parker, S.; Harris, M., Glyphosate-trimesium: Uptake and metabolism in USA grape vines
15.11.1991, RJ1002B

Guidelines: none

Deviations: not applicable

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study conducted in California (USA) glyphosate-trimesium, the trimethylsulfonium salt of glyphosate, was applied to the ground below vine grapes (Chenin Blanc variety) or used as overspray on selected parts of the plants. The active substance was ^{14}C-radiolabelled either in the glyphosate- or in the trimethylsulfonium-ion. For the ground treatment two sprayings with a total target rate equivalent to 8.0 kg as/ha were applied. The overspray was performed with a standard solution without relation to actual GAPs. Samples of grapes were collected 14 days after the application and for the soil treatment also one year later.

Grape samples collected after soil treatment werde extracted with water. The samples following simulated overspray were first washed with water to remove surface residues and afterwards also extacted with water. Total radioactive residues were determined using LSC. For the identification of metabolites HPLC-UV and TLC were used and the results compared with reference substances.

Findings:
In grape samples collected after ground treatment TRR levels for the glyphosate moiety were 0.007 mg eq/kg two weeks after the final application as well as after one year. In untreated control samples the natural backgroud was between 0.0012 mg eq/kg and 0.0055 mg eq/kg. No further identification of the residue was conducted.

Following simulated overspraying TRR levels were much higher with 1.25 mg eq/kg for the ^{14}C-glyphosate- and 1.15 mg eq/kg for the ^{14}C-trimethylsulfonium-ion. In the following table the radioactive residues found are summarised.

Table B.7.1-10: Extraction and identification of radioactive residues following simluated overspraying of ^{14}C-glyphosate to grapes in the USA

Matrix/Compound	^{14}C-glyphosate-label in mg eq/kg (% TRR)	^{14}C-trimethylsulfonium-label in mg eq/kg (% TRR)
Application rate	14.3 mg/vine	13.2 mg/vine
TRR	1.25	1.15
Extrated (water extract)	1.20 (96)	1.01 (87.5)
Glyphosate	0.964 (77.1)	-
AMPA	0.031 (2.5)	-
Trimethylsulfonium-ion	-	0.959 (83.4)
Unextracted	0.05 (4)	0.14 (12.5)

Conclusions:
In this study its was demonstrated that the translocation of glyposate applied as ground treatment in vineyards into the grapes is negligable. TRR levels in the fruits after two weeks and one year were in the same order of magnitude like the background levels in control samples.
In simulated overspraying most of the radioactivity was recovered as unchanged parent glyphosate. AMPA was the only metabolite identified being present at 2.5 % of the TRR.

Reference: OECD KIIA 6.2.1, RIP9500017

Report: Wilkinson, M. J.; Joseph, R. S. I.
ICIA0224: Uptake and metabolism in grape-vines
28.02.1990, RJ 0815B

Guidelines: none

Deviations: not applicable

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study grape vines (Müller Thurgau variety) were grown in UK (Berkshire) and treated with ^{14}C-glyphosate-trimesium labelled either in the glyphosate- or the trimethylsulfonium-ion. The application was made as a soil drench to the bases of the trunks at rates equivalent to 8 kg as/ha (glyphosate-label) and 7 kg as/ha (trimethylsulfonium-label). Samples of grapes, leaves and stems were collected 7 days after the application.

Grapes were homogenised and extracted with methanol and water. Stems, leaves and stalks were only analysed for the total radioactive residues. Total radioactive residues were determined using LSC. For the identification of metabolites HPLC-UV and TLC were used and the results compared with reference substances.

Findings:
After application of ^{14}C-glyphosate-trimesium to the base of vine, TRR levels in the grapes did not exceed 0.006 mg eq/kg for the glyphosate-label and 0.003 mg eq/kg for the trimethylsulfonium-label. No further analysis on the composition of radioactive residues was performed.

Table B.7.1-11: Total radioactive residues in grape samples following ground treatment at 7 or 8 kg as/ha (7 DAT)

Matrix	TRR in mg eq/kg (glyphosate-label)	TRR in mg eq/kg (trimethylsulfonium-label)
Grapes	<0.006	<0.003
Stems	<0.023	<0.009
Leaves	≤0.023	0.031[a]
Stalks	<0.023	0.010[a]

a: based on dry-matter

Conclusions:
The information provided by this study is limited. It can be concluded that within seven days after ground treatment no significant translocation of glyphosate residues into grape vines has to be expected.

Reference: OECD KIIA 6.2.1

Report: Rueppel, M. L.; Suba, L. A.; Moran, S. J.
CP 67573 residue and metabolism Part 20: The metabolism of CP 67573 in grape plants
March 1973, Report No. 355, RIP9501191

Guidelines: none

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this sutdy the uptake and metabolism of ^{14}C-glyphosate and ^{14}C-AMPA was investigated in grapes (Concord, Sauvignon Blanc and Thompson Seedless varieties). A set of different application techniques (soil treatment, trunk application, hydroponic application and foliar treatment) was used to characterise relevant mechanism for the uptake.

For the soil treatment stocking solutions were sprayed at rates equivalent to 3.4 kg ^{14}C-glyphosate per ha or 1.7 kg ^{14}C-AMPA per ha. For the uptake via the trunk an amount of 40 µg/tree was applied and covered to prevent splashing of the area during watering. After 8 and 42 days samples were collected (leaves and stems, treated trunk, untreated trunk and branches and roots). After 6 and 12 weeks samples were collected for analysis.
The behaviour od glyphosate following foliar application was investigated on vines three weeks after end of dormancy. Leaves were treated either with 10, 60 µg or 120 µg ^{14}C-glyphosate and covered with a plastic bags after drying. The radioactivity was applied either as single treatment or as 7 day pulse. After 7 to 70 days samples of treated leaves, leaves and stem above above treatment, stem connecting treated leaves, leaves and stem below treatment, other new growth, trunk and roots were collected.
For the hydroponic treatment solutions of 5, 10, 20 and 40 ppm ^{14}C-glyphosate were used. Samples were collected after 10-42 days.

All samples collected were homogenised and extracted with water or 0.5N NH_4OH. Total radioactive residues were determined using LSC. For the identification of metabolites GC-MS, TLC and NMR were used and the results compared with reference substances.

Findings:
In the following table the recovered radioactivity following soil and trunk treatment are summarised.

Table B.7.1-12: Recovered radioactivity in grape vine samples following soil or trunk treatment with ^{14}C-glyphosate and ^{14}C-AMPA

Variety	Treatement	Sample	% of applied activity recovered	
			42 DAT	84 DAT
Concord	Control	vine	0.031[a]	0.041[a]
	^{14}C-glyphosate, soil treatment	vine	0.067	0.083
		dead leaves	0.087	NP
	^{14}C-glyphosate, trunk treatment	vine	0.738	0.98
		treated stem	82.72	67.12
		dead leaves	0.13	NP
	^{14}C-AMPA, soil treatment	vine	0.068	0.12
		dead leaves	0.006	NP
Sauvingon blanc	Control	vine	0.061[a]	0.066[a]
	^{14}C-glyphosate, soil treatment	vine	0.075	0.078
	^{14}C-glyphosate, trunk treatment	vine	0.267	1.57
		treated stem	93.3	34.31
		grapes	0.0026	NP
	^{14}C-AMPA, soil treatment	vine	0.094	0.049
		grapes	0.0053	NP

a: based on target activity applied
NP: not performed

After foliar treatment the recovered radioactivity was:

Table B.7.1-13: Recovered radioactivity in grape vine samples treated with ^{14}C-glyphosate in foliar application

Sample	DAT	% AR				
		Treated leaves	Above treatment	Others incl. grapes	Stem and roots	Total
Concord grapes	7	72.2	8.7	0.5	15.9	97.3
	14	60.9	6.8	0.5	7.9	76.1
	28	54.9	4.9	0.7	18.8	79.3
Sauvignon blanc	7	57.8	0.8	0.8	33.4	92.8
	14	62.2	3.1	3.5	12.8	81.6
	28	46.7	1.6	0.7	16.2	65.2
Thompson Seedless	7	65.4	1.1	0.4	12.8	79.7
	14	57.7	5.0	0.8	12.1	75.6
	28	43.3	0.7	0.3	11.8	56.1
Concord (pulse treatment)	7	71.5	4.9	-	14.4	90.8
	14	70.1	11.5	-	23.4	105.0
	28	62.7	3.9	-	16.2	82.8
	42	77.1	3.6	-	11.7	92.4
	56	80.9	4.1	-	6.7	91.7
	70	71.3	8.1	-	8.2	88.1

Matrices from foliar treated grape vines were also extracted and analysed for the composition of the radioactivity.

Table B.7.1-14: Extracted radioactivity and identification of the residue in grape vine samples treated with ^{14}C-glyphosate in foliar application

Experiment	Sample	DAT	% extracted	% extracted as		
				glyphosate	AMPA	other
Concord	treated leaves	7	78.7	87.8	3.9	-
	new growth	7	78.3	74.3	-	3.3
	treated leaves	14	72.0	70.5	1.5	-
	new growth	14	82.9	78.1	-	4.2
	treated leaves	28	83.2	78.8	4.4	-
	new growth	28	105.1	100.5	-	1.0
	roots and old stock	28	87.6	87.6	-	-
	new growth[a]	28	83.2	74.6	-	4.3
	grapes[a]	28	64.6	64.6	-	-
Sauvingon blanc	treated leaves	7	90.5	87.7	2.8	-
	new growth	7	112.1	103.1	-	7.9
	treated leaves	14	91.1	88.2	2.9	-
	new growth	14	95.5	80.1	1.0	7.8
	treated leaves	28	93.5	84.3	9.2	-
	new growth	28	103.3	84.3	2.0	7.1
	grapes	28	88.0	79.5	1.0	6.9
	roots and old stock	28	90.2	90.2	-	-
Thompson seedless	treated leaves	7	101.1	97.1	-	-
	new growth	7	98.0	88.5	1.2	1.7
	treated leaves	14	89.6	89.6	-	-
	new growth	14	102.1	89.3	<2.0	6.3
	treated leaves	28	72.4	70.6	1.8	-
	new growth	28	94.4	74.4	<1.0	9.7
	roots and old stock	28	87.7	87.7	-	-
Concord, pulse treatment	treated leaves	7	89.0	85.6	3.4	-
	new growth	7	107.6	98.5	1.0	5.7
	treated leaves	14	96.3	93.8	2.5	-
	new growth	14	89.8	82.9	1.0	4.1
	new growth	28	90.5	77.1	1.0	5.3
	new growth	42	89.7	82.8	1.0	1.2
	new growth	56	82.0	70.4	1.0	5.0
	new growth	70	65.1	58.5	1.0	4.8

a: different experiment using 150 plants instead of 12

After application of the active substance via hydroponic solution the radioactivity found in the different plant parts is summarised in the following table:

Table B.7.1-15: Recovered radioactivity in grape vine plants treated hydroponically for 10 to 42 days

Hydroponic concentration	Sample	% AR recovered		
		10 days	21 days	42 days
5 ppm	Root	9.82	15.03	12.37
	Trunk	0.04	0.09	0.09
	Stem	0.05	0.13	0.37
	Leaf	0.12	0.21	0.21
	Hydroponic solution	70.14	42.3	56.9
	Rinse	2.51	2.6	2.5
	Total	82.68	60.36	72.44
10 ppm	Root	6.45	7.77	18.66
	Trunk	0.04	0.04	0.07
	Stem	0.03	0.07	0.29
	Leaf	0.07	0.16	0.21
	Hydroponic solution	75.69	67.5	41.4
	Rinse	1.42	2.6	4.5
	Total	83.7	78.14	64.83
20 ppm	Root	6.91	6.0	9.12
	Trunk	0.02	0.13	0.07
	Stem	0.04	0.06	0.13
	Leaf	0.09	0.13	0.19
	Hydroponic solution	78.4	78.3	67.9
	Rinse	2.32	1.3	1.7
	Total	87.78	85.92	79.11
40 ppm	Root	4.73	10.15	14.67
	Trunk	0.09	0.06	0.19
	Stem	0.06	0.1	0.14
	Leaf	0.11	0.2	0.3
	Hydroponic solution	87.17	64.2	63.4
	Rinse	2.11	3.6	2.9
	Total	94.27	78.31	81.6

Conclusions:
Following soil or stem treatment, the uptake and translocation of ^{14}C-glyphosate into grapes was minimal. After foliar application again most of the applied activity was recovered from the treated plant parts. However some translocation into stem and roots and into plant parts above the treated leaves was observed. The residues were good extractable with water (65-105 % AR). Identification revealed mainly unchanged glyphosate (58-103 % AR).
After hydroponic treatment only roots showed significant amounts of radioactivity. Most of the applied activity was still present in the hydroponic solution.

Reference: OECD KIIA 6.2.1

Report: Hasegawa, L. S.; Kumamoto, J.; Jordan, L. S.
Degradation of Glyphosate in avocado fruit
10.04.1995, L365, ASB2011-13642

Guidelines: no

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be additional information.

Material and Methods:
In this study conducted in a research project supported by the California Avocado Advisory Board the bahaviour of ^{14}C-radiolabelled glyphosate was investigated in avocado.
The study contained two parts. First a selected leaf was painted with ^{14}C-glyphosate. After up to 10 days samples of leaves near the treated leaf were analysed for radioactive residues.
In the second part of the experiment a mature avocado fruit was picked and a cavity was drilled into the end of the peduncle. The cavity was filled with an aqueous solution containing ^{14}C-glyphosate (453000 cpm) and then kept filled with destilled water for the remainder of 10 days. The fruit was placed in a respiration chamber, monitoring the formation of $^{14}CO_2$.
Analysis of the radioactivity was conducted by LSC and ion exchange HPLC with radiodetection agains glyphosate and AMPA as reference substances.

Findings:
In the first part of the experiment no translocation of radioactivity from the treated leaf into other parts of the plant was observed.
The treated avocado fruit was separated into its different segments and analysed for radioactivity. Results are presented in the following table:

Table B.7.1-16: Recovery of radiolabel from ^{14}C-glyphosate treated avocado fruit

Matrix (weight)	Glyphosate	AMPA	Unasssigned
	cpm (%)	cpm (%)	cpm (%)
Avocado, mesocarp (190.4 g)	290378 (64.1)	1179 (0.26)	4330 (0.9)
Avocado, exocarp (24.9 g)	31029 (6.8)	0 (0)	363 (<0.1)
Avocado, seed (53.9 g)	202 (<0.1)	0 (0)	2107 (0.47)
Avocado, peduncle (2.6 g)	69057 (15.2)	340 (<0.1)	2162 (0.48)
Filter paper	4225 (0.9)	0 (0)	98 (<0.1)
Total recovered	394891 (87.1)	1519 (0.34)	9060 (2)
CO_2	-	-	16239 (3.6)

Conclusions:
The information provided by this study are limited. No translocation of radioactivity from one treated leaf into other parts of the branch were observed. In one avocado fruit directly treated with glyphosate via a drilled cavity in the peduncle, most of radioactivity was present in the mesocarp and peduncle. AMPA was present in very low amounts <1% of the TRR.

B.7.1.1.2 Root and tuber vegetables

Reference: OECD KIIA 6.2.1

Report: Nadeau, R. G.
CP 67573 residue and metabolism Part 26: The metabolism of CP 67573 in potato plants
March 1974, Report No. 376, RIP9501193

Guidelines: no

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be additional information.

Material and Methods:
The metabolism and uptake of ^{14}C-glyphosate in potatoes was investigated following soil and foliar treatment.
For the soil treatment potato plants were grown in two planting pots per label containing 10 kg soil each. Either ^{14}C-glyphosate (23.8 mg per pot, 5.75 x 10^8 dpm), ^{14}C-AMPA (23.4 mg per pot, 5.2 x 10^9 dpm) or ^{14}C-$NaHCO_3$ (8.23 x 10^8 dpm per pot) were directly applied to the soils and thoroughly mixed. In addition into two of the pots ^{14}C-glyphosate treated weeds were incorperated to simulate ploughing (23.3 mg to the weeds, 3 weeks before incorperation, 5.75 xy 10^8 dpm). After 9,15 and 25 days leaf samples were collected from the plants. Tubers were collected after 128 days.
The application of ^{14}C-glyphosate onto the foliage was conducted by applying 100 µg radiolabelled active substance to leaves of 40 day old plants. Plants were harvested 1, 3, 14 and 34 days after treatment and separated into the top part and the tubers.

Total radioactive residues were analysed using LSC methods. The quantification and identification following 0.1 HCl or 1N NaOH/pyridine buffer (1:9, v/v) extraction was performed by GC/MS following derivatisation to N-trifluoroacetyltrimethyl-esters. ion exchange chromatography or high voltage electrophoresis (HVE) against reference substances. In additon $^{14}CO_2$ released during the soil treatment was collected.

Findings:
In the following table the radioactivity recovered in samples after soil treatement are summarised.

Table B.7.1-17: Recovered radioactivty in potato plants and tubers following soil treatment

Label	applied activity (dpm)	Recovered activity (dpm)					
		plant (9 DAT)	plant (15 DAT)	plant (25 DAT)	tuber (128 DAT)	Tops (128 DAT)	roots (128 DAT)
Control	-	2.0 1.2	0.7 0.4	1.1 1.1	0.5 0.5	0.8 0.6	0.1 0
^{14}C-HCO_3	8.23 x 10^8	1.2 3.7	0.6 2.8	2.4 2.1	0.2 0.6	2.8 1.1	0.6 1.5
^{14}C-glyphosate	5.75 x 10^8	53.2 67.0	27.2 35.8	18.4 20.5	5.3 7.5	13.5 15.1	33.9 48.5
^{14}C-glyphosate weed	5.75 x 10^8	NP	21.2 24.2	11.0 18.2	8.4 9.4	10.8 13.0	42.0 27.3
^{14}C-AMPA	5.2 x 10^9	9.2 8.9	13.2 16.2	11.7 12.0	10.9 16.1	13.4 12.7	30.7 20.8

NP: not performed

For potato tuber the radioactive residues were analysed by HVE using different medias. In all experiments AMPA was the major residue in tubers, found in relative amounts of 50-69 % after ^{14}C-glyphosate treatment and 65-80 % after ^{14}C-AMPA treatment. Parent glyphosate was also found, but at lower levels (2-18 %).

Following foliar application the recovered radioactivity in the samples is presented in the following table. According to the study report glyphosate was the only residue identified, however the identification was based on electrophoresis with radiodetection not capable of finding individual substances <5 % of the TRR.

Table B.7.1-18: Uptake and translocation of radioactivity following foliar treatment of potato plants

Sample	% AR			
	DAT 1	DAT 3	DAT 14	DAT 34
Treated leaf	80.8	81.5	56.8	72.3
leaves above	0.25	0.45	0.6	0.35
Leaves below	0.1	0.15	0.7	0.2
Stem above	0.055	0.15	0.1	0.1
Stem below	0.35	0.25	0.15	0.15
Tubers	4.6	6.3	12.4	10.2
Roots	0.2	0.15	0.15	0.1
Foliage, untreated[a]	0.1	0.05	0.11	0.15
tubers, untreated[a]	0.15	0.05	3.8	3.3
Roots, untreated[a]	0.015	0.01	0.05	0.06
Seed potato[a]	0.06	0.16	0.01	not determined
Total	86.7	89.2	74.7	86.7

a: some plants had 2 stems, the 2nd stem did not hold treated leaves

Conclusions:
In this experiment the uptake of radioactivity from soil following application of ^{14}C-glyphosate or ^{14}C-AMPA was limited. In tubers the identification of the radioactivity revealed AMPA as major residue. Unchanged glyphosate residues were present, however at much lower levels.

Following foliar application most of the radioactivity was recovered in the treated leaves. In adjected tuber second highest radioactive residues were found amounting up to 12.4 % of the AR. No identification of the radioactivity was conducted.

Reference: OECD KIIA 6.2.1

Report: Malik, J. M.; Brightwell, B. B.
CP 67573 residue and metabolism Part 29: The metabolism of CP 67573 in sugar beets
March 1976, Report No. 394, RIP9501195

Guidelines: no

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study the uptake of radioactivity into sugar beets grown in soil treated either with ^{14}C-glyphosate or ^{14}C-AMPA was investigated. Both radiolabelled analytes were applied to the soil of planting pots at rates of 8 mg per pot (corresponding to 4.5 kg as/ha). Directly afterwards the pots were covered with plastic bages and kept in greenhouse for 4, 6 or 8 weeks until sampling. In parallel untreated control samples were grown.

Total radioactive residues in the different samples were analysed by LSC. No further identification of the residue was reported.

Findings:
In the following table the uptake of radioactivity by sugar beet roots and the translocation into the leaves is summarised.

Table B.7.1-19: Uptake of radioactivity into sugar beet roots and leaves following soil treatment with ^{14}C-glyphosate or ^{14}C-AMPA at rates equivalent to 4.5 kg as/ha

Analyte applied	Interval	Sample	% AR recovered
^{14}C-glyphosate	4 weeks	Leaves, treated	0.033
		Leaves, control	0.003
		Roots, treated	0.037
		Roots, control	0.003
	6 weeks	Leaves, treated	0.038
		Leaves, control	0.004
		Roots, treated	0.023
		Roots, control	0.005
	8 weeks	Leaves, treated	0.040
		Leaves, control	0.003
		Roots, treated	0.030
		Roots, control	0.003
^{14}C-AMPA	4 weeks	Leaves, treated	0.033
		Leaves, control	0.002
		Roots, treated	0.015
		Roots, control	0.002
	6 weeks	Leaves, treated	0.124
		Leaves, control	0.003
		Roots, treated	0.042
		Roots, control	0.002
	8 weeks	Leaves, treated	0.151
		Leaves, control	0.002
		Roots, treated	0.149
		Roots, control	0.003

Conclusions:
In this experiment the observed uptake of radioactivity into roots or leaves of sugar beets was minimal. Less than 0.2% of the applied activity following soil treatment with ^{14}C-glyphosate or ^{14}C-AMPA were recovered in the plant samples. No identification of the radioactivity was conducted.

B.7.1.1.3 Pulses and oilseeds

Reference: OECD KIIA 6.2.1

Report: Tambling, D. R.
[^{14}C-Anion] ICIA0224: Nature of the residue: Soybeans
29.05.1992, RR 91-092B, RIP9500015

Guidelines: EPA §171-4(a): "Nature of Residues in Plants"

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study ^{14}C-glyphosate-trimesium radiolabelled in the glyphosate-ion was applied to soil at planting of soya beans. The plants were cultivated in pots approximately 40 cm wide. Within 2h after planting a drench application equivalent to 8.4 kg as/ha was applied to the young soya bean plants. The Plants remained in greenhouse until harvested. Samples of immature plants (31 DAT) and straw, hulls, hay and seeds (97 DAT) were collected and analysed for residues.

The extraction of the samples was performed by water follwed by methanol. Purification of the extracts was achied with cation-exchange columns and HPLC. After extraction the remaining 14C was hydrolysied with 1N HCl for 2h and 20 % NaOH for 24h. In addition defatted soya bean (extracted with hexane) were used to isolate the proteins. Total radioactive residues were determined using LSC. For the identification of metabolites HPLC-UV and TLC were used and the results compared with reference substances.

Findings:
In the following table TRR levels found, the extactability of radioactive residues and the amounts of glyphosate, AMPA and extracted natural products are summarised.

Table B.7.1-20: TRR, extractability and amounts of glyphosate, AMPA and natural products in soya beans treated with ^{14}C-glyphosate-trimesium at planting

Sample	TRR in mg eq/kg	Total extracted in % TRR	Glyphosate in mg eq/kg (% TRR)	AMPA in mg eq/kg (% TRR)	Natural products in mg eq/kg (% TRR)	Unxectracted in mg eq/kg (% TRR)	Total recovered in % TRR
Forage (31 DAT)	1.76	45.0	0.058 (3.3)	0.1 (5.7)	0.634 (36)	1.051 (59.7)	104.7
Straw (97 DAT)	0.859	37.4	0.005 (0.6)	0.023 (2.7)	0.293 (34.1)	0.497 (57.9)	95.3
Hulls (97 DAT)	0.487	47.9	0.02 (4.1)	0.007 (1.5)	0.206 (42.3)	0.222 (45.7)	93.6
Seeds, yellow (97 DAT)	1.31	53.1~~48.5~~[a]	0.034 (2.6)	0.021 (1.6)	0.641 (48.9)	0.613 (46.8)	99.9
Seeds, green (97 DAT)	0.772	53.1~~48.5~~[a]	0.02 (2.6)[b]	0.012 (1.6)[b]	0.378 (48.9)[b]	0.361 (46.8)[b]	99.9
Hay (97 DAT)	0.854	48.5~~53.1~~	0.02 (2.1)	0.02 (2.0)	0.42 (44.4)	0.47 (50)	98.4

a: sum of hexane and aqueous extract
b: not fractionated, derived by analogy from yellow seeds

The unextracted residue was further characterised by hydrolysis or fractionating methods and/or analytical methods such as HPLC, chemical derivatisation and dialysis. In the following table the attributed products are presented.

Table B.7.1-21: Characterisation of unextracted residues in soya bean matrices treated with ^{14}C-glyphosate-trimesium at planting

Sample	Unxectracted in mg eq/kg (% TRR)	Carbohydrate in mg eq/kg (% TRR)	Protein in mg eq/kg (% TRR)	Lignin in mg eq/kg (% TRR)	Cellulose in mg eq/kg (% TRR)	Bound in mg eq/kg (% TRR)
Forage (31 DAT)	1.051 (59.7)	0.445 (25.3)	0.303 (17.2)	0.025 (1.43)	0.278 (15.8)	-
Straw (97 DAT)	0.497 (57.9)	0.148 (17.2)	0.137 (16.0)	0.025 (2.9)	0.187 (21.8)	-
Hulls (97 DAT)	0.222 (45.7)	0.082 (16.9)	0.071 (14.5)	NP	0.037 (7.6)	-
Seeds, yellow (97 DAT)	0.613 (46.8)	NP	0.314 (24)	NP	NP	0.299 (22.8)
Seeds, green (97 DAT)	0.361 (46.8)	NP	0.185 (24)	NP	NP	0.176 (22.8)
Hay (97 DAT)	0.47 (50)	0.06 (8.4)	0.2 (20)	0.01 (1.0)	0.07 (8.6)	0.13 (11.9)

Conclusions:
In soya beans treated with glyphosate-trimesium at the glyphosate-ion at planting only minor levels of glyphosate or AMPA were found in the various plant parts. Most of the radioactivity was incorperated into natural products like carbohydrates and proteins.

Reference: OECD KIIA 6.2.1

Report: Rueppel, M. L.; Suba, L. A.
CP 67573, Residue and Metabolism Part 10: The Metabolism of CP 67573 in soybeans, cotton, wheat, and corn
02.07.1973, Report No. 304, RIP9600099

Guidelines: none

Deviations: not applicable

GLP: no~~yes~~

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study the uptake and metabolism of ^{14}C-glyphosate and ^{14}C-AMPA in soya beans, cotton, wheat and mauze were investigated following soil or hydroponic treatment.
In the first part of the study the crops were planted in pots and the soil was treated with application rates equivalent to either 4.5 kg glyphosate or 1.7 kg AMPA per ha. Samples of plants were harvested after 4, 6 and 8 weeks. In parallel control samples were kept to check for uptake of $^{14}CO_2$ from soil metabolism.
In a second set of experiments plants growing in sand culture were hydroponically treated with an aqueous solution containing ^{14}C-glyphosate equivalent to 2.24 kg as/ha. Plant samples as well as the sand was collected after 4, 10 and 18 days and analysed for radioactive residues.
In the third experiment plants were grown in a hydroponic sand culture. An amount of ^{14}C-glyphosate equivalent to 2.24 kg as/ha was applied to the sand. After 4, 10 and 18 days

samples collected and the radioactivity in the sand was washed using water followed by sequential extraction with 1N NH_4OH.
The fourth experiment was a priliminary study to investigate the uptake of ^{14}C-glyphosate from hydroponic solution. Plants were kept in hydroponic solution containing an activity of 1.1 x 10^7 dpm for 3 days. After this interval the plants were separated into aerial parts and roots and analysed for the radioactivity taken up.
The final set of experiments included various hydroponic studies to investigate the metabolism of ^{14}C-glyphosate. The plants were kept in hydroponic solution to a maximum of 10 to 56 days with samples of plants and hydroponic solution collected in between..

Total radioactive residues were determined using LSC. For the identification of metabolites GC-MS, TLC and NMR were used and the results compared with reference substances.

Findings:
In the following table the recovered radioactivty from plants samples grown in treated soil are summarised.

Table B.7.1-22: Recovered radioactivity in maize, cotton, soya beans and wheat following soil treatment equivalent to 4.5 kg glyphosate per ha

Crop	Weeks after treatment	Treatment	% AR	mg eq/kg
Maize	4	control	0.0070	0.068
		treated	0.0310	0.21
	6	control	0.0361	0.089
		treated	0.0395	0.1434
	8	control	0.0131	0.0218
		treated	0.0472	0.0785
Cotton	4	control	0.0015	0.04
		treated	0.0180	0.26
	6	control	0.0051	0.020
		treated	0.0586	0.21
	8	control	0.2001	0.27
		treated	0.2760	0.42
Soya beans	4	control	0.0013	0.0294
		treated	0.0237	0.202
	6	control	0.0629	0.109
		treated	0.138	0.293
	8	control	0.0355	0.0453
		treated	0.0726	0.0755
Wheat	4	control	0.00085	0.008
		treated	0.0204	0.20
	6	control	0.0030	0.015
		treated	0.0335	0.18
	8	control	0.0220	0.061
		treated	0.1159	0.35

Table B.7.1-23: Recovered radioactivity in maize, cotton, soya beans and wheat following soil treatment equivalent to 1.7 kg AMPA per ha

Crop	Weeks after treatment	Treatment	% AR	mg eq/kg
Maize	4	control[a]	0.003	0.011
		treated	0.007	0.021
	6	control[a]	0.006	0.014
		treated	0.017	0.032
	8	control[a]	0.009	0.011
		treated	0.044	0.031
Cotton	4	control[a]	0.0033	0.071
		treated	0.0008	0.024
	6	control[a]	0.0012	0.01
		treated	0.0046	0.044
	8	control[a]	0.0036	0.011
		treated	0.0077	0.032
Soya beans	4	control[a]	0.003	0.018
		treated	0.014	0.094
	6	control[a]	0.0055	0.014
		treated	0.017	0.053
	8	control[a]	0.0105	0.0105
		treated	0.033	0.041
Wheat	4	control[a]	0.0115	0.0185
		treated	0.0035	0.075
	6	control[a]	0.0026	0.028
		treated	0.0049	0.084
	8	control[a]	0.00575	0.042
		treated	0.0076	0.058

a: mean of two samples

Following hydroponic treatment of maize, cotton, soya beans and wheat grown in sand the recovered radioactivity in plant samples and sand was:

Table B.7.1-24: Recovered radioactivity following hydroponic application of ^{14}C-glyphosate to maize, cotton, soya beans and wheat grown in sand at rates equivalent to 2.24 kg as/ha

Treated crop	Sample	% AR		
		4 DAT	10 DAT	18 DAT
Soya beans	Plant, total	0.03	0.02	-
	Plant, aerial part	-	-	0.07
	Plant, roots	-	-	0.22
	Sand wash[a]	84.7	82.0	82.9
	Total recovered	84.9	82.0	83.2
Wheat	Plant, total	0.09	0.16	-
	Plant, aerial part	-	-	0.03
	Plant, roots	-	-	0.37
	Sand wash[a]	93.9	92.2	85.8
	Total recovered	94.0	92.4	86.2
Cotton	Plant, total	0.09	0.06	-
	Plant, aerial part	-	-	0.03
	Plant, roots	-	-	0.16
	Sand wash[a]	86.2	83.4	62.4
	Total recovered	86.3	83.5	62.6
Maize	Plant, aerial part	0.87	1.45	11.29
	Plant, roots	2.73	0.49	2.24
	Sand wash[a]	72.83	62.8	52.0
	Total recovered	76.5	64.9	65.5

a: sum of extracts

The results of the priliminary hydroponic experiment were:

Table B.7.1-25: Uptake of radioactivity after 3 days in hydroponic solution

Crop	% AR recovered (1.1 x 10^7 dpm)			
	Maize	Cotton	Wheat	Soya beans
Aerial part	19.0	33.40	26.21	1.66
Roots	7.31	10.09	7.24	1.75
Hydroponic solution	67.48	62.15	63.16	84.75
Root wash	11.90	8.21	4.77	4.08
Total recovered	105.69	113.85	101.38	92.24

Following hydroponic uptake of glyphosate for the purpose of identifying metabolites resuilts were:

Table B.7.1-26: Uptake and identified metabolites of ^{14}C-glyphosate in soya bean plants grown in hydroponic solution

Time (days)	Matrix	% AR recovered	% AR extracted	% recovered expessed as TRR (expressed as % extracted)			
				glyphosate	AMPA	N-methyl-AMPA	others
99 plants in 20 L hydroponic solution, 50 mg ^{14}C-methane-glyphosate (5.15 x 10^9 dpm)							
1	Hydroponic solution	119	NA	>97	-	-	-
6	Hydroponic solution	96.5	NA	>98	-	-	-
12	Hydroponic solution	82.3	NA	95.4	4.6	-	-
20	Hydroponic solution	68.3	NA	93.4	6.6	-	-
28	Hydroponic solution	66.4	NA	92.8	7.2	-	-
	Aerial parts	4.19	72.3	63.1 (87.3)	9.2 (12.7)	-	-
	Roots	10.8	42.3	35.4 (83.6)	3.0 (7.0)	0.3 (0.7)	3.6 (8.6)
24 plants in 5 L hydroponic solution, 12 mg ^{14}C-methane-glyphosate (1.25 x 10^9 dpm)							
1	Hydroponic solution	93.94	NA	>97	-	-	-
6	Hydroponic solution	80.22	NA	>97	-	-	-
	Aerial parts	0.93	69.2	NP	NP	NP	NP
	Roots	3.68	37.6	29.3 (77.9)	2.3 (6.2)	1.0 (2.7)	5.0 (13.3)
12	Hydroponic solution	67.49	NA	96.8	3.2	-	-
	Aerial parts	1.12	70.3	65.4 (93.2)	5.4 (6.8)	-	-
	Roots	6.71	59.7	54.1 (90.6)	5.6 (9.4)	-	-
20	Hydroponic solution	55.96	NA	86.1	13.9	-	-
	Aerial parts	1.42	82.3	78.5 (95.4)	3.8 (4.6)	-	-
	Roots	9.34	59.0	50.2 (92.4)	3.5 (6.5)	-	0.6 (1.1)
28	Hydroponic solution	43.88	NA	88.5	11.5	-	-
	Aerial parts	1.71	80.3	70.3 (87.5)	5.1 (6.3)	-	5.0 (6.2)
	Roots	8.64	59.0	44.3 (75.1)	1.4 (2.4)	-	13.3 (22.5)
24 plants in 5 L hydroponic solution, 12 mg ^{14}C-glycine-1-glyphosate (1.76 x 10^9 dpm)							
1	Hydroponic solution	107.51	NA	>97	-	-	-
6	Hydroponic solution	77.47	NA	>99	-	-	-
	Aerial parts	1.46	53.1	53.1 (100)	-	-	-
	Roots	2.97	35.0	35.0 (100)	-	-	-
12	Hydroponic solution	58.45	NA	>99	-	-	-
	Aerial parts	1.10	65.8	65.8 (100)	-	-	-
	Roots	6.85	73.0	73.0 (100)	-	-	-
20	Hydroponic solution	43.95	NA	>99	-	-	-
	Aerial parts	1.56	72.0	69.6 (96.7)	-	-	2.4 (3.3)
	Roots	13.88	59.3	59.3 (100)	-	-	-
28	Hydroponic solution	37.44	NA	>99	-	-	-
	Aerial parts	1.79	82.6	82.6 (100)	-	-	-
	Roots	12.26	70.0	65.0 (92.8)	-	-	5.0 (7.2)
24 plants in 5 L hydroponic solution, 12 mg ^{14}C-glycine-2-glyphosate (1.63 x 10^9 dpm)							
1	Hydroponic solution	109.9	NA	>97	-	-	-
6	Hydroponic solution	81.22	NA	>98	-	-	-
	Aerial parts	0.64	66.8	66.8 (100)	-	-	-
	Roots	4.25	38.4	30.3 (78.8)	-	2.3 (6.0)	4.0 (10.5)
12	Hydroponic solution	73.09	NA	>99	-	-	-
	Aerial parts	1.21	78.6	78.6 (100)	-	-	-
	Roots	5.3	62.2	53.7 (86.4)	-	-	8.6 (13.6)
20	Hydroponic solution	67.2	NA	>99	-	-	-
	Aerial parts	2.0	73.9	71.6 (96.9)	-	-	2.2 (3.1)
	Roots	8.56	41.5	28.3 (68.3)	-	-	13.2 (31.7)

Time (days)	**Matrix**	**% AR recovered**	**% AR extracted**	**% recovered expessed as TRR (expressed as % extracted)**			
25	Hydroponic solution	57.36	NA	>98	-	-	-
	Aerial parts	2.09	91.8	85.5 (93.1)	-	-	6.4 (7.0)
	Roots	10.3	43.5	21.5 (49.5)	-	-	21.9 (50.4)[a]
28	Hydroponic solution	58.43	NA	>99	-	-	-
198 plants in 20 L hydroponic solution, 2.96 mg ^{14}C-methane- & 50 mg ^{13}C-methane-glyphosate (3.1 x 10^8 dpm)							
1	Hydroponic solution	98.23	NA	>97	-	-	-
6	Hydroponic solution	84.5	NA	92	8	-	-
	Aerial parts	0.41	85	60.6 (71.3)	16.6 (19.5)	-	-
	Roots	1.71	NP	NP	NP	NP	NP
12	Hydroponic solution	77.13	NA	>98	-	-	-
	Aerial parts	0.72	63	54.4 (86.4)	7.3 (11.6)	-	-
	Roots	3.49	NP	NP	NP	NP	NP
20	Hydroponic solution	77.35	NA	92	8	-	-
	Aerial parts	4.96	66	56.2 (85.1)	5.2 (7.8)	-	-
	Roots	3.47	NP	NP	NP	NP	NP
26	Hydroponic solution	73.8	NA	>94	-	-	-
	Aerial parts	7.7	67	55.1 (82.2)	7.8 (11.6)	-	-
	Roots	5.48	NP	NP	NP	NP	NP
24 plants in 5 L hydroponic solution, 12 mg ^{14}C-methane-glyphosate (1.24 x 10^9 dpm), pulse treatment for first 6 days only							
1	Hydroponic solution	99.76	NA	>98	-	-	-
6	Hydroponic solution	93.02	NA	98	2	-	-
	Aerial parts	0.29	67.4	55.3 (82.1)	8.8 (13.1)		3.2 (4.8)
	Roots	2.41	NP	NP	NP	NP	NP
12	Aerial parts	0.29	55.0	46.5 (84.6)	4.7 (8.6)		3.7 (6.8)
	Roots	2.68	NP	NP	NP	NP	NP
20	Aerial parts	0.39	50.8	39.3 (77.3)	5.5 (10.9)		6.0 (11.8)
	Roots	1.98	NP	NP	NP	NP	NP
28	Aerial parts	0.3	43.2	29.4 (68.1)	5.8 (13.3)		7.2 (16.6)
	Roots	0.8	NP	NP	NP	NP	NP
42	Aerial parts	0.45	NP	NP	NP	NP	NP
	Roots	1.35	NP	NP	NP	NP	NP
56	Aerial parts	0.78	NP	NP	NP	NP	NP
	Roots	1.63	NP	NP	NP	NP	NP

NP: not performed
NA: not applicable
a: origin: 10.0 % TRR (23.1 % extracted) plus more than 6 unknown (11.9 % TRR, 27.4 % extracted)

Table B.7.1-27: Uptake and identified metabolites of ^{14}C-glyphosate in maize, cotton and wheat plants grown in hydroponic solution

Time (days)	Matrix	% AR recovered	% AR extracted	% recovered expessed as TRR (expressed as % extracted)			
				glyphosate	AMPA	N-methyl-AMPA	others
Maize, 24 plants in 5 L hydroponic solution, 3 mg ^{14}C-methane-glyphosate (3.13 x 10^8 dpm)							
1	Hydroponic solution	104.5	NA	84	-	-	-
6	Hydroponic solution	79.49	NA	93	-	-	-
	Aerial parts	0.96	70.6	70.6 (100)	-	-	-
	Roots	3.39	68.6	61.1 (89.1)	7.4 (10.9)	-	-
12	Hydroponic solution	66.69	NA	>90	-	-	-
	Aerial parts	3.8	58.2	19.7 (33.9)	16.2 (27.9)	4.2 (7.3)	18 (30.9)
	Roots	8.43	66.7	56.6 (84.9)	10.1 (15.1)	-	-
20	Hydroponic solution	56.93	NA	84	16	-	-
	Aerial parts	7.01	61.6	23.7 (38.5)	22.2 (36.0)	1.9 (3.1)	13.8 (22.4)
	Roots	9.74	70.6	45.5 (64.4)	8.4 (11.9)	0.6 (0.8)	16.2 (22.9)
28	Hydroponic solution	44.76	NA	80	20	-	-
	Aerial parts	4.73	76.9	28.1 (36.5)	27.0 (35.1)	2.0 (2.6)	19.3 (25.8)
	Roots	9.74	55.5	40.8 (73.5)	8.0 (14.4)	-	5.6 (10.1)
Wheat, 72 plants in 5 L hydroponic solution, 3 mg ^{14}C-methane-glyphosate (3.13 x 10^8 dpm)							
1	Hydroponic solution	103.26	NA	>86	-	-	-
6	Hydroponic solution	81.81	NA	NP	-	-	-
	Aerial parts	1.28	77.3	69.3 (89.6)	8.0 (10.4)	-	-
	Roots	1.39	42.3	33.5 (79.3)	8.8 (20.7)	-	-
10	Hydroponic solution	58.31	NA	>89	-	-	-
	Aerial parts	2.46	77.3	70.7 (91.5)	6.6 (8.5)	-	-
	Roots	2.52	54.5	38.5 (70.7)	5.2 (9.6)	2.0 (3.7)	6.8 (12.5)
12	Hydroponic solution	63.92	NA	>90	-	-	-
20	Hydroponic solution	55.94	NA	79	21	-	-
28	Hydroponic solution	50.0	NA	72	28	-	-
Cotton, 24 plants in 5 L hydroponic solution, 12 mg ^{14}C-methane-glyphosate (1.25 x 10^9 dpm)							
1	Hydroponic solution	109.0	NA	>95	-	-	-
6	Hydroponic solution	87.81	NA	95	5	-	-
	Aerial parts	0.4	73.8	73.8 (100)	-	-	-
	Roots	1.32	38.8	38.8 (75.7)	8.9 (17.4)	-	3.5 (6.8)
13	Hydroponic solution	83.12	NA	>98	-	-	-
	Aerial parts	1.28	84.6	80.0 (94.5)	4.6 (5.5)	-	-
	Roots	3.43	30.8	21.3 (69.0)	4.2 (13.5)	0.8 (2.5)	4.5 (14.8)
20	Hydroponic solution	72.32	NA	93	7	-	-
	Aerial parts	2.98	78.8	70.8 (89.8)	5.3 (6.7)	-	2.7 (3.5)
	Roots	7.98	32.8	24.5 (74.8)	4.4 (13.3)	0.2 (0.7)	3.8 (11.4)
28	Hydroponic solution	58.17	NA	90	10	-	-
	Aerial parts	2.15	89.2	70.5 (79.0)	8.0 (9.0)	-	2.8 (3.1)
	Roots	19.34	17.3	11.1 (64.2)	3.0 (17.6)	0.4 (2.6)	2.6 (14.9)

NA: not applicable

Conclusions:

In this study the uptake of glyphosate and AMPA via the roots was investigated using different scenarios. All experiments involving soil or sand only gave a very limited uptake of radioactivity, normally being <1 % of the AR except for maize giving up to 11.3 % AR in aerial parts.

Following that results hydroponic treatment without soil or sand was selected for the investigation of the metabolism in soya beans, maize, wheat and cotton. For soya beans and cotton the uptake into the plants after 28 days was approximately 10-15 % AR. Identification

revealed mainly unchanged glyphosate 20-100 % of the TRR, followed by AMPA (up to 16.6 % TRR). N-methyl-AMPA was also identified, but at rates <10 % TRR.
In principle wheat and maize gave comparable results to soya beans and cotton, however AMPA was found at higher levels being present up to 27% of the TRR.
Especially at higher DAT extraction rates in the roots were relatively low, going from 70 % of the TRR down to 17.3 % in cotton roots. Acidic and bsic treatment did not release a significant additional amount of radioactivity.

B.7.1.1.4 Cereal grains

Reference: OECD KIIA 6.2.1

Report: Stuart, C.; Parker, S.; Joseph, R. S. I.
ICIA0224: Metabolism on wheat following a preharvest foliar spray
06.12.1989, RJ0778B, RIP9500014

Guidelines: none

Deviations: not applicable

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
The study was performed to determine the uptake and metabolism of glyphosate-trimesium (the trimethylsulfonium salt of glyphosate) in wheat grown in the UK. The active substance was ^{14}C-radiolabelled either in the glyphosate- or the trimethylsulfonium-moiety and applied at a rate equivalent to 6 kg as/ha to wheat close to harvest (<20 % moisture in grain). After 7 days samples of straw, grain and chaff were collected and analysed for radioactive residues.

The extraction of samples was performed with water for the ^{14}C-glyphosate-label and with methanol followed by water for the ^{14}C-trimethylsulfonium-label. Total radioactive residues were determined using LSC. For the identification of metabolites RP-HPLC-UV and TLC were used and the results compared with reference substances.

Findings:
The unchanged glyphosate moiety was the major residue detected in the wheat samples following treatment with ^{14}C-glyphosate-label. A small quantitiy of AMPA was detected. After application of ^{14}C-trimethylsulfonium-label, the trimethylsulfonium-moiety was the only residue identified. In the following table the results of this study are summarised.

Table B.7.1-28: Residues in wheat matrices following application of ^{14}C-glyphosate-trimesium at rates equivalent to 6 kg as/ha 7 days before harvest

Matrix/Compound	Residues following ^{14}C-glyphosate-label in mg eq/kg (% TRR)[a]			Residues following ^{14}C-trimethylsulfonium-label in mg eq/kg (% TRR)[a]		
	grain	chaff	straw	grain	chaff	straw
TRR	2.68 (100)	327.5 (100)	124.2 (100)	8.22 (100)	363.9 (100)	151.2 (100)
Extracted[b]	2.52 (94.2)	310.8 (94.9)	114.8 (92.4)	8.04 (97.8)	291.2 (80)	121.1 (80.1)
Glyphosate	2.43 (90.8)	278.4 (85)	102.6 (82.6)	NA	NA	NA
AMPA	0.08 (2.8)	12.8 (3.9)	4.1 (3.3)	NA	NA	NA
trimethylsulfonium	NA	NA	NA	7.83 (95.3)	277.3 (76.2)	116.4 (77)
unknown	0.01 (0.5)	6.55 (2.0)	2.2 (1.8)	-	2.6 (0.72)	0.3 (0.2)
unassigned background	0.03 (1.3)	5.6 (1.7)	3.7 (3.0)	0.03 (0.42)	2.1 (0.58)	1.3 (0.9)
Unextracted	0.12 (4.5)	14.7 (4.5)	12.4 (10)	0.34 (4.2)	76.1 (20.9)	30.8 (20.4)
Total recovered	100 %	100 %	100 %	99.9%	110 %	89.9 %

a: expressed as glyphosate-trimesium
b: ^{14}C-glyphosate: aqueous extract, ^{14}C-trimethylsulfonium-label: combined aqueous and methanol extract
NA: not applicable

Conclusions:
The application of glyphosate-trimesium to wheat 7 days before harvest mainly results in unchanged TMS-ions in all matrices investigated. For the glyphosate-moiety most of the residue was present as unchanged parent, with minor amounts of AMPA (2.8-3.9 % TRR) detectable. For the trimethylsulfonium-moiety no metbolites could be identified.

Reference: OECD KIIA 6.2.1

Report: Suba, L. A.; Georgieff, M. K.
CP 67573 residue and metabolism Part 22: The metabolism of N-phosphonomethylglycine in barley, oats, rice and sorghum
Dec. 1974, Report No. 341, RIP9501189

Guidelines: none

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study the uptake and metabolism of ^{14}C-glyphosate in cereal grain (barley, oats, rice and corghum) following soil treatment or application via hydroponic solution was invesitigated. The soil was treated with application rates equivalent to 9, 4.5 or 2.3 kg as/ha. For the hydroponic application a solution containing 0.183 mg ^{14}C-glyphosate per mL was

used. Samples were collected after 7 to 28 days for the hydroponic application and separated into roots and aerial parts.Following soil treatment plant samples were taken after 4,6 and 8 weeks.

Total radioactive residues were determined using LSC. For the identification and quantification of metabolites TLC in combination with radiodetection, GC-FPD and GC-MS were used and the results compared with reference substances (glyphosate, AMPA and N-methyl-AMPA).

Findings:
After soil application only samples treated at rates equivalent to 4.5 kg as/ha were further analysed. In the following tables the ^{14}C levels found in plant samples after 4-8 weeks as well as the results following hydroponic application are summarised.

Table B.7.1-29: Radioactivity found in sorghum, rice, oats and barley grown in soil treated with ^{14}C-glyphosate at rates equivalent to 4.5 kg as/ha

Matrix	Weeks after treatment	^{14}C in plants	
		% applied	mg eq/kg
Sorghum, whole plant	4 (control)	0.06	0.048
	4 (treated)	0.08	0.073
	6 (control)	0.05	0.03
	6 (treated)	0.06	0.085
	8 (control	0.05	0.024
	8 (treated)	0.08	0.085
Rice, whole plant	4 (control)	0.04	0.23
	4 (treated)	0.03	0.11
	6 (control)	0.01	0.038
	6 (treated)	0.04	0.077
	8 (control	0.01	0.061
	8 (treated)	0.04	0.157
Oats, whole plant	4 (control)	0.06	0.05
	4 (treated)	0.09	0.062
	6 (control)	0.05	0.017
	6 (treated)	0.07	0.029
	8 (control	0.04	0.018
	8 (treated)	0.13	0.05
Barley, whole plant	4 (control)	0.07	0.084
	4 (treated)	0.08	0.093
	6 (control)	0.03	0.026
	6 (treated)	0.07	0.078
	8 (control	0.03	0.024
	8 (treated)	0.05	0.059

Table B.7.1-30: Radioactivity found in sorghum, rice, oats and barley treated hydroponically with 0.183 mg ^{14}C-glyphosate per mL

Matrix	Days after treatment	Roots, ^{14}C in plants		Tops, ^{14}C in plants	
		% applied	mg eq/kg	% applied	mg eq/kg
Sorghum	7 (control)	0.012	0.003	0.016	0.005
	7 (treated)	1.94	1.1	0.08	0.037
	14 (control)	0.11	0.020	0.042	0.004
	14 (treated)	5.64	1.597	0.48	0.073
	28 (control)	0.112	0.004	0.119	0.004
	28 (treated)	13.4	1.711	2.7	0.216
Rice	7 (control)	0.004	0.003	0.002	0.003
	7 (treated)	2.88	2.505	0.77	0.534
	14 (control)	0.02	0.010	0.008	0.008
	14 (treated)	5.59	3.304	1.98	1.545
	20 (control)	0.08	0.078	0.19	0.266
	20 (treated)	12.36	10.749	5.58	8.787
	28 (control)	NP	NP	NP	NP
	28 (treated)	6.53	3.684	4.68	2.816
Oats	7 (control)	0.006	0.003	0.012	0.004
	7 (treated)	2.1	1.797	0.59	0.25
	14 (control)	0.03	0.014	0.04	0.013
	14 (treated)	4.96	2.98	3.21	1.0
	28 (control)	0.05	0.007	0.10	0.008
	28 (treated)	13.76	6.482	3.49	0.707
Barley	7 (control)	0.004	0.009	0.003	0.004
	7 (treated)	3.36	4.542	0.51	0.27
	14 (control)	0.01	0.005	0.02	0.009
	14 (treated)	6.31	7.982	1.62	1.179
	28 (control)	0.026	0.004	0.04	0.007
	28 (treated)	23.05	6.128	2.87	0.571

NP: not performed

Samples collected after 28 days were analyed for indentification of the residue using extraction by water and TLC methods.

Table B.7.1-31: Extractability and identification of radioactive residues in barley, oats, rice and sorghum roots and tops following hydroponic treatment with 0.183 mg ^{14}C-glyphosate per mL

Matrix	% TRR extracted (H_2O)	% TRR					
		Glyphosate	AMPA	N-methyl-AMPA	origin	unextracted	indeterminate
Barley, tops	100	73.3	14.0	3.5	-	-	9.3
Barley, roots	61.23	52.6	3.8	0.4	-	38.8	4.4
Oats, tops	89.0	76.6	6.5	1.7	4.4	11.0	-
Oats, roots	40.8	35.7	2.5	1.1	1.0	59.2	0.5
Rice, tops	85.36	73.7	8.6	1.4	1.9	14.6	-
Rice, roots	33.23	19.1	7.4	1.6	5.0	66.8	0.1
Sorghum, tops	100	76.2	12.7	5.4	-	-	5.7
Sorghum, roots	49.57	44.8	2.2	0.5	-	50.4	2.1

Conclusions:
In cereal grains (barley, oats, rice and sorghum) the uptake of radioactivity from treated soil was very limited. Less than 0.1 % of the applied radioactivity was found in the plants. Following application of glpyhosate via hydroponic solution glyphosate gave a better uptake into the plants. Although roots showed higher residues than the tops of the plants, up to 5.88 % of the applied radioactivity was recovered in the tops. The identification of the radioactivity revealed unchanged parent glyphosate as the major residue (19.1-76.6 % TRR), followed by AMPA (2.2-14 % TRR).

~~**Reference:**~~ ~~OECD KIIA 6.2.1~~

~~**Report:**~~ ~~Michaux, M., CP 67573: Determination of crop residues in winter wheat, spring wheat and spring barley~~
~~07.01.1973, Report A1, RIP9501209~~

~~**Guidelines:**~~ ~~none~~

~~**Deviations:**~~ ~~not applicable~~

~~**GLP:**~~ ~~no~~

~~**Acceptability:**~~ ~~The study is considered to be informative.~~

~~**Material and Methods:**~~
~~In this study the uptake of glyphosate and AMPA residues into cereal grain matrices following pre-emergent soil applicaton of either 2.24 or 4.48 kg unlabelled active substance was investigated. In several locations in Northern Europe spring barley, spring wheat and winter wheat were grown and harvested at maturity to analyse the samples for glyphosate and AMPA.~~

~~Analysis was performed by aqueous extraction, followed by clean up, derivatisation with N-trifluoro-acetyl methyl and GC-FID. However validation data only gave mean recoveries of 59 % for glyphosate and 65 % for AMPA.~~

~~**Findings:**~~
~~In 18 field trials involving treatment with 0, 2.24 or 4.48 kg as/ha to bare soil before emergence of cereals (9 x winter wheat, 3 x spring wheat and 6 x spring barley), most of the residue for both glyphosate and AMPA were <0.05 mg/kg. Glyphosate and AMPA were determined at the LOQ in 3 and 4 straw samples, respectively. No residues at or above the LOQ were found in the grain.~~

~~**Conclusions:**~~
~~This study is considered only as additional information, since analysical methods involved did not perform with acceptable recovery for data generation purposes. However the data indicate the the uptake of residues from soil was low. Apart from few detects at the LOQ, no residues above 0.05 mg/kg were found in straw or grain of barley and wheat.~~

B.7.1.1.5 Miscellaneous crops

Reference: OECD KIIA 6.2.1

Report: Malik, J. M.
CP 67573 residue and metabolism Part 24: The metabolism of CP 67573 in coffee plants
February 1975, Report No. 344, RIP9501192

Guidelines: none

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study the uptake and translocation of ^{14}C-radiolabelled glyphosate and ^{14}C-AMPA were investigated following foliar, stem, hydroponic or soil treatment to coffee plants.
For the soil treatment stocking solutions were sprayed at rates equivalent to 4.5 kg glyphosate or AMPA per ha. After 4, 6 and 8 weeks plant samples were collected.
Hydroponic treatment of coffee plants was conducted with 1.1, 3.6 or 11.1 ppm ^{14}C-glyphosate in the hydroponic solution. The treated plants were grown for 21 days before samples were collected.
For the uptake via the stem an ^{14}C-glyphosate solution was applied to the stem. The plant was kept in a hydroponic solution for 5 weeks. After this timeframe samples of leaves, untreated stems, treated stems and roots were collected.
The behaviour of glyphosate following foliar application was investigated by treating eight leaves with a solution of ^{14}C-glyphosate. After 3-5 weeks treated and untreated leaves, stems and roots were samples. In a second experiment leaves of bean producing coffee plants were treated. Each 4 weeks samples of coffee beans were collected and analysed for radioactive residues.

All samples collected were homogenised and extracted with water or 0.5N NH_4OH. Total radioactive residues were determined using LSC. For the identification of metabolites GC-MS, TLC and NMR were used and the results compared with reference substances.

Findings:
In the following table the uptake of ^{14}C-glyphosate and ^{14}C-AMPA from treated soil are summarised.

Table B.7.1-32: Uptake of applied radioactivity by coffee plants from soil treated with ^{14}C-glyphosate or ^{14}C-AMPA

Time (weeks)	Sample	^{14}C-glyphosate		^{14}C-AMPA	
		dpm	% of AR	dpm	% of AR
4	control	12,662	0.0063[a]	39,302	0.0060
	treated	6,614	0.0033	102,924	0.0157
6	control	13,663	0.0068 [a]	39,027	0.0060
	treated	22,670	0.0113	145,402	0.0222
8	control	22,970	0.0115 [a]	77,915	0.0119
	treated	33,823	0.0169	255,560	0.0381

a: compared to applied dose
AR: applied radioactivity

Following stem application the recovered radioactivity was 87.2 % of the AR in the stem, 0.54 % AR in leaves, 1.68% AR in untreated stem parts, 0.5 % AR in the roots and 1.14 % AR in the hydroponic solution. In total 91.58 % of the AR were recovered.

After hydroponic treatment for three weeks most of the AR was recovered in roots and in the remaining hydroponic solution. Analysis of the solution showed that a significant perentage of the active substance was degraded into AMPA and N-methyl-AMPA (1.1 ppm: up to 85 % AR after 55 days, 3.6 ppm:up to 10% AR after 33 days and 11.1 ppm: up to 21% AR after 33 days).
Samples of roots and aerial parts collected after three weeks were also extracted and analysed for the composition of the radioactivity. Extraction rates were between 74-90 % using methanol water and between 38-48 % using water only. Glyphosate was the major residue representing 80-91 % of the extracted radioactivity. In roots and aerial parts AMPA was also found in all samples, ranging from 9-20 % of the AR.

Table B.7.1-33: Recovery of AR following treatment via hydroponic solution with 1.1, 3.6 or 11.1 ppm ^{14}C-glyphosate for three weeks

Treatment	% AR recovered				
	Roots	Aerial parts	Root wash	hydroponic solution	Total
1.1 ppm	11.7	0.1	38.7	23.5	74.0
3.6 ppm	4.3	0.1	15.2	83.8	104.0
11.1 ppm	5.0	0.2	10.5	77.5	93.2

Table B.7.1-34: Identification of radioactive residues from hydroponically treated coffee plants after 21 days

Sample	Treatment	% TRR extracted[a]	% extracted as glyphosate (% of extract)	% extracted as glyphosate (% of extract)	% extracted as N-methyl-AMPA (% of extract)
Aerial	1.1 ppm	86	74 (86)	12 (14)	-
	3.6 ppm	74	60 (81)	14.1 (19)	-
	11.1 ppm	48	38.4 (80)	9.6 (20)	-
Roots	1.1 ppm	90	82 (91)	8.1 (9)	-
	3.6 ppm	87	79.2 (91)	7.8 (9)	-
	11.1 ppm	38	31.9 (84)	6.1 (16)	-
Hydroponic solution	1.1 ppm	not appplicable	37	58	5
	3.6 ppm	not appplicable	92	8	-
	11.1 ppm	not appplicable	87	13	-

a: based on total dpm measured compared to extracted dpm measured. Equivalents parent substance per kg sample was not reported.

For the foliar uptake of glyphosate several experiments with ^{13}C- and ^{14}C-glyphosate were used with different formulations and application techniques. For the experiment using a mixture of ^{13}C- and ^{14}C-glyphosate identification of the residue revealed only unchanged parent. An identification of the residue was only conducted following application of a $^{13/14}$C-mixture to coffee plants. In all samples glyphosate was the only residue present (>99 % of the TRR). A summary of these experiments and the results are presented below.

Table B.7.1-35: Recovery of AR following leaf treatment of coffee plants

Treatment	% AR recovered				
	treated leaves	roots	stems	untreated leaves	total
^{14}C-glyphosate, 7.7 x 10^6 dpm, upper leaf surface treated, 3 weeks DAT	86.3	1.3	6.2	2.7	96.5
^{14}C-glyphosate, 7.7 x 10^6 dpm, lower leaf surface treated, 3 weeks DAT	65.2	8.5	17.9	0.8	92.4
^{14}C-glyphosate, 1.54 x 10^7 dpm, upper and lower leaf surface treated, 3 weeks DAT	73.3	10.4	8.0	0.6	92.3
^{14}C-glyphosate, 1.54 x 10^7 dpm, upper and lower leaf surface treated, 5 weeks DAT	75.1	10.2	13.8	4.1	103.2
^{14}C-glyphosate (unformulated), 1.54 x 10^7 dpm, upper and lower leaf surface treated, 5 weeks DAT	93.8	4.3	2.1	1.8	102.0
^{13}C- & ^{14}C-glyphosate, 3.9 x 10^7 dpm, upper and lower leaf surface treated, 5 weeks DAT	43.6	26.5	11.5	2.8	87.9[a]

a: including wilted lower leaves (0.1% AR) and hydroponic solution (3.4% AR)

In a final experiment bean carrying coffee trees were treated with ^{14}C-glyphosate. The beans were grown to maturity within 23 weeks after the treatment and analysed for the recovered radioactivity and its composition. In the beans the % AR recovered increased from 0.02-0.05 % AR in immature beans to 0.94% AR in green beans and 0.62 % AR in ripe beans. Identication of the extracted radioactivity (96-99% extraction rate) gave 95 % of the residue as glyphosate and 5 % as AMPA.

Conclusions:

In coffee plants treated via soil, , stem, hydroponic or foliar application the uptake and translocation of radioactivity was minimal. Identification of the recovered radioactivity

consisted mainly of unchanged parent, followed by AMPA at much lower levels (1/4th of present glyphosat or less).

Reference: OECD KIIA 6.2.1

Report: Sutherland, M. L.; Banduhn, M. C.
The metabolism of Glyphosate in pasture crops
18.05.1976, Report No. 404, RIP9501197

Guidelines: none

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study the behaviour of ^{14}C-glyphosate in pasture crops was investigated in four experiments.
In the first experiment soil treated at rates equivalent to 4.5 kg as/ha was used for seeding descue, timothy bromegrass, alfalfa and white clover. Samples were collected after 6, 12, 18, 24 and 32 weeks.
The second layout involved treatment of quackgrass with 1.7 kg as/ha. One week after treatment the weeds were incorperated. ffescue and alfalfa were seeded one month after the initial treatment. Samples were collected after 6, 12, 18 and 24 weeks.
In the third experiment established fescue and alfalfa plants were foliar treated with 1.1 kg ^{14}C-glyphosate. After 9, 15 and 23 weeks the regrowth was samples and analysed.
The fourth experiment simulated pre-harvest use. Established fescue and alfalfa plants were treated with 1.1 kg ^{14}C-glyphosate per ha one week before harvest and allowed to air-dry.

Total radioactive residues were analysed by LSC following combustion.

Findings:
In the following table the recovered radioactivity in pasture samples grown in treated soil are summarised.

Table B.7.1-36: Recovered radioactivity in pasture crops planted after soil treatment with ^{14}C-glyphosate at rates equivalent to 4.5 kg as/ha

Sample	% AR after treatment (dpm measured)				
	6 weeks	12 weeks	18 weeks	24 weeks	32 weeks
Fescue, treated	0.08 (74,664)	0.03 (32,440)	0.05 (25,501)	0.03 (9,884)	0.03 (7,209)
Fescue, control	- (7,440)	- (2,780)	- (2,486)	- (1,071)	- (704)
Alfalfa, treated	0.03 (129,087)	0.02 (29,636)	0.02 (26,838)	<0.01 (10,194)	<0.01 (6,049)
Alfalfa, control	- (8,095)	- (3,180)	- (2,370)	- (1,104)	- (732)
Timothy bromegrass, treated	0.06 (88,384)	0.04 (37,912)	0.06 (22,,888)	0.04 (9,238)	0.03 (5,894)
Timothy bromegrass, control	- (11,337)	- (3,767)	- (1,694)	- (7,268)	- (450)
White clover, treated	<0.01 (133,621)	<0.01 (49,820)	<0.01 (23,257)	<0.01 (6,266)	<0.01 (5,024)
White clover, control	- (11,617)	- (2,176)	- (1,536)	- (468)	- (401)

After treatment of quackgrass and its incorperation into the soil recovered radioactivity in fescue and alfalfa was.

Table B.7.1-37: Recovered radioactivity in fescue and alfalfa planted after treatment of quackgrass with ^{14}C-glyphosate at rates equivalent to 1.7 kg as/ha and incorperation into the soil

Sample	% AR after treatment			
	6 weeks	12 weeks	18 weeks	24 weeks
Fescue	0.09	0.05	0.02	0.09
Alfalfa	0.01	0.01	<0.01	0.04

The recovered radioactivity in foliar treated alfalfa and fescue and the residue in the regrowth was:

Table B.7.1-38: Recovered radioactivity in fescue and alfalfa foliar treated with ^{14}C-glyphosate at rates equivalent to 1.1 kg as/ha and the residue in the regrowth

Sample	% AR after treatment			
	1 week (treated foliage)	9 weeks (regrowth)	15 weeks (regrowth)	23 weeks (regrowth)
Fescue	69	0.17	0.18	0.05
Alfalfa	55	0.20	0.19	0.06

For the fourth experiment air-dried pasture foliar treated with 1.1 kg ^{14}C-glyphosate per contained 41.8 % of the AR for fescue and 63.7 % of the AR for alfalfa.

In all experiments no identification of the radioactivity was conducted.

Conclusions:
This study provided limited information on the uptake and translocation of residues following application of glyphosate to soil, weeds or pasture foliage. In all experiments the uptake was very limited, no exceeding 0.1 % of the AR. In directly treated foliage 41.8-69 % of the AR were recovered after one week.

Reference: OECD KIIA 6.2.1

Report: Anonymous
Glyphosate residue and metabolism studies in sugarcane and soils
01.03.1976, Report No. RD93, RIP9501198

Guidelines: none

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be informative.

Material and Methods:
In this study the behaviour of ^{14}C-glyphosate in sugar cane following different methods of treatment was investigated in the USA. The active substance was applied either before pre-planting (11.2 kg as/ha), as an interline broadcast treatment (3.4 or 6.7 kg as/ha) or post-emergent treatment along the field edges (5.6 or 11.2 kg as/ha). Samples collected after different intervals were processed into bagasses. molasses, sugar and tops & leaves. All samples were analysed for residues of glyphosate and AMPA.

Samples were analysed using GC/NPD following aqueous extraction und derivatisation with TFAA. Recovery experiments using fortification levels between 0.05 mg/kg to 4 mg/kg were unsatisfactoring, provided normally less than 70 % recovered.

Findings:
In the following table the residues found in sugar cane after pre-planting treatment are summarised.

Table B.7.1-39: Residues in sugar cane and ist processed fractions following soil treatment before planting with ^{14}C-glyphosate at 11.2 kg as/ha

Location	kg as/ha	DAT	mg eq/kg							
			sugarcane		bagasse		molasses		sugar	
			glyphosate	AMPA	glyphosate	AMPA	glyphosate	AMPA	glyphosate	AMPA
Kunia	0	195	<0.05	<0.05	-	-	-	-	-	-
	11.2	195	<0.05	<0.05	-	-	-	-	-	-
Oahu	0	354	<0.05	<0.05	<0.05	<0.05	<0.5	<0.5	<0.05	<0.05
	11.2	354	<0.05	<0.05	<0.05	<0.05	<0.5	<0.5	<0.05	<0.05

After interline broadcast spraying residues found were:

Table B.7.1-40: Residues in sugar cane and its processed fractions following interline broadcast spraying with ^{14}C-glyphosate at 1.4 or 3.6 kg as/ha

Location	kg as/ha	DAT	mg eq/kg							
			sugarcane		bagasse		molasses		sugar	
			glyphosate	AMPA	glyphosate	AMPA	glyphosate	AMPA	glyphosate	AMPA
Oahu	0	0	0.1	<0.05						
		91	<0.05	<0.05						
		183	<0.05	<0.05	<0.05	<0.05	<0.5	<0.5	<0.05	<0.05
	1.4	0	0.74	<0.05						
		91	0.13	<0.05						
		183	<0.05	<0.05	<0.05	<0.05	<0.5	<0.5	<0.05	<0.05
	6.7	0	1.85	<0.05						
		91	0.11	<0.05						
		183	<0.05	<0.05	<0.05	<0.05	<0.5	<0.5	<0.05	<0.05
Mauna	0	0	0.15	<0.05						
		73	0.08	<0.05						
		165	<0.05	<0.05	<0.05	<0.05	<0.5	<0.5	<0.05	<0.05
	1.4	0	3.0	<0.05						
		73	0.40	<0.05						
		165	<0.05	<0.05	<0.05	<0.05	<0.5	<0.5	<0.05	<0.05
	6.7	0	6.9	<0.05						
		73	0.48	<0.05						
		165	0.09	<0.05	<0.05	<0.05	0.96	<0.5	0.08	<0.05

In the final part of the study the residues in sugar cane from the fields edges following application glyphosate were analysed. Residues found were:

Table B.7.1-41: Residues in sugar cane and its processed fractions following field edge spraying with ^{14}C-glyphosate at 5.6 or 11.2 kg as/ha

Location	kg as/ha	mg eq/kg									
		sugar cane		bagasse		molasses		sugar		tops and leaves	
		glyp.	AMPA	glyp.	AMPA	glyp.	AMPA	glyp.	AMPA	glyp.	AMPA
Pahokee (40 DAT)	0	<0.05	<0.05	<0.05	<0.05	-	-	-	-	0.1	<0.05
	5.6	0.14	<0.05	0.09	<0.05	3.0	<0.5	0.08	-	0.30	<0.05
	11.2	0.28	<0.05	0.14	<0.05	4.5	<0.5	0.13	<0.05	1.9	<0.05
Clewiston (44 DAT)	0	<0.05	<0.05	<0.05	<0.05	<0.5	<0.5	<0.05	<0.05	0.20	<0.05
	5.6	0.24	<0.05	0.21	<0.05	1.6	<0.5	<0.05	<0.05	1.0	<0.05
	11.2	0.34	<0.05	0.25	<0.05	3.0	<0.5	0.14	<0.05	2.0	<0.05
Franklin (47 DAT)	0	<0.05	<0.05	<0.05	<0.05	<0.5	<0.5	-	-	<0.05	<0.05
	5.6	<0.05	<0.05	<0.05	<0.05	<0.5	<0.5	<0.05	<0.05	<0.05	<0.05
	11.2	<0.05	<0.05	<0.05	<0.05	<0.5	<0.5	<0.05	<0.05	<0.05	<0.05
Waialua (42 DAT)	0	<0.05	<0.05	<0.05	<0.05	<0.5	<0.5	<0.05	<0.05	-	-
	5.6	<0.05	<0.05	<0.05	<0.05	<0.5	<0.5	<0.05	<0.05	-	-
	11.2	<0.05	<0.05	<0.05	<0.05	<0.5	<0.5	<0.05	<0.05	-	-
Oahu (44 DAT)	0	<0.05	<0.05	<0.05	<0.05	<0.5	<0.5	<0.05	<0.05	-	-
	5.6	<0.05	<0.05	<0.05	<0.05	<0.5	<0.5	<0.05	<0.05	-	-
	11.2	<0.05	<0.05	<0.05	<0.05	<0.5	<0.5	<0.05	<0.05	-	-

glyp. glyphosate

Conclusions:

This study is considered only as additional information, since analysical methods involved did not perform with acceptable recovery for data generation purposes. However the data indicate the the uptake of residues from soil was low. In addition processing of sugar cane following commercial practive indicated that glyphosate residues being present after interline or field

edge spraying are mainly found in molasses. AMPA residues were not detected, even following high application rates of up to 11.2 kg as/ha.

Conclusion non-tolerant crops
The uptake of glyphosate and AMPA via the rooots was very limited in the studies submitted. In presence of soil or sand only minimal amount (normally <1 % of the AR) were recovered in plant matrices. Following hydroponic cultivation without substrate up to 10 % of the radioactivity were found in the plants.
After foliar application the translocation into untreated parts of the plants were also limited. Some translocation was observed, however >80 % of the radioactivity were normally present in the treated compartiments.
Indentification of the radioactivity mainly revealed glyphosate as major residue. Translocated residues also showed AMPA, however its relative levels were normally below 10 % of the TRR. Following higher PHIs a larger part of the radioactivity was present in natural products, either by direct incorperation or by soil degradation and uptake of $^{14}CO_2$.

B.7.1.2 Tolerant crops (CP4 EPSPS and GOX modification)

B.7.1.2.1 Oilseeds

Reference:	OECD KIIA 6.2.1
Report:	Rogers, M. D.; Saeman Bleeke, M.; Goure, W. F.; George, C.; Nadeau, R. G. Nature of Glyphosate residues in Roundup herbicide tolerant canola 08.06.1994, MSL-13318, RIP9800118
Guidelines:	EPA §171-4(a): "Nature of Residues in Plants"
Deviations:	none
GLP:	yes
Acceptability:	The study is considered to be acceptable.

Material and Methods:
In this study two lines of genetically modified rape (canola) were treated with a mixture of ^{14}C-, ^{13}C and ^{12}C-glyphosate-isopropyl, labelled in the phosphonomethyl-moiety. The study involved two lines of tolerant rape: GT73 and GT200, including CP4 EPSPS and two varinats of the glyphosate oxidoreductase (GOX). Plant were treated under two regimens, either using a broadcast spraying equivalent to 0.455 kg as/ha at 2-4 leaf stage (14 day after planting) or two broadcast sprayings equivalent to 0.9 kg as/ha each at 2-4 leaf stage (14 days after planting) and 6-leaf stage (22 days after planting). Corresponding applications of ^{12}C-glyphosate were conducted as control plots for the radiodetection to abvoid contamination. The only commodity harvested were mature seeds after 87 days and 79 days following one or two applications, respectively.
Total radioactive residues (TRR) were analysed using combustion with LSC. Homogenised samples were extracted with three times with hexane for the oil compartiment, followed by aqueous extraction at pH2.5 with 6N HCl, leaving the extracted rape meal. The extracted

meals was further extracted involving enzymic, acidic and basic hydrolysis. For the identification of residues a broad pattern of analytical methods involving HPLC (normal phase, reverse phase and ion exchange) and GC in combination with mass spectrometry was used against reference substances.

Findings:

In treated rape seeds the following TRR levels (expressed as glyphosate) were found:

Table B.7.1-42: TRR in rape seeds (GT73, CP4 EPSPS and GOX modified) measured by LSC

Rape seed line	Application rate in kg as/ha	Test substance	TRR in mg eq/kg
GT73	0.455	^{14}C-Glyphosate	0.483
	0.455	^{12}C-Glyphosate (control)	0.027
	2 × 0.9	^{14}C-Glyphosate	8.093
	2 × 0.9	^{12}C-Glyphosate (control)	0.027
GT200	0.455	^{14}C-Glyphosate	0.845
	0.455	^{12}C-Glyphosate (control)	0.075
	2 × 0.9	^{14}C-Glyphosate	4.876
	2 × 0.9	^{12}C-Glyphosate (control)	0.077

The extraction of the samples was only conducted for the GT73 line, which was selected by the manufacturer for commercial marketing and released 21.4-30.8 % of the TRR, mainly in the aqueous extract. Most of the radioactivity was still presented in the extracted rape meal. In the following table the results of the seed extraction for the samples treated with ^{14}C-labelled active substance are summarised.

Table B.7.1-43: Extractability of radioactive residues in rape seeds (GT73, CP4 EPSPS and GOX modified)

Fraction	GT73 (1 × 0.455 kg as/ha)		GT73 (2 × 0.9 kg as/ha)	
	% TRR	mg eq/kg	% TRR	mg eq/kg
Seed (combustion)	100	0.483	100	8.093
Oil (hexane extract)	4.6	0.022	1.8	0.141
Aqueous extract	20.9	0.101	21.7	1.757
Extracted meal	69.2	0.334	78.6	6.364
Total	94.7	0.457	102.1	8.262

The identification of radioactivity in the extractes revealed that the hexane phase contained natural occuring fatty acids. Saponification of the extract showed a pattern of fatty acids characteristic to rape. No glyphosate or structural related metabolites could be identified.
In the aqueous extract AMPA was the dominant residue, found at 7.1-7.7 % TRR, followed by N-Glyceryl-AMPA (3.4-3.9 % TRR) and N-acetyl-AMPA (0.7-0.9 % TRR). Another large part of the radioactivity was identified as natural products including sucrose (4.9-5.3 % TRR), following incorperation of ^{14}C. A summary of the composition in the aqueous extract is presented below:

Table B.7.1-44: Characterisation and identification of the aqueous extract of rape seed (GT73, CP4 EPSPS and GOX modified)

	GT73 (1 × 0.455 kg as/ha)		GT73 (2 × 0.9 kg as/ha)	
	% TRR	mg eq/kg	% TRR	mg eq/kg
Total aqueous extract	20.9	0.101	21.7	1.76
AMPA	7.7	0.037	7.1	0.58
N-glyceryl-AMPA	3.4	0.017	3.9	0.31
N-acetyl-AMPA	0.9	0.004	0.7	0.06
natural products (sucrose)	4.9 (<2)	0.024 (<0.01)	5.3 (1.6)	0.43 (0.13)
Total identified or characterised	16.9	0.082	17.0	1.38

The extracted meal of the sample treated twice at 0.9 kg as/ha was further investigated using enzymic, acidic and basic hydrolysis. The only metabolite identified was AMPA (up to 5.8 % TRR) following NaOH hydrolysis. Other radioactivity was present in natural products like proteins, starch, cellulose, lignin, amino acids and formate.

Table B.7.1-45: Hydrolysis of rape meal (GT73, CP4 EPSPS and GOX modified, 2 × 0.9 kg as/ha)

Fraction	% TRR	mg eq/kg	Association to
Extracted meal	78.6	6.36	
Sequential enzyme hydrolyses*			
Protease	5.9	0.48	proteins
Amylase	0.9	0.07	starch
Cellulase	1.7	0.14	cellulose
Extraction with Dioxane and water*			
Bjorkman lignin	1.7	0.14	free lignin
Acidolysis lignin	3.0	0.25	bound lignin
unassigned	11.0	0.89	
Hydrolysis with 6N HCl*			
Total	13.3	1.08	
Derivatised with n-butanol	11.6	0.94	amino and organic acids
0.1N NaOH hydrolysis*			
Total	39.9	3.23	
unknown	3.5	0.28	
Formate	16.5	1.34	
AMPA	5.8	0.47	
2.5N NaOH hydrolysis*			
Total	63.6	5.15	
unknown	5.6	0.45	
Formate	16.5	1.34	
AMPA	3.7	0.3	
natural products	14.9	1.21	
Insoluble biopolymers	19.7	1.59	
Unextracted*	5.8	0.47	

* not performed subsequently on the same sample

Conclusions:

In GOX modified rape seed treated at early growth stages parent glyphosate was completely degraded, leaving minor levels of AMPA, N-glyceryl-AMPA and N-acetyl-AMPA as metabolites identified. The major part of the radioactivity applied was incorperated into natural products like fatty acids, protein, starch, lignin and cellulose.

Reference: OECD KIIA 6.2.1

Report: Goure, W. F.
Nature of Glyphosate residues in soybeans tolerant to Roundup herbicide
07.07.1994, MSL-13520, RIP9800117

Guidelines: EPA §171-4(a): "Nature of Residues in Plants"

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
For the investigation of the metabolism of glyphosate in CP4 EPSPS modified soya beans the plants were treated with ^{14}C-glyphosate-isopropyl, labelled in the phosphonomethyl-moiety. The study was conducted in a controlled environment growth chamber.

The treatment consisted of three regimens. In pre-emergence a rate equivalent to 5.38 kg as/ha was applied to bare soil immediately before planting of soya bans. For post-emergence the first application was at a rate equivalent to 0.84 kg as/ha, applied 21 days after planting (BBCH 23). A second plot was conducted including also a second application at a rate equivalent to 1.68 kg as/ha 43 days after planting (BBCH 51). Approximately two weeks after each application, the foliage of the plants was washed to remove surface residues on nonabsorbed ^{14}C-glyphosate. Crop samples (soya bean forage, hay and seeds) were collected to simulate agricultural practice.
In parallel control plots were treated with ^{12}C-glyphosate. The plants were either kept in the same growth chamber with ^{14}C-treated plants to investigate the uptake of $^{14}C\text{-}CO_2$ formed by degradation in soil or in separate chambers as control.

Total radioactive residues (TRR) were analysed using combustion with LSC. Forage and hay samples were extracted with water. Seed samples were first extracted with hexane to remove the oil, then with acetonitrile/water (v/v 1:1) and finally with water.
For the identification of residues a broad pattern of analytical methods involving HPLC (normal phase, reverse phase and ion exchange) and GC in combination with mass spectrometry was used against reference substances.

Findings:
In the following table the TRR levels (expressed as glyphosate) and the extractablility following the different treatment regimens are summarised. For plants treated with ^{12}C-glyphosate in separate growth chambers, no radioactivity above the LOQ of 0.001 mg eq/kg was observed.

Table B.7.1-46: TRR levels and extractability of soya bean forage, hay and grain following pre- and post-emergence treatment (CP4 EPSPS modified)

Matrix	DALT	TRR in mg eq/kg	mg eq/kg (% TRR) Hexane extract	Aqueous extract	Unextracted	% Total
Pre-emergence (5.38 kg as/ha)						
Forage (^{12}C control)[a]	56	0.135	np	0.024 (17.7)	0.113 (83.7)	101.4
Forage (^{14}C)	56	0.239	np	0.056 (23.5)	0.175 (73.2)	96.6
Hay (^{12}C control)[a]	84	0.121	np	0.029 (24.3)	0.094 (77.9)	102.2
Hay (^{14}C)	84	0.205	np	0.063 (30.8)	0.153 (74.4)	105.2
Seeds (^{12}C control)[a]	104	0.445	0.076 (17.0)	0.092 (20.6)	0.269 (60.5)	98.0
Seeds (^{14}C)	104	0.748	0.106 (14.2)	0.207 (27.6)	0.420 (56.1)	98.0
Post-emergence (1 × 0.84 kg as/ha)						
Forage (^{12}C control)[a]	35	0.014	np	np	np	np
Forage (^{14}C)	35	0.863	np	0.818 (94.8)	0.04 (4.7)	99.5
Hay (^{12}C control)[a]	63	0.034	np	np	np	np
Hay (^{14}C)	63	0.546	np	0.436 (79.9)	0.048 (8.9)	88.8
Seeds (^{12}C control)[a]	83	0.193	0.041 (21.2)	0.049 (25.2)	0.102 (52.8)	99.2
Seeds (^{14}C)	83	0.406	0.037 (9.0)	0.20 (49.2)	0.148 (36.5)	94.6
Post-emergence (1 × 0.84 kg as/ha + 1 × 1.68 kg as/ha)						
Forage (^{12}C control)[a]	13	0.014	np	np	np	np
Forage (^{14}C)	13	23.651	np	24.637 (104.2)	0.908 (3.8)	108.0
Hay (^{12}C control)[a]	41	0.033	np	np	np	np
Hay (^{14}C)	41	10.416	np	8.015 (77.0)	0.787 (7.6)	84.5
Seeds (^{12}C control)[a]	61	0.224	np	np	np	np
Seeds (^{14}C)	61	17.459	0.086 (0.5)	14.545 (83.3)	2.020 (11.6)	95.4

np: not performed
a: samples collected from plants in the same growth chamber as ^{14}C-treated plants

Further analysis for the composition of radioactivity was only conducted for the post-emergence samples treated twice (1 × 0.84 kg as/ha + 1 × 1.68 kg as/ha). The results for forage, hay and grain are presented in the following tables:

Table B.7.1-47: Composition of radioactivity in soya bean forage (EPSPS modified, 1 × 0.84 kg as/ha + 1 × 1.68 kg as/ha)

Soya bean forage	mg eq/kg	% TRR
Total	23.651	100
Aqueous extract	24.637	104.2
glyphosate	21.078	89.1
AMPA	1.619	6.8
N-methyl-AMPA	0.140	0.6
natural products	0.618	2.6
Unextracted	0.908	3.8
Total recovered	23.455	99.1

Table B.7.1-48: Composition of radioactivity in soya bean hay (EPSPS modified, 1 × 0.84 kg as/ha + 1 × 1.68 kg as/ha)

Soya bean hay	mg eq/kg	% TRR
Total	10.416	100
Aqueous extract	8.015	77.0
glyphosate	5.582	53.6
AMPA	1.328	12.8
N-methyl-AMPA	0.130	1.3
N-glyceryl-AMPA	0.084	0.8
unknown	0.059	0.6
natural products	0.274	2.6
Unextracted	0.787	7.6
Total recovered	7.457	71.7

Table B.7.1-49: Composition of radioactivity in soya bean seeds (EPSPS modified, 1 × 0.84 kg as/ha + 1 × 1.68 kg as/ha)

Soya bean seeds	mg eq/kg	% TRR
Total	17.459	100
Hexane extract	0.159	0.9
saponifiable fatty acids	0.137	0.8
Aqueous extract	15.383	88.1
glyphosate	4.402	25.2
AMPA	8.579	49.1
N-methyl-AMPA	0.131	0.8
N-glyceryl-AMPA	0.278	1.6
AMPA conjugate	0.177	1.0
N-acetyl-AMPA	0.235	1.4
N-malonyl-AMPA	0.309	1.8
natural products	0.468	2.7
HCl extract	1.007	5.8
amino acids and natual organic acids	0.897	5.1
Unextracted	0.143	0.8
Total recovered	15.613	89.5

Conclusions:

In CP4-EPSPS modified soya beans parent glyphosate was the major residue in plant parts directly affected by the application (forage, hay). The major metabolite present was AMPA, ranging from 6.8-12.8 % of the TRR. Further metabolites present were N-methyl-AMPA and N-glyceryl-AMPA, both at 1.3 % of the TRR or less.

In soya bean seeds TRR levels were in the same order of magnitude as in forage and hay, although soya beans were treated before formation of the pods. Parent glyphosate was present at 25.2 % of the TRR, however AMPA posed the major residue with 49.1 % TRR. In addition N-methyl-AMPA, N-glyceryl-AMPA, AMPA conjugates, N-acetyl-AMPA and N-malonyl-AMPA were indentified at levels between 0.8 % and 1.8 % of the TRR.

The results obtained suggest that glyphosate is systemically available in soya bean plants and also present in plant parts not directly affected by the application. In CP4-EPSPS modified plants glyphosate is still the major residue in treated plant parts. Translocation in the plants is also observed for parent glyphosate, however AMPA was the dominant residue found. An additional part of the study showed that glyphosate is degraded in soil into $^{14}CO_2$, which is again taken up by the plants and incorperated into natrual products.

Reference: OECD KIIA 6.2.1

Report:	Bleeke, M. S. Nature of Glyphosate residues in cotton plants tolerant to Roundup herbicide. March 1997, Report MSL-14113, RIP9700619
Guidelines:	EPA §171-4(a): "Nature of Residues in Plants"
Deviations:	none
GLP:	yes
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Cotton plants tolerant to glyphosate by expressing the CP4-EPSPS protein was treated with ^{14}C-radiolabelled active substance in two treatments with 0.93 kg as/ha at the 3-4 leaf stage (42 days after planting) and with 1.26 kg as/ha at the 5-6 leaf stage (51 days after planting). Forage samples were collected 27 days, mature cotton seeds 158 days after the final application. To distinguish between foliar and root uptake duplicate experiments were conducted either covering the soil (protected) or not (unprotected). In parallel replicate plots in the same greenhouse treated with unlabelled glyphosate at identical rates were conducted to measure the incorperation of $^{14}CO_2$ formed in soil metabolism.

Total radioactive residues were analysed by combustion and LSC. Forage samples were rinsed and extracted using water. Seed samples were initially extracted with hexane to remove the oil and afterwards with acetonitrile/water (1:1, v/v). For identification of radioactive residues exchange chromatography and HPLC were used for the isolation of metabolites, followed by mass spectrometry and comparison with know reference substances using HPLC and TLC. Unextracted residues after solvent extraction were additionally extracted using water, acetonitrile, 0.1 N HCl, 0.1 N NaOH, 1% sodium lauryl sulfate and acetone/water (7:3, v/v).

Findings:
In the following table the total radioactive residues following application of ^{14}C- and ^{12}C-glyphosate to cotton are summarised:

Table B.7.1-50: Total radioactive residues in tolerant cotton matrices and soil following foliar application of ^{14}C-glyphosate

Matrix	**mg eq/kg (^{14}C-glyphosate) treated**		**mg eq/kg (^{12}C-glyphosate treated[a])**	
	unprotected soil	protected soil	unprotected soil	protected soil
Plant, after 1st application	289	180	0.005	0.006
Plant, after 2nd application	352	316	0.014	0.005
Forage (27 DAT)				
total[b]	15.2	30.4	0.039	0.008
rinsed	6.28	6.98	-	-
rinse	11.2	15.9	-	-
Mature stalk (158 DAT)	0.179	0.105	0.047	0.008
Seed (158 DAT)	0.181	0.107	0.070	0.018
Cotton lint (158 DAT)	0.140	0.083	0.057	0.016
Soil, before 1st application	0-15 cm: <0.001 15-30 cm: <0.001	0-15 cm: <0.001 15-30 cm: <0.001	not analysed	not analysed
Soil, after 1st application	0-15 cm: 0.472 15-30 cm: 0.01	0-15 cm: 0.002 15-30 cm: <0.001	not analysed	not analysed
Soil, before 2nd application	0-15 cm: 0.569 15-30 cm: -	0-15 cm: 0.001 15-30 cm: 0.003	not analysed	not analysed
Soil, after 2nd application	0-15 cm: 0.569 15-30 cm: 0.002	0-15 cm: 0.004 15-30 cm: <0.001	not analysed	not analysed
Soil, forage harvest	0-15 cm: 1.07 15-30 cm: 0.018	0-15 cm: 0.013 15-30 cm: <0.001	not analysed	not analysed
Soil, seed harvest	0-15 cm: 0.701 15-30 cm: 0.009	0-15 cm: 0.016 15-30 cm: 0.023	not analysed	not analysed

a: treatment with unlabelled material to measure incorperation of ^{14}C formed in soil metabolism
b: measured by combustion

The extractability of residues and their composition was:

Table B.7.1-51: Extractability and compositon of radioactive residues in tolerant cotton forage and seeds following foliar application with ^{14}C-glyphosate

Fraction	Residues in forage as mg eq/kg (% TRR)		Residues in seeds as mg eq/kg (% TRR)	
	unprotected	protected	unprotected	protected
TRR	15.2 (100)	30.4 (100)	0.181 (100)	0.107 (100)
Hexane extracted oil	NP	NP	0.027 (14.7)	0.012 (11.3)
saponifiable fatty acids	NP	NP	0.022 (12.3)	0.011 (10.4)
Aqueous extract	14.7 (96.9)	30.0 (98.5)	0.034 (18.6)	0.034 (31.9)
glyphosate	13.9 (91.5)	29.1 (95.7)	0.022 (12.0)	0.025 (23.7)
AMPA	0.243 (1.6)	0.201 (0.66)	<0.002 (<1%)	0.001 (1.38)
glyphosate-conjugate	0.082 (0.54)	0.087 (0.29)	-	-
natural products	0.127 (0.83)	0.123 (0.4)	0.011 (5.83)	0.007 (6.93)
Unextracted	0.708 (4.7)	0.447 (1.47)	0.136 (75.4)	0.058 (54.1)
Total recovered	15.4 (101.6)	30.4 (100)	0.197 (108.7)	0.104 (97.4)
Identified/characterised	14.4 (94.5)	29.5 (97.1)	0.054 (30.1)	0.045 (42.4)

NP: not performed

Unextracted residues in cotton seeds from protected soil samples were further characterised by extraction with water, acetonitrile, 0.1 N HCl, 0.1 N NaOH, 1% sodium lauryl sulfate and acetone/water (7:3, v/v).

Table B.7.1-52: Additional radioactivity released from tolerant cotton seeds by extraction with different solvents

Solvent	Unextracted residue in mg eq/kg (% TRR)	additional ^{14}C extracted		final unextracted residue
		% TRR	mg eq/kg	mg eq/kg
Protected seed	0.058 (54%)			
Water		4.42	0.005	0.053
Acetonitrile		1.61	0.002	0.056
0.1N HCl		8.09	0.009	0.049
0.1 N NaOH		10.72	0.011	0.047
1 % Na-Laurylsulfate		11.77	0.013	0.045
70 % acetone/water		7.88	0.008	0.050

Conclusions:

In this study on tolerant cotton modified with CP4 EPSPS most of the residue recovered after foliar treatment were identified as unchanged glyphosate. In the forage approximated 2/3 of the TRR were water rinsable surface residues. The total residue in forage consisted of glyphosate (91.5-95.7 % TRR), with traces on AMPA also being present (0.66-1.6 % TRR).

In the seeds, which had not formed at the point of application, TRR levels were relatively low (0.107-0.181 mg eq/kg). Glyphosate was the major residue extracted (12-23.7 % TRR). AMPA was only a minor component of the residue (<1-1.38 % TRR). The largest part of the radioactivity remained unextracted, even after intensive extraction including acidid and basic solvents.

B.7.1.2.2 Root and tuber crops

Reference:	OECD KIIA 6.2.1
Report:	Mehrsheikh, A. Metabolism of Glyphosate in Roundup Ready Sugarbeet 08.08.2000, MSL-16247, RIP2001-906 & Mehrsheikh, A. Protocol - Metabolism of Glyphosate in Roundup Ready Sugarbeet 01.09.1999, 99-63-M-7, RIP2003-1134
Guidelines:	EU Guidance to Dir. 91/414/EEC
Deviations:	none
GLP:	yes
Acceptability:	The study is considered to be acceptable.

Material and Methods:
In this study a mixture of ^{12}C-. ^{13}C- and ^{14}C-glyphosate (labelled in the phosphonomethyl-moiety) was applied to sugar beet line 77, which was modified to express CP4 EPSPS. The sugar beets were grown outdoor in screened enclosures and treated either pre-emergence with 0.9 kg as/ha one day before planting or post-emergence with 1.08 kg as/ha at 2-4 true leaf stage (BBCH 14, 35 d after planting) followed by 1.08 kg as/ha at 12-14 true leaf stage (BBCH 19, 68 days after planting).
Samples of sugar beets were collected at maturity 159 days after planting (160 DAT for pre-emergence application and 91 DAT for post-emergence application).

Total radioactive residues (TRR) were analysed using combustion with LSC. Samples were extracted with water.
For the identification of residues a broad pattern of analytical methods involving HPLC (normal phase, reverse phase and ion exchange) and GC in combination with mass spectrometry was used against reference substances. In addition to reference substances ratios of ^{12}C and ^{13}C were used to identify incorperated glyphosate residues or degradation products.

Findings:
In the following table the TRR levels (expressed as glyphosate) and the extractablility following the pre- or post-emergence application are summarised.

Table B.7.1-53: TRR levels and extractablity of radioactive residues in sugar beets (EPSPS modified) treated pre- or post-emergence

Matrix	DAT	TRR treated	TRR control	Aqueous extract* mg eq/kg (% TRR)	Unextracted* mg eq/kg (% TRR)	Total recovered* % TRR
Tops, pre-emergence	160	0.005	<0.001	0.003 (59)	0.003 (50	109
Roots, pre-emergence	160	0.008	0.002	0.007 (86)	0.002 (20)	106
Tops, post-emergence	91	3.437	<0.001	2.978 (86.7)	0.062 (1.8)	88.5
Roots, post-emergence	91	1.396	0.001	1.442 (103.3)	0.018 (1.32)	104.6

*: based on treated samples

The composition of radioactivity was only investigated for sugar beets treated post-emergent:

Table B.7.1-54: Identification and characterisation of radioactivity in sugar beets (EPSPS modified) treated post-emergence

Compound	Tops		Roots	
	mg eq/kg	% TRR	mg eq/kg	% TRR
Total residue	3.437	100	1.396	100
Aqueous extract	2.978	86.7	1.442	103.3
Glyphosate	2.470	76.7	1.330	95.3
AMPA	0.06	1.84	0.05	3.79
Natural products	0.05	1.38	0.02	1.22
Glyphosate/AMPA acetylated conjugates	0.03	0.8	0.01	0.55
Total identified/characterised	2.880	83.67	1.41	100.87

Conclusions:
In EPSPS-modified sugar beets treated pre-emergence only minor TRR was found in samples collected at maturity. Following post-emergence treatment TRR levels were much higher, with approximately three times higher residues in sugar beet tops compared to the roots. In both matrices glyphosate was the major residue, being present at 76.7 % TRR in tops and 95.3 % TRR in roots. Other metabolites identified were AMPA or acetylated conjugates of glpyhosate and AMPA, each amounts 0.06 mg eq/kg or less (up to 3.79% TRR).
In summary it can be concluded that no glyphosate is taken up from soil by sugar beets. Residues were present in treated parts of the plants and a translocation into the roots is observed. In CP4 EPSPS sugar beets the metabolism of glyphosate was limited with unchanged parent posing the major residue >75% TRR in all matrices.

B.7.1.2.3 Cereal grains

Reference: OECD KIIA 6.2.1

Report: George, Ch.
Nature of Glyphosate residues in corn plants which are tolerant to Roundup herbicide
12.06.1995, Report MSL-14018, RIP9700618

Guidelines: EPA §171-4(a): “Nature of Residues in Plants”

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study "Roundup Ready™" maize expressing both CP4 EPSPS and GOX proteins was treated in greenhouse with ^{14}C-glyphosate in two foliar applications at rates equivalent to 0.93 kg as/ha (5-6 leaf stage) and 0.84 kg as/ha (10-12 leaf stage, 4 weeks later). Samples were collected immediately after each treatment and in the forage (3 DAT), silage (49 & 53 DAT) and maturity growth stage (grain & fodder, 83 DAT). To distinguish between foliar and root uptake duplicate experiments were conducted either covering the soil (protected) or not (unprotected). In parallel replicate plots in the same greenhouse treated with unlabelled glyphosate at identical rates were conducted to measure the incorperation of $^{14}CO_2$ formed in soil metabolism.

Total radioactive residues were analysed by combustion and LSC. Forage and silage samples collected were rinsed using water. Plant, forage, silage and fodder samples were extracted using water. Grain samples were initially extracted with hexane to remove the oil and afterwards extracted with water. For identification of radioactive residues exchange chromatography and HPLC were used for the isolation of metabolites, followed by mass spectrometry and comparison with know reference substances using HPLC and TLC. Unextracted residues after solvent extraction in grain were additionally hydrolysed using protease, amylase, cellulase and finally 2N HCl (4h reflux).

Findings:
In the following table the total radioactive residues following application of ^{14}C- and ^{12}C-glyphosate to maize are summarised:

Table B.7.1-55: Total radioactive residues in tolerant maize matrices and soil following foliar application of ^{14}C-glyphosate

Matrix	**mg eq/kg (^{14}C-glyphosate) treated**		**mg eq/kg (^{12}C-glyphosate treated[a])**	
	unprotected soil	protected soil	unprotected soil	protected soil
Plant, after 1st application	62.9	34.4	0.0128	0.00525
Plant, after 2nd application	12.2	8.96	0.0140	0.0037
Forage (3 DAT)				
total[b]	10.8	13.3	0.0165	0.00213
rinsed	5.35	4.87	-	-
rinse	3.66	4.58	-	-
Silage (49 & 53 DAT)				
total[b]	9.59	9.11	0.0486	0.0107
rinsed	5.44	4.53	-	-
rinse	1.24	0.711	-	-
Grain (83 DAT)	1.04	0.685	0.0639	0.0146
Fodder (83 DAT)	19.1	14.9	0.129	0.0433
Soil, before 1st application	0-15 cm: <0.001 15-30 cm: <0.001	0-15 cm: <0.001 15-30 cm: <0.001	not analysed	not analysed
Soil, after 1st application	0-15 cm: 0.051 15-30 cm: <0.001	0-15 cm: 0.001 15-30 cm: 0.002	not analysed	not analysed
Soil, before 2nd application	0-15 cm: 0.169 15-30 cm: 0.002	0-15 cm: 0.015 15-30 cm: 0.002	not analysed	not analysed
Soil, after 2nd application	0-15 cm: 0.294 15-30 cm: 0.002	0-15 cm: 0.022 15-30 cm: 0.002	not analysed	not analysed
Soil, forage harvest	0-15 cm: 0.049 15-30 cm: 0.002	0-15 cm: 0.019 15-30 cm: 0.002	not analysed	not analysed
Soil, silage harvest	0-15 cm: 0.057 15-30 cm: 0.004	0-15 cm: 0.026 15-30 cm: 0.014	not analysed	not analysed
Soil, grain & fodder harvest	0-15 cm: 0.067 15-30 cm: 0.037	0-15 cm: 0.024 15-30 cm: 0.016	not analysed	not analysed

a: treatment with unlabelled material to measure incorporation of ^{14}C formed in soil metabolism
b: measured by combustion

In parallel to the rinsing experiment samples were extracted using water (plus hexane for grain) followed by identification of the residue. In the following table the results are summarised:

Table B.7.1-56: Extractability and identification of radioactive residues in maize matrices following foliar application of ^{14}C-glyphosate

Matrix	mg eq/kg (% TRR)							
	TRR	AMPA	Glyphosate	Natural products after hydrolysis	N-glyceryl-AMPA	Glyphoate-conjugates	Total characterised/ identified	Unextracted by solvent
Protected soil								
Forage (3 DAT)	13.3	1.25 (9.4)	10.8 (80.9)	0.25 (1.9)	0.05 (0.4)	0.05 (0.4)	12.4 (92.9)	0.38 (2.9)
Silage (53 DAT)	9.11	0.82 (9.0)	7.09 (77.9)	0.23 (2.6)	0.11 (1.2)	0.06 (0.7)	8.32 (91.4)	0.4 (4.4)
Fodder (83 DAT)	14.9	0.73 (4.9)	12.4 (83.3)	0.36 (2.4)	0.17 (1.2)	0.2 (1.3)	13.8 (93.1)	0.68 (4.5)
Grain (83 DAT)	0.685	0.37 (54.1)	0.05 (7.4)	0.16[a] (23.2)	0.05 (6.9)	-	0.63 (91.8)	0.14 (20.9)
Unprotected soil								
Forage (3 DAT)	10.8	1.72 (15.9)	7.77 (71.9)	0.24 (2.2)	0.06 (0.5)	0.04 (0.4)	9.8 (91.0)	0.31 (2.8)
Silage (49 DAT)	9.59	1.26 (13.1)	6.43 (67.1)	0.34 (3.5)	0.14 (1.5)	0.04 (0.4)	8.2 (85.6)	0.43 (4.5)
Fodder (83 DAT)	19.1	2.13 (11.2)	14.27 (74.8)	0.65 (3.4)	0.31 (1.6)	0.36 (1.9)	17.7 (92.8)	1.0 (5.4)
Grain (83 DAT)	1.04	0.63 (60.3)	0.03 (2.6)	0.27[b] (25.5)	0.07 (6.9)	-	1.0 (95.3)	0.24 (23.2)

a: 0.009 mg eq/kg saponifiable fatty acids and 0.13 mg eq/kg starch
b: 0.01 mg eq/kg saponifiable fatty acids and 0.22 mg eq/kg starch

Conclusions:
In this study involving treatment of "RoundUp Ready™" maize expressing both CP4 EPSPS and GOX proteins with ^{14}C-glyphosate, it was demonstrated that the uptake via the foliage is the dominant route. After three days approximated 50% of the applied radioactivity could be removed by rinsing. This rate declined 20% after 49-53 days in silage. In the grain radioactive residues were present following translocation, however the levels were minor compared to the rest of the plant. Control samples kept in the same greenhouse showed minimal radioactivity, not suggesting incorperation of $^{14}CO_2$ as an relevant source of radioactive residues.
The identification of the residues showed unchanged parent glyphosate as the major residue in all plant parts directly affected. AMPA was also found, however its relative levels were always below 10 % of the TRR. In grain the ratio was different. Most of the residue (54-60 % TRR) was identified as AMPA. Parent glyphosate was also present, but at very low levels of 2.6-7.4 % TRR. The remaining radioactivity was incorperated mainly in starch and to a minor extinct in saponifiable fatty acids.

Conclusion GOX and CP4 EPSPS modified crops:
Depending on the type of glyphosate tolerance the residue pattern to be expected may differ strongly. Modifications affecting the CP4 EPSPS result in a metabolism pathway identical to non-tolerant crops, mainly consisting of unchanged parent glyphosate with minor levels of AMPA. The tolerance is mainly based on the highly reduced sensitivity of the protein against glyphosate. However expression of the GOX protein strongly accelerates the biotransformation of glyphosate into AMPA, making it the major residue in all commodities investigated. Glyphosate was still present, but at much lower levels normally contributing less than 10% to the TRR.

B.7.1.3 Tolerant crops (GAT)

Reference: OECD KIIA 6.2.1

Report: Chapleo, S.; McLachlan, T.
The metabolism of [^{14}C]Glyphosate in 0827 canola
29.04.2010, DuPont-26109, ASB2011-13744

Guidelines: Nature of the Residue - Plants and the recommendations of EU Commission Directive 96/68/EC Annex II, Section 8.1 (21. October 1996)
US-EPA: OPPTS 860.1300 – Nature of the Residue – Plants
Canadian PMRA Residue Chemistry Test Guidelines Dir 98-02, Section 2

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
This study examined the metabolic fate of [^{14}C]-glyphosate in 0827 rape (canola) modified to express the glyphosate N-acetyltransferase (GAT) gene. Magnitude and nature of the residues in rape were examined following an application regime comprising one pre-emergence soil application (4.5 kg as/ha) and three foliar applications (at 2 and 5 leaf stages and at 1 week before maturity, each 1.9 kg as/ha) of [^{14}C]-glyphosate.
Rape plants were harvested midway between the second and third foliar application (foliage, Harvest 1), immediately prior to the final application (pods with seeds, foliage) and at maturity (seeds). Tissues were homogenized and extracted with 0.1 % formic acid (aqueous):methanol (96:4 v/v). Post extraction solids of foliage and seeds were extracted further with α-amylase (pH 7, 2 x 72 H, 50 °C), a mixture of amyloglucosidase and cellulose (pH 5, 2 x 48 h, 50 °C), NaOH (0.1 N, 60 °C, 2 x 6 h) and HCl (1.0 N, 60 °C, 2 x 6 h). The TRR was determined as the sum of the total dpm in the extractable and unextracted residues expressed as mg/kg equivalents of the parent glyphosate. Extracts containing ≥0.01 mg/kg were analysed by HPLC and the identification of residues was accomplished with reference to authenticated reference standards.

Findings:
The total radioactive residue (TRR) in seeds was 2.155 mg/kg, those in foliage and immature pods were 1.550 and 1.273 mg/kg, respectively. The majority (≥78.4% of the TRR) of the foliage, pod and seeds residues was readily extracted with 0.1 % formic acid (aqueous) : methanol (96:4 v/v). The further extractions released an additional 0.9-11.7 % TRR from mature seeds and an additional 0.4-1.7 % TRR from foliage. Terminal unextracted residues were 2.7-3.6 % TRR with the exception of immature pods (20.4 % TRR).

Table B.7.1-57: Total Radioactive Residues (TRRs) in rape (GAT modified)

Matrix	Timing and Applic. No.	PHI (days)	TRR	
			mg eq/kg	% TRR
Foliage	BBCH 69, end of flowering	38 day after 2nd post-emergence application	Extracted: 5.818 Unextracted: 0.161 Total: 5.979	Extractable: 97.3 Unextracted: 2.7 Total: 100
Foliage	BBCH 87	prior final application	Extractable: 1.494 Unextracted: 0.056 Total: 1.550	Extractable: 96.4 Unextracted: 3.6 Total: 100
Immature pods	BBCH 87	prior final application	Extractable: 1.013 Unextracted: 0.260 Total: 1.273	Extractable: 79.6 Unextracted: 20.4 Total: 100
Mature seeds	BBCH 89	7 days	Extractable: 2.080 Unextracted: 0.075 Total: 2.155	Extractable: 96.5 Unextracted: 3.5 Total: 100

The composition of radioactivity in rape foliage, immature pods and seeds was:

Table B.7.1-58: Composition of radioactive residues in rape foliage, immature pods and seeds (GAT modified)

Metabolite Fraction	Foliage (BBCH 69)		Immature pods (BBCH 87)		Foliage (BBCH 87)		Seeds	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR	100	5.979	100	1.273	100	1.550	100	2.155
Glyphosate	3.0	0.179	ND	ND	ND	ND	20.8	0.448
AMPA	1.4	0.084	ND	ND	ND	ND	1.9	0.041
N-acetyl AMPA	3.4	0.203	ND	ND	ND	ND	14.7	0.316
N-acetyl Glyphosate	89.5	5.351	79.6	1.013	93.0	1.442	51.1	1.101
Unidentified	ND	ND	ND	ND	ND	ND	7.1	0.152
Unextracted	2.7	0.161	20.4	0.260	3.6	0.056	3.5	0.075

ND: not detected

Conclusions:

In GAT modified rape N-acetyl-glyphosate was the major residue found at levels between 51.1-93 % of the TRR. In foliage and imature pods, samples prior to the next application, no or only minor residues of unchanged parent glyphosate were found. Application of glyphosate 7 days before harvest resulted in 20.8 % of the TRR still being present in mature seeds. In addition AMPA and N-acetyl-AMPA were found in early foliage and seeds, ranging up to 14.7 % of the TRR in seeds one week after the final treatment.

In summary it can be concluded that GAT modified rape quickly degrades glyphosate into N-acetyl-glyphosate. After more than one week only minor levels of glyphosate remain in unchanged. AMPA and N-acetyl-AMPA are only present in minor amounts except for N-acetyl-AMPA in seeds treated one week before harvest (14.7 % TRR).

Reference: OECD KIIA 6.2.1

Report: MacDonald, A. M. G.
The metabolism of [^{14}C]Glyphosate in GAT/GM-HRA (DP-356Ø43-5, PHP20163a) soybeans
16.10.2007, DuPont-19530, ASB2008-2658

Guidelines: Nature of the Residue - Plants and the recommendations of EU Commission Directive 96/68/EC Annex II, Section 8.1 (21. October 1996)
US-EPA: OPPTS 860.1300 – Nature of the Residue – Plants
Canadian PMRA Residue Chemistry Test Guidelines Dir 98-02, Section 2

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
For soybeans ^{14}C-glyphosate was applied four times to GAT modified plants. The first application of 3.4 kg as/ha (acid equivalents) was conducted to the soil before emergence of the soya beans. Subsequent foliar application of 1.5 kg as/ha (about 10 days before mid-full bloom), 2.4 kg as/ha (full bloom) and 0.9 kg as/ha (14 days before maturity) followed.
Samples of the plants were taken at four times: soya bean forage was collected 36 days after the soil treatment immediately before the first foliar treatment was applied; soya bean hay was sampled 4 days after the first foliar treatment. Foliage (with pods and grain) was collected 82 days after the second foliar application and immediately before the final treatment. Mature plants (foliage, pods and grain) were harvested 14 days after the final treatment.

All samples were homogenized and extracted using 0.1 % formic acid (aqueous):methanol (96:4 v/v) followed by enzyme (α-amylase then amyloglucosidase and cellulose), alkaline (NaOH 0.1 N, 60 °C, 6h) then acid (HCl, 1 N, 60 °C, 6h) digestion. The total radioactive residue (TRR) was determined as the sum of the total radioactivity by LSC. Extracts containing more the 0.01 mg/kg TRR were analyzed by HPLC (UV, RAD) using authenticated reference standards. In addition TLC was used as confirmation method.

Findings:
In early samples following the soil application 42.9 % of the TRR remained unextracted using aqueous methanol, followed by enzymic, basic and acidic hydrolysis. TRR levels in samples following soil application were 0.428 mg eq/kg. After foliar application samples (hay, foliage, pods and seeds) gave higher extraction rates between 98.3-99.2% of the TRR (1.905-22.087 mg eq/kg). An overview of the radioactive residues found in soya bean matrices is presented below:

Table B.7.1-59: TRR levels and extractability of residues in soya bean samples (GAT modified)

Extract	Forage (a) mg eq/kg (% TRR)	Hay (b) mg eq/kg (% TRR)	Seeds (c) mg eq/kg (% TRR)	Foliage (c) mg eq/kg (% TRR)	Seeds (d) mg eq/kg (% TRR)	Pods (d) mg eq/kg (% TRR)	Foliage (d) mg eq/kg (% TRR)
Extracted residue	0.245 (57.1)	13.323 (99.1)	1.874 (98.3)	11.079 (98.7)	3.101 (98.7)	17.611 (99.2)	21.844 (98.9)
0.1% formic acid (aqueous):methanol (96:4 v/v)	0.123 (28.7)	12.893 (95.9)	1.694 (88.9)	9.676 (86.2)	2.765 (88)	15.639 (88.1)	19.481 (88.2)
α-amylase	0.063 (14.8)	0.309 (2.3)	0.128 (6.7)	1.123 (10)	0.239 (7.6)	1.385 (7.8)	1.833 (8.3)
amyloglucosidase and cellulase	0.026 (6.0)	0.081 (0.6)	0.038 (2.0)	0.236 (2.1)	0.075 (2.4)	0.444 (2.5)	0.442 (2.0)
NaOH (0.1N)	0.019 (4.4)	0.027 (0.2)	0.008 (0.4)	0.022 (0.2)	0.013 (0.4)	0.107 (0.6)	0.044 (0.2)
HCl (1N)	0.014 (3.2)	0.013 (0.1)	0.006 (0.3)	0.022 (0.2)	0.009 (0.3)	0.036 (0.2)	0.044 (0.2)
Unextracted residue	0.184 (42.9)	0.121 (0.9)	0.032 (1.7)	0.146 (1.3)	0.047 (1.5)	0.124 (0.7)	0.243 (1.1)
Total	0.428 (100)	13.444 (100)	1.905 (100)	11.225 (100)	3.142 (100)	17.751 (100)	22.087 (100)

a: sampling 36 d after pre-emergence soil application (pre-emergence: 3.4 kg as/ha)
b: sampling 4 days after first foliar spraying (pre-emergence: 3.4 kg as/ha + post-emergence: 1.5 kg as/ha)
c: sampling 82 days after second foliar spraying (pre-emergence: 3.4 kg as/ha + post-emergence: 1 x 1.5 kg as/ha & 1 x 2.4 kg as/ha)
d: sampling 14 days after third foliar spraying (pre-emergence: 3.4 kg as/ha + post-emergence: 1 x 1.5 kg as/ha & 1 x 2.4 kg as/ha & 1 x 0.9 kg as/ha)

Identification of the radioactivity in the various matrices gave the following results.

Table B.7.1-60: Identification of radioactive residues in soya bean forage (GAT modified, pre-emergence: 3.4 kg as/ha, 36 DAT)

Extract	Glyphosate mg eq/kg (% TRR)	AMPA mg eq/kg (% TRR)	N-acetyl-AMPA mg eq/kg (% TRR)	N-acetyl-glyphosate mg eq/kg (% TRR)	Unidentified mg eq/kg (% TRR)
0.1 % formic acid (aqueous):methanol (96:4 v/v)	0.033 (7.6)	0.081 (19.3)	-	0.003 (0.6)	0.002 (0.4)
α-amylase	0.006 (1.5)	0.054 (12.9)	-	-	-
amyloglucosidase and cellulase	-	0.017 (4.0)	-	-	-
NaOH (0.1N)	-	0.002 (0.6)	-	0.006 (1.3)	0.001 (0.2)
HCl (1N)	-	0.012 (2.5)	-	-	-
Total extracted	0.039 (9.1)	0.166 (39.3)	-	0.009 (1.9)	0.003 (0.6)
Unextracted	0.184 (42.9)				

Table B.7.1-61: Identification of radioactive residues in soya bean hay (GAT modified, pre-emergence: 3.4 kg as/ha + post-emergence: 1.5 kg as/ha, 4 DAT)

Extract	Glyphosate mg eq/kg (% TRR)	AMPA mg eq/kg (% TRR)	N-acetyl-AMPA mg eq/kg (% TRR)	N-acetyl-glyphosate mg eq/kg (% TRR)	Unidentified mg eq/kg (% TRR)
0.1 % formic acid (aqueous):methanol (96:4 v/v)	9.678 (72)	0.408 (3.0)	0.096 (0.7)	2.574 (19.2)	0.064 (0.4)[a]
α-amylase	0.045 (0.4)	0.230 (1.7)	-	-	0.015 (<0.1)[b]
amyloglucosidase and cellulase	0.017 (0.1)	0.043 (0.4)	-	0.003 (<0.1)	-
NaOH (0.1N)	-	0.011 (0.1)	-	0.004 (<0.1)	-
HCl (1N)	-	0.012 (0.1)	-	-	-
Total extracted	9.740 (72.5)	0.704 (5.3)	0.096 (0.7)	2.581 (19.2)	0.079 (0.5)
Unextracted	0.121 (0.9)				

a: 5 components, each <0.3%TRR and <0.04 mg eq/kg
b: 8 components, each <0.1%TRR and <0.005 mg eq/kg

Table B.7.1-62: Identification of radioactive residues in soya bean seeds (GAT modified, pre-emergence: 3.4 kg as/ha + post-emergence: 1 x 1.5 kg as/ha & 1 x 2.4 kg as/ha, 82 DAT)

Extract	Glyphosate mg eq/kg (% TRR)	AMPA mg eq/kg (% TRR)	N-acetyl-AMPA mg eq/kg (% TRR)	N-acetyl-glyphosate mg eq/kg (% TRR)	Unidentified mg eq/kg (% TRR)
0.1 % formic acid (aqueous):methanol (96:4 v/v)	0.433 (22.7)	0.071 (3.7)	-	1.089 (57.2)	0.022 (1.2)
α-amylase	-	0.028 (1.4)	-	0.060 (3.1)	0.009 (0.5)[a]
amyloglucosidase and cellulase	0.001 (<0.1)	0.004 (0.2)	-	0.007 (0.3)	0.001 (<0.1)
NaOH (0.1N)	not profiled				
HCl (1N)	not profiled				
Total extracted	0.434 (22.7)	0.103 (5.3)	-	1.156 (60.6)	0.032 (1.7)
Unextracted	0.032 (1.7)				

a: 5 components, each <0.4 %TRR and <0.007 mg eq/kg

Table B.7.1-63: Identification of radioactive residues in soya bean foliage (GAT modified, pre-emergence: 3.4 kg as/ha + post-emergence: 1 x 1.5 kg as/ha & 1 x 2.4 kg as/ha, 82 DAT)

Extract	**Glyphosate** mg eq/kg (% TRR)	**AMPA** mg eq/kg (% TRR)	**N-acetyl-AMPA** mg eq/kg (% TRR)	**N-acetyl-glyphosate** mg eq/kg (% TRR)	**Unidentified** mg eq/kg (% TRR)
0.1 % formic acid (aqueous):methanol (96:4 v/v)	4.402 (39.2)	0.596 (5.3)	0.229 (2.0)	4.262 (38.0)	0.148 (1.3)[a]
α-amylase	0.456 (4.1)	0.107 (1.0)	-	0.397 (3.6)	-
amyloglucosidase and cellulase	0.028 (0.2)	0.09 (0.8)	0.026 (0.2)	0.04 (0.4)	0.03 (0.1)[b]
NaOH (0.1N)	0.008 (0.1)	0.005 (0.1)	<0.001 (<0.1)	<0.001 (<0.1)	<0.001 (<0.1)
HCl (1N)	-	0.021 (0.2)	-	-	-
Total extracted	4.894 (43.6)	0.819 (7.4)	0.255 (2.2)	4.699 (42.0)	0.179 (1.6)
Unextracted	0.146 (1.3)				

a: 9 components, each <0.4 %TRR and <0.04 mg eq/kg
b: 8 components, each <0.1 %TRR and <0.007 mg eq/kg

Table B.7.1-64: Identification of radioactive residues in soya bean seeds (GAT modified, pre-emergence: 3.4 kg as/ha + post-emergence: 1 x 1.5 kg as/ha & 1 x 2.4 kg as/ha & 1 x 0.9 kg as/ha, 14 DAT)

Extract	**Glyphosate** mg eq/kg (% TRR)	**AMPA** mg eq/kg (% TRR)	**N-acetyl-AMPA** mg eq/kg (% TRR)	**N-acetyl-glyphosate** mg eq/kg (% TRR)	**Unidentified** mg eq/kg (% TRR)
0.1 % formic acid (aqueous):methanol (96:4 v/v)	0.094 (3.0)	0.191 (6.1)	0.738 (23.5)	1.742 (55.4)	-
α-amylase	0.008 (0.2)	0.12 (3.8)	-	0.037 (1.2)	0.032 (1.0)[a]
amyloglucosidase and cellulase	-	0.03 (1.0)	-	0.008 (0.2)	0.003 (0.1)
NaOH (0.1N)	-	0.005 (0.2)	-	0.001 (0.1)	<0.001 (<0.1)
HCl (1N)	-	0.005 (0.1)	-	-	-
Total extracted	0.102 (3.2)	0.351 (11.2)	0.738 (23.5)	1.788 (56.9)	0.035 (1.1)
Unextracted	0.047 (1.5)				

a: 2 components, each <0.9 %TRR and <0.029 mg eq/kg

Table B.7.1-65: Identification of radioactive residues in soya bean pods (GAT modified, pre-emergence: 3.4 kg as/ha + post-emergence: 1 x 1.5 kg as/ha & 1 x 2.4 kg as/ha & 1 x 0.9 kg as/ha, 14 DAT)

Extract	Glyphosate mg eq/kg (% TRR)	AMPA mg eq/kg (% TRR)	N-acetyl-AMPA mg eq/kg (% TRR)	N-acetyl-glyphosate mg eq/kg (% TRR)	Unidentified mg eq/kg (% TRR)
0.1 % formic acid (aqueous):methanol (96:4 v/v)	9.821 (55.3)	0.641 (3.6)	0.532 (3.0)	4.645 (26.2)	-
α-amylase	0.19 (1.1)	0.845 (4.8)	0.017 (0.1)	0.206 (1.2)	0.108 (0.6)[a]
amyloglucosidase and cellulase	0.082 (0.5)	0.215 (1.2)	0.017 (0.1)	0.051 (0.3)	0.051 (0.1)[b]
NaOH (0.1N)	0.008 (<0.1)	0.066 (0.4)	0.008 (0.1)	0.004 (<0.1)	0.006 (<0.1)
HCl (1N)	-	0.027 (0.2)	-	-	-
Total extracted	10.101 (56.9)	1.794 (10.2)	0.574 (3.3)	4.906 (27.7)	0.165 (0.7)
Unextracted	0.124 (0.7)				

a: 9 components, each <0.1 %TRR and <0.021 mg eq/kg
b: 14 components, each <0.1 %TRR and <0.008 mg eq/kg

Table B.7.1-66: Identification of radioactive residues in soya bean foliage (GAT modified, pre-emergence: 3.4 kg as/ha + post-emergence: 1 x 1.5 kg as/ha & 1 x 2.4 kg as/ha & 1 x 0.9 kg as/ha, 14 DAT)

Extract	Glyphosate	AMPA	N-acetyl-AMPA	N-acetyl-glyphosate	Unidentified
	mg eq/kg (% TRR)	mg eq/kg (% TRR)	mg eq/kg (% TRR)	mg eq/kg (% TRR)	mg eq/kg (% TRR)
0.1 % formic acid (aqueous):methanol (96:4 v/v)	11.195 (50.7)	1.177 (5.3)	0.236 (1.1)	6.629 (30)	0.18 (0.9)[a]
α-amylase	0.545 (2.5)	0.743 (3.4)	0.022 (0.1)	0.368 (1.7)	0.123 (0.6)[b]
amyloglucosidase and cellulase	0.046 (0.2)	0.256 (1.2)	0.05 (0.2)	0.042 (0.2)	0.031 (<0.1)[c]
NaOH (0.1N)	0.005 (<0.1)	0.032 (0.2	<0.001 (<0.1)	<0.001 (<0.1)	<0.001 (<0.1)
HCl (1N)	-	0.042 (0.2)	-	-	-
Total extracted	11.791 (53.4)	2.25 (10.3)	0.308 (1.4)	7.039 (31.9)	0.243 (1.1)
Unextracted	0.243 (1.1)				

a: 4 components, each <0.5%TRR and <0.108 mg eq/kg
b: 9 components, each <0.1%TRR and <0.024 mg eq/kg
c: 8 components, each <0.1%TRR and <0.007 mg eq/kg

Conclusions:

In soya beans modified with GAT unchanged parent glyphosate was the major residue in all plant parts directly affected by the application. After soil treatment only a minor uptake into the plants was observed, mainly consisting of AMPA and glyphosate. Following foliar application seeds protected by the pods mainly showed N-acetyl-glyphosate (56.9-60.6 % TRR) with lower residues of parent glyphosate (3.2-22.7 % TRR), AMPA (5.3-11.2 % TRR) and N-acetyl-AMPA (up to 23.5 % TRR). In soya bean hay and foliage N-acetyl-AMPA was

also the second highest contributor to the overall residue, being present at levels of 19.2-42 % of the TRR.

Reference: OECD KIIA 6.2.1

Report: Green, M. A.
The metabolism of [^{14}C]Glyphosate in Optimum GAT (Event DP-Ø9814Ø-6) field corn
25.10.2007, DuPont-19529, ASB2008-2657

Guidelines: Nature of the Residue - Plants and the recommendations of EU Commission Directive 96/68/EC Annex II, Section 8.1 (21. October 1996)
US-EPA: OPPTS 860.1300 – Nature of the Residue – Plants
Canadian PMRA Residue Chemistry Test Guidelines Dir 98-02, Section 2

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
For maize genetically modified with GAT the metabolism was investigated using a single application of ^{14}C-glyphosate at pre-emergence with 4.3 kg as/ha (acid equivalents) to the soil followed by three foliar applications (2 x between BBCH 31-39 plus 1 x at BBCH 87, each 1.1 kg as/ha). Samples of immature foliage (48 days after soil treatment, before first foliar application), forage (59 days after the second foliar application) and mature plants separated into stover, cob and grain (7 days after final application) were collected.

All samples were homogenized and extracted using 0.1% formic acid (aqueous):methanol (96:4 v/v) followed by enzyme (α-amylase then amyloglucosidase and cellulose), alkaline (NaOH 0.1 N, 60 °C, 6h) then acid (HCl, 1 N, 60 °C, 6h) digestion. The total radioactive residue (TRR) was determined as the sum of the total radioactivity by LSC. Extracts containing more the 0.01 mg/kg TRR were analysed by HPLC (UV, RAD) using authenticated reference standards. In addition TLC was used as confirmation method.

Findings:
In the samples analysed TRR values ranging from 12.2 mg/kg for stover down to 0.275 mg/kg for grain were found. For all matrices more than 90 % of the radioactivity was extracted and identified or characterised. Immature foliage samples (48 days after soil treatment) contained very low total radioactive residues (0.022 mg eq/kg) and were not further analysed. A summary of the results is presented in the following table.

Table B.7.1-67: TRR levels and extractability of residues in maize samples (GAT modified)

Extract	**Forage (a)** mg eq/kg (% TRR)	**Stover (b)** mg eq/kg (% TRR)	**Cobs (b)** mg eq/kg (% TRR)	**Grain (b)** mg eq/kg (% TRR)
Extracted residue	3.35 (96.4)	12.304 (100.5)	0.583 (84.9)	0.234 (84.9)
0.1 % formic acid (aqueous):methanol (96:4 v/v)	3.204 (87)	10.406 (85)	0.475 (69.3)	0.195 (71)
α-amylase	0.316 (9.1)	1.359 (11.1)	0.141 (20.5)	0.048 (17.6)
amyloglucosidase and cellulase	0.045 (1.3)	0.159 (1.3)	0.023 (3.3)	0.007 (2.4)
NaOH (0.1N)	0.035 (1.0)	0.135 (1.1)	0.019 (2.7)	0.003 (1.0)
HCl (1N)	0.021 (0.6)	0.086 (0.7)	NP	NP
Unextracted residue	0.031 (0.9)	0.11 (0.9)	0.029 (4.2)	0.022 (7.9)
Total (TRR)	3.476 (100)	12.242 (100)	0.686 (100)	0.275 (100)

a: sampling 48 d after pre-emergence soil application (pre-emergence: 4.3 kg as/ha)
b: sampling 7 days after third foliar spraying (pre-emergence: 4.3 kg as/ha + post-emergence: 3 x 1.1 kg as/ha)
NP: not performed

Table B.7.1-68: Identification of radioactive residues in maize forage (GAT modified, pre-emergence: 4.3 kg as/ha, 48 DAT)

Extract	**Glyphosate** mg eq/kg (% TRR)	**AMPA** mg eq/kg (% TRR)	**N-acetyl-AMPA** mg eq/kg (% TRR)	**N-acetyl-glyphosate** mg eq/kg (% TRR)	**Unidentified** mg eq/kg (% TRR)
0.1 % formic acid (aqueous):methanol (96:4 v/v)	1.852 (53.3)	0.118 (3.4)	0.056 (1.6)	0.845 (24.3)	0.034 (1.0)[a]
α-amylase	0.143 (4.1)	0.022 (0.6)	0.004 (0.1)	0.072 (2.1)	0.017 (0.3)[b]
amyloglucosidase and cellulase	0.021 (0.6)	-	-	0.015 (0.4)	-
NaOH (0.1N)	-	-	-	0.005 (0.2)	0.006 (0.3)[c]
HCl (1N)	not profiled				
Total extracted	2.016 (58)	0.14 (4.0)	0.06 (1.7)	0.937 (27)	0.057 (1.6)
Unextracted	0.184 (4.3~~42.9~~)				

a: 6 components, one 0.4 % TRR (0.013 mg/kg), all other <0.013%TRR and <0.007 mg eq/kg
b: 7 components, each <0.2 %TRR and <0.009 mg eq/kg
c: 18 components, each <0.1 %TRR and <0.004 mg eq/kg

Table B.7.1-69: Identification of radioactive residues in maize stover (GAT modified, pre-eme rgence: 4.3 kg as/ha + post-emergence: 3 x 1.1 kg as/ha, 7 DAT)

Extract	**Glyphosate** mg eq/kg (% TRR)	**AMPA** mg eq/kg (% TRR)	**N-acetyl-AMPA** mg eq/kg (% TRR)	**N-acetyl-glyphosate** mg eq/kg (% TRR)	**Unidentified** mg eq/kg (% TRR)
0.1 % formic acid (aqueous):methanol (96:4 v/v)	8.174 (66.8)	0.368 (3.0)	0.134 (1.1)	1.899 (15.5)	0.061 (0.4)[a]
α-amylase	0.836 (6.8)	0.054 (0.4)	0.018 (0.2)	0.249 (2.0)	0.027 (0.2)[b]
amyloglucosidase and cellulase	0.09 (0.7)	-	-	0.026 (0.2)	0.002 (<0.1)[c]
NaOH (0.1N)	0.066 (0.6)	-	-	0.014 (0.1)	0.002 (<0.1)[d]
HCl (1N)	not profiled				
Total extracted	9.166 (74.9)	0.422 (4.4)	0.152 (1.3)	2.188 (17.8)	0.092 (0.8)
Unextracted	0.11 (0.9)				

a: 5 components, one 0.2 % TRR (0.025 mg/kg), all other <0.1 %TRR and <0.016 mg eq/kg
a: 5 components, each <0.1 %TRR and <0.009 mg eq/kg
c: single component
d: 2 components, each <0.1 %TRR and <0.001 mg eq/kg

Table B.7.1-70: Identification of radioactive residues in maize cobs (GAT modified, pre-emergence: 4.3 kg as/ha + post-emergence: 3 x 1.1 kg as/ha, 7 DAT)

Extract	**Glyphosate** mg eq/kg (% TRR)	**AMPA** mg eq/kg (% TRR)	**N-acetyl-AMPA** mg eq/kg (% TRR)	**N-acetyl-glyphosate** mg eq/kg (% TRR)	**Unidentified** mg eq/kg (% TRR)
0.1 % formic acid (aqueous):methanol (96:4 v/v)	-	-	0.028 (4.0)	0.394 (57.5)	0.003 (0.5)[a]
α-amylase	-	-	0.006 (0.9)	0.03 (4.6)	0.069 (9.9)[b]
amyloglucosidase and cellulase	-	-	<0.001 (<0.1)	0.002 (0.4)	<0.001 (2.3)[c]
NaOH (0.1N)	-	-	-	0.009 (1.3)	<0.001 (<0.1)[d]
HCl (1N)	not profiled				
Total extracted	-	-	0.034 (5.0)	0.435 (63.8)	0.074 (12.8)
Unextracted	0.029 (4.2)				

a: single component
b: 12 components, one at 2 % TRR (0.014 mg eq/kg), all others each <1.4 %TRR and <0.009 mg eq/kg
c: 13 components, each <0.3 %TRR and <0.001 mg eq/kg
d: 2 components, each <0.1 %TRR and <0.001 mg eq/kg

Table B.7.1-71: Identification of radioactive residues in maize grain (GAT modified, pre-emergence: 4.3 kg as/ha + post-emergence: 3 x 1.1 kg as/ha, 7 DAT)

Extract	Glyphosate mg eq/kg (% TRR)	AMPA mg eq/kg (% TRR)	N-acetyl-AMPA mg eq/kg (% TRR)	N-acetyl-glyphosate mg eq/kg (% TRR)	Unidentified mg eq/kg (% TRR)
0.1% formic acid (aqueous):methanol (96:4 v/v)	-	0.005 (2.0)	0.026 (9.1)	0.138 (50.1)	0.007 (2.6)
α-amylase	<0.001 (0.1)	0.011 (4.1)	<0.001 (0.3)	0.003 (1.1)	0.034 (8.3)[b]
amyloglucosidase and cellulase	not profiled				
NaOH (0.1N)	not profiled				
HCl (1N)	not profiled				
Total extracted	<0.001 (<0.1)	0.016 (6.1)	0.026 (9.4)	0.141 (51.2)	0.041 (10.9)
Unextracted	0.022 (7.9)				

a: single component
b: 14 components, each <1.3 %TRR and <0.006 mg eq/kg

Conclusions:
In genetically modified maiez with the GAT gene most of the radioactivity found in the samples was unchanged glyphosate at early PHIs and N-acetyl-glyphosate at later PHIs. Neither AMPA nor n-acetyl-AMPA (which may be formed from AMPA and N-acetyl-glyphosate) was found at levels above 10 % of the TRR. Although treated before emergence, early forage samples already contained substantial levels of radioactivity, mainly identified as glyphosate and N-acetyl-glyphosate, suggesting an uptake via the roots. In mature cobs and grain, which are protected by hull leaves, most of the residue was present as N-acetyl-glyphosate. In view of the last treatment being conducted seven days before harvest, it can be concluded that the major part of the residue was translocated and metabolisied following previous sprayings.

Conclusion GAT modified plants:
In GAT modified rape, soya beans and maize the metabolism of glyphosate was comparable. After foliar appplication glyphosate remains more or less stable on treated plant parts and is taken up into the plants. Then the GAT modification results in the formation of N-acetyl-glyphosate and minor amounts of N-acetyl-AMPA and AMPA, which are translocated into non-affected parts like seeds and grain.
Following pre-emergence application a minor uptake of parent glyphosate from the soil was observed for maize. In soya beans the uptake was minimal. In Figure B.7.1-4 a proposed metabolic pathway of glyphosate in GAT modified plants is presented.

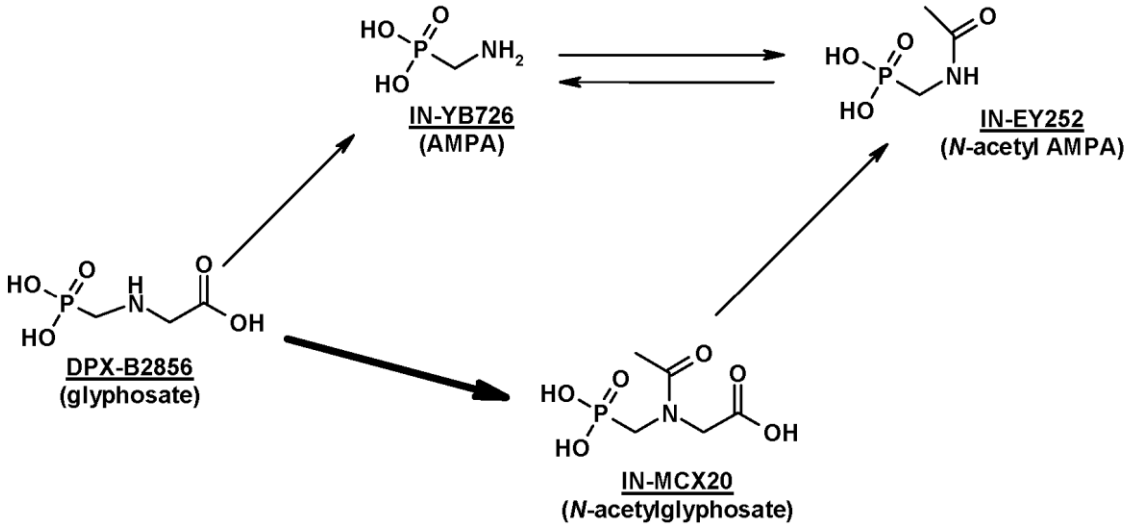

Figure B.7.1-4: Proposed metabolic pathway of glyphosate in GAT modified plants

B.7.1.4 Public literature

In several studies the effect of glyphosate on the concentration of secondary plant metabolites was investigated. Bresnahn et al (2003, ASB2012-12365) investigated the levels of shikimic acid, which is involved in the main mode of action for glyphosate in plants, in wheat. It was observed that following glyphosate application an approximately 2-fold increase in the shikimic acid concentration in the grain was observed compared to control samples or wheat treated with other herbicides.
In studies published by Bohm et al (2008, ASB2012-12366) and Duke et al (2003, ASB2012-12400) the effect of glyphosate to isoflavones in soya beans was investigated. No significant influence was identified.
The activity of gluthathione-S-transferase in maize was investigated by Cataneo et al (2003, ASB2012-12384). The results indicate an increase in the activity of the molecule, probably due to its role in the plant metabolism of the active substance.

In a literature review Duke (2011, ASB2012-12401) comapred the mode of glyphosate resistance in weed, showing that only a minor share of the weeds express a GOX gene. Most of the weeds contained a unchanged glyphosate, suggesting a modification of the EPSPS-enzyme. However, the level of resistance in weeds was found to be lower than in genetically modified crops.
Further investigations on secondary plant metabolites in organic, conventional and GM-soya beans and the residues of glyphosate and AMPA were published by Bøhn et al. (2014, ASB2014-6353). Organic soybeans showed a nutritional profile with more sugars, such as glucose, fructose, sucrose and maltose, significantly more total protein, zinc and less fibre than both conventional and GM-soya. Organic soybeans also contained less total saturated fat and total omega-6 fatty acids than both conventional and GM-soya. The ration of residues of glyphosate and AMPA (mean 3.3 and 5.7 mg/kg, respectively) confirmed the findings from ^{14}C-radiolabelled plant metabolism studies provided by the applicant.

The metabolism of glyphosate in *Mucuna pruriens var. utilis* (velvet beans) was investigated by Rojano-Delgado et al (2012, ASB2012-12462). The velvet beans are a plant species

exhibiting an innate, very high resistance to glyphosate. Using ^{14}C-radiolabelled glyphosate, the uptake and metabolism in velvet beans compared to *Amaranthus retroflexus* was observed. It was shown that velvet beans had a much lower uptake of the radioactivity via the leaves. While amaranthus accumulated up to 94 % of the applied dose within 24h, an uptake of less than 40 % was observed for the velvet beans. After 72 hours 52 % of the applied dose were taken up.
Besides the investigation of the uptake, degradation products found in the velvet bean leaves were analysed to identify the mode of tolerance for this species. In treated plants glyphosate, AMPA, sarcosine and formaldehyde were identified. In comparision to non-tolerant amaranthus, where mainly glyphosate and traces of AMPA were found, a secondary metabolic pathway via sarcosine, which is normally found in bacteria only, was postulated by the author:

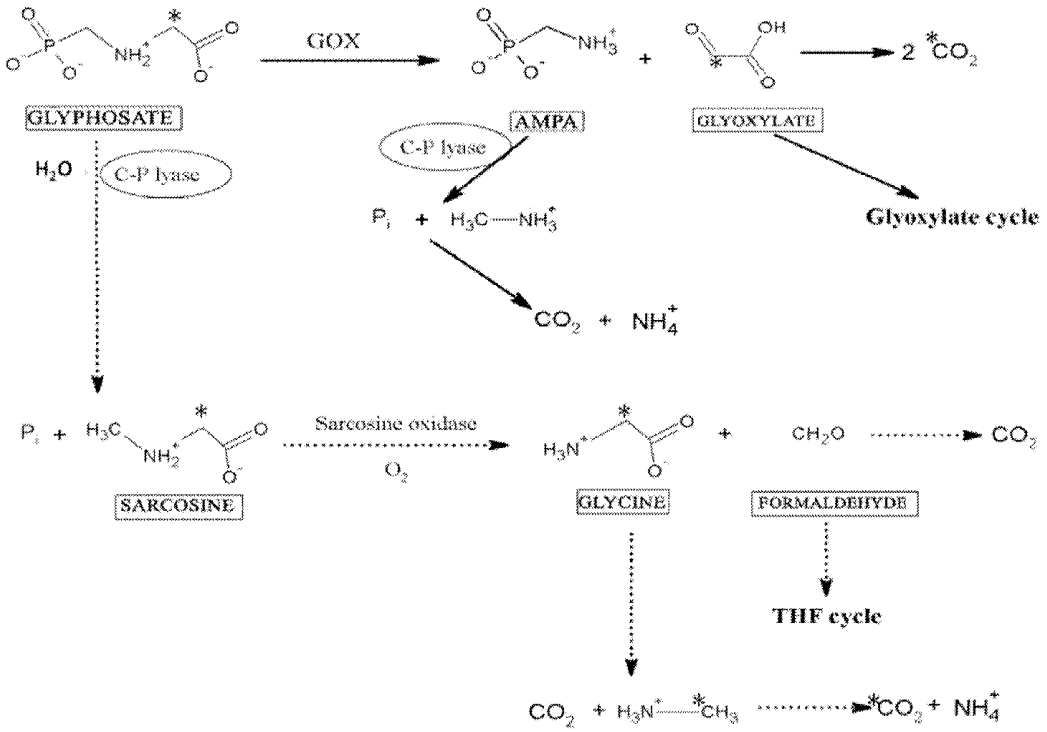

Fig. 6. Potential degradation pathway for glyphosate (⟶ primary pathway in plants and soils, ⇢ primary pathway in bacteria).

In summary it was concluded, that the natural tolerance of velvet beans is based on three modes of action: a natural GOX modification resulting in AMPA, a high-tolerance EPSPS enzyme and the additional degradation into sarcosine, finally resulting in glycine, formaldehyde and their natural products.
In view of the residue situation the observation of an additional metabolic pathway is relevant. However, all degradation products observed in this pathway are identical to already known metabolites (AMPA) or represent natural products commonly available in bichemical cycles (sarcosine, glycine, formaldehyde, glyoxylate). Further investigation in addition to the information already available are not necessary to describe the metabolism of glyphosate in terms of residues.

The sensitivity of different plant species to glyphosate was investigated by Reddy et al (2008, ASB2012-12463). Besides these phytotoxic effects it was investigated, if the metabolism of glyphosate into AMPA is a common factor in the natural resistance of plants against glyphosate. Although non-tolerant crops (especially soya beans) showed increased

concentrations of shikimic acid, no correlation of glyphosate or AMPA concentration to the tolerance were found.

The uptake of glyphosate into maize seedlings was investigated by Wagner et al. (2003, ASB2012-12484) using ^{14}C-radiolablled active substance. The seedlings were grown in ^{14}C-glyphosate solutions with concentrations of 0-30 mg as/kg). After 26h of exposure the plants were transferred into fresh nutrient solution and grown for 5 additional days. The glyphosate uptake was observed to be 11 % of the theoretical mass flow. If more than 0.6µg/g glyphosate were observed, a decrease in the growth was observed. It could be shown, that radioactivity taken up by the plants was mainly located in the new leaves, suggesting symplastic distribution in the plants.

B.7.2 Metabolism, distribution and expression of residues in livestock (Annex IIA 6.2.2 to 6.2.4; Annex IIIA 8.2)

The metabolism of glyphosate, AMPA and N-acetyl glyphosate in livestock animals was investigated in lactating goats and laying hens.

B.7.2.1 Lactating goats

Reference: OECD KIIA 6.2.3

Report: Powles, P.
(^{14}C-Glyphosate): Absorption, distribution, metabolism and excretion following repeated oral administration to the dairy goat, 07.11.1994, Report No. 676/9-1011, RIP9501207

Guidelines: EPA Nature of the residues in Livestock (171-4)

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study ^{14}C-glyphosate radiolabelled in the phosphonomethyl-moiety was administered to two lactaing goats for a period of up to 5 consecutive days. Each animal received nominal doses of 200 mg/kg feed via capsule twice per day. The first goat was dosed with 355 mg per day (50 kg bw, 7.1 mg ^{14}C-glyphosate per kg bw) for 5 days before feeding but after milk collection. A second animal received 400 mg per day (50 kg, 8 mg ^{14}C-glyphosate per kg bw) for three consecutive days. Excreta and milk were collected at 24 hour intervals following the initial dose. Selective tissues such as liver, kidney, skeletal muscle (hind and forequarter) and fat (omental and kidney) were sampled for analysis after sacrafice at 23.5 hours after the final dose. In addition blood samples were taken 1, 2, 3, 4, 6, 8 and 12 hours after the initial dose.

The total radioactivity was determined by combustion and/or LSC. Radioactivity was extracted from each amtrix adding chloroform/HCl for homogenosation, and after

centrifugation the aqueous phase was transferred to a chelating ion exchange resin and the radioactivity was eluted with HCl. After chromatography using a strong anion exchange resin the residue was solubilized in water. The methods of analysis used for identification involved HPLC and TLC by comparision with reference substances.

Findings:

In the following table the total recovered radioactivity following administration of ^{14}C-glyphosate to lacataing goats is summarised.

Table B.7.2-1: Total recovered radioactivity following administration of ^{14}C-glyphosate to lactating goats at nominal feed levels of 200 mg/kg feed

Tissue	% AR	
	Goat 1 (7.1 mg/kg bw, 5 days)	Goat 2 (8 mg/kg bw, 3 days)
Urine	9.44	4.7
Faeces	78.16	52.58
Cage wash	0.48	0.29
Cage debris	1.74	-
Whole milk	0.03	0.03
Tissues	0.05	-
Total	89.9	57.64

In the milk residues reached a plateau quickly within the first three days. Recovered TRR levels in whole milk were:

Table B.7.2-2: TRR levels in whole milk following administration of ^{14}C-glyphosate to lactating goats at nominal feed levels of 200 mg/kg feed

Days	TRR in mg eq/kg	
	Goat 1 (7.1 mg/kg bw, 5 days)	Goat 2 (8 mg/kg bw, 3 days)
1	0.036	0.040
2	0.060	0.066
3	0.064	0.086
4	0.072	NA
5	0.041	NA

NA: not applicable

After sacrifice of the animals TRR levels in tissues from both animal were measured.

Table B.7.2-3: TRR levels in tissues following administration of ^{14}C-glyphosate to lactating goats at nominal feed levels of 200 mg/kg feed

Tissue	TRR in mg eq/kg	
	Goat 1 (7.1 mg/kg bw, 5 days)	Goat 2 (8 mg/kg bw, 3 days)
Fat	<0.028	<0.036
Kidney	3.852	12.15
Liver	0.404	0.225
Muscle	0.035	0.061

For goat 2 dosed over three days concentrations of ^{14}C in blood plasma was monitored for 12 hours after the administration:

Table B.7.2-4: TRR levels in blood plasma following administration of ^{14}C-glyphosate to lactating goats at nominal feed levels of 200 mg/kg feed

Time (hours)	mg eq/kg
1	0.051
2	0.059
3	0.069
4	0.080
6	0.102
8	0.101
12	0.094

The identification of radioactive residues was only conducted for samples collected from goat 2. In milk, fat and muscle the TRR levels were below the limit required for an identification. The results from urine, faeces, liver and kidney were:

Table B.7.2-5: Identification of radioactive residues in urine, faeces, liver and kidney of lactating goats following administration of ^{14}C-glyphosate to lactating goats at nominal feed levels of 200 mg/kg feed

Sample	% TRR			
	glyphosate	AMPA	unknown	total
Urine (24-48h), radio-HPLC	95.86	-	-	95.86
Urine (24-48h), TLC system 1	71.74	5.55	22.34	99.63
Urine (24-48h), TLC system 2, sample + reference[a]	84.66	5.09	9.93	99.68
Urine (24-48h), TLC system 2, pure sample	79.74	4.68	15.46	99.88
Faeces (24-48h), radio-HPLC	94.06	-	3.23	97.29
Faeces (24-48h), TLC system 1	92.68	3.95	2.12	98.74
Faeces (24-48h), TLC system 2	94.0	2.23	3.55	99.78
Liver , radio-HPLC	95.52	-	-	95.52
Liver, TLC system 1	96.64	-	-	96.64
Liver, TLC system 2	95.89	-	-	95.89
Kidney, radio-HPLC	96.93	-	-	96.93
Kidney, TLC system 1	91.59	8.01	-	99.6
Kidney, TLC system 1	94.08	4.90	-	98.97

a: sample extract and unlabelled reference substances spotted on the same location

Conclusions:

Following administration of ^{14}C-glyphosate to lactating goats most of the radioactivity was recovered in the faeces (52.6-78.2 % AR). In milk (0.03 % AR) and tissues (0.05 % AR) only minor amounts of the administered radioactivity were found. Absolute TRR levels were 0.036-0.086 mg eq/kg in whole milk, <0.036 mg/kg in fat, 0.035-0.061 mg/kg in muscle, 0.225-0.404 mg/kg in kidney and 3.852-12.15 mg/kg in liver. Concentrations of radioactivity in blood plasma peaked within 12 hours, suggesting a quick uptake and excretion of the residue. Identification of the radioactivity in urine, faeces, kidney and liver mainly revealed unchanged glyphosate (71.7-96.9 % TRR). The only other metabolite found was AMPA at levels up to 8 % of the TRR.

Reference: OECD KIIA 6.2.3

Report: Bodden, R. M.
Metabolism study of synthetic $^{13}C/^{14}C$-labeled Glyphosate and Aminomethylphosphonic acid in lactating goats. Part I
30.12.1987, Report: MSL 7586, RIP9501203

Guidelines: not stated

Deviations: not applicable

GLP: yes

Acceptability: The study is considered to be acceptable.

Reference: OECD KIIA 6.2.3

Report: Patanella, J. E.; Feng, P.
Metabolism study of synthetic 13C/14C-labeled Glyphosate and Aminomethylphos-phonic acid in lactating goats. Part II
February 1988, Report: MSL-7458, RIP9501204

Guidelines: not stated

Deviations: not applicable

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
The current study the metabolism of glyhosate an AMPA was investigated in lactating goats using a 9:1 mixture of ^{14}C-radiolabelled substances (phosphonomethyl-moiety label). Three goats received a target dose of 120 mg/kg feed (216 mg glyphosate and 24 mg AMPA per animal and day, 4.1 mg glyphosate and 0.45 mg AMPA per kg bw and day) for a period of five consecutive days. Two of the goats were sacrificed 22-24 h after the last doing, the thrid goat after additional five days for depuration. In parallel one goat was kept as control.
Milk samples, the total urine and the faeces excreted by each animal were collected twice daily in the morning and afternoon. After sacrifice, the following tissues and organs were collected from each animal for analysis: kidney, liver, round muscle, fat (approximately 1:1 renal and omental fat) and the gastrointestinal tract with contents.

The analysis of radioactivity was carried out by combustion or LSC. Tissues were extracted with water/chloroform, milk samples were mixed with HCl to precipitate proteins, after centrifugation the supernatants were concentrated to dryness and the residues solubilized in water. Metabolites were characterised and identified by HPLC and GC/MS.

Findings:
In the following table the total radioactivity recovered from the treated goats are summarised:

Table B.7.2-6: Total radioactivity recovered from lactating goats following administration of 216 mg ^{14}C-glyphosate and 24 mg ^{14}C-AMPA per day and animal

Matrix	% AR recovered		
	Animal 1	Animal 2	Animal 3 (including 5 day depuration)
Kidney	0.13	0.05	<0.01
Liver	0.04	0.03	0.03
Muscle	<0.01	<0.01	<0.01
Fat	<0.01	<0.01	<0.01
Milk	<0.01	<0.01	<0.01
Urine	23.6	20.3	20.1
Pan rinse	0.2	0.4	0.06
Faeces	58.2	47.2	66.3
Rumen contents	0.59	1.55	<0.01
Gastrointestinal tract contents	3.32	11.5	0.15
Total	86.1	81.1	86.7

TRR levels found in milk reached a plateau after 2-3 days for all animals. In the goat kept for depuration a quick decline of the TRR found in milk was observed after dosing. Total radioactive residues found in milk and tissues were:

Table B.7.2-7: TRR in milk from lactating goats following administration of 216 mg ^{14}C-glyphosate and 24 mg ^{14}C-AMPA per day and animal

Days	TRR recovered in milk (mg eq/kg)		
	Animal 1	Animal 2	Animal 3 (including 5 day depuration)
Predose	ND	ND	ND
1	0.020	0.049	0.019
2	0.033	0.076	0.030
3	0.034	0.086	0.038
4	0.035	0.080	0.037
5	0.031	0.078	0.038
6	-	-	0.025
7	-	-	0.013
8	-	-	0.007
9	-	-	0.006

Table B.7.2-8: TRR in tissues and blood from lactating goats following administration of 216 mg ^{14}C-glyphosate and 24 mg ^{14}C-AMPA per day and animal

Matrix	TRR recovered in tissues (mg eq/kg)		
	Animal 1	Animal 2	Animal 3 (including 5 day depuration)
Kidney	10.5	3.49	0.505
Liver	0.529	0.457	0.381
Muscle	0.028	0.026	0.009
Fat	0.011	0.009	0.004
Blood	0.129	0.082	0.016

The analysis of the radioactive residues following extraction was reported in the second part of the study. Kidney, liver, fat and muscle samples were investigated for their composition of glyphosate and AMPA.

Table B.7.2-9: Composition of radioactive residues in goats kidney following administration of 216 mg ^{14}C-glyphosate and 24 mg ^{14}C-AMPA per day and animal

Matrix	Animal 1 TRR: 10.5 mg eq/kg	Animal 2 TRR: 3.49 mg eq/kg	Animal 3 (including 5 day depuration) TRR: 0.505 mg eq/kg
	% TRR	% TRR	% TRR
Total extracted	103.6	100.7	100.3
chloroform extract	<0.1	<0.1	<0.1
water extract	102.6	96.7	97.2
unextracted	1.0	4.0	3.1
Analysis of water extract	% of water extract	% of water extract	% of water extract
Recovery after deproteination	99.5 (102.1 % TRR)	98.7 (95.4 % TRR)	98.7 (95.9 % TRR)
Recovery after concentration	98.0 (100.5 % TRR)	94.4 (91.3 % TRR)	96.5 (93.8 % TRR)
Ion Pair HPLC			
AMPA	4.0 (4.1 % TRR)	9.1 (8.8 % TRR)	15.8 (15.4 % TRR)
glyphosate	95.4 (97.9 % TRR)	90.4 (87.4 % TRR)	83.2 (80.9 % TRR)
Cation exchange HPLC			
AMPA	3.9 (4 % TRR)	8.7 (8.4 % TRR)	13.8 (13.4 % TRR)
glyphosate	94.7 (97.2 % TRR)	89 (86.1 % TRR)	84.2 (81.8 % TRR)

Table B.7.2-10: Composition of radioactive residues in goats liver following administration of 216 mg ^{14}C-glyphosate and 24 mg ^{14}C-AMPA per day and animal

Matix	Animal 1 TRR: 0.529 mg eq/kg	Animal 2 TRR: 0.457 mg eq/kg	Animal 3 (including 5 day depuration) TRR: 0.381 mg eq/kg
	% TRR	% TRR	% TRR
Total extracted	103.9	103.4	103.5
chloroform extract	0.1	0.7	0.3
water extract	99.5	95.7	98.0
unextracted	4.3	7.0	5.2
Analysis of water extract	% of water extract	% of water extract	% of water extract
Recovery after deproteination	85.3 (84.9 % TRR)	79.5 (76.1 % TRR)	87.5 (85.8 % TRR)
Recovery after concentration	90.6 (90.1 % TRR)	96.6 (92.4 % TRR)	92.5 (90.7 % TRR)
Ion Pair HPLC			
AMPA	18.8 (18.7 % TRR)	19.2 (18.4 % TRR)	36.0 (35.3 % TRR)
glyphosate	79.4 (79 % TRR)	78.6 (75.2 % TRR)	62.0 (60.8 % TRR)
Cation exchange HPLC			
AMPA	15.5 (15.4 % TRR)	11.4 (10.9 % TRR)	32.4 (31.8 % TRR)
glyphosate	82.4 (82 % TRR)	82.8 (79.2 % TRR)	64.8 (63.5 % TRR)

Table B.7.2-11: Composition of radioactive residues in goats fat following administration of 216 mg ^{14}C-glyphosate and 24 mg ^{14}C-AMPA per day and animal

Matix	Animal 1 TRR: 0.011 mg eq/kg	Animal 2 TRR: 0.009 mg eq/kg	Animal 3 (including 5 day depuration) TRR: 0.004 mg eq/kg
	% TRR	% TRR	% TRR
Total extracted	99.4	104.8	85.4
chloroform extract	2.6	4.5	9.7
water extract	95.8	96.7	72.3
unextracted	1.0	3.6	3.4
Analysis of water extract	% of water extract	% of water extract	% of water extract
Recovery after deproteination	100.1 (95.9 % TRR)	103.8 (100.4 % TRR)	93.5 (67.6 % TRR)
Recovery after concentration	93.5 (89.6 % TRR)	89.6 (86.6 % TRR)	111.4 (80.5 % TRR)
Ion Pair HPLC			
AMPA	9.4 (9 % TRR)	16.8 (16.2 % TRR)	14.5 (10.5 % TRR)
glyphosate	85.8 (82.2 % TRR)	82.6 (79.9 % TRR)	82.7 (59.8 % TRR)
Cation exchange HPLC			
AMPA	9.0 (8.6 % TRR)	10.5 (10.2 % TRR)	10.5 (7.6 % TRR)
glyphosate	90.3 (86.5 % TRR)	80.4 (77.7 % TRR)	89.6 (64.8 % TRR)

Table B.7.2-12: Composition of radioactive residues in goats muscle following administration of 216 mg ^{14}C-glyphosate and 24 mg ^{14}C-AMPA per day and animal

Matix	Animal 1 TRR: 0.028 mg eq/kg	Animal 2 TRR: 0.026 mg eq/kg	Animal 3 (including 5 day depuration) TRR: 0.009 mg eq/kg
	% TRR	% TRR	% TRR
Total extracted	107.8	114.1	106.5
chloroform extract	0.4	0.8	<0.1
water extract	98.7	96.4	78.1
unextracted	8.7	16.9	28.4
Analysis of water extract	% of water extract	% of water extract	% of water extract
Recovery after deproteination	100.4 (99.1 % TRR)	98 (94.5 % TRR)	99.7 (77.9 % TRR)
Recovery after concentration	67.9 (67 % TRR)	70.8 (68.3 % TRR)	63.2 (49.4 % TRR)
Ion Pair HPLC			
AMPA	4.7 (4.6 % TRR)	12.5 (12.1 % TRR)	14.2 (11.1 % TRR)
glyphosate	90.8 (89.6 % TRR)	77.9 (75.1 % TRR)	70.7 (55.2 % TRR)
Cation exchange HPLC			
AMPA	4.3 (4.2 % TRR)	10.6 (10.2 % TRR)	10.4 (8.1 % TRR)
glyphosate	83.2 (82.1 % TRR)	65.7 (63.3 % TRR)	51.8 (40.5 % TRR)

Conclusions:
Following administration of ^{14}C-glyphosate and ^{14}C-AMPA (9:1), only a minor transfer of residues into milk and tissues was observed. Most of the radioactivity was recovered in faeces (47.2-66.3 % AR) and urine (20.1-23.6 % AR). In animal products highest TRR levels were found in the main excretion organs kidney (3.49-10.5 mg eq/kg) and liver (0.457-0.529 mg eq/kg). In milk, muscle and fat residues were much lower, giving highest concentrations of 0.086 mg eq/kg, 0.028 mg eq/kg and 0.011 mg eq/kg, respectively.
In all matrices glyphosate and AMPA were the only metabolites indentified. In kidney, fat and muscle the ratio was in the same range as administered (9:1). Only in liver slightly higher amounts of AMPA were found, ranging up to 36 % of the TRR in comparison to glyphosate (62 % of the TRR).
In summary it can be concluded that both glyphosate and AMPA are taken up by the animals and translocated into animal tissues. Target tissues are kidney, since urine is one major route for excretion, and liver. Except for liver, giving slightyl elevated AMPA concentrations, both analytes were present unchanged and in the ratio administered.

Reference: OECD KIIA 6.2.3

Report: Ericson, J. L.
The nature of residues of orally administered [Phosphonomethylene-^{14}C] glyphosate-trimesium in goat tissues and milk
14.03.1994, Report No: RR 93-062B, RIP9500022

Guidelines: EPA Nature of the residues in Livestock (171-4)

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study one lactaing goat was dosed with ^{14}C-glyphosate-trimesium radiolabelled in the phosphonomethyl-glycine-anion for a period of seven consecutive days. The animal was administered daily doses via capsule equivalent to 64 mg glyphosate per kg feed (136.4 mg per animal, 2.6 mg/kg bw and day). A second gaot was kept as control, receiving only placebo capsules. Over the whole dosing period excreta and milk were sampled. 12-15 hours after the final administration the animals were sacrificed and tissue samples were collected.

The analysis of radioactivity was carried out by combustion and/or LSC. Tissues were extracted with chloroform/0.1N HCl. For fat samples water and chloroform were used, followed by hydrolysis with HCl. Milk samples were extracted with chloroform after precipitation of proteins using acetic acid. The extracts were purified with an inon exchange column and characterised by HPLC and TLC. Further Characterisation and identification of radioactive residues was conducted following deriviatisation using GC/MS against reference substances.

Findings:
Residues found in the control animal were always below the detection limits of the analytical methods use. Therefore only results of treated animal are presented.

In the following table the TRR levels found in milk and tissues, expressed as glyphosate equivalents, and their extractability are summarised. In milk TRR reached a plateau after 4 days.

Table B.7.2-13: TRR and extractablity in tissues and milk from a lactating goat dosed with 2.6 mg ^{14}C-glyphosate-anion (formulated as glyphosate-trimesium) for seven consecutive days

Tissue	Glyphosate in mg eq/kg (% TRR)			
	TRR by combustion	Water soluble	Organo soluble	unextracted
Milk, day 1	0.00255	not performed		
Milk, day 2	0.0139	not performed		
Milk, day 3	0.0189	not performed		
Milk, day 4	0.0217	not performed		
Milk, day 5	0.0212	not performed		
Milk, day 6	0.0211	not performed		
Milk, day 7	0.0222	0.014 (58.7)	0.004 (20.5)	0.004 (21.2)
Milk, day 8	0.0225	not performed		
Muscle	0.0256	0.025 (95.6)	0.001 (3.1)	<0.001 (1.2)
Liver	0.234	0.214 (91.7)	0.013 (5.7)	0.006 (2.7)
Fat	0.0175	0.031 (99.0)	<0.001 (<0.1)	<0.001 (1.0)
Kidney	5.58	5.56 (99.6)	0.014 (0.2)	0.007 (0.1)

For tissues and milk the extracts were characterised and metabolites present identified. The composition of radioactivity was:

Table B.7.2-14: Characterisation and identification of radioactive residues in tissues and milk from a lactating goat dosed with 2.6 mg ^{14}C-glyphosate-anion (formulated as glyphosate-trimesium) for seven consecutive days

Tissue	mg eq/kg (% TRR)					
	Glyphosate	AMPA	Lactose	triglycerides	post-extraction solids	Total characterised/ identified
Kidney	4.82 (86.3)	0.42 (7.5)	not performed			5.23 (93.8)
Liver	0.14 (59.4)	0.05 (21.4)	not performed			0.19 (80.8)
Muscle	0.022 (87.1)	0.0016 (6.3)	not performed			0.02 (93.5)
Fat	0.03 (91.3)	0.001 (4.7)	not performed			0.03 (96.0)
Milk, day 7	0.0054 (22.3)	0.0004 (2.4)	0.005 (25.2)	0.005 (20.4)	0.004 (21.1)[a]	0.015 (70.3)

a: post-extraction solids present in the remaining protein-pellet

Conclusions:

Residues of the phosphonomethyl-glycine-anion in tissues and milk following administration of glyphosate-trimesium were generally low (0.004-0.14 mg eq/kg) except for kidney (4.82 mg eq/kg), showing the highest concentration. The radioactive residues found in tissues consisted mainly of the unchanged glyphosate anion itself (59.4-91.3 % TRR) and the metabolite AMPA (4.7-21.4 % TRR). The major radioactive residue in milk were natural products in the form of lactose (25.2 % TRR), triglycerides (20.4 % TRR) and probably proteins present as post-extraction pellet (21.1 % TRR). However, the glyphosate anion (22.3 % TRR) and AMPA (2.4 % TRR) were also detected, but at lower levels compared to tissues.

Reference:	OECD KIIA 6.2.3
Report:	Lowrie, Ch. Metabolism of [^{14}C]-N-Acetylglyphosate (IN-MCX20) in the lactating goat 26.10.2007, Report No.: DuPont-19796, ASB2008-2660
Guidelines:	EPS Residue test guideline OPPTS 860.1300 (Nature of the Residue – Livestock) and FAO Guidelines as recommended by the EU Commission Directive 96/68/EC Annex I
Deviations:	none
GLP:	yes
Acceptability:	The study is considered to be acceptable.

Material and Methods:
In this study the metabolism of ^{14}C-N-acetyl-glyphosate was investigated in lactating goats. On lactating goat of 30 kg bodyweight was administered a dose of 205.4 mg/kg of labelled N-acetyl-glyphosate in the diet via capsule for 5 consecutive days (6.8 mg/kg bw and day). During the whole period excreta and milk were sampled. The animal was sacrificed 12 h after the last dose and the radioactive residues in bile, liver, kidney, muscle and fat (omental, renal and subcutaneous) were determined.

Tissue samples were treated with 0.2 N hydrochloric acid followed by an extraction using hexane to remove fatty material. For liver, kidney, muscle and omental fat an additional enzyme treatment (pepsin and protease) was applied to release additional radioactivity. All extracts were analysed by HPLC in comparison to authenticated reference standards.

Findings:
The total recovery of the administered dose in excreta, milk and tissues was 87.83 %. Most of the radioactivity was found in faeces (74.17 %), urine (11.45 %) and cagewash (2.12 %). Milk, liver and kidney each contained about 0.03 % of the total dose administered. The TRR in muscle, fat, kidney and liver were 0.047, 0.089, 4.689 and 0.715 mg/kg respectively.

Table B.7.2-15: TRR levels and total radioactivity recovered from a lactating goat administered ^{14}C-N-acetyl-glyphosate for 5 consecutive days

Sample	TRR in mg eq/kg	% AR
Faeces	-	74.17
Urine	-	11.45
Cage wash	-	2.12
Bile	0.013	-
Milk, day 1	0.03	0.025
Milk, day 2	0.033	
Milk, day 3	0.032	
Milk, day 4	0.033	
Milk, day 5	0.036	
Liver	0.715	0.03
Kidney	4.689	0.03
Muscle	0.047	-
Omental fat	0.065	-
Renal fat	0.093	-
Subcutaneous fat	0.108	-
Total	-	87.83

In general most of the radioactivity found was identified as unchanged N-acetyl-glyphosate. Further metabolites were glyphosate, AMPA and N-acetyl-AMPA. The extraction rates were, except for muscle, above 75 %. In muscle only 42 % of the TRR could be released after acid and enzyme treatment. The total radioactive residues and the identified metabolites are summarised in the following table:

Table B.7.2-16: Identification of radioactive residues in tissues and milk following administration of N-acetyl-glyphosate to lactating goats

Component	Radioactive residues in mg eq/kg (% TRR)						
	Milk	Liver	Kidney	Muscle	Omental fat	Renal fat	Subcutaneous fat
TRR	0.027 (100)	0.804 (100)	4.852 (100)	0.086 (100)	0.187 (100)	0.107 (100)	0.139 (100)
Extracted	0.021 (76.85)	0.669 (83.21)	4.708 (97.03)	0.036 (42.03)	0.065 (34.81)	0.1 (93.57)	0.128 (92.41)
AMPA	0.001 (3.4)	0.068 (8.45)	-	-	0.001 (0.5)	0.001 (1.2)	0.007 (4.77)
Glyphosate	0.001 (3.6)	0.118 (14.71)	0.242 (4.98)	-	0.011 (6.03)	0.005 (5.02)	0.004 (2.65)
N-Acetyl-AMPA	-	-	-	-	0.007 (4.31)	0.001 (0.59)	0.021 (14.86)
N-Acetyl-glyphosate	0.011 (39.98)	0.446 (55.51)	3.742 (77.12)	0.014 (16.7)	0.040 (21.43)	0.078 (73.19)	0.090 (64.73)
unknowns	-	0.004 (0.52)	0.386 (7.97)[a]	0.009 (11.13)[b]	<0.001 (1.86)	0.009 (8.31)	-
Total identified	0.013 (46.92)	0.636 (78.67)	4.37 (90.07)	0.023 (27.83)	0.065 (34.16)	0.094 (86.29)	0.122 (87.01)
Pepsin digest	-	0.055 (6.9)	0.225 (4.64)	<0.001 (<0.01)	0.053 (28.45)	-	-
Protease digest	-	<0.001 (<0.01)	<0.001 (<0.01)	<0.001 (<0.01)	<0.001 (<0.01)	-	-
Unextracted	0.006 (23.15)	0.08 (9.89)	<0.001 (<0.01	0.05 (57.97)	0.122 (65.19)	0.007 (6.43)	0.011 (7.59)

a up to 7 components each accounting <2.13 % TRR (<0.103 mg eq/kg)
b up to 3 components each accounting <6 % TRR (<0.005 mg eq/kg)
n.d. not detected

Conclusions:
The metabolism of N-acetyl-glyphosate in lactating goats was more or less comparable to parent glyphosate. The molecule remained mainly unchanged, giving highest residues in kidney (4.68 mg eq/kg), confirming a major excretion route via the urine. Liver gave second highest residues (0.804 mg eq/kg). Other tissues and milk only gave low residues not exceeding 0.2 mg eq/kg. Further metabolites identifed were glyphosate and AMPA. N-acetyl-AMPA was also detected, but at very low amounts, suggesting preferred de-acetylation of N-acetyl-glyphosate instead of degradation into N-acetyl-AMPA. In milk a plateau of radioactive residues was reached within 24 hours.

B.7.2.2 Laying hens

Reference: OECD KIIA 6.2.2

Report: Powles, P.
(^{14}C-Glyphosate): Absorption, distribution, metabolism and excretion following repeated oral administration to the laying hen
07.11.1994, Report No. 676/8-1011, RIP9501208

Guidelines: EPA Nature of the residues in Livestock (171-4)

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study ^{14}C-glyphosate radiolabelled in the phosphonomethyl-moiety was administered to laying hens (2 groups with five animals each) for a period of 5 or 7 consecutive days. Each animal received nominal doses of 200 mg/kg feed via capsule twice per day (29.5 mg per animal and day, 18.2 mg/kg bw and day).
The first group was dosed for 7 days , the second group for 5 days. Excreta and eggs were collected at 24 hour intervals following the initial dose. Selective tissues were sampled for analysis after sacrifice at 23 hours after the final dose. In addition blood samples were taken 1, 2, 3, 4, 6, 8 and 12 hours after the initial dose.

The total radioactivity was determined by combustion and/or LSC. Radioactivity was extracted from each matrix adding chloroform/HCl for homogenisation, and after centrifugation the aqueous phase was transferred to a chelating ion exchange resin and the radioactivity was eluted with HCl. After chromatography using a strong anion exchange resin the residue was solubilised in water. The methods of analysis used for identification involved HPLC and TLC by comparison with reference substances.

Findings:
In the following table the total recovered radioactivity following administration of ^{14}C-glyphosate to laying hens is summarised.

Table B.7.2-17: Total recovered radioactivity following administration of ^{14}C-glyphosate to laying hens at nominal feed levels of 200 mg/kg feed

Tissue	**% AR (mean of dose group)**	
	Group 1 (18.2 mg/kg bw, 7 days)	Group 2 (18.2 mg/kg bw, 5 days)
Excreta	76.45	63.65
Egg white	<0.01	<0.01
Egg yolk	<0.01	<0.01
Cage washings	2.98	0.81
Cage begris	0.88	0.75
Tissues	0.02	0.02
Total	80.34	65.23

In the egg white residues reached a plateau quickly within the five days. For egg yolk residues were increasing until day 7. Recovered TRR levels in egg white and yolk were:

Table B.7.2-18: TRR levels in egg white and yolk following administration of ^{14}C-glyphosate to laying hens at nominal feed levels of 200 mg/kg feed

Days	TRR in mg eq/kg (mean of dose group)			
	Group 1 (18.2 mg/kg bw, 7 days)		Group 2 (18.2 mg/kg bw, 5 days)	
	egg white	egg yolk	egg white	egg yolk
1	<0.024	<0.062	<0.01	<0.011
2	0.029	<0.062	0.023	<0.011 (0.006)
3	0.043	0.090	0.044	0.075
4	0.038	0.198	0.056	0.164
5	0.049	0.318	0.072	0.228
6	0.059	0.365	-	-
7	0.053	0.484	-	-

After sacrifice of the animals TRR levels in tissues from both dose groups were measured.

Table B.7.2-19: TRR levels in tissues following administration of ^{14}C-glyphosate to laying hens at nominal feed levels of 200 mg/kg feed

Tissue	TRR in mg eq/kg (mean/highest)	
	Group 1 (18.2 mg/kg bw, 7 days)	Group 2 (18.2 mg/kg bw, 5 days)
Skin	0.212 / 0.428	0.359 / 0.409
Fat	0.153 / 0.217	0.083 / 0.218
Muscle	<0.043 / <0.043	0.041 / 0.049
Liver	1.242 / 1.886	1.080 / 1.633

For goat 2 dosed over three days concentrations of ^{14}C in blood plasma was monitored for 12 hours after the administration:

Table B.7.2-20: TRR levels in blood plasma following administration of ^{14}C-glyphosate to laying hens at nominal feed levels of 200 mg/kg feed

Time (hours)	mg eq/kg (mean of dose group)
1	0.475
2	0.402
3	0.321
4	0.187
6	0.172
8	0.099
12	0.050

The identification of radioactive residues was only conducted for samples collected from the second dose group (5 days of dosing). In egg yolk, liver, skin, fat, muscle and excetas the results were:

Table B.7.2-21: Identification of radioactive residues in urine, faeces, liver and kidney of laying hens following administration of ^{14}C-glyphosate to lactating goats at nominal feed levels of 200 mg/kg feed

Sample	% TRR			
	glyphosate	AMPA	unknown	total
Excreta (48-96h), radio-HPLC	95.07	-	-	95.07
Excreta (48-96h), TLC system 1	97.19	2.34	-	99.53
Excreta (48-96h), TLC system 2	98.44	1.06	-	99.51
Liver, radio-HPLC	61.38	36.8	-	98.18
Liver, TLC system 1	89.41	9.94	-	99.33
Liver, TLC system 2	83.72	14.26	-	97.98
Skin , radio-HPLC	98.65	-	-	98.65
Skin, TLC system 1	95.34	0.79	3.23	99.36
Skin, TLC system 2, sample + reference[a]	78.78	1.76	19.08	99.62
Skin, TLC system 2, pure sample	56.84	1.48	41.35	99.68
Fat, radio-HPLC	98.94	-	-	98.94
Fat, TLC system 1	62.21	-	34.86	97.07
Fat, TLC system 1	66.55	-	31.69	98.24
Muscle, radio-HPLC	97.81	-	-	97.81
Muscle, TLC system 1	97.91	-	-	97.91
Muscle, TLC system 1	45.15	-	-	45.15
Egg yolk, radio-HPLC	96.13	-	-	96.13
Egg yolk, TLC system 1	72.60	-	23.83	96.43
Egg yolk, TLC system 1	24.23	-	72.93	97.16

a: sample extract and unlabelled reference substances spotted on the same location

Conclusions:
Following administration of ^{14}C-glyphosate to laying hens most of the radioactivity was recovered in the excreta (64-76 % AR). In eggs (<0.01 % AR) and tissues (0.02 % AR) only minor amounts of the administered radioactivity were found. Mean TRR levels were <0.01-0.072 mg eq/kg in egg white, <0.011-0.484 mg eq/kg in egg yolk, 0.083-0.153 mg eq/kg in fat, 0.041 mg eq/kg in muscle, 1.08-1.24 mg eq/kg in liver and 0.21-0.36 mg eq/kg in skin. In egg yolk no plateau of the residue was reached within the maximum dosage period of 7 consecutive days.
Concentrations of radioactivity in blood plasma gave the highest residue directly after administration and declined to approximated 10 % after 12 h, suggesting a quick uptake and excretion of the residue.
Identification of the radioactivity in revealed mainly unchanged glyphosate (24-98.7 % TRR). In liver also AMPA was found at relative rates between 9.9-36.8 % TRR, depending on the analytical system. In fatty tissues (skin, fat and egg yolk) a significant amount of radioactivity remained unidentified (3-72.9 % TRR).

Reference: OECD KIIA 6.2.2

Report: Bodden, R. M.
Metabolism study of synthetic ^{13}C/^{14}C-labeled Glyphosate and Aminomethylphos-phonic acid in laying hens. Part I
01.02.1988 Report No: MSL-7591, RIP9501205

Guidelines: not stated

Deviations: not applicable

GLP: yes

Acceptability: The study is considered to be acceptable.

Reference: OECD KIIA 6.2.2

Report: Patanella, J. E.; Feng, P.
Metabolism study of synthetic 13C/14C-labeled Glyphosate and Aminomethylphosphonic acid in laying hens. Part II
February 1988, Report No: MSL-7420, RIP9501206

Guidelines: not stated

Deviations: not applicable

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study laying hens were administed a mixture of ^{14}C-glyphosate and ^{14}C-AMPA (approx. 9:1) for a period of 7 consecutive days. The dose rates were based on the average daily feed consuption resulting in 120 mg/kg feed (15 mg glyphosate and 1.6 mg AMPA per animal and day, 9.7 and 1.03 mg/kg bw, respectively) or 400 mg/kg feed (49.9 mg glyphosate and 5.3 mg AMPA per animal and day, 32.2 and 3.4 mg/kg bw, respectively).
In total five groups consisting of 5 hens each were defined: one control group receiving placebo, 2 groups receiving 120 mg/kg in the feed for 7 days, one group receiving 400 mg/kg in the feed for 7 days and one group receiving 120 mg/kg in the feed for 7 days with additional 10 days for depuration.
Eggs were collected at 24 hour intervals following the initial dose. Selective tissues were sampled for analysis after sacrafice at 23 hours after the final dose.

The analysis of radioactivity was carried out by combustion or LSC. Tissues were extracted with water/chloroform, milk samples were mixed with HCl to precipitate proteins, after centrifugation the supernatants were concentrated to dryness and the residues solubilized in water. Metabolites were characterised and identified by HPLC and GC/MS.

Findings:
Residues found in the control group (group 1) were always below the detection limits of the analytical methods use. Therefore only results of treated does groups are presented.

In the following table the total recovered radioactivity in eggs, tissues and excreta are summarised:

Table B.7.2-22: Total radioactivity recovered following administration of ^{14}C-glyphosate and ^{14}C-AMPA to laying hens for 7 consecutive days

Matrix	% AR			
	Group 2 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 3 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 4 (49.9 mg glyphosate and 5.3 mg AMPA per kg bw and days, 7 days)	Group 5 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days + 10 days depuration)
Kidney	0.02	0.02	0.02	<0.01
Liver	0.02	0.02	0.02	<0.01
Thight muscle	<0.01	<0.01	<0.01	<0.01
Breast muscle	<0.01	<0.01	<0.01	<0.01
Fat	<0.01	<0.01	<0.01	<0.01
Ovaries	0.02	0.02	0.01	<0.01
GI tract with contends	1.65	2.11	1.30	<0.01
Egg yolk	0.01	0.01	0.01	0.02
Egg white	<0.01	<0.01	<0.01	<0.01
Gizzard	<0.01	<0.01	<0.01	<0.01
Excreta	81.8	83.6	81.0	90.5
Pan rins	0.06	0.08	0.06	0.01
Total	83.6	85.9	82.4	90.5

Eggs were separately analysed for radioactive residues in egg white and egg yolk. A plateau level in eggs white was reached after approximately 6 days. Residues in egg yolk increased until the end of dosing. TRR levels expressed as parent glyphosate were:

Table B.7.2-23: TRR levels found in egg white following administration of ^{14}C-glyphosate and ^{14}C-AMPA to laying hens for 7 consecutive days

Days	TRR in egg white (mg glyphosate eq/kg)			
	Group 2 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 3 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 4 (49.9 mg glyphosate and 5.3 mg AMPA per kg bw and days, 7 days)	Group 5 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days + 10 days depuration)
1	<0.0013	<0.0013	<0.005	<0.0013
2	<0.0013	<0.0013	<0.005	<0.0013
3	0.004	0.004	0.010	0.003
4	0.008	0.012	0.026	0.008
5	0.010	0.014	0.030	0.009
6	0.011	0.017	0.032	0.010
7	0.011	0.016	0.026	0.010
8	0.011	0.015	0.027	0.010
9 (depuration)	-	-	-	0.007
10 (depuration)	-	-	-	0.008
11 (depuration)	-	-	-	0.006
12 (depuration)	-	-	-	0.006
13 (depuration)	-	-	-	0.002
14 (depuration)	-	-	-	0.002
15 (depuration)	-	-	-	0.001
16 (depuration)	-	-	-	<0.0013
17 (depuration)	-	-	-	<0.0013

Table B.7.2-24: TRR levels found in egg yolk following administration of ^{14}C-glyphosate and ^{14}C-AMPA to laying hens for 7 consecutive days

Days	TRR in egg yolk (mg glyphosate eq/kg)			
	Group 2 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 3 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 4 (49.9 mg glyphosate and 5.3 mg AMPA per kg bw and days, 7 days)	Group 5 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days + 10 days depuration)
1	<0.0013	<0.0013	<0.005	<0.0013
2	<0.0013	<0.0013	<0.005	<0.0013
3	0.002	0.002	<0.005	0.002
4	0.033	0.020	0.096	0.028
5	0.077	0.050	0.252	0.071
6	0.118	0.096	0.444	0.121
7	0.170	0.133	0.625	0.172
8	0.229	0.191	0.753	0.196
9 (depuration)	-	-	-	0.224
10 (depuration)	-	-	-	0.236
11 (depuration)	-	-	-	0.206
12 (depuration)	-	-	-	0.173
13 (depuration)	-	-	-	0.128
14 (depuration)	-	-	-	0.089
15 (depuration)	-	-	-	0.060
16 (depuration)	-	-	-	0.038
17 (depuration)	-	-	-	0.019

In other matrices collected after sacrifice of the animals TRR levels were:

Table B.7.2-25: TRR levels found in samples collected at sacrifice following administration of ^{14}C-glyphosate and ^{14}C-AMPA to laying hens for 7 consecutive days

Matrix	TRR in mg glyphosate eq/kg			
	Group 2 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 3 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 4 (49.9 mg glyphosate and 5.3 mg AMPA per kg bw and days, 7 days)	Group 5 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days + 10 days depuration)
Kidney	1.81	1.75	7.0	0.069
Liver	0.560	0.511	1.91	0.079
Thight muscle	0.026	0.026	0.090	0.008
Breast muscle	0.018	0.019	0.055	0.006
Fat	0.020	0.015	0.063	0.005
GI tract with contents	18.8	23.9	52.7	0.038
Gizzard	0.352	0.361	1.134	0.032
Ovaries	0.264	0.271	0.939	0.016
Whole blood	0.135	0.146	0.524	0.049

The analysis of the radioactive residues following extraction was reported in the second part of the study. Kidney, liver, gizzard, fat, muscle and egg yolk samples were investigated for their composition of glyphosate and AMPA. Egg white samples were not further analysed.

Table B.7.2-26: Composition of radioactive residues in laying hens kidney following administration of ^{14}C-glyphosate and ^{14}C-AMPA to laying hens for 7 consecutive days

Matix	Group 2 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 3 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 4 (49.9 mg glyphosate and 5.3 mg AMPA per kg bw and days, 7 days)	Group 5 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days + 10 days depuration)
	% TRR	% TRR	% TRR	% TRR
Total extracted	110.9	109.5	106.8	98.9
chloroform extract	0.1	0.1	0.1	0.3
water extract	109.2	106.6	104.6	94.3
unextracted	1.6	2.8	2.1	4.3
Analysis of water extract	% of water extract	% of water extract	% of water extract	% of water extract
Recovery after deproteination	-	-	-	95.7 (90.2 % TRR)
Recovery after concentration	-	-	-	89.1 (84.0 % TRR)
Ion Pair HPLC		97.0 (103.4 % TRR)		
AMPA	4.9 (5.4 % TRR)	not performed	4.1 (4.3 % TRR)	9.8 (9.2 % TRR)
glyphosate	93.3 (101.9 % TRR)	not performed	94.2 (98.5 % TRR)	89.7 (84.6 % TRR)
Cation exchange HPLC				
AMPA	4.7 (5.1 % TRR)	5.4 (5.8 % TRR)	4.3 (4.5 % TRR)	10.3 (9.7 % TRR)
glyphosate	93.5 (102.1 % TRR)	93.9 (100.1 % TRR)	95.2 (99.6 % TRR)	87.9 (82.9 % TRR)

Table B.7.2-27: Composition of radioactive residues in laying hens liver following administration of ^{14}C-glyphosate and ^{14}C-AMPA to laying hens for 7 consecutive days

Matix	**% TRR**			
	Group 2 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 3 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 4 (49.9 mg glyphosate and 5.3 mg AMPA per kg bw and days, 7 days)	Group 5 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days + 10 days depuration)
	% TRR	% TRR	% TRR	% TRR
Total extracted	107.6	108.5	109.4	101.6
chloroform extract	0.5	0.5	0.7	<0.1
water extract	105.3	105.1	105.8	99.0
unextracted	1.8	2.9	2.9	2.6
Analysis of water extract	% of water extract	% of water extract	% of water extract	% of water extract
Recovery after deproteination	95.3 (100.4 % TRR)	101.3 (106.5 % TRR)	99.9 (105.7 % TRR)	98.7 (97.7 % TRR)
Recovery after concentration	88.7 (93.4 % TRR)	81.6 (85.8 % TRR)	96.2 (101.8 % TRR)	78.4 (77.6 % TRR)
Ion Pair HPLC				
AMPA	29.6 (31.2 % TRR)	29.3 (30.8 % TRR)	34.2 (36.2 % TRR)	55.3 (54.7 % TRR)
glyphosate	69.4 (73.1 % TRR)	69.8 (73.4 % TRR)	65.0 (68.8 % TRR)	42.1 (41.7 % TRR)
Cation exchange HPLC				
AMPA	27.3 (28.7 % TRR)	29.0 (30.5 % TRR)	32.8 (34.7 % TRR)	54.5 (54 % TRR)
glyphosate	70.1 (73.8 % TRR)	68.7 (72.2 % TRR)	66.1 (69.9 % TRR)	42.0 (41.6 % TRR)

Table B.7.2-28: Composition of radioactive residues in laying hens gizzard following administration of ^{14}C-glyphosate and ^{14}C-AMPA to laying hens for 7 consecutive days

Matix	**% TRR**			
	Group 2 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 3 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 4 (49.9 mg glyphosate and 5.3 mg AMPA per kg bw and days, 7 days)	Group 5 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days + 10 days depuration)
	% TRR	% TRR	% TRR	% TRR
Total extracted	100.3	108.5	105.4	104.7
chloroform extract	0.1	0.2	<0.1	0.5
water extract	98.7	105.4	103.0	90.2
unextracted	1.5	2.9	2.4	14.0
Analysis of water extract	% of water extract	% of water extract	% of water extract	% of water extract
Recovery after deproteination	not performed	not performed	not performed	not performed
Recovery after concentration	62.7 (61.9 % TRR)	64.4 (67.9 % TRR)	69.2 (71.3 % TRR)	72.4 (65.3 % TRR)
Ion Pair HPLC				
AMPA	40.3 (39.8 % TRR)	39.6 (41.7 % TRR)	39.5 (40.7 % TRR)	15.6 (14.1 % TRR)
glyphosate	59.7 (58.9 % TRR)	60.4 (61.9 % TRR)	60.5 (62.3 % TRR)	84.4 (76.1 % TRR)
Cation exchange HPLC				
AMPA	38.0 (37.5 % TRR)	41.3 (43.5 % TRR)	40.6 (41.8 % TRR)	19.6 (15.2 % TRR)
glyphosate	60.6 (59.8 % TRR)	56.9 (60 % TRR)	58.3 (60 % TRR)	77.4 (69.8 % TRR)

Table B.7.2-29: Composition of radioactive residues in laying hens fat following administration of ^{14}C-glyphosate and ^{14}C-AMPA to laying hens for 7 consecutive days

Matix	**% TRR**			
	Group 2 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 3 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 4 (49.9 mg glyphosate and 5.3 mg AMPA per kg bw and days, 7 days)	Group 5 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days + 10 days depuration)
	% TRR	% TRR	% TRR	% TRR
Total extracted	101.9	106.6	104.9	101.6
chloroform extract	17.5	17.3	12.6	58.2
water extract	84.4	89.3	92.3	43.4
unextracted	<0.1	<0.1	<0.1	<0.1
Analysis of water extract	% of water extract	% of water extract	% of water extract	% of water extract
Recovery after deproteination	95.8 (80.9 % TRR)	87.1 (77.8 % TRR)	87.4 (80.7 % TRR)	82.0 (35.6 % TRR)
Recovery after concentration	100.1 (84.5 % TRR)	102.5 (91.5 % TRR)	100.7 (92.9 % TRR)	101.8 (44.2 % TRR)
Ion Pair HPLC				
AMPA	15.3 (12.9 % TRR)	15.1 (13.5 % TRR)	14.1 (13.0 % TRR)	35.1 (15.2 % TRR)
glyphosate	82.7 (69.8 % TRR)	83.9 (74.9 % TRR)	85.2 (78.6 % TRR)	64.9 (28.2 % TRR)
Cation exchange HPLC				
AMPA	13.1 (11.1 % TRR)	14.0 (12.5 % TRR)	11.5 (10.6 % TRR)	33.0 (14.3 % TRR)
glyphosate	86.4 (72.9 % TRR)	83.8 (74.8 % TRR)	86.5 (79.8 % TRR)	65.8 (28.6 % TRR)

Table B.7.2-30: Composition of radioactive residues in laying hens thight muscle following administration of ^{14}C-glyphosate and ^{14}C-AMPA to laying hens for 7 consecutive days

Matix	**% TRR**			
	Group 2 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 3 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 4 (49.9 mg glyphosate and 5.3 mg AMPA per kg bw and days, 7 days)	Group 5 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days + 10 days depuration)
	% TRR	% TRR	% TRR	% TRR
Total extracted	109.1	111.2	95.0	93.6
chloroform extract	2.4	2.0	1.2	1.5
water extract	106.7	109.2	93.8	92.1
unextracted	not analysed	not analysed	not analysed	not analysed
Analysis of water extract	% of water extract	% of water extract	% of water extract	% of water extract
Recovery after deproteination	97.7 (104.2 % TRR)	93.9 (102.5 % TRR)	95.9 (90 % TRR)	70.2 (64.7 % TRR)
Recovery after concentration	76.4 (81.5 % TRR)	66.5 (72.6 % TRR)	71.8 (67.3 % TRR)	79.5 (73.2 % TRR)
Ion Pair HPLC				
AMPA	not performed	not performed	not performed	not performed
glyphosate	not performed	not performed	not performed	not performed
Cation exchange HPLC				
AMPA	13.0 (13.9 % TRR)	13.4 (14.6 % TRR)	15.0 (14.1 % TRR)	33.1 (30.5 % TRR)
glyphosate	69.6 (74.3 % TRR)	69.5 (75.9 % TRR)	75.8 (71.1 % TRR)	58.8 (54.2 % TRR)
unknown	9.2 (9.8 % TRR)	10.1 (11 % TRR)	5.3 (5 % TRR)	2.8 (2.6 % TRR)

Table B.7.2-31: Composition of radioactive residues in laying hens breast muscle following administration of ^{14}C-glyphosate and ^{14}C-AMPA to laying hens for 7 consecutive days

Matix	**% TRR**			
	Group 2 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 3 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 4 (49.9 mg glyphosate and 5.3 mg AMPA per kg bw and days, 7 days)	Group 5 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days + 10 days depuration)
	% TRR	% TRR	% TRR	% TRR
Total extracted	104.5	99.7	96.6	95.2
chloroform extract	1.1	0.8	1.5	5.7
water extract	103.4	98.9	95.1	89.5
unextracted	not analysed	not analysed	not analysed	not analysed
Analysis of water extract	% of water extract	% of water extract	% of water extract	% of water extract
Recovery after deproteination	not performed	not performed	not performed	not performed
Recovery after concentration	not performed	not performed	not performed	not performed
Ion Pair HPLC				
AMPA	not performed	not performed	not performed	not performed
glyphosate	not performed	not performed	not performed	not performed
Cation exchange HPLC				
AMPA	14.0 (14.5 % TRR)	11.7 (11.6 % TRR)	17.5 (16.6 % TRR)	44.9 (40.2 % TRR)
glyphosate	63.8 (66 % TRR)	62.6 (61.9 % TRR)	72.0 (68.5 % TRR)	43.7 (39.1 % TRR)
unknown	11.7 (12.1 % TRR)	16.3 (16.1 % TRR)	4.4 (4.2 % TRR)	2.6 (2.3 % TRR)

Table B.7.2-32: Composition of radioactive residues in egg yolk following administration of ^{14}C-glyphosate and ^{14}C-AMPA to laying hens for 7 consecutive days

Matix	**% TRR**			
	Group 2 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 3 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days)	Group 4 (49.9 mg glyphosate and 5.3 mg AMPA per kg bw and days, 7 days)	Group 5 (9.7 mg glyphosate and 1.03 mg AMPA per kg bw and days, 7 days + 10 days depuration)
	% TRR	% TRR	% TRR	% TRR
Total extracted	94.9	94.3	99.1	98.1
chloroform extract	9.5	9.7	8.7	9.3
water extract	76.2	70.5	76.5	70.5
unextracted	9.2	14.1	13.9	18.3
Analysis of water extract	% of water extract	% of water extract	% of water extract	% of water extract
Recovery after deproteination	108.9 (83 % TRR)	101.3 (71.4 % TRR)	97.3 (74.4 % TRR)	97.4 (68.7 % TRR)
Recovery after concentration	99.6 (75.9 % TRR)	98.7 (69.6 % TRR)	101.3 (77.5 % TRR)	107.2 (75.6 % TRR)
Ion Pair HPLC				
AMPA	20.5 (15.6 % TRR)	23.4 (16.5 % TRR)	19.1 (14.6 % TRR)	18.6 (13.1 % TRR)
glyphosate	79.3 (60.4 % TRR)	76.6 (54 % TRR)	80.7 (61.7 % TRR)	81.4 (57.4 % TRR)
Cation exchange HPLC				
AMPA	16.3 (12.4 % TRR)	19.1 (13.5 % TRR)	17.8 (13.6 % TRR)	15.5 (10.9 % TRR)
glyphosate	83.7 (63.8 % TRR)	80.6 (56.8 % TRR)	82.0 (62.7 % TRR)	84.3 (59.4 % TRR)

Conclusions:

Following administration of a 9:1 mixture of ^{14}C-glyphosate and ^{14}C-AMPA to laying hens most of the radioactivity was recovered in the excreta (81.0-90.5 % AR). In eggs yolk (0.01-0.02 % AR), egg white (<0.01 % AR) and tissues (up to 0.02 % AR) only minor amounts of the administered radioactivity were found. Most of radioactivity was found in the kidney 0.069-7.0 mg eq/kg), followed by liver (0.079-1.91 mg eq/kg) and egg yolk (0.229-0.753 mg eq/kg). Muscle, fat and egg white gave much lower residues, normally not exceeding 0.1 mg eq/kg. In egg yolk no plateau of the residue was reached within the maximum dosage period of 7 consecutive days.

Identification of the radioactivity in revealed no significant degradation of the two administered substances. Except for an unidentified compound in muscle (up to 16.3 % TRR but <0.01 mg eq/kg) only glyphosate and AMPA were found. Except for liver and gizzard the ratio of glyphosate to AMPA was comparable to the 9:1 ratio administered. In gizzard and liver AMPA was present at higher relative amounts, giving a 2:1 ratio. In samples obtained after depuration of 10 days also a higher relative amount of AMPA was found (up to 1:1 in egg yolk), however the absolute concentration decreased significantly compared to animals sacrificed 23 h after dosing.

Reference: OECD KIIA 6.2.2

Report: Bowler, D. T.
[^{14}C-PMG] Glyphosate-trimesium: Nature of the residue in tissues and eggs of laying hens
28.02.1994, Report No: RR-93-064B, RIP9500020

Guidelines: EPA Nature of the residues in Livestock (171-4)

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study laying hens (leghorn) were dosed with ^{14}C-glyphosate-trimesium radiolabelled in the phosphonomethyl-glycine-anion for a period of 10 consecutive days. The animals were administered daily doses equivalent to 62.4 mg glyphosate per kg feed (6.8 mg per animal, 4.1 mg/kg bw and day). Over the whole dosing period excreta and eggs were sampled. 12-15 hours after the final administration the animals were sacrificed and tissue samples were collected.

The analysis of radioactivity was carried out by combustion and/or LSC. Tissues and eggs were extracted with chloroform/0.1N HCl. The extracts were purified with an inon exchange column and characterised by HPLC and TLC. Further Characterisation and identification of radioactive residues was conducted following deriviatisation using GC/MS against reference substances. Unextracted residues were additionally hydrolysed using 6N HCl for up to 9h.

Findings:
In the following table the TRRs found in egg white and egg yolk is presented:

Table B.7.2-33: TRR found in egg white and egg yolk following administration of 4.1 mg/kg bw of ^{14}C-glyphosate-trimesium radiolabelled in the phosphonomethyl-glycine-anion for 10 consecutive days

Day	TRR in eggs (mg phosphonomethyl-glycine-anion per kg)	
	Egg yolk	Egg white
1	<0.001	<0.001
2	0.0014	0.00374
3	0.0254	0.0106
4	0.0641	0.0105
5	0.11	0.0133
6	0.153	0.0121
7	0.194	0.0144
8	0.211	0.0144
9	0.229	0.0171
10	0.238	0.0169

The radioactivity found in extractes of eggs and tissues was analysed for its composition:

Table B.7.2-34: TRR in tissues and characterisation/identification of radioactive residues following administration of 4.1 mg/kg bw of ^{14}C-glyphosate-trimesium radiolabelled in the phosphonomethyl-glycine-anion to laying hens for 10 consecutive days

Metabolite	mg phosphonomethyl-glycine-anion equivalents per kg (% TRR)					
	Egg yolk	Egg white	Thight muscle	Breast muscle	Liver	Fat
TRR	0.24 (100)	0.0189 (100)	0.0401 (100)	0.0292 (100)	0.4402 (100)	0.0293 (100)
Glyphosate	0.1429 (59.54)	0.0043 (21.48)	0.0245 (61)	0.0114 (39.05)	0.2684 (60.97)	0.0119 (40.66)
AMPA	0.0055 (2.28)	0.0002 (0.82)	0.0016 (4.06)	0.0015 (5.0)	0.0992 (22.53)	0.001 (3.31)
Unknown 1	0.0033 (1.37)	0.0001 (0.49)	0.0015 (3.79)	0.0004 (1.21)	0.0224 (5.08)	0.0004 (1.46)
Organo-soluble	-	0.0002 (1.06)	0.0009 (2.19)	0.0002 (0.65)	0.0133 (3.01)	-
Phospholipids	0.0178 (7.4)	-	-	-	-	-
Non-polar lipids	0.0415 (17.3)	-	-	-	-	0.0129 (44.1)
Polar conjugate	0.0076 (3.22)	0.0073 (38.36)	0.0067 (16.59)	0.0063 (21.54)	0.0014 (0.31)	0.0006 (2.01)
Hydrol. released fraction	0.0162 (6.73)	0.0071 (37.83)	0.0044 (10.84)	-	0.008 (1.82)	-
HCl hydrolysate	-	-	-	0.0087 (29.68)	-	-
Unextracted	0.0053 (2.19)	-	0.0006 (1.49)	0.0008 (2.86)	0.0277 (6.28)	0.0025 (8.45)
Total characterised/identified	0.24 (99.98)	0.0189 (100)	0.0401 (99.96)	0.0292 (99.99)	0.4402 (100)	0.0293 (99.99)

Conclusions:
Glyphosate-trimesium radiolabelled in the phosphonomethyl-glycine-anion was the major residue in all matrices investigated. Highest total radioactive residues were found in liver, followed by egg yolk. In egg yolk no plateau of total radioactive residues was reached within 10 days. However a major part of the radioactivity was characterised in the non-polar lipid fraction, suggesting incorporation into natural products. Significant amounts of AMPA were only found in liver, ranging up to 22.5 % of the TRR. No additional metabolites besides the glyphosate anion and AMPA were identified.

Reference: OECD KIIA 6.2.2

Report: Lowrie, Ch.
The metabolism of [^{14}C]-N-Acetylglyphosate (IN-MCX20) in laying hens
08.11.2007, Report No.: DuPont-19795, ASB2008-2659

Guidelines: EPS Residue test guideline OPPTS 860.1300 (Nature of the Residue – Livestock) and FAO Guidelines as recommended by the EU Commission Directive 96/68/EC Annex I

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
Laying hens (5 animals) were dosed for 7 consecutive days with 63 mg/kg ^{14}C-N-acetyl-glyphosate in the feed via oral administration (8.3 mg/animal and day, 4.5 mg/kg bw and day). Excreta and eggs were samples during the whole period of time once or twice daily respectively. After the dosing the animals were sacrificed and tissues (liver, breast and tight muscle and abdominal fat) were analysed for radioactive residues.

Total radioactivity was determined by combustion of the samples and detection of the resulting $^{14}CO_2$. Eggs were extracted using 0.2N hydrochloric acid followed by a partitioning with dichloromethane and chloroform (eggs white only). Tissue samples were also treated with 0.2 N hydrochloric acid followed by an extraction using hexane to remove fatty materials. For egg yolk and liver an additional enzyme treatment (pepsin and protease) was applied to release additional radioactivity. All extracts were analyzed by HPLC in comparison to authenticated reference standards.

Findings:
The total recovery of the radioactivity administered was 90.18%. Most of the radioactivity (90.08 %) was found in the excreta of the hens. In animal tissues TRR values ranged from 0.01 mg/kg for egg white up to 0.505 mg/kg for liver.

Table B.7.2-35: TRR levels and total radioactivity recovered from a laying hens administered ^{14}C-N-acetyl-glyphosate for 7 consecutive days

Sample	TRR in mg eq/kg	% AR
Excreta	-	84.14
Cage wash	-	5.94
Egg white / Egg yolk, day 1	0.001 / <0.001	0.005 / 0.035
Egg white / Egg yolk, day 2	0.009 / 0.044	
Egg white / Egg yolk, day 3	0.013 / 0.093	
Egg white / Egg yolk, day 4	0.015 / 0.197	
Egg white / Egg yolk, day 5	0.015 / 0.294	
Egg white / Egg yolk, day 6	0.015 / 0.295	
Egg white / Egg yolk, day 7	0.015 / 0.342	
Liver	0.511	0.05
Muscle	0.039	-
Abdominal fat	0.051	-
Total	-	90.18

In all matrices about 90% of the radioactivity was extracted and identified. In general most of the radioactivity found was identified as glyphosate, indicating a removal of the N-acetyl-group. Further metabolites were AMPA and N-acetyl-AMPA as well as unchanged N-acetyl-glyphosate. The total radioactive residues and the identified metabolites are summarized in the following table:

Table B.7.2-36: Identification of radioactive residues in tissues and eggs following administration of ^{14}C-N-acetyl-glyphosate to laying hens for 7 consecutive days

Component	Radioactive residues in mg eq/kg (% TRR)				
	Egg white	Egg yolk	Liver	Muscle	Abdominal fat
TRR	0.10 (100)	0.229 (100)	0.505 (100)	0.033 (100)	0.057 (100)
Extracted	0.009 (94.33)	0.187 (81.47)	0.483 (95.56)	0.029 (87.47)	0.053 (92.42)
AMPA	-	0.002 (0.91)	0.034 (6.74)	0.005 (16.69)	0.007 (11.29)
Glyphosate	0.001 (10.9)	0.013 (5.69)	0.084 (16.34)	0.002 (7.19)	0.023 (39.43)
N-Acetyl-AMPA	<0.001 (4.34)	0.003 (1.1)	0.02 (4.04)	0.001 (1.89)	0.006 (10.18)
N-Acetyl-glyphosate	0.004 (41.48)	0.157 (68.4)	0.323 (63.82)	0.009 (25.22)	0.014 (23.45)
unknowns	<0.001 (3.4)	-	-	0.006 (14.86)[a]	0.001 (1.37)
Total identified	0.005 (60.12)	0.175 (76.1)	0.461 (90.94)	0.023 (65.85)	0.051 (85.72)
Pepsin digest	-	0.027 (11.61)	0.019 (3.81)	-	-
Protease digest	-	0.007 (3.1)	0.001 (0.27)	-	-
Unextracted	0.001 (5.67)	0.008 (3.82)	0.002 (0.36)	0.004 (12.53)	0.004 (7.58)

a up to 4 components each accounting <8.95 % TRR (<0.003 mg eq/kg)

Conclusions:
In laying hens administered N-acetyl-glyphosate the unchanged molecule was the major residue in all matrices (25.22-68.4 % TRR) except for abdominal fat (23.45 % TRR). Glyphosate was also present, normally representing the second highest residue (5.69-10.9 % TRR) and the highest one in abdominal fat (39.4 % TRR). AMPA was found as major metabolite in muscle (16.69 % TRR) and abdominal fat (11.29 % TRR). N-acetyl-AMPA was only present at minor amounts of 1.11-10.18 % TRR, suggesting preferred de-acetylation of N-acetyl-glyphosate instead of degradation into N-acetyl-AMPA. In egg whites a plateau for the TRR was reached after 4 days, in egg yolk the TRR increased until the final day of the study.

B.7.2.3 Fish

No metabolism data for glyphosate or its metabolites in fish was provided.

B.7.3 Definition of the residue (Annex IIA 6.7; Annex IIIA 8.7)

B.7.3.1 Residue definition for plant commodities

The metabolism of glyphosate was investigated in numerous crops, including tolerant varieties with CP4-EPSPS, GOX and GAT modifications. In some of the experiments glyphosate-trimesium, which contains the glyphosate-anion, was used. Since the glyphosate-trimesium salt dissociates quickly, these studies also provide valuable information on the metabolism of glyphosate formulated as sodium- or isopropylamine-salt. The metabolism data does not show differences in the behaviour of the glyphosate-anion related to the cations used, making all variants of the active substance equivalent.

B.7.3.1.1 Non-tolerant crops

In non-tolerant crops the following crop groups were investigated using different methods of application (see brackets):

Fruits
- Mandarins (soil, foliar, hydroponic)
- Almond, walnut and pecan (soil, foliar)
- Apples (soil, foliar, trunk)
- Grapes (soil, foliar, trunk, hydroponic)
- Avocado (foliar, direct fruit treatment)

Root and tuber crops
- Potato (soil, foliar)
- Sugar beets (soil)
- Serradelle, turnips (soil)

Pulses and oilseeds
- Cotton (soil, hydroponic)
- Soya beans (soil, hydroponic)

Cereal grains
- Barley (soil, hydroponic)
- Maize (soil, hydroponic)
- Oats (soil, hydroponic)
- Rice (soil, hydroponic)
- Sorghum (soil, hydroponic)
- Wheat (soil, hydroponic, foliar - dessication)

Miscelleneous crops
- Coffee (soil, foliar, stem, hydroponic)
- Pasture (soil, foliar)
- Sugar cane (soil, foliar)

Rotational crops
- Beets, carrots, radish
- Lettuce, cabbage
- Peas

- Soya beans
- Barley, wheat

For all investigated crops, soley the soil applications before planting match the conditions of the intended uses. All other experiments investigating the metabolism of glyphosate in non-tolerant plants were performed without GAP relation to achieve an optimal identification of the residue under laboratory conditions.

The metabolism of glyphosate was similar in all crops investigated, however partly depending on the application technique.

B.7.3.1.1.1 Soil treatment (non-tolerant crops)

In soil treatment experiments conducted in all crop groups very minor amounts of the applied radioactivity were recovered in the crop samples, normally representing <1 % of the AR. Untreated control plants kept in the same greenhouse environment indicate an uptake of $^{14}CO_2$ released during the quick degradation of glyphosate in the soil. Most of the total radioactivity was found in underground parts, while the translocation into upper plant parts was low. TRR found in treated plants were at the levels as in untreated control plants.
In soya beans, which was the only study involving identification of the residue, exceptionally high total radioactive residue were found (0.487 mg eq/kg to 1.76 mg eq/kg). However, identified levels of glyphosate and AMPA each were 0.1 mg eq/kg or less in all matrices. Most of the radioactivity was characterised as natural components (mainly proteins, carbohydrates, lignin, cellulose), probably following incorperation of $^{14}CO_2$.

Table B.7.3-1: Overview of glyphosate residues and its metabolites following soil application

Crop (Refrence)	amount applied	DALT	TRR recovered	Glyphosate mg eq/kg (% TRR)	AMPA mg eq/kg (% TRR)	other mg eq/kg (% TRR)	Comments
Fruit crops							
Mandarin (Sutherland, 1974, RIP9501194)	2.24 kg as eq/ha	1 to 4 months	0.08-0.41 % AR (no data on absolute concentrations provided)	n.a.	n.a.	n.a.	63.82-64.67 % AR were recovered from soil. TRR too low for identification
Tree nuts, (Nadeau et al, 1976, RIP9501196)	12.9 µg per pot (cal. 5.1 kg as eq/ha)	16 weeks	0.08-0.36 % AR (no data on absolute concentrations provided)	n.a.	n.a.	n.a.	Control plants kept in the same environment contained 0.05-0.29 % AR due to CO_2 uptake. TRR too low for identification
Apples, Rueppel et al, 1974, RIP9501190)	1.7 kg as eq/ha	6 and 12 weeks	Leaves: 0.0016-0.059 % AR Branches & trunk: 0.0018-0.011 % AR	n.a.	n.a.	n.a.	Control plants kept in the same environment contained 0.0095-0.02 % AR in leaves and 0.0043-0.009 % AR in branches and trunk due to CO_2 uptake. TRR too low for identification
	3.4 kg as eq/ha	6 and 12 weeks	Leaves: 0.0013-0.093 % AR Branches & trunk: 0.002-0.041 % AR	n.a.	n.a.	n.a.	
Grapes, (Parker et al, 1991, RIP9500012)	2 x 8.0 kg as eq/ha	2 weeks and 1 year	Grapes: 0.007 mg eq/kg	n.a.	n.a.	n.a.	TRR too low for identification
Grapes, (Wilkinson et al, 1990, RIP9500017)	Glyphosate-label: 8 kg as eq/ha	7 days	Grapes: <0.006 mg eq/kg Stems: <0.023 mg eq/kg Leaves: <0.023 mg eq/kg Stalks: <0.023 mg eq/kg	n.a.	n.a.	n.a.	TRR too low for identification
	TMS-label: 7 kg as eq/ha	7 days	Grapes: <0.003 mg eq/kg Stems: <0.009 mg eq/kg Leaves: 0.031 mg eq/kg (dry-matter based) Stalks: 0.01 mg eq/kg (dry-matter based)	n.a.	n.a.	n.a.	

Crop (Refrence)	**amount applied**	**DALT**	**TRR recovered**	**Glyphosate mg eq/kg (% TRR)**	**AMPA mg eq/kg (% TRR)**	**other mg eq/kg (% TRR)**	**Comments**
Grapes, (Rueppel et al, 1974, RIP9501191)	3.4 kg as eq/ha	42 or 84 days	Vines: 0.067-0.083 % AR Dead leaves: 0.087 % AR (no data on absolute concentrations provided)	n.a.	n.a.	n.a.	TRR too low for identification
Root and tuber crops							
Sugar beets, Malik et al, 1976, RIP9501195)	4.5 kg as eq/ha	4, 6 or 8 weeks	Roots: 0.023-0.037 % AR Leaves: 0.033-0.040 % AR (no data on absolute concentrations provided)	n.a.	n.a.	n.a.	Control plants kept in the same environment contained 0.003-0.005 % AR in roots and 0.003-0.004 % AR in leaves due to CO_2 uptake.
Pulses and oilseeds							
Soya beans, (Tambling, 1992, RIP9500015)	8.4 kg as eq/ha to seedlings	Forage: 31 days other: 97 days	Forage: 1.76 mg eq/kg Straw: 0.859 mg eq/kg Hulls: 0.487 mg eq/kg Seeds (yellow): 1.31 mg eq/kg Seeds (green): 0.772 mg eq/kg Hay: 0.854 mg eq/kg	0.058 (3.3%) 0.005 (0.6%) 0.02 (4.1%) 0.034 (2.6%) 0.02 (2.6%) 0.02 (2.3%)	0.1 (5.7%) 0.023 (2.7%) 0.007 (1.4%) 0.021 (1.6%) 0.012 (1.6%) 0.02 (2.3%)	1.685 (95.7%) 0.79 (92%) 0.428 (88%) 1.254 (95.7%) 0.739 (95.7%) 0.89 (94.4%)	other metabolites: extractable natural products and radioactivity associated to unextracted carbohydrates, proteins, lignin and cellulose. Bound residues after hydrolysis: 0.299 mg eq/kg (22.8% TRR) for yellow seeds, 0.176 mg eq/kg (22.8% TRR) for green seeds and 0.13 mg eq/kg (11.9% TRR) for hay. In other matrices no radioactivity remained uncharacterised.
Soya beans, (Rueppel, 1973, RIP9600099)	4.5 kg as eq/ha	4, 6 or 8 weeks	Plant: 0.0755 – 0.293 mg eq/kg	n.a.	n.a.	n.a.	Control plants kept in the same environment contained 0.0294-0.109 mg eq/kg due to CO_2 uptake.
Cotton, (Rueppel, 1973, RIP9600099)	4.5 kg as eq/ha	4, 6 or 8 weeks	Plant: 0.21-0.42 mg eq/kg	n.a.	n.a.	n.a.	Control plants kept in the same environment contained 0.02-0.27 mg eq/kg due to CO_2 uptake.

Crop (Refrence)	amount applied	DALT	TRR recovered	Glyphosate mg eq/kg (% TRR)	AMPA mg eq/kg (% TRR)	other mg eq/kg (% TRR)	Comments
Cereals							
Barley, (Suba et al, 1974, RIP9501189)	4.5 kg as eq/ha	4, 6 or 8 weeks	Plant: 0.059-0.093 mg eq/kg	n.a.	n.a.	n.a.	Control plants kept in the same environment contained 0.024-0.084 mg eq/kg due to CO_2 uptake.
Maize, (Rueppel, 1973, RIP9600099)	4.5 kg as eq/ha	4, 6 or 8 weeks	Plant: 0.0785 to 0.21 mg eq/kg	n.a.	n.a.	n.a.	Control plants kept in the same environment contained 0.0218-0.089 mg eq/kg due to CO_2 uptake.
Oats, (Suba et al, 1974, RIP9501189)	4.5 kg as eq/ha	4, 6 or 8 weeks	Plant: 0.029-0.05 mg eq/kg	n.a.	n.a.	n.a.	Control plants kept in the same environment contained 0.017-0.05 mg eq/kg due to CO_2 uptake.
Rice, (Suba et al, 1974, RIP9501189)	4.5 kg as eq/ha	4, 6 or 8 weeks	Plants: 0.077-0.157 mg eq/kg	n.a.	n.a.	n.a.	Control plants kept in the same environment contained 0.038-0.23 mg eq/kg due to CO_2 uptake.
Sorghum, (Suba et al, 1974, RIP9501189)	4.5 kg as eq/ha	4, 6 or 8 weeks	Plant: 0.073-0.085 mg eq/kg	n.a.	n.a.	n.a.	Control plants kept in the same environment contained 0.024-0.048 mg eq/kg due to CO_2 uptake.
Wheat, (Rueppel, 1973, RIP9600099)	4.5 kg as eq/ha	4, 6 or 8 weeks	Plant: 0.18-0.35 mg eq/kg	n.a.	n.a.	n.a.	Control plants kept in the same environment contained 0.008-0.061 mg eq/kg due to CO_2 uptake.
Miscellaneous crops							
Coffee, (Malik, 1975, RIP9501192)	4.5 kg as eq/ha	4, 6 or 8 weeks	Plant: 0.0033-0.0169 % AR (no data on absolute concentrations provided)	n.a.	n.a.	n.a.	Control plants kept in the same environment contained 0.0063-0.0115 % AR due to CO_2 uptake.
Pasture, (Sutherland et al, 1976, RIP9501197)	4.5 kg as eq/ha	6, 12, 18, 24 or 32 weeks	Plant: <0.01-0.08 % AR (no data on absolute concentrations provided)	n.a.	n.a.	n.a.	

n.a. not analysed

B.7.3.1.1.2 Hydroponic treatment (non-tolerant crops)

Hydroponic studies conducted on all crop groups except root and tuber vegetables were the key studies to identify the metabolism of glyphosate in plants. Generally the uptake of radioactivity into plants was very low. Normally less than 5 % of the AR were recovered in aerial parts while root compartiments may contain up to 20 % of the AR after longer treatment intervals.
Without soil present as substrate, in most cases no significant degradation of glyphosate was observed in the hydroponic solution. In single studies a partial degradation of glyphosate into AMPA in the hydroponic solution was oberved.
The identification of the radioactivity in samples after hydroponic treatment revealed in most samples unchanged glyphosate as major residue (50-100 % of the TRR). Occasionally AMPA was found, mainly at levels of 4 to 10 % of the TRR. Since a small degradation of glyphosate into AMPA was also observed in the hydroponic solutions, it is unclear wether AMPA levels in plant matrices were formed by plant metabolism or taken up via the roots.
In addition to glyphosate and AMPA, N-methyl-AMPA was present in several root samples, but the relative amounts were between 0.3 to 5.4 % of the TRR.

Table B.7.3-2: Overview of glyphosate residues and its metabolites following hydroponic treatment

Crop (Refrence)	amount applied	DALT	TRR recovered	Glyphosate mg eq/kg (% TRR)	AMPA mg eq/kg (% TRR)	N-methyl-AMPA mg eq/kg (% TRR)	Comments
Fruit crops							
Mandarin, (Sutherland, 1974, RIP9501194)	10 ppm	1 or 2 weeks	Leaves: 1.2-1.3 % AR Stem: 0.3-0.4 % AR Roots: 2.6-4.2 % AR (no data on absolute concentrations provided)	n.a.	n.a.	n.a.	
Grapes, (Rueppel et al, 1973, RIP9501191)	5, 10, 20 or 40 ppm	10, 21 or 42 days	Root: 4.73-18.66 % AR Trunk: 0.02-0.19 % AR Stem: 0.03-0.37 % AR Leaf: 0.07-0.3 % AR Hydroponic solution: 41.4-87.17 % AR Rinse: 1.3-4.5 % AR	n.a.	n.a.	n.a.	Relative radioactivity recovered independent on concentration in hydroponic solution.
Pulses and oilseeds							
Soya beans, (Rueppel, 1973, RIP9600099)	2.5 mg/L (methane-label)	28 days	Aerial parts: 4.19 % AR Roots: 10.8 % AR	63.1 % TRR 35.4 % TRR	9.2 % TRR 3.0 % TRR	- 0.3 % TRR	
	2.4 mg/L (methane-label)	6 days	Aerial parts: 0.93 % AR Roots: 3.68 % AR	n.a. 29.3 % TRR	n.a. 2.3 % TRR	- 1.0 % TRR	
		12 days	Aerial parts: 1.12 % AR Roots: 6.71 % AR	65.4 % TRR 54.1 % TRR	5.4 % TRR 5.6 % TRR	- -	
		20 days	Aerial parts: 1.42 % AR Roots: 9.34 % AR	78.5 % TRR 50.2 % TRR	3.8 % TRR 3.5 % TRR	- -	
		28 days	Aerial parts: 1.71 % AR Roots: 8.64 % AR	70.3 % TRR 44.3 % TRR	5.1 % TRR 1.4 % TRR	- -	

Crop (Refrence)	**amount applied**	**DALT**	**TRR recovered**	**Glyphosate mg eq/kg (% TRR)**	**AMPA mg eq/kg (% TRR)**	**N-methyl-AMPA mg eq/kg (% TRR)**	**Comments**
	2.4 mg/L (glycine-label)	6, 12, 20 or 28 days	Aerial parts: 1.1-1.79 % AR Roots: 2.97-13.88 % AR	96.7-100 % TRR 92.8-100 % TRR	- -	- -	
	2.4 mg/L (glycine-label)	6, 12, 20 or 25 days	Aerial parts: 0.64-2.09 % AR Roots: 4.25-10.3 % AR	66.8-85.5 % TRR 21.5-53.7 % TRR	- -	- 2.3 % TRR (day 6 only)	
	2.65 mg/L (mixture ^{14}C and ^{13}C methane-label)	6 days 12 days 20 days 26 days	Aerial parts: 0.41 % AR Roots: 1.71 % AR Aerial parts: 0.72 % AR Roots: 3.49 % AR Aerial parts: 4.96 % AR Roots: 3.47 % AR Aerial parts: 7.7 % AR Roots: 5.48 % AR	60.6 % TRR n.a. 54.4 % TRR n.a. 56.2 % TRR n.a. 55.1 % TRR n.a.	16.6 % TRR n.a. 7.3 % TRR n.a. 5.2 % TRR n.a. 7.8 % TRR n.a.	- n.a. - n.a. - n.a. - n.a.	
	2.4 mg/L (pulse, first 6 days only)	6 days 12 days 20 days 28 days	Aerial parts: 0.29 % AR Roots: 2.41 % AR Aerial parts: 0.29 % AR Roots: 2.68 % AR Aerial parts: 0.39 % AR Roots: 1.98 % AR Aerial parts: 0.3 % AR Roots: 0.8 % AR	55.3 % TRR n.a. 46.5 % TRR n.a. 39.3 % TRR n.a. 29.4 % TRR n.a.	8.8 % TRR n.a. 4.7 % TRR n.a. 5.5 % TRR n.a. 5.8 % TRR n.a.		AMPA and N-methyl-AMPA measured together.
Cotton, (Rueppel, 1973,	2.4 mg/L	6 days	Aerial parts: 0.4 % AR Roots: 1.32 % AR	73.8 % TRR 38.8 % TRR	- 8.9 % TRR	- -	Extractability in roots 17.3-38.8 %.

Crop (Refrence)	amount applied	DALT	TRR recovered	Glyphosate mg eq/kg (% TRR)	AMPA mg eq/kg (% TRR)	N-methyl-AMPA mg eq/kg (% TRR)	Comments
RIP9600099)		13 days	Aerial parts: 1.28 % AR Roots: 3.43 % AR	80.0 % TRR 21.3 % TRR	4.6 % TRR 4.2 % TRR	- 0.8 % TRR	
		20 days	Aerial parts: 2.98 % AR Roots: 7.98 % AR	70.8 % TRR 24.5 % TRR	5.3 % TRR 4.4 % TRR	- 0.2 % TRR	
		28 days	Aerial parts: 2.15 % AR Roots: 19.34 % AR	70.5 % TRR 11.1 % TRR	8.0 % TRR 3.0 % TRR	- 0.4 % TRR	
Cereals							
Barley, (Suba eta al, 1974; RIP9501189)	0.183 mg/L	7 days	Tops: 0.27 mg eq/kg (0.51 % AR) Roots: 4.542 mg eq/kg (3.36 % AR)	n.a. n.a.	n.a. n.a.	n.a. n.a.	
		14 days	Tops: 1.179 mg eq/kg (1.62 % AR) Roots: 7.982 mg eq/kg (6.31 % AR)	n.a. n.a.	n.a. n.a.	n.a. n.a.	
		28 days	Tops: 0.571 mg eq/kg (2.87 % AR) Roots: 6.128 mg eq/kg (23.05 % AR)	73.3 % TRR 52.6 % TRR	14.0 % TRR 3.8 % TRR	3.5 % TRR 0.4 % TRR	
Maize, (Rueppel, 1973, RIP9600099)	0.6 mg/L	6 days	Aerial parts: 0.96 % AR Roots: 3.39 % AR	70.6 % TRR 61.1 % TRR	- 7.4 % TRR	- -	
		12 days	Aerial parts: 3.8 % AR Roots: 8.43 % AR	19.7 % TRR 56.6 % TRR	16.2 % TRR 10.1 % TRR	4.2 % TRR -	
		20 days	Aerial parts: 7.01 % AR Roots: 9.74 % AR	23.7 % TRR 45.5 % TRR	22.2 % TRR 8.4 % TRR	1.9 % TRR 0.6 % TRR	
		28 days	Aerial parts: 4.73 % AR Roots: 9.74 % AR	28.1 % TRR 40.8 % TRR	27.0 % TRR 8.0 % TRR	2.0 % TRR -	
Oats, (Suba et al, 1974, RIP9501189)	0.183 mg/L	7 days	Tops: 0.25 mg eq/kg (0.59 % AR) Roots: 1.797 mg eq/kg (2.1 % AR)	n.a. n.a.	n.a. n.a.	n.a. n.a.	
		14 days	Tops: 1.0 mg eq/kg (3.21 % AR) Roots: 2.98 mg eq/kg (4.96 % AR)	n.a. n.a.	n.a. n.a.	n.a. n.a.	

Crop (Refrence)	amount applied	DALT	TRR recovered	Glyphosate mg eq/kg (% TRR)	AMPA mg eq/kg (% TRR)	N-methyl-AMPA mg eq/kg (% TRR)	Comments
		28 days	Tops: 0.707 mg eq/kg (3.49 % AR) Roots: 6.482 mg eq/kg (13.76 % AR)	76.6 % TRR 35.7 % TRR	6.5 % TRR 2.5 % TRR	1.7 % TRR 1.1 % TRR	
Rice, (Suba et al, 1974, RIP9501189)	0.183 mg/L	7 days	Tops: 0.534 mg eq/kg (0.77 % AR) Roots: 2.505 mg eq/kg (2.88 % AR)	n.a. n.a.	n.a. n.a.	n.a. n.a.	
		14 days	Tops: 1.545 mg eq/kg (1.98 % AR) Roots: 3.304 mg eq/kg (5.59 % AR)	n.a. n.a.	n.a. n.a.	n.a. n.a.	
		20 days	Tops: 8.787 mg eq/kg (5.58 % AR) Roots: 10.749 mg eq/kg (12.36% AR)	n.a. n.a.	n.a. n.a.	n.a. n.a.	
		28 days	Tops: 2.816 mg eq/kg (4.68 % AR) Roots: 3.684 mg eq/kg (6.53 % AR)	73.7 % TRR 19.1 % TRR	8.6 % TRR 7.4 % TRR	1.4 % TRR 1.6 % TRR	
Sorghum, (Suba et al, 1974, RIP9501189)	0.183 mg/L	7 days	Tops: 0.037 mg eq/kg (0.08 % AR) Roots: 1.1 mg eq/kg (1.94 % AR)	n.a. n.a.	n.a. n.a.	n.a. n.a.	
		14 days	Tops: 0.073 mg eq/kg (0.48 % AR) Roots: 1.597 mg eq/kg (5.64 % AR)	n.a. n.a.	n.a. n.a.	n.a. n.a.	
		28 days	Tops: 0.216 mg eq/kg (2.7 % AR) Roots: 1.711 mg eq/kg (13.4 % AR)	76.2 % TRR 44.8 % TRR	12.7 % TRR 2.2 % TRR	5.4 % TRR 0.5 % TRR	
Wheat, (Rueppel, 1973, RIP9600099)	0.6 mg/L	6 days	Aerial parts: 1.28% AR Roots: 1.39 % AR	69.3 % TRR 33.5 % TRR	8.0 % TRR 8.8 % TRR	- -	
		13 days	Aerial parts: 2.46 % AR Roots: 2.52 % AR	70.7 % TRR 38.5 % TRR	6.6 % TRR 5.2 % TRR	- 2.0 % TRR	

Crop (Refrence)	**amount applied**	**DALT**	**TRR recovered**	**Glyphosate mg eq/kg (% TRR)**	**AMPA mg eq/kg (% TRR)**	**N-methyl-AMPA mg eq/kg (% TRR)**	**Comments**
Miscellaneous crops							
Coffee, (Malik, 1975, RIP9501192)	1.1 ppm	21 days	Aerial parts: 0.1 % TRR Roots: 11.7 % TRR Root wash: 38.7 % TRR Hydroponic solution: 23.5 % TRR	74 % TRR 82 % TRR n.a. 37 % TRR	12 % TRR 8.1 % TRR n.a. 58 % TRR	- - n.a. 5 % TRR	Control plants kept in the same environment contained 0.0063-0.0115 % AR due to CO_2 uptake.
	3.6 ppm		Aerial parts: 0.1 % TRR Roots: 4.3 % TRR Root wash: 15.2 % TRR Hydroponic solution: 83.8 % TRR	60 % TRR 79.2 % TRR n.a. 92 % TRR	14.1 % TRR 7.8 % TRR n.a. 8 % TRR	- - n.a. -	
	11.1 ppm		Aerial parts: 0.2 % TRR Roots: 5.0 % TRR Root wash: 10.5 % TRR Hydroponic solution: 77.5 % TRR	38.4 % TRR 31.9 % TRR n.a. 87 % TRR	9.6 % TRR 6.1 % TRR n.a. 13 % TRR	- - n.a. -	

n.a. not analysed
- not detected

B.7.3.1.1.3 Stem or trunk direct treatment (non-tolerant crops)

Application of glyphosate to the stem or trunk of fruit trees as spot treatment showed, that the applied radioactivity stayed local without significant translocation. In the fruit trees investigated less than 1 % of the AR was present in adjected leaves of fruits. The radioactivity was not further investigated for their composition.

B.7.3.1.1.4 Foliar treatment (non-tolerant crops)

For foliar treatment most of the studies submitted involved direct treatment of selected leaves under laboratory conditions. The composition of the residue was investigated for the fruit group, cereal group and for miscellaneous crops..
In these experiments the translocation as well as the composition of the radioactivity were investigated. It was demonstrated that glyphosate was not significantly translocated within the plants. Normally >80 % of the AR were recovered from treated parts except for potatoes, where up to 12.4 % of the AR were found in the tubers.
The composition of the residue revealed unchanged glyphosate as the major residue in all samples, normally posing >75 % of the TRR. AMPA was also frequently detected, but at very low levels up to 9.2 % of the TRR.
For the desiccation of wheat by foliar spraying, which was conducted under conditions equivalent to the field with an application rate of 6 kg as/ha, the previous results were confirmed. Parent glyphosate was the major residue, amounting 90.8 % of the TRR in grain (2.43 mg eq/kg), 85 % of the TRR in chaff (278.4 mg eq/kg) and 82.6 % of the TRR in straw (102.6 mg eq/kg). The only other metabolite identified was AMPA at levels of 2.8-3.9 % of the TRR (0.075 mg eq/kg to 12.8 mg eq/kg).

Table B.7.3-3: Overview of glyphosate residues and its metabolites following foliar treatment

Crop (Refrence)	amount applied	DALT	TRR recovered	Glyphosate mg eq/kg (% TRR)	AMPA mg eq/kg (% TRR)	other mg eq/kg (% TRR)	Comments
Fruit crops							
Mandarin, (Sutherland, 1974, RIP9501194)	4 mg per leaf	1 to 8 weeks	Treated leaves: 76.6 % AR → 10.3 % AR Non-treated leaves: 1.8 % AR → 2.6 % AR Stems: 1.3 % AR → 2.2 % AR Fruit: <0.01 % AR → 9.8 % AR	n.a.	n.a.	n.a.	Accountability: 79.7 % AR → 13.5 % AR
Tree nuts, (Nadeau et al, 1976, RIP9501196)	100 µg per leaf	14 days	Walnut, treated leaves: 45-60.5 % AR Walnuts, untreated tops: 6.2-7.3 % AR Walnut, roots: 10.4-23.5 % AR	92 % TRR 96 % TRR 97 % TRR	3 % TRR 2 % TRR 2 % TRR	5 % TRR 1 % TRR 1 % TRR	other metabolites not identified
		35 days	Walnut, treated leaves: 47.7-64.5 % AR Walnuts, untreated tops: 5.8-8.2 % AR Walnut, roots: 14.8-27.4 % AR	71.1 % TRR 70.3 % TRR 87.0 % TRR	8 % TRR 4.6 % TRR 4 % TRR	9 % TRR - -	
		35 days	Almond, treated leaves: 61.1-71.1 % AR Almond, untreated tops: 12.6-14.5 % AR Almond, roots: 6.6-8.2 % AR	77.0 % TRR 52.7 % TRR 79.3 % TRR	8 % TRR 9.5 % TRR 5.9 % TRR	10.1 % TRR - -	
		35 days	Pecan, treated leaves: 53.1-54 % AR Pecan, untreated tops: 4.2-10 % AR Pecan, roots: 3.3-16.6 % AR	86.2 % TRR 88.2 % TRR 95.0 % TRR	6.0 % TRR 2.0 % TRR -	6.3 % TRR - -	

Crop (Refrence)	**amount applied**	**DALT**	**TRR recovered**	**Glyphosate mg eq/kg (% TRR)**	**AMPA mg eq/kg (% TRR)**	**other mg eq/kg (% TRR)**	**Comments**
Apples, (Rueppel et al, 1974, RIP9501190)	5.356 µg per shoot (4-5 leaves)	7 days	Treated leaves: 64.86 % AR New growth above treatment: 3.24 % AR Other new growth: 0.33 % AR	88.7 % TRR 85.9 % TRR n.a.	0.69 % TRR 0.47 % TRR n.a.		AMPA and N-methyl-AMPA analysed together.
		21 days	Treated leaves: 75.06 % AR New growth above treatment: 2.32 % AR Other new growth: 3.87 % AR	81.3 % TRR 101 % TRR 87.3 % TRR	0.64 % TRR 0.67 % TRR 0.69 % TRR		
		28 days	Treated leaves: 45.06 % AR New growth above treatment: 3.56 % AR Other new growth: 1.68 % AR Roots: 3.17 % AR Trunk & branches: 1.68 % AR	96.1 % TRR 94.9 % TRR 66.8 66.4 % TRR 64.4 % TRR	4.0 % TRR 0.21 % TRR 1.0 % TRR - -		
		49 days	Treated leaves: 60.03 % AR New growth above treatment: 4.13 % AR Other new growth: 2.84 % AR	90.6 % TRR 87.4 % TRR 92.2 % TRR	5.6 % TRR 2.5 % TRR 2.9 % TRR		
		70 days	Treated leaves: 60.51 % AR New growth above treatment: 5.03 % AR Other new growth: 1.47 % AR	83.6 % TRR 87.1 % TRR 95.1 % TRR	6.5 % TRR 5.0 % TRR 2.7 % TRR		
Grapes, Parker et al, 1991, RIP9500012)	14.3 mg/vine	14 days	Grapes: 1.25 mg eq/kg	0.964 (77)	0.031 (2.5)		

Crop (Refrence)	**amount applied**	**DALT**	**TRR recovered**	**Glyphosate mg eq/kg (% TRR)**	**AMPA mg eq/kg (% TRR)**	**other mg eq/kg (% TRR)**	**Comments**
Grapes, Rueppel et al, 1973, RIP9501191)	20 µg/leaf (120 µg per plant)	7 days	Treated leaves: 72.2 % AR New growth: 8.7 % AR	87.8 % TRR 74.3 % TRR	3.9 % TRR -	- 3.3 % TRR	Concord variety "Other" residues not identified. *: different set of plants
		14 days	Treated leaves: 60.9 % AR New growth: 6.8 % AR	70.5 % TRR 78.1 % TRR	1.5 % TRR -	- 4.2 % TRR	
		28 days	Treated leaves: 54.9 % AR New growth: 4.9 % AR Roots and old stock: 18.8 % AR New growth*: 9.4 % AR Grapes*: 0.004 % AR	78.8 % TRR 101 % TRR 87.6 % TRR 74.6 % TRR 64.6 % TRR	4.4 % TRR - - - -	- 1.0 % TRR - 4.3 % TRR -	
	20 µg/leaf (120 µg per plant)	7 days	Treated leaves: 57.8 % AR New growth: 0.8 % AR	87.7 % TRR 103 % TRR	2.8 % TRR -	- 7.9 % TRR	Sauvignon blanc variety "Other" residues not identified.
		14 days	Treated leaves: 62.2 % AR New growth: 3.1 % AR	88.2 % TRR 80.1 % TRR	2.9 % TRR 1.0 % TRR	- 7.8 % TRR	
		28 days	Treated leaves: 46.7 % AR New growth: 1.6 % AR Roots and old stock: 16.2 % AR Grapes: 0.7 % AR	84.3 % TRR 84.3 % TRR 90.2 % TRR 79.5 % TRR	9.2 % TRR 2.0 % TRR - 1.0 % TRR	- 7.1 % TRR - 6.9 % TRR	
	20 µg/leaf (60 µg per plant)	7 days	Treated leaves: 65.4 % AR New growth: 1.1 % AR	97.1 % TRR 88.5 % TRR	- 1.2 % TRR	- 1.7 % TRR	Thompson Seedless variety "Other" residues not identified.
		14 days	Treated leaves: 57.7 % AR New growth: 5.0 % AR	89.6 % TRR 89.3 % TRR	- <2.0 % TRR	- 6.3 % TRR	
		28 days	Treated leaves: 43.3 % AR New growth: 0.7 % AR Roots and old stock: 11.8 % AR	70.6 % TRR 74.4 % TRR 87.7 % TRR	1.8 % TRR <1.0 % TRR -	- 9.7 % TRR -	

Crop (Refrence)	**amount applied**	**DALT**	**TRR recovered**	**Glyphosate mg eq/kg (% TRR)**	**AMPA mg eq/kg (% TRR)**	**other mg eq/kg (% TRR)**	**Comments**
	20 µg/leaf (120 µg per plant), pulse	7 days	Treated leaves: 71.5 % AR New growth:4.9 % AR	85.6 % TRR 98.5 % TRR	3.4 % TRR 1.0 % TRR	- 5.7 % TRR	Concord variety "Other" residues not identified.
		14 days	Treated leaves: 70.1 % AR New growth: 11.5 % AR	93.8 % TRR 82.9 % TRR	2.5 % TRR 1.0 % TRR	- 4.1 % TRR	
		28 days	New growth: 3.9 % AR	77.1 % TRR	1.0 % TRR	5.3 % TRR	
		42 days	New growth: 3.6 % AR	82.8 % TRR	1.0 % TRR	1.2 % TRR	
		56 days	New growth: 4.1 % AR	70.4 % TRR	1.0 % TRR	5.0 % TRR	
		70 days	New growth: 8.1 % AR	58.5 % TRR	1.0 % TRR	4.8 % TRR	
Root and tuber crops							
Potato, (Nadeau, 1974, RIP9501193)	100 µg/plant	1 day	Treated leaves: 80.8 % AR Tubers: 4.6 % AR	see comment	see comment	see comment	According to the study report, soley glyphosate was recovered from all samples following foliar treatment. However, the analysis was performed by electrophoresis, not capable of identifying metabolites being present at levels <5% of the TRR. This study is considered as additional information only.
		3 days	Treated leaves: 81.5 % AR Tubers: 6.3 % AR				
		14 days	Treated leaves: 56.8 % AR Tubers: 12.4 % AR				
		34 days	Treated leaves: 72.3 % AR Tubers: 10.2 % AR				
Cereals							
Wheat, (Stuart et al, 1989, RIP9500014)	6 kg as eq/ha (glyphosate-label)	7 days	Chaff: 327.5 mg eq/kg Straw: 124.2 mg eq/kg Grain: 2.68 mg eq/kg	278.4 (85) 102.6 (82.6) 2.43 (90.8)	12.8 (3.9) 4.1 (3.3) 0.075 (2.8)	-	

Crop (Refrence)	**amount applied**	**DALT**	**TRR recovered**	**Glyphosate mg eq/kg (% TRR)**	**AMPA mg eq/kg (% TRR)**	**other mg eq/kg (% TRR)**	**Comments**
Miscellaneous crops							
Coffee, (Malik, 1975, RIP9501192)	3.9 x 10^7 dpm	5 weeks	Treated leaves: 43.6 % AR Roots: 26.5 % AR Stems: 11.5 % AR Untreated leaves: 2.8 % AR	99 % TRR 99 % TRR 99 % TRR 99 % TRR	<1% TRR <1% TRR <1% TRR <1% TRR	-	Absolute application rate not stated. Only samples with identified residues presented.
	7.12 x 10^8 dpm	4 weeks 8 weeks 23 weeks	Immature beans: 0.02 % AR Immature beans: 0.017 % AR Ripe beans: 0.66 % AR Ripe pods: 0.68 % AR	99 % TRR 99 % TRR 95 % TRR 95 % TRR	<1% TRR <1% TRR 5 % TRR 5 % TRR	-	Absolute application rate not stated. Only samples with identified residues presented.
Pasture, (Sutherland et al, 1976, RIP9501197)	1.1 kg as eq/ha	7 days	Fescue: 69 % AR Alfalfa: 55 % AR	n.a.	n.a.	n.a.	no identification of the residue performed

B.7.3.1.1.5 Rotational crops (non-tolerant)

The metabolism of glyphosate in rotational crops was investigated in several confined studies involving application rates to bare soil equivalent to 3.87 - 6.5 kg as/ha.
The investigation of soil samples in these studies demonstrated the quick degradation of the parent substance, leaving AMPA as major degradation product with relative levels up to 10 times higher than the glyphosate remaining.
Samples obtained from rotational crops often contained substancial total radioactive residues, equivalent to glyphosate concentrations of up to 4.4 mg eq/kg. However, as demonstrated in soil treatment metabolism studies, most of the radioactivity remained unextracted due to incorporation of $^{14}CO_2$ from the degradation of glyphosate in soil.
In extracts, glyphosate levels depended on the interval between treatment and sampling. After short intervals of up to 14 weeks glyphosate levels found were generally higher than AMPA (glyphosate: 19.6 – 62 % of the TRR, AMPA: 2.3 - 15.6 % of the TRR). In samples collected after longer intervals, unchanged glyphosate was only present in minor amounts of 10 % of the TRR or less (absolute levels <0.001 mg eq/kg to 0.026 mg eq/kg), while AMPA was the dominant residue with up to 20 % of the TRR (up to 0.05 mg eq/kg). Further metabolites were not identified.

Table B.7.3-4: Overview of glyphosate residues and its metabolites in rotational crops (confined)

Reference	Application rate	PBI	Sample	TRR in mg eq/kg	Glyphosate in mg eq/kg (% TRR)	AMPA in mg eq/kg (% TRR)	Others in mg eq/kg (% TRR)	Comments
Nicholls, 1990, RIP9501201 & McMUllan et al, 1990, RIP9501202	4.16 kg as/ha (bare soil)	30 days	Lettuce, 70 DAT	0.108	0.0041 (3.8)	0.0158 (14.6)	0.0309 (28.6)	Others characterised as glucose/fructose
			Lettuce, 90 DAT	0.048	ND	0.0039 (8.1)	0.0162 (33.8)	
			Lettuce, 105 DAT	0.097	0.0028 (2.9)	0.0137 (28.5)	0.0327 (33.7)	
			Barley, straw 125 DAT	0.175	0.0018 (1.0)	0.0065 (3.7)	0.0294 (16.8)	
			Barley, grain 125 DAT	0.188	0.0184 (9.8)	0.0336 (17.9)	0.0144 (7.7)	
			Carrot, tops 154 DAT	0.051	ND	0.0007 (1.4)	0.0136 (26.7)	
			Carrot, root 154 DAT	0.037	ND	0.0041 (11.1)	0.0151 (40.8)	
		120 days	Lettuce, 147 DAT	0.059	ND	0.0027 (4.6)	0.0147 (24.9)	
			Lettuce, 167 DAT	0.055	0.0009 (1.6)	0.0050 (9.1)	0.0083 (15.1)	
			Lettuce, 181 DAT	0.037	ND	0.0046 (12.4)	0.0087 (23.5)	
			Barley, straw 314 DAT	0.056	ND	0.0054 (9.6)	0.0046 (8.2)	
			Barley, grain 314 DAT	0.078	ND	0.0111 (14.2)	0.0049 (6.3)	
			Carrot, tops 210 DAT	0.028	ND	0.0003 (1.1)	0.0049 (17.5)	
			Carrot, root 210 DAT	0.017	ND	0.0014 (8.2)	0.0039 (22.9)	
		365 days	Lettuce, 399 DAT	0.057	ND	0.0076 (13.3)	0.0182 (31.9)	
			Lettuce, 425 DAT	0.043	ND	0.0045 (10.5)	0.0080 (18.6)	
			Lettuce, 455 DAT	0.028	ND	0.0056 (20.0)	0.0079 (28.2)	
			Barley, forage 412 DAT	0.056	ND	0.0093 (16.6)	0.0082 (14.6)	
			Barley, straw 482 DAT	0.061	ND	0.0047 (7.7)	0.0068 (11.1)	
			Barley, grain 482 DAT	0.047	ND	0.0074 (15.7)	0.0031 (6.6)	
			Carrot, tops 482 DAT	0.018	NA	NA	NA	
			Carrot, root 482 DAT	0.0096	NA	NA	NA	
Hatterman, 1998, RIP2003-1112	6.5 kg as/ha (bare soil)	30 days	Radish, leaf 75 DAT	4.8	<0.05	<0.05	-	
			Radish, root 75 DAT	0.24	<0.05	<0.05	-	
			Lettuce, leaf 75 DAT	0.34	<0.05	<0.05	-	
			Wheat, forage 120 DAT	1.3	<0.05	0.2 (15)	-	
			Wheat, chaff 120 DAT	1.6	<0.05	0.4 (25)	-	
			Wheat, grain 120 DAT	2.0	<0.05	0.3 (15)	-	

Reference	Application rate	PBI	Sample	TRR in mg eq/kg	Glyphosate in mg eq/kg (% TRR)	AMPA in mg eq/kg (% TRR)	Others in mg eq/kg (% TRR)	Comments
		120 days	Radish, leaf 165 DAT	0.17	<0.05	<0.05	-	
			Radish, root 165 DAT	0.15	<0.05	<0.05	-	
			Lettuce, leaf 165 DAT	0.25	<0.05	<0.05	-	
			Wheat, forage 210 DAT	1.4	0.4 (28.6)	0.1 (7)	-	
			Wheat, chaff 210 DAT	1.0	0.3 (33.3)	0.2 (20)	-	
			Wheat, grain 210 DAT	0.7	<0.05	0.2 (28.6)	-	
		365 days	Radish, leaf 410 DAT	0.02	<0.05	<0.05	-	
			Radish, root 410 DAT	0.05	<0.05	<0.05	-	
			Lettuce, leaf 410 DAT	0.02	<0.05	<0.05	-	
			Wheat, forage 455 DAT	0.08	<0.05	<0.05	-	
			Wheat, chaff 455 DAT	0.19	0.06 (31.6)	<0.05	-	
			Wheat, grain 455 DAT	0.16	<0.05	<0.05	-	
Suba, 1976, RIP9501199	4.48 kg as/ha (bare soil)	4 weeks	Peas (primary)	0.22	0.137 (62)	0.013 (5.9)	0.01 (4.7)	Soil treatment before planting of primary crop. After harvest of primary crop, rotational crops grown in same soil. Other not further identified.
		14 weeks	Carrot, roots	0.094	0.018 (19.6)	0.004 (4.1)	0.004 (3.8)	
		14 weeks	Carrot, leaves	0.086	0.018 (21.5)	0.002 (2.3)	0.004 (4.6)	
		14 weeks	Cabbage	0.056	0.026 (46.4)	0.003 (4.5)	0.005 (8.4)	
		6.5 weeks	String beans (primary)	0.13	0.07 (54.2)	0.008 (6.0)	0.009 (7.2)	
		16.5 weeks	Maize, forage	0.04	0.005 (12.0)	0.004 (11.1)	0.005 (12.0)	
		22 weeks	Maize, forage	0.09	0.005 (5.6)	0.008 (8.9)	0.007 (7.6)	
		22 weeks	Maize, kernel	0.06	NA	NA	NA	
		22 weeks	Maize, cob	0.05	0.002 (3.3)	0.005 (9.0)	0.005 (10.8)	
		7 weeks	Carrot roots	0.11	0.038 (34.5)	0.006 (5.0)	0.006 (5.0)	
		7 weeks	Carrot leaves	0.17	0.051 (30.2)	0.005 (3.2)	0.006 (3.6)	
		13.5 weeks	String bean leaves	0.08	0.019 (23.8)	0.003 (3.5)	0.004 (5.0)	
		13.5 weeks	String bean pod	0.04	0.016 (40.1)	0.003 (7.9)	0.004 (8.9)	
		24.5 weeks	Cabbage	0.045	0.004 (8.3)	0.003 (6.7)	0.004 (9.3)	
		11 weeks	Cabbage, leaves	0.08	0.026 (32.1)	0.006 (7.0)	0.003 (4.2)	
		11 weeks	Cabbage, head	NQ	(18.5)	(3.3)	(2.0)	
		17.5 weeks	Pea leaves	0.19	0.026 (13.6)	0.021 (11.1)	0.01 (5.5)	
		17.5 weeks	Pea pods	0.28	0.025 (9.0)	0.044 (15.6)	0.013 (4.8)	
		28.5 weeks	Carrot roots	0.05	0.022 (43.2)	0.004 (7.6)	0.004 (8.5)	
		28.5 weeks	Carrot leaves	0.08	0.014 (17.9)	0.002 (2.9)	0.007 (8.6)	

Reference	Application rate	PBI	Sample	TRR in mg eq/kg	Glyphosate in mg eq/kg (% TRR)	AMPA in mg eq/kg (% TRR)	Others in mg eq/kg (% TRR)	Comments
Brightwell et al, 1978, RIP9501200	4.48 kg as/ha (bare soil) plus additional 4.48 kg as/ha after 30 days for emergency	Primary	Cabbage	0.10	0.005 (4.8)	0.008 (8.0)	-	Primary: 2 day PBI Emergency: after 30 days the first rotation was destroyed and additional 4.48 kg as/ha were sprayed crop replanted
		Emergency	Cabbage	0.11	0.003 (3.1)	0.007 (6.7)	-	
		4 month	Cabbage	0.07	0.005 (7.6)	0.008 (11.7)	-	
		1 year	Cabbage	0.03	0.002 (6.7)	0.003 (9.5)	-	
		Primary	Wheat, forage	3.39	0.116 (4.9)	0.362 (10.7)	-	
		Emergency	Wheat, forage	1.08	0.128 (11.9)	0.046 (4.3)	-	
		4 month	Wheat, forage	0.86	0.003 (3.6)	0.014 (16.5)	-	
		1 year	Wheat, forage	0.14	<0.001 (4.0)	<0.001 (4.8)	-	
		Primary	Beet, foliage	0.36	0.021 (5.9)	0.008 (2.3)	-	
		Emergency	Beet, foliage	0.21	0.005 (2.7)	0.0035 (1.6)	-	
		4 month	Beet, foliage	0.04	0.001 (3.0)	<0.001 (0.9)	-	
		1 year	Beet, foliage	0.04	0.002 (4.2)	<0.001 (2.2)	-	
		Primary	Beet, root	0.24	0.030 (12.4)	0.022 (9.0)	-	
		Emergency	Beet, root	0.33	0.039 (12.2)	0.015 (4.7)	-	
		4 month	Beet, root	0.08	0.009 (10.9)	0.002 (2.3)	-	
		1 year	Beet, root	0.04	0.004 (10.4)	0.002 (3.8)	-	
Spillner et al, 1993, RIP9500018	3.87 kg as/ha (bare soil)	35 days	Lettuce	0.072	0.001 (0.7)	0.015 (20.4)	0.003 (4.4)	Others: sum of glyphosate and AMPA released in hydrolysis
			Radish, roots	0.02	0.0004 (1.8)	0.002 (8.7)	-	
			Radish, tops	0.02	0.0002 (0.9)	0.002 (12.3)	-	
			Wheat, forage	0.024	0.0001 (0.5)	0.005 (20.5)	-	
			Wheat, straw	0.05	0.0002 (0.4)	0.006 (11.7)	-	
			Wheat, grain	0.076	<0.001	0.026 (34)	0.003 (3.7)	
	4.38 and 0.74 kg as/ha with one month interval	63 days	Lettuce	0.127	0.001 (0.9)	0.024 (18.5)	0.006 (4.9)	Others: sum of glyphosate and AMPA released in hydrolysis
			Radish, roots	0.022	0.0004 (1.7)	0.002 (11.0)	-	
			Radish, tops	0.021	0.002 (1.1)	0.002 (9.5)	-	
			Wheat, forage	0.033	<0.001	0.007 (20.5)	-	
			Wheat, straw	0.063	0.0002 (0.3)	0.008 (12.7)	-	
			Wheat, grain	0.092	0.002 (2.3)	0.024 (25.8)	0.003 (2.3	
	4.38, 1.4 and 0.74 kg as/ha with monthly interval	308 days	Lettuce	0.017	NP	NP	0.003 (19.6)	Others: sum of glyphosate and AMPA in aqueous extract
			Radish, roots	0.01	NP	NP	0.001 (7.4)	
			Radish, tops	0.016	NP	NP	0.002 (10)	
			Wheat, forage	0.017	NP	NP	NP	
			Wheat, straw	0.031	NP	NP	0.003 (8.5)	
			Wheat, grain	0.038	NP	NP	NP	

PBI Plant-back interval; NA not analysed; ND not detected; NQ not quantified

B.7.3.1.1.6 Information from supervised field trials (non-tolerant)

In supervised field trials on non-tolerant crops glyphosate was the only residue relevant. AMPA was normally not found at levels above the LOQ of the analytical methods used (0.01-0.2 mg/kg), independent on the point of application (pre-emergence or pre-harvest).

B.7.3.1.2 Glyphosate tolerant crops

For glyphosate tolerant crops multiple plant metabolism studies were submitted for the different genetic modifications:

Oilseeds
- Rape/canola (CP4-EPSPS & GOX, GAT)
- Soya beans (CP4-EPSPS, GAT)
- Cotton (CP4-EPSPS)

Root and tubers
- Sugarbeet (CP4-EPSPS)

Cereal grains
- Maize (CP4-EPSPS & GOX, GAT)

In comparison to non-tolerant crops the major difference in the use pattern for glyphosate is the option to apply the active substance during the growing season without causing severe damage to the target crop.
Currently cultivation of glyphosate tolerant crops is not authorised within the EU. However, future authorisations of tolerant crops in the EU or an import of commodities obtained from glyphosate tolerant crops are probable. To provide harmonised residue definitions for all plant commodities present on the European market, also metabolism information of tolerant crops also need to be considered for the current assessment.

B.7.3.1.2.1 CP4-EPSPS modified crops

In CP4-EPSPS modified crops the glyphosate tolerance is based on a modified 5-enolpyruvylshikimate-3-phosphate synthase, which is much less susceptible to glyphosate than the enzyme natural occuring in plants. Metabolism studies involving treatment of tolerant crops soley modified with CP4-EPSPS are available for soya bean, cotton and sugar beets.

For soya beans the metabolic pattern in forage and hay was comparable to non-tolerant crops. Parent glyphosate was the major residue, amounting 53.6-89.1 % of the TRR (5.582 – 21.078 mg eq/kg). AMPA was present at much lower amounts of 6.8 to 12.8 % of the TRR.
In soya beans seeds, which were not developed at the point of the last application, AMPA was the major residue with 49.1 % of the TRR (8.579 mg eq/kg). Glyphosate was present with 25.2 % of the TRR (4.402 mg eq/kg). Further metabolites identified were N-methyl-AMPA, N-glyceryl-AMPA, N-acetyl-AMPA and N-malonyl-AMPA, none of them exceeding 3 % of the TRR (or 0.3 mg/kg).

In tolerant cotton forage glyphosate was the major residue with 91.5 % of the TRR (13.9 mg eq/kg). AMPA was also detected, but at very low levels of 1.6 % of the TRR (0.243 mg eq/kg).
In the seeds again unchanged glyphosate gave the highest residue with 12 % of the TRR (0.022 mg eq/kg), while AMPA was not detectable (<1% of the TRR, <0.002 mg eq/kg). However, a high percentage of the TRR in seeds remained unextracted (54 – 75 %).

Finally, in sugarbeet mature tops and roots glyphosate was the only relevant residue present at 76.7 % of the TRR (2.47 mg eq/kg) and 95.3 % of the TRR (1.33 mg eq/kg), respectively. The only other metabolite idenfied was AMPA, with 1.8 % of the TRR in sugar beet tops (0.06 mg eq/kg) and 3.8 % of the TRR in the roots (0.05 mg eq/kg).

B.7.3.1.2.2 GOX ("glyphosate oxidoreductase") modified crops

For the GOX modification, which is often used in combination with CP4-EPSPS, an alternative protein obtained from baceteria – the glyphosate oxidoreductase – is expressed in the plants, causing an accelerated degradation of glyphosate into AMPA.

In tolerant oilseed rape TRR levels found in the seeds were between 0.483–0.845 mg eq/kg for after single treatment and 4.876-8.093 mg eq/kg after double treatment. As expected from the results for non-tolerant crops, the extraction rates were relatively low for the rape seeds, releasing 20.9-21.7% of the TRR by aqueous extraction. In the extracts, AMPA was the major residue with 7.1-7.7% of the TRR, followed by N-glyceryl-AMPA (3.4-3.9% of the TRR) and N-acetyl-AMPA (0.7-0.9% of the TRR).
No parent glyphosate was detected in the seeds extract. Hydrolysis of the post extraction solids released most of the radioactivity remaining, leaving 5.8% of the TRR unextracted. The residue was mainly characterised as proteins, carbohydrates, lignin and amino- or organic acids. In total, additional 9.5% of the TRR were released as AMPA.

In tolerant maize TRR levels found were 10.8 mg eq/kg in the forage, 9.59 mg eq/kg in the silage, 19.1 mg eq/kg in the fodder and 1.04 mg eq/kg in the grain.
Aqueous extraction released most of the radioactivity in forage, silage and fodder (2.8-4.5% of the TRR unextracted), however in grain 20.9-23.2 % of the radioactivity remained unextracted. The identification of the extracts showed parent glyphosate as the major residue in forage, silage and fodder with relative amounts between 67.1-83.3% of the TRR. AMPA was frequently detected at levels of 4.9-15.9% of the TRR.
In the grain, which was not developed at the point of the last treatment, AMPA was the major residue with 54.1-60.3% of the TRR (0.37-0.63 mg eq/kg). Parent glyphosate was also found, but at very low levels of 2.6-7.4% of the TRR (0.03-0.05 mg eq/kg). In addition N-glyceryl-AMPA and glyphosate conjugates were found, but at levels between 0.4-1.9% of the TRR in forage, silage and fodder and of 6.9% of the TRR in grain (N-glyceryl-AMPA only). In the grain a large part of the unextracted radioactivity (23.2-25.5% of the TRR) was characterised as natural products (starch and saponifiable fatty acids).

Table B.7.3-5: Overview of glyphosate residues and its metabolites in CP4-EPSPS and GOX modified crops

Crop (Refrence)	amount applied	DALT	TRR recovered	Glyphosate mg eq/kg (% TRR)	AMPA mg eq/kg (% TRR)	N-glyceryl-AMPA mg eq/kg (% TRR)	N-acetyl-AMPA mg eq/kg (% TRR)	other mg eq/kg (% TRR)	Comments
Sugar beets, (Mehrsheikh, 2000, RIP2001-906 & 1999, RIP2003-1134)	0.9 kg as/ha (pre-emergence)	160 days	Tops: 0.005 mg eq/kg Roots: 0.008 mg eq/kg	NP NP	NP NP	NP NP	NP NP	NP NP	Line 77 (CP4-EPSPS modified)
	2 x 1.08 kg as/ha (BBCH 14 & BBCH 19)	91 days	Tops: 3.437 mg eq/kg Roots: 1.396 mg eq/kg	2.47 (76.7) 1.33 (95.3)	0.06 (1.8) 0.05 (3.8)	ND ND	ND ND	ND ND	
Rape (canola), (Rogers et al, 1994, RIP9800118)	0.455 kg as/ha (BBCH 14)	87 days	Seeds (GT 73): 0.483 mg eq/kg Aqueous extract: 0.101 mg eq/kg (20.9 % TRR) Meal (hydrolysed): NP	ND NP	0.037 (7.7) NP	0.017 (3.4) NP	0.004 (0.9) NP	ND NP	Line GT73 (CP4-EPSPS & GOX) other radioactivity characterised as natural products
	2 x 0.9 kg as/ha (14d after planting & BBCH 16)	79 days	Seeds (GT 73): 8.093 mg eq/kg Aqueous extract: 1.76 mg eq/kg (21.7 % TRR) Meal (hydrolysed): 6.36 mg eq/kg (78.6 % TRR)	ND NP	0.58 (7.1) 0.77 (9.5)	0.31 (3.9) ND	0.06 (0.7) ND	ND NP	
Soya beans, (Goure, 1994, RIP9800117)	5.38 kg as/ha (bare soil, pre-emergence)	56 days 84 days 104 days	Forage: 0.239 mg eq/kg Hay: 0.205 mg eq/kg Seeds: 0.748 mg eq/kg	NP NP NP	NP NP NP	NP NP NP	NP NP NP	NP NP NP	CP4-EPSPS modified

Crop (Refrence)	**amount applied**	**DALT**	**TRR recovered**	**Glyphosate mg eq/kg (% TRR)**	**AMPA mg eq/kg (% TRR)**	**N-glyceryl-AMPA mg eq/kg (% TRR)**	**N-acetyl-AMPA mg eq/kg (% TRR)**	**other mg eq/kg (% TRR)**	**Comments**
	0.84 kg as/ha (BBCH 23)	35 days 63 days 83 days	Forage: 0.863 mg eq/kg Hay: 0.546 mg eq/kg Seeds: 0.406 mg eq/kg	NP NP NP	NP NP NP	NP NP NP	NP NP NP	NP NP NP	
	0.84 kg as/ha + 1.68 kg as/ha (BBCH 23 + BBCH 51)	13 days 41days 61 days	Forage: 23.651 mg eq/kg Hay: 10.416 mg eq/kg Seeds: 17.459 mg eq/kg	21.078 (89.1) 5.582 (53.6) 4.402 (25.2)	1.619 (6.8) 1.328 (12.8) 8.579 (49.1)	ND 0.084 (0.8) 0.278 (1.6)	ND ND 0.235 (1.4)	N-methyl-AMPA, N-malonyl-AMPA & AMPA conjugates : 0.6-1.8 % TRR each	
Cotton, (Bleeke, 1997, RIP9700619)	0.93 & 1.26 kg as/ha (BBCH 14 & BBCH 51)	27 days 158 days	Forage: 15.2 mg eq/kg Seeds: 0.181 mg eq/kg	13.9 (96.9) 0.022 (12)	0.243 (1.6) <0.002	ND ND	ND ND	ND ND	CP4-EPSPS modified
Maize, (George, 1995, RIP9700618)	0.93 & 0.84 kg as/ha (BBCH 16 & BBCH 19)	3 days 49-53 days 83 days	Forage: 10.8 mg eq/kg Silage: 9.59 mg eq/kg Fodder: 19.1 mg eq/kg Grain: 1.04 mg eq/kg	7.77 (71.9) 6.43 (67.1) 14.27 (74.8)~~0.03 (2.6)~~ 0.03 (2.6)~~14.27 (74.8)~~	1.72 (15.9) 1.26 (13.1) 2.13 (11.2)~~0.63 (60.3)~~ 0.63 (60.3)~~2.13 (11.2)~~	0.06 (0.5) 0.14 (1.5) 0.31 (1.6)~~0.07 (6.9)~~ 0.07 (6.9)~~0.31 (1.6)~~	ND ND ND ND	0.04 (0.4) 0.04 (0.4) 0.36 (1.9)~~ND~~ ND~~0.36 (1.9)~~	“RoundUp Ready” (CP4-EPSPS & GOX-modification)

NP not performed; ND not detected

B.7.3.1.2.3 GAT ("glyphosate N-acetyltransferase") modified crops

The GAT modification was implemented as an additional mode of glyphosate tolerance by expressing the enzyme "glyphosate N-acetyltransferase" (GAT). The new enzyme favours a metabolic pathway normally not observed in plants by acetylation of glyphosate and AMPA into N-acetyl-glyphosate and N-acetyl-AMPA, both not showing herbicidal activity.

For oilseed rape TRR levels found were 5.979 mg eq/kg for the early foliage (BBCH 69), 1.273 mg eq/kg for immature pods (BBCH 87), 1.55 mg eq/kg for late foliage (BBCH 87) and 2.155 mg eq/kg for the seeds.
In all matrices N-acetyl-glyphosate was the dominant residue being present between 51.1-93% of the TRR. Significant amounts of parent glyphosate were only detected in the seeds with at levels of 20.8% of the TRR (0.448 mg eq/kg) in addition to N-acetyl-AMPA at 14.7% of the TRR (0.316 mg eq/kg).
AMPA was soley detected in foliage and seeds with 1.4 and 1.9 % of the TRR, respectively.

In GAT modified soya bean forage following an initial soil treatment AMPA was the major residue, representing 39.3% of the TRR (0.166 mg eq/kg), followed by glyphosate (9.1% TRR, 0.039 mg eq/kg) and N-acetyl-glyphosate (1.9% TRR, 0.009 mg/kg).
In plant parts directly affected by foliar treatment (foliage, hay and pods), glyphosate always was the major residue being present at rates of 43.6-72.5 % of the TRR. At higher DATs the amount of N-acetyl-glyphosate increases, showing levels between 19.2-42 % of the TRR. AMPA was also found in most samples, however at levels of 11.2% of the TRR or less. N-acetyl-AMPA did not exceed 3.3% of the TRR.
In the seeds N-acetyl-glyphosate was the major residue, representing 56.9-60.6 % of the TRR (1.156-1.788 mg eq/kg). Glyphosate and AMPA were present at lower levels amounting 22.7 % and 5.3 % of the TRR in seeds sampled before the last application and 3.2 % and 11.2 % of the TRR in seeds samples 14 days after desiccation. In latter seeds N-acetyl-AMPA was also found as a major metabolite, representing 23.5% of the TRR (0.738 mg eq/kg).

For maize forage (DAT 58) and stover (DAT 7) unchanged glyphosate was the major residue, representing 58% (1.852 mg eq/kg) and 74.9 % of the TRR (8.174 mg eq/kg), respectively. However, especially for stover the short interval of 7 days between application and sampling has to be taken into account leaving not much time for metabolic degradation. N-acetyl-glyphosate was the only other major metabolite with levels of 27% TRR in forage (0.937 mg eq/kg) and 17.8% TRR in stover (2.188 mg eq/kg). AMPA and N-acetyl-AMPA were detected in both matrices, however at lower levels between 4.0-4.4% TRR and 1.3-1.7% TRR, respectively.
In the cobs relevant for sweet-corn neither glyphosate nor AMPA were detected. The major residue was N-acetyl-glyphosate with 63.8% of the TRR (0.435 mg eq/kg). In minor amounts N-acetyl-AMPA was identified with 5.0% of the TRR (0.034 mg eq/kg).
In the grain the metabolic pattern was similar to cobs, mainly showing N-acetyl-glyphosate at levels of 0.141 mg eq/kg (51.2% of the TRR). Parent glyphosate was detected but present below the LOQ of 0.001 mg eq/kg. AMPA and N-acetyl-AMPA did not exceed a levels of 10% of the TRR (6.1% of the TRR (0.016 mg eq/kg) and 9.4% of the TRR (0.026 mg eq/kg), respectively).

Table B.7.3-6: Overview of glyphosate residues and its metabolites in GAT modified crops

Crop (Refrence)	amount applied	DALT	TRR recovered	Glyphosate mg eq/kg (% TRR)	AMPA mg eq/kg (% TRR)	N-acetyl-glyphosate mg eq/kg (% TRR)	N-acetyl-AMPA mg eq/kg (% TRR)	other mg eq/kg (% TRR)	Comments
Rape, (Chapleo, 2010, ASB2011-13744)	4.5 kg as/ha (pre-emergence) +	38 days after 2nd foliar appl.	Foliage: 5.979 mg eq/kg	0.179 (3.0)	0.084 (1.4)	5.351 (89.5)	0.203 (3.4)	ND	
	3 x 1.9 kg as/ha (BBCH 12, 15 and 7 days before harvest)	before last appl.	Foliage: 1.55 mg eq/kg	ND	ND	1.013 (79.6)	ND	ND	
			Immature pods: 1.273 mg eq/kg	ND	ND	1.442 (93.0)	ND	ND	
		7 days after last appl.	Seeds: 2.155 mg eq/kg	0.448 (20.8)	0.041 (1.9)	1.101 (51.1)	0.316 (14.7)	ND	
Soya beans, (MacDonald, 2007, ASB2008-2658)	3.4 kg as/ha (pre-emergence) +	36 days after soil appl.	Forage: 0.428 mg eq/kg	0.039 (9.1)	0.166 (39.3)	0.009 (1.9)	ND	0.003 (0.6)	other: multiple components each below quantification limit
	1.5, 2.4 & 0.9 kg as/ha (BBCH 61, 65 and 14 days before harvest)	4 days after 1st foliar appl.	Hay: 13.444 mg eq/kg	9.74 (72.5)	0.704 (5.3)	2.581 (19.2)	0.096 (0.7)	0.079 (0.5)	
		before last appl.	Seeds: 1.905 mg eq/kg	0.434 (22.7)	0.103 (5.3)	1.156 (60.6)	ND	0.032 (1.7)	
			Foliage: 11.225 mg eq/kg	4.894 (43.6)	0.819 (7.4)	4.699 (42.0)	0.255 (2.2)	0.179 (1.6)	
		14 days	Seeds: 3.142 mg eq/kg	0.102 (3.2)	0.351 (11.2)	1.788 (56.9)	0.738 (23.5)	0.035 (1.1)	
			Pods: 17.751 mg eq/kg	10.101 (56.9)	1.794 (10.2)	4.906 (27.7)	0.574 (3.3)	0.165 (0.7)	
			Foliage: 22.087 mg eq/kg	11.791 (53.4)	2.25 (10.3)	7.039 (31.9)	0.308 (1.4)	0.243 (1.1)	
Maize, (Green, 2007, ASB2008-2657)	4.3 kg as/ha (pre-emergence) +	48 days after soil appl.	Forage: 3.476 mg eq/kg	2.016 (58)	0.14 (4.0)	0.937 (27)	0.06 (1.7)	0.057 (1.6)	other: multiple components each below quantification limit
	3 x 1.1 kg as/ha (2 x BBCH 31-39 and 7 days before harvest)	7 days	Stover: 12.242 mg eq/kg	9.166 (74.9)	0.422 (4.4)	2.188 (17.8)	0.152 (1.3)	0.092 (0.8)	
			Cobs: 0.686 mg eq/kg	ND	ND	0.435 (63.8)	0.034 (5.0)	0.074 (12.8)	
			Grain: 0.275 mg eq/kg	<0.001	0.016 (6.1)	0.141 (51.2)	0.026 (9.4)	0.041 (10.9)	

ND: not detected; NP: not performed

B.7.3.1.3 Conclusion for plant matrices

In non-tolerant crops glyphosate was the only significant residue following direct treatment via foliar, trunk, stem or hydroponic treatment.
In presence of soil as substrate and in rotational crops AMPA was found at relative rates comparable or even higher than parent glyphosate. However, the overall uptake of both glyphosate and AMPA via the roots was very low, rarely exceeding a level of 0.05 mg/kg following pre-emergence application or in rotational crops after exaggerated treatment.
In supervised field trials involving desiccation, glyphosate was the only residue detected in significant amounts. AMPA was rarely present and only at levels at least one order of magnitude lower than the parent substance. After pre-emergence or pre-sowing application, normally no residues of glyphosate or AMPA above the LOQ were found.
For monitoring purposes glyphosate is a suitable marker compound for setting and enforcing MRLs in non-tolerant plant commodities.
~~Parent glyphosate is on low acute toxicity, making the allocation of an ARfD unnecessary. The toxicity of AMPA is not higher than of glyphosate, also without showing acute toxic effects.~~ Based on the toxicological profile, the toxicity of AMPA is not higher than of glyphosate, both being covered by the derived ADI and ARfD values. However, since AMPA may be present at comparable levels to glyphosate in root and tuber compartiments, it should also be taken into acocunt for the dietary intake assessment. In summary it can be concluded that for non-tolerant crops glyphosate and AMPA are the significant residue for dietary intake.

In glyphosate tolerant crops the metabolism may different significantly compared to unmodified crops. Depending on the kind of modification AMPA (GOX), N-acetyl-glyphosate (GAT) and N-acetyl-AMPA (GAT) may become major metabolites in plant commodities, often being present at equal or higher levels than the unchanged parent substance.
While CP4-EPSPS does not affect the metabolic pattern, the ratio between glyphosate and AMPA in GOX modified plants was approximately 1:1.
For GAT modified plants glyphosate was the major residue in the foliage (forage, hay, stover). However mature soya bean seeds contained little glyphosate remaining (3 % TRR), while in maize cobs and grain no glyphosate above the LOQ was found. In oilseed rape approx. 20 % of the TRR were identified as glyphosate. In supervised field trials the average ratios between glyphosate and its metabolites (AMPA; N-acetyl-glyphosate and N-acetyl-AMPA) were ~~between 1:1.05 (lentils)~~ up to 1:17.7 (soya beans).
For monitoring purposes, glyphosate is a suitable marker in CP4-EPSPS-modified plants, since no change in the metabolic pathway is induced. For GOX-modified plants, appoximately 50% of the resiude is still presnet as glyphosate, also representing a sufficient amount for analysis. However, in GAT modified plants, parent glyphosate was a minor metabolite, sometimes not exceeding the LOQ in food relevant commodities like maize cobs or grain. Since glyphosate tolerant commodities are also imported into the EU, parent glyphosate is a poor marker for monitoring purposes in GAT-modified crops. Therefore it is proposed to also included N-acetyl-glyphosate, which is a major residue in all GAT-modified crops, into the residue definition for enforcement purposes. However, since only GAT-modified crops contain N-acetyl-glyphosate, this addition is limited to ~~lentils,~~ soya beans, sweet corn and maize
Both N-acetyl-glyphosate and N-acetyl-AMPA are found to be of no higher toxicity than parent glyphosate ~~without the necessity to establish an ARfD~~. Since both metabolites are

significant metabolites in GAT-modified crops, the residue definition for dietary intake purposes should be defined as the sum of glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA, all expressed as glyphosate equivalents. All metabolites are covered by the ADI and ARfD values derived for parent glyphosate.

Under processing conditions and storage glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA were stable.

In 2009, following EFSA Reasoned Option concerning import tolerances for glyphosate tolerant commodities (EFSA Journal 2009; 7(9):1310, ASB2012-3480), it was concluded that the import of GAT-modified commodities does not require a change of the established "glyphosate only" residue definition for monitoring the current MRLs are based on. In the meantime new CXLs were established on Codex level by WHO/FAO, taking N-acetyl-glyphosate into account for the residue definition for monitoring purposes in soya beans and maize (which were the only crops evaluated with a GAT modification at that time).
Analytical methods measuring glyphosate, AMPA, N-acetyl-glyphosate and N-acetly-AMPA by LC-MS/MS-methods were reported in EFSAs Reasoned Option as well as in the 2011 JMPR Report proposing the CXL change. However, the data owner of these methods is no member of the Task Force providing information for the inclusion of glyphosate into Annex I of Reg. (EC) 1107/2009.
Nevertheless, for a better harmonisation of MRLs on global scale and to avoid a underestimation of the residue for GAT-modified commodities, it is proposed to include N-acetyl-glyphosate into the residue definition for monitoring purposes for all commodities, which may be obtained from GAT-modified crops. In contrast to the previous evaluation of GAT-modified crops on European level, the expanding field of uses demand consideration of N-acetyl-glyphosate also for monitoring purposes of glyphosate.

Residue definition for enforcement purposes in sweet corn, oilseeds rape, soya beans and maize (non-tolerant and tolerant, all modifications):
sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate

Residue definition for enforcement purposes in other plant commodities:
glyphosate

Residue definition for dietary intake purposes in plant commodities:
sum of glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA, all expressed as glyphosate equivalents

For a better understanding the historical development of the residue definitions in plant commodities is summaried in the following table:

Table B.7.3-7: Historical overview of the residue defintion for monitoring of plant commodities in the EU and on Codex level

Year	**European Union**		**Codex Alimentarius**		**Comment**
Residue definition:	Monitoring	Risk assessment	Monitoring	Risk assessment	
Up to 2009	Glyphosate	Sum of glyphosate and AMPA, expressed as glyphosate	Glyphosate	Sum of glyphosate and AMPA, expressed as glyphosate	Residue definition for non-tolerant and CP4-EPSPS- & GOX-modified crops
2009	no change	**All plant commodities**: sum of glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA, all expressed as glyphosate equivalents	no change	no change	EFSA RO on GAT-crops (2009: 7(9):1310). Import tolerance on soya beans and maize.
2011 to 2011	no change	no change	**Maize and soya beans**: Sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate **Other plant commodities**: glyphosate	**All plant commodities**: sum of glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA, all expressed as glyphosate equivalents	JMPR assessment of GAT-crops & CXL change.
Current evaluation (proposal)	**Sweet corn, oilseeds rape, soya beans and maize**: Sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate **Other plant commodities**: glyphosate	no change	no change	no change	Proposal to adopt Codex residue definition for the EU. On EU level pending import tolerances for GAT modified ~~lentils and~~ oilseed rape into the EU.

For the future generation of residue data N-acetly-glyphosate and N-acetyl-AMPA are only mandatory analytes in GAT-modified crops.

In non-tolerant crops glyphosate is the only significant residue, making conversion factors unnecessary. In glyphosate tolerant crops the ratio of glyphosate and its metabolites strongly depends on the type of modification (CP4-EPSPS, GOX or GAT) and the point of application (pre-emergence, post-emergence and/or desiccation). In addition no GAP information are available to identify the critical scenario for the specific crops to derive conversion factors.
In summary the range of concentrations for glyphosate and its metabolites in non-tolerant and modified crops is to variable to derive reliable conversion factors from monitoring to dietary intake assessment.

B.7.3.2 Residue definition for animal commodities

In livestock animals the metabolism of glyphosate (sodium- or trimesium-salt), AMPA and N-acetyl-glyphosate were investigated in lactating goats and laying hens.

B.7.3.2.1 Administration of glyphosate (incl. trimesium) and AMPA

For glyphosate and AMPA the active substances were either applied as a single compound or as a 9:1 mixture (glyphosate : AMPA). The degradation of both analytes was minimal in all animals investigated.

Unchanged glyphosate and AMPA were the only analytes identified. When applied simultaneously the relative levels in tissues, milk and eggs corresponded to the relative amounts administered. Only in hens gizzard and liver (goats and hens) a slight shift of the ratio towards AMPA was observed.

Following feeding levels up to 200 mg/kg feed, the transfer of radioactivity into muscle, milk and fat was very low, often not exceeding TRR levels of 0.05 mg eq/kg.
Substantial radioactive residues were found in kidney, which is explained by urine being the major route of excretion. The composition of the residue in kidney was mainly identified as glyphosate or as a mixture with AMPA, if administered simultaneously.
In liver, residues were also higher compared to muscle and fat (approx. one order of magnitude higher). Glyphosate was the major residue followed by AMPA, which was found at relative amounts up to 33.5 % of the TRR.
In egg yolk the TRR did not reach a plateau within the interval investigated in the metabolism studies submitted. The identification of the residues at sacrifice (days 7-8) revealed mainly unchanged glyphosate as dominant residue (64.3-81.4 % TR). Poultry feeding studies for 28 days indicated that a plateau for glyphosate and AMPA in eggs is reached after 14 days.

B.7.3.2.2 Administration of N-acetyl-glyphosate

For N-acetyl-glyphosate, which is the major plant metabolite in GAT modified crops, the behaviour in lactating goats and laying hens was comparable to parent glyphosate.

Milk, muscle and fat contained low residues while kidney, followed by liver, gave higher concentrations in goats. The composition of the TRR was mainly unchanged N-acetyl-glyphosate, contributing 16.7-77% to the TRR. In addition glyphosate (3.6-14.7% TRR) and AMPA (0.5-8.5 % TRR) were present in significant amounts. N-acetyl-glyphosate was rarely detected except for fat, reaching a relative level of 6.6 % TRR in goats.

In laying hens muscle and fat showed TRR levels of 0.039 mg eq/kg and 0.051 mg eq/kg, respectively. Highest residues were found in hens liver (0.511 mg eq/kg) while the kidneys were not investigated. Total radioactive residues in eggs increased during the whole dosing period of 7 days without reaching a plateau. Again, a plateau was observed in laying hens feeding studies after approximately 14 days of administration.
The composition of the residue in hen tissues was generally comparable to goats. N-acetyl-glyphosate (23.5-68.4 % TRR) was the major residue besides glyphosate (5.7-39.4% TRR) and AMPA (0.9-16.7% TRR). In contrast to goats, N-acetyl-AMPA was a major metabolite in hens fat (10.2% of the TRR), but not in other tissues (up to 4.0 % TRR).

Table B.7.3-8: Overview of metabolism studies for glyphosate, AMPA and N-acetyl-glyphosate in livestock animal

Animal, Reference	Substance administered	Sample and TRR found	Glyphosate mg eq/kg (% TRR)	AMPA mg eq/kg (% TRR)	N-acetyl-glyphosate mg eq/kg (% TRR)	N-acetyl-AMPA mg eq/kg (% TRR)	other mg eq/kg (% TRR)	Comments
Lactating goat, (Powles, 1994, RIP9501207)	Glyphosate 7.1 & 8 mg/kg bw 5 days	Whole milk: 0.036-0.086 mg eq/kg Fat: <0.028-<0.036 mg eq/kg Kidney: 3.852-12.15 mg eq/kg Liver: 0.225-0.404 mg eq/kg Muscle: 0.035-0.061 mg eq/kg	NP NP 94.2 % TRR 96 % TRR NP	NP NP 6.5 % TRR ND NP	NP NP NP NP NP	NP NP NP NP NP	ND ND ND ND ND	Average of different detection systems and animals
Lactating goat, (Bodden, 1987, RIP9501203 & Patanella et al, 1988, RIP9501204)	Glyphosate/AMPA (9:1) 4.1 mg glyphosate and 0.45 mg AMPA per kg bw 5 days	Whole milk: 0.019-0.049 mg eq/kg Fat: 0.009-0.011 mg eq/kg Kidney: 3.49-10.5 mg eq/kg Liver: 0.457-0.529 mg eq/kg Muscle: 0.026-0.028 mg eq/kg	NP 81.6 % TRR 92.1 % TRR 78.9 % TRR 77.5 % TRR	NP 11.0 % TRR 6.3 % TRR 15.9 % TRR 7.8 % TRR	NP NP NP NP NP	NP NP NP NP NP	ND ND ND ND ND	Average of different detection systems and animals
Lactating goat, (Ericson, 1994, RIP9500022)	Glyphosate-trimesium 2.6 mg/kg bw 7 days	Whole milk: 0.0026-0.0225 mg eq/kg Fat: 0.0175 mg eq/kg Kidney: 5.58 mg eq/kg Liver: 0.234 mg eq/kg Muscle: 0.0256 mg eq/kg	0.0054 (22.3) 0.03 (91.3) 4.82 (86.3) 0.14 (59.4) 0.022 (87.1)	0.0004 (2.4) 0.001 (4.7) 0.42 (7.5) 0.05 (21.4) 0.0016 (6.3)	NP NP NP NP NP	NP NP NP NP NP	see comment NP NP NP NP	Milk: 0.005 (25.2 % TRR) Lactose; 0.005 (20.4 % TRR) triglycerides; 0.004 (21.1 % TRR) unextracted
Lactating goat, (Lowrie, 2007, ASB2008-2660)	N-acetyl-glyphosate 6.8 mg/kg bw 5 days	Whole milk: 0.013-0.036 mg eq/kg Fat: 0.065-0.108 mg eq/kg Kidney: 4.689 mg eq/kg Liver: 0.715 mg eq/kg Muscle: 0.047 mg eq/kg	0.001 (3.6) 0.007 (4.6) 0.242 (4.98) 0.118 (14.7) ND	0.001 (3.4) 0.003 (2.2) ND 0.068 (8.45) ND	0.011 (40) 0.069 (53.1) 3.742 (77.1) 0.446 (55.5) 0.014 (16.7)	ND 0.01 (6.6) ND ND ND	ND ND ND ND ND	Fat: mean of omental, renal and subcutaneous fat

Animal, Reference	**Substance administered**	**Sample and TRR found**	**Glyphosate mg eq/kg (% TRR)**	**AMPA mg eq/kg (% TRR)**	**N-acetyl-glyphosate mg eq/kg (% TRR)**	**N-acetyl-AMPA mg eq/kg (% TRR)**	**other mg eq/kg (% TRR)**	**Comments**
Laying hens, (Powles, 1994, RIP9501208)	Glyphosate 18.2 mg/kg bw 5 or 7 days	Egg white: <0.01-0.072 mg/kg Egg yolk: <0.011-0.484 mg eq/kg Fat: 0.083-0.218 mg eq/kg Muscle: 0.041-0.049 mg eq/kg Liver: 1.08-1.886 mg eq/kg Skin: 0.212-0.428 mg eq/kg	NP 64.3 % TRR 75.9 % TRR 80.3 % TRR 78.2 % TRR 89.1 % TRR	NP ND ND ND 20.3 % TRR 1.3 % TRR	NP NP NP NP NP	NP NP NP NP NP	ND ND ND ND ND	Average of different detection systems and animals, no plateau in egg yolk reached
Laying hens, (Bodden, 1988, RIP9501205 & Patanella et al, 1988, RIP9501206)	Glyphosate/AMPA (9:1) 9.7 mg glyphosate and 1.03 mg AMPA per kg bw 7 days	Egg white: <0.0013-0.017 mg/kg Egg yolk: <0.0013-0.229 mg eq/kg Fat: 0.015-0.02 mg eq/kg Muscle: 0.018-0.026 mg eq/kg Liver: 0.511-0.56 mg eq/kg Kidney: 1.75-1.81 mg eq/kg	NP 58.8 % TRR 73.1 % TRR 69.5 % TRR 73.1 % TRR 101.4 % TRR	NP 14.5 % TRR 12.5 % TRR 13.7 % TRR 30.3 % TRR 5.4 % TRR	NP NP NP NP NP NP	NP NP NP NP NP NP	NP ND ND ND ND ND	Average of different detection systems and animals, no plateau in egg yolk reached
	Glyphosate/AMPA (9:1) 49.9 mg glyphosate and 5.3 mg AMPA per kg bw 7 days	Egg white: <0.005-0.027 mg/kg Egg yolk: <0.005-0.753 mg eq/kg Fat: 0.063 mg eq/kg Muscle: 0.073 mg eq/kg Liver: 1.91 mg eq/kg Kidney: 7.0 mg eq/kg	NP 62.2 % TRR 79.2 % TRR 69.8 % TRR 69.4 % TRR 99.1 % TRR	NP 14.1 % TRR 11.8 % TRR 15.4 % TRR 35.5 % TRR 4.42 % TRR	NP NP NP NP NP NP	NP NP NP NP NP NP	NP ND ND ND ND ND	
Laying hens, (Bowler, 1994, RIP9500020)	Glyphosate-trimesium 4.1 mg/kg bw 10 days	Egg white: <0.001-0.0171 mg/kg Egg yolk: <0.001-0.238 mg eq/kg Fat: 0.02936 mg eq/kg Muscle: 0.0292-0.0401 mg eq/kg Liver: 0.4402 mg eq/kg	0.0043 (21.5) 0.1429 (59.5) 0.0119 (40.7) 0.018 (50) 0.268 (61)	0.0002 (0.8) 0.0055 (2.3) 0.001 (3.3) 0.0016 (4.5) 0.099 (22.5)	NP NP NP NP NP	NP NP NP NP NP	0.0001 (0.5) 0.0033 (1.4) 0.0004 (1.5) 0.001 (2.5) 0.0224 (5.1)	no plateau in egg yolk reached
Laying hens, (Lowrie, 2007, ASB2008-2659)	N-acetyl-glyphosate 4.5 mg/kg bw 7 days	Egg white: 0.001-0.015 mg/kg Egg yolk: <0.001-0.342 mg eq/kg Fat: 0.057 mg eq/kg Muscle: 0.033 mg eq/kg Liver: 0.5050 mg eq/kg	0.001 (10.9) 0.013 (5.69) 0.023 (39.43) 0.002 (7.19) 0.084 (16.34)	ND 0.002 (0.91) 0.007 (11.29) 0.005 (16.69) 0.034 (6.74)	0.004 (41.48) 0.157 (68.4) 0.014 (23.45) 0.009 (25.22) 0.323 (63.82)	<0.001 0.003 (1.1) 0.006 (10.2) 0.001 (1.9) 0.02 (4.0)	ND ND ND ND ND	no plateau in egg yolk reached

NP: not performed, ND: not detected

Conclusion

In livestock animals glyphosate, AMPA and N-acetyl-AMPA are degraded to a minor extent. Most of the residues was recovered as administered, suggesting that the metabolic pattern in animal tissues, milk and eggs will be identical to the residue found in the feed crops.

For non-tolerant crops, glyphosate is the major residue in potential feed items. Since most of the crops are not genetically modified, the unchanged parent will also be the only residue found in animal tissues after utilisation as feedstuff.

However, tolerant crops may contain high amounts of AMPA or N-acetyl-AMPA. AMPA was found at the same levels as glyphosate in GOX-tolerant crops, leaving parent glyphosate as a sufficient marker component in animal matrices. In contrast N-acetyl-glyphosate was the dominant residue in GAT-modified crops, leaving only minor amounts of glyphosate in potential feed items, especially for soya beans.

On Codex level the residue definition for monitoring purposes was concluded to be the sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate for animal commodities, taking into account the new residues situation after introduction of the GAT-modification. For the EU it was concluded (EFSA Reasoned Option concerning import tolerances for glyphosate tolerant commodities (EFSA Journal 2009; 7(9):1310, ASB2012-3480)) that the impact of imported GAT-modified feed crops are of low significance in the livestock animals dietary burden compared to non-tolerant feed commodities grown in the EU (mainly triggered by forage and fodder crops no traded internationally).

Analytical methods measuring glyphosate, AMPA, N-acetyl-glyphosate and N-acetly-AMPA by LC-MS/MS-methods were reported in EFSAs Reasoned Option from 2009 as well as in the 2011 JMPR Report proposing the CXL change. However, the data owner of these methods is no member of the Task Force providing information for the inclusion of glyphosate into Annex I of Reg. (EC) 1107/2009.

In spite of a better global harmonisation of EU MRLs with Codex CXLs it is suggest to also included N-acetyl-glpyhosate into the residue definition for enforcement purposes for animal commodities.

For dietary intake assessement glyphosate and N-acetyl-glyphosate may be the major residue in animal commodities, depending on the composition of the feed. In addition AMPA and N-acetly-AMPA may appear as major metabolites in animal commodities. AMPA, N-acetyl-glyphosate and N-acetyl-AMPA are found to be of no higher toxicity than parent glyphosate ~~without the necessity to establish an ARfD~~. It is proposed to included all three metabolites in to the residue definition for risk assessment of animal commodities.

Residue definition for enforcement purposes in animal commodities:
sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate

Residue definition for dietary intake purposes in animal commodities:
sum of glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA, all expressed as glyphosate equivalents

The composition of the residue in animal commodities strongly depends on the feed items, the amounts of genetically modified feed commodities in the livestock animals dietary burden and their residues. High amounts of e.g. soya products may result in a totally different residue pattern compared to an animals diet containing mainly forage, straw or cereal grain grown in the EU. Therefore the calculation of conversion factors would not cover the true residue situation and result in unreliable estimations for the dietary intake.

Glyphosate and its three major metabolites are mainly found in kidney and liver. In muscle and fat the residues were generally very low and at comparable levels. In summary no accumulation of the residue in fatty compartiments was observed, indicating that the residue is not fat soluble.

For the calculation of the dietary burden for the purpose of MRL setting, only glyphosate and N-acetyl-glyphosate need to considered, since the reformation of both analytes from AMPA or N-acetly-AMPA is unlikely. For dietary intake purposes glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA need to be taken into account, however N-acetyl-metabolites will only be relevant for GAT-modfied feed commodities.

Table B.7.3-9: Historical overview of the residue defintion for monitoring of animal commodities in the EU and on Codex level

Year	**European Union**		**Codex Alimentarius**		**Comment**
Residue definition:	Monitoring	Risk assessment	Monitoring	Risk assessment	
Up to 2009	Glyphosate	Sum of glyphosate and AMPA, expressed as glyphosate	Glyphosate	Sum of glyphosate and AMPA, expressed as glyphosate	
2009	no change	sum of glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA, all expressed as glyphosate equivalents	no change	no change	EFSA RO on GAT-crops (2009: 7(9):1310). ASB2012-3480 Import tolerance on soya beans and maize.
2011 to 2011	no change	no change	Sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate	sum of glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA, all expressed as glyphosate equivalents	JMPR assessment of GAT-crops & CXL change.
Current evaluation (proposal)	Sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate	sum of glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA, all expressed as glyphosate equivalents	no change	no change	Proposal to adopt Codex residue definition for the EU. On EU level pending import tolerances for GAT modified ~~lentils and~~ oilseed rape into the EU.

B.7.4 Use pattern

Representative uses for the Renewal of Approval (AIR 2) according to Commission Regulation (EC) N° 1141/2010

Table B.7.4-1: List of representative uses (glyphosate)

Crop and/ or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Formulation**		**Application**				**Application rate per treatment**			**PHI (days)** (l)	**Remarks:** (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	L/ha product min max	water L/ha min max	kg as/ha min max		
All crops** (all seeded or transplanted crops)	EU		F	Emerged annual, perennial and biennial weeds	SL	360g /L	Spray	Pre planting of crop	1-2	21d (see remark)	1-6	100-400	0.36-2.16		Spring & autumn after harvest (incl. stubble and/or seedbed prep.) For all crops: Maximum application rate 4.32 kg/ha glyphosate in any 12 month period across use categories, equivalent to the sum of pre-plant, pre-harvest and post harvest stubble applications The interval between applications is dependent on new
All crops** (all seeded crops)	EU		F	Emerged annual, perennial and biennial weeds	SL	360g /L	Spray	Post planting/ pre emergence of crop	1		1-3	100-400	0.36-1.08		

Crop and/ or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Formulation**		**Application**				**Application rate per treatment**			**PHI (days)** (l)	**Remarks:** (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	L/ha product min max	water L/ha min max	kg as/ha min max		
															weed emergence after the first treatment, relative to the time of planting the crop.
Cereals (pre-harvest) wheat, rye, triticale,	EU		F	Emerged annual, perennial and biennial weeds	SL	360g /L	Spray	Crop maturity < 30 % grain moisture	1		2-6	100-400	0.72-2.16	7	Maximum application rate 4.32 kg/ha glyphosate in any 12 month period across use categories, equivalent to the sum of pre-plant, pre-harvest and post harvest stubble applications. Pre-harvest uses in all crops include uses for weed control (higher doses) and harvest aid, sometimes
Cereals (pre-harvest) barley and oats	EU		F	Emerged annual, perennial and biennial weeds	SL	360g /L	Spray	Crop maturity < 30 % grain moisture	1		2-6	100-400	0.72-2.16	7	
Oilseeds (pre-harvest) rapeseed, mustard seed, linseed	EU		F	Emerged annual, perennial and biennial weeds	SL	360g /L	Spray	Crop maturity < 30 % grain moisture	1		2-6	100-400	0.72-2.16	14	

Crop and/ or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Formulation**		**Application**				**Application rate per treatment**			**PHI (days)** (l)	**Remarks:** (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	L/ha product min max	water L/ha min max	kg as/ha min max		
															referred to as desiccation (lower doses). The critical GAP is the high dose recommended used for weed control.
Orchard crops, vines, including citrus & tree nuts	EU		F	Emerged annual, perennial and biennial weeds	SL	360g /L	Spray	Post emergence of weeds	1-3	28d	2-8	100-400	0.72-2.88	N/A	Applications to avoid contact with tree branches. Maximum cumulative application rate 4.32 kg/ha glyphosate in any 12 month period <u>Note:</u> Because applications are made to the intra-rows (inner strips between the trees within a row), application rates

Crop and/ or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Formulation**		**Application**				**Application rate per treatment**			**PHI (days)** (l)	**Remarks:** (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	L/ha product min max	water L/ha min max	kg as/ha min max		
															per ha are expressed per 'unit of treated surface area' the actual application rate per ha orchard or vineyard will roughly only be 33%
Orchard crops, vines, including citrus & tree nuts	EU		F	Emerged annual, perennial and biennial weeds	SL	360g /L	(ULV) Sprayer or Knapsack use (spot treatment)	Post emergence of weeds	1-3	28d	2-8	0-400	0.72-2.88		Applications made round base of trunk [0.0 L/ha water addresses ULV application of the undiluted product] Maximum cumulative application rate 4.32 kg/ha glyphosate in any 12 month period Note: Because

Crop and/ or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Formulation**		**Application**				**Application rate per treatment**			**PHI (days)** (l)	**Remarks:** (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	L/ha product min max	water L/ha min max	kg as/ha min max		
															applications are made round base of trunk and to the intra-rows , (inner strips between two trees within a row), application rates per ha are expressed per 'unit of treated surface area' the actual application rate per ha orchard or vineyard will roughly only be 33%-50%

Crop and/ or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Formulation**		**Application**				**Application rate per treatment**			**PHI (days)** (l)	**Remarks:** (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	L/ha product min max	water L/ha min max	kg as/ha min max		

Remarks:

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*eg.* fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) eg. biting and suckling insects, soil born insects, foliar fungi, weeds
(d) eg. wettable powder (WP), watersoluble granule (WG)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, eg. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, eg. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(i) g/kg or g/l
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) The minimum and maximum number of application possible under practical conditions of use must be provided
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

B.7.5 Identification of critical GAPs

Based on the uses presented in Table B.7.4-1 the following critical GAPs were identifed:

Table B.7.5-1: Critical GAPs for glyphosate

Crop and/ or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Formulation**		**Application**				**Application rate per treatment**			**PHI (days)** (l)	**Remarks:** (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	L/ha product min max	water L/ha min max	kg as/ha min max		
All crops** (all seeded or transplanted crops)	EU		F	Emerged annual, perennial and biennial weeds	SL	360g /L	Spray	Pre planting of crop	1-2	21d (see remark)	1-6	100-400	0.36-2.16		For all crops: Maximum application rate 4.32 kg/ha glyphosate in any 12 month period.
Cereals (pre-harvest) wheat, rye, triticale,	EU		F	Emerged annual, perennial and biennial weeds	SL	360g /L	Spray	Crop maturity < 30 % grain moisture	1		2-6	100-400	0.72-2.16	7	Maximum application rate 4.32 kg/ha glyphosate in any 12 month period
Cereals (pre-harvest) barley and oats	EU		F	Emerged annual, perennial and biennial weeds	SL	360g /L	Spray	Crop maturity < 30 % grain moisture	1		2-6	100-400	0.72-2.16	7	
Oilseeds (pre-harvest) rapeseed,	EU		F	Emerged annual, perennial and	SL	360g /L	Spray	Crop maturity < 30 %	1		2-6	100-400	0.72-2.16	14	

Crop and/ or situation (a)	**Member State or Country**	**Product name**	**F G or I (b)**	**Pests or Group of pests controlled (c)**	**Formulation**		**Application**				**Application rate per treatment**			**PHI (days) (l)**	**Remarks: (m)**
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	L/ha product min max	water L/ha min max	kg as/ha min max		
mustard seed, linseed				biennial weeds				grain moisture							
Orchard crops, vines, including citrus & tree nuts	EU		F	Emerged annual, perennial and biennial weeds	SL	360g /L	(ULV) Sprayer or Knapsack use (spot treatment)	Post emergence of weeds	1-3	28d	2-8	0-400	0.72-2.88	N/A	Applications made round base of trunk. Maximum cumulative application rate 4.32 kg/ha glyphosate in any 12 month period <u>Note:</u> Because applications are made to the intra-rows , application rates per ha are expressed per 'unit of treated surface area'. The actual application rate per ha will roughly only be 33%-50%

Crop and/ or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Formulation**		**Application**				**Application rate per treatment**			**PHI (days)** (l)	**Remarks:** (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	L/ha product min max	water L/ha min max	kg as/ha min max		

Remarks:

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (eg. fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) eg. biting and suckling insects, soil born insects, foliar fungi, weeds
(d) eg. wettable powder (WP), watersoluble granule (WG)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, eg. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, eg. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated

(i) g/kg or g/l
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) The minimum and maximum number of application possible under practical conditions of use must be provided
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

B.7.6 Residues resulting from supervised trials (Annex IIA 6.3; Annex IIIA 8.3)

For a detailed description of all supervised field trials, including reference via the BVL registration number, please refer to the Appendix B7.18 at the end of the residue section.
Unless specifically reported in the remarks of the residue tables, all supervised field trials were conducted in line with the current EU guidance for supervised field trials (SANCO 7029/VI/95 rev.5, 22.07.1997). Analytical methods were validated with satisfactory recoveries (70-110%, <20% SD). Residues in control samples were generelly below the LOQ of the analytical methods, unless reported. In the supervised field trials tables, multiple analyses of identical samples were reported as arithmetic means, replicate samples or results from replicate plots as individual values.
All residues presented in the supervised field trial tables were not corrected for recoveries.

For dietary intake purposes the total residue was calculated according to the following equations:

Non-tolerant crop
In plant metabolism studies for glyphosate and AMPA it was clearly shown that parent glyphosate is the major residue in all plant matrices. Even after soil treatment or in rotational crops only very minor amounts of AMPA compared to the overall residues of parent glyphosate were found. The additional metabolites N-acetyl-glyphosate and N-acetyl-AMPA, which are only formed in GAT-modified crops, are no relevant for non-tolerant crops. Since AMPA is expected to be present at much lower levels than glyphosate, addition of LOQs in case of non-detectable residues would represent an overestimation of the true residue. Therefore, only in case of finite residues for one of the analytes the results are added according to the following procedure:

Table B.7.6-1: Example for the calculation of the total residue for dietary intake purposes for glyphosate non-tolerant plant commodities

Glyphosate in mg/kg	AMPA in mg/kg	Total residue in mg/kg	Comment
<0.05	<0.05	<0.05	no addition, since AMPA is expected at much lower levels than glyphosate
0.05	<0.05	0.13	0.05 mg/kg glyphosate + 0.05 mg/kg AMPA x 1.5 (equivalence factor AMPA to glyphosate)
<0.05	0.05	0.13	0.05 mg/kg glyphosate + 0.05 mg/kg AMPA x 1.5 (equivalence factor AMPA to glyphosate)
0.1	0.1	0.25	0.1 mg/kg glyphosate + 0.1 mg/kg AMPA x 1.5 (equivalence factor AMPA to glyphosate)

Equivalence factor: AMPA (111 g/mol) → Glyphosate (169 g/mol) = 1.5

Tolerant crops are not within the scoop of this document. A brief summary of the information presented in the first DAR for glyphosate are presented at the end of this chapter. Recent evaluations for import tolerances of genetically modified crops were also reported by EFSA (EFSA Journal 2009; 7(9):1310, ASB2012-3480).

B.7.6.1 Orchards and vineyards

For orchards and vineyards the critical GAP was identified to involve up to three gound directed sprayings against weeds. Since the target crop is not directly affected by the spraying, no PHI was considered necessary.
Orchard crops and vines are not genetically modified. Therefore only glyphosate and AMPA are considered for the evaluation of the residue.

The critical GAP does not specify growth stages for the treatment of the crop. To accommodate for a conservative situation, supervised field trial results were selected according to the following criteria:

- Target application rate 2.88 kg as/ha ± 25 % (2.16 – 3.6 kg as/ha) or higher, when the data suggests a nil-residue situation.
- Last application at macrostadium BBCH 7 or later to ensure fruits being present
- the number of applications are not considered relevant. If residue occur, the source will normally be unintended drift, which can be avoided by careful application of the active substance. The only exception will be grapes and olives. Grapes may contain low hanging fruits being more susceptible to drift contamination. For olives common agricultural practice involves ground picking of the fruits, making the total amount applied relevant for the residue levels found.
- Since no PHI was defined, all supervised field trials matching the criteria listed before are taken into account.
- The critical GAP is identical for whole Europe. Data will be selected for Northern and Southern Europe individually.
- The formulation and the active ingredient (sodium salt or isopropylamine salt) are not expected to influence the residue for glyphosate.

For the use of glyphosate in orchards and vineyards the following residues were identified:

Table B.7.6-2: Glyphosate and AMPA residues after application to orchards and vineyards

Commo-dity	Region (a)	Outdoor/ Indoor	Individual trial results (mg/kg)		STMR (mg/kg) (b)	HR (mg/kg) (c)	Median CF (d)
			Enforcement (glyphosate)	Risk assessment (total (e))			
Treenuts (hazelnut)	SEU	outdoor	<0.05(4)	<0.05(4)	0.05	0.05	1
Pome fruit (apples & pears)	NEU	outdoor	<0.02, <0.05(3)	<0.02, <0.05(3)	0.05	0.05	1
	SEU	outdoor	<0.05(17~~3~~)	<0.05(17~~3~~)	0.05	0.05	1
~~Pome fruit (pears)~~	~~SEU~~	~~outdoor~~	~~<0.05(4)~~	~~<0.05(4)~~	~~0.05~~	~~0.05~~	~~1~~
Stone fruit (cherries)	NEU	outdoor	<0.05, <0.05	<0.05, <0.05	0.05	0.05	1
Stone fruit (peaches)	SEU	outdoor	<0.05, <0.05	<0.05, <0.05	0.05	0.05	1
Grapes	NEU	outdoor	<0.05(6), 0.07(f), 0.3(f)	<0.05(6), 0.07(f), 0.3(f)	0.05	0.3	1
Olives	SEU	outdoor	tree-picked: <0.05(12) & <0.05(6)(g) ground-picked: 0.11, 0.14, 0.53, 0.93 ~~<0.05(4), 0.11, 0.11, 0.12, 0.13, 0.14, 0.53, 0.93, 0.93~~	tree-picked: <0.05(12) & <0.05(6)(g) ground-picked: 0.11, 0.14, 0.53, 0.93 ~~<0.05(4), 0.11, 0.11, 0.12, 0.13, 0.14, 0.53, 0.93, 0.93~~	0.335~~0.19~~ (table olives only)	0.93~~1.0~~ (table olives only)	not applicable, AMPA always <0.05 mg/kg, CF not reliable

Underline median value from trial results (based on DoR for risk assessment)

(a): NEU, SEU, EU or Import (country code). In the case of indoor uses there is no necessity to differentiate between NEU and SEU.
(b): Median value of the individual trial results according to the risk assessment residue definition.
(c): Highest value of the individual trial results according to the risk assessment residue definition.
(d): The median conversion factor for enforcement to risk assessment is obtained by calculating the median of the individual conversion factors for each residues trial.
(e): sum of glyphosate and AMPA, expressed as glyphosate (see Table B.7.6-1)
(f): low hanging fruits treated at lower rates of 2 x 0.72 kg as/ha
(g): additional data, analytical recovery <70%

The data presented for the ground directed application of glyphosate in orchards (citrus fruit, tree nuts, pome fruit and stone fruit) demonstrate that no residues of glyphosate or AMPA above the LOQ have to be expected. Since the crop is not directly affected, the data for all tree crops can be used for mutual support.

For grapes low hanging fruits may be affect by ground directed spaying of the weeds, resulting in glyphosate residues up to 0.3 mg/kg. Under practical conditions the probability of such a contamination strongly depends on the application technique (e.g. shielded spraying) and the size of the vines. To accommodate for this variablity, the residues in low hanging fruits are taken into account for the evaluation.

For olives the data indicates a clear distinction between tree picked and ground picked fruits. While no residues above the LOQ of 0.05 mg/kg for both analytes were found for tree picked olives, most of the ground picked olives showed glyphosate residues up to a maximum of 0.93 mg/kg. To accommodate for the common practice of harvesting ground laying olives, these results are taken into account to the evaluation of glyphosate residues following ground treatment. Since olives for oil production are considered as a major crop in SEU, the data provided for ground picked fruits are insufficient for a assessment. Therefore only table olives, which are a minor crop in the EU, are evaluated.

B.7.6.2 All crops with pre-emergence or pre-planting treatment

For all crops treated at pre-emergence or pre-planting the critical GAP was identified to involve up to two treatment with a maximum of 2.16 kg as/ha each. No PHI was considered necessary, since the pre-harvest interval is covered by the plant growth.

The applicant submitted several supervised field trials on annual crops for an extrapolation to all other crops. The supervised field trial results were selected according to the following criteria:

- Target application rate 2.16 kg as/ha ± 25 % (1.6 – 2.7 kg as/ha) or higher, when the data suggests a nil-residue situation.
- Last application at BBCH 00 to BBCH 09
- the number of applications are not considered relevant. Glyphosate degrades quickly in soil and is not relevant for rotational crops. The proposed treatment interval of at least 21 days suggets that the final application soley contributes to the residue.
- Since no PHI was defined, all supervised field trials matching the criteria listed before are taken into account.
- The critical GAP is identical for whole Europe. Data will be selected for Northern and Southern Europe individually.
- The formulation and the active ingredient (sodium salt or isopropylamine salt) are not expected to influence the residue for glyphosate.

The field trial data available does not involve treatment of genetically modified crops. Therefore only glyphosate and AMPA residues are taken into account for dietary intake purposes.

Table B.7.6-3: Glyphosate and AMPA residues after application to crops pre-emergent or pre-planting

Commodity	Region (a)	Outdoor/ Indoor	Individual trial results (mg/kg)		STMR (mg/kg) (b)	HR (mg/kg) (c)	Median CF (d)
			Enforcement (glyphosate)	Risk assessment (total (e))			
Roots crops	NEU	outdoor	<0.05(2)	<0.05(2)	0.05	0.05	1
(carrots)	SEU	outdoor	<0.05(2)	<0.05(2)			
Root crops	NEU	outdoor	<0.05(2)	<0.05(2)	0.05	0.05	1
(potato)	SEU	outdoor	<0.05(2)	<0.05(2)			
Bulb vegetables	NEU	outdoor	<0.05(6)	<0.05(6)	0.05	0.05	1
(bulb onoins)	SEU	outdoor	<0.05(2)	<0.05(2)			
Fruiting vegetables (tomato)	NEU	outdoor	<0.05(2)	<0.05(2)	0.05	0.05	1
Fruiting vegetables (cucumber)	SEU	outdoor	<0.05	<0.05	0.05	0.05	1
Fruiting vegetables	NEU	outdoor	<0.05	<0.05	0.05	0.05	1
(zucchini)	SEU	outdoor	<0.05	<0.05			
Brassica vegetables	NEU	outdoor	<0.05(2)	<0.05(2)	0.05	0.05	1
(cauliflower)	SEU	outdoor	<0.05(2)	<0.05(2)			
Brassica vegetables	NEU	outdoor	<0.05(2)	<0.05(2)	0.05	0.05	1
(head cabbage)	SEU	outdoor	<0.05(2)	<0.05(2)			
Leafy vegetables	NEU	outdoor	<0.05(2)	<0.05(2)	0.05	0.05	1
(lettuce, head + cutting)	SEU	outdoor	<0.05(2)	<0.05(2)			
Stem vegetables	NEU	outdoor	<0.05(2)	<0.05(2)	0.05	0.05	1
(leek)	SEU	outdoor	<0.05(2)	<0.05(2)			
Barley, grain	NEU	outdoor	<0.05(5)	<0.05(5)	0.05	0.05	1
Barley, straw	NEU	outdoor	<0.05(4)	<0.05(3), 0.125	0.05	0.05	1
Wheat, grain	NEU	outdoor	<0.05(10), 0.05	<0.05(10), 0.125			
Wheat, straw	NEU	outdoor	<0.05(12)	<0.05(11), 0.125			

Commodity	**Region** (a)	**Outdoor/ Indoor**	**Individual trial results (mg/kg)**		**STMR (mg/kg)** (b)	**HR (mg/kg)** (c)	**Median CF** (d)
			Enforcement (glyphosate)	Risk assessment (total (e))			
Sugar beet, roots	NEU	outdoor	<0.05(6)	<0.05(6)	0.05	0.05	1
	SEU	outdoor	<0.05(2)	<0.05(2)			
Sugar beet, tops	NEU	outdoor	<0.05(6)	<0.05(6)	0.05	0.05	1
	SEU	outdoor	<0.05(2)	<0.05(2)			

Underline median value from trial results (based on DoR for risk assessment)
(a): NEU, SEU, EU or Import (country code). In the case of indoor uses there is no necessity to differentiate between NEU and SEU.
(b): Median value of the individual trial results according to the risk assessment residue definition.
(c): Highest value of the individual trial results according to the risk assessment residue definition.
(d): The median conversion factor for enforcement to risk assessment is obtained by calculating the median of the individual conversion factors for each residues trial.
(e): sum of glyphosate and AMPA, expressed as glyphosate (see Table B.7.6-1)

For the application of glyphosate pre-emergence or before transplanting numerous supervised field trials were submitted covering commodities from all non-permanent crop groups. In none of the samples treated according to the intended use residues of glyphosate or AMPA above the LOQ were found except for a single samples of barley and wheat with residue at the LOQ of 0.05 mg/kg.

B.7.6.3 Linseed, mustard seed and rape seed

For linseeds, mustard seeds and rape seeds the critical GAP was identified for the desiccation of the crops, involving one pre-harvest treatment with up to 2.16 kg as/ha and a PHI of 14 days. The supervised field trial results were selected according to the following criteria:

- Target application rate 2.16 kg as/ha ± 25 % (1.6 – 2.7 kg as/ha).
- PHI of at least 14 days
- one single pre-harvest treatment
- The critical GAP is identical for whole Europe. Data will be selected for Northern and Southern Europe individually.
- The formulation and the active ingredient (sodium salt or isopropylamine salt) are not expected to influence the residue for glyphosate.
- Rape forage is no relevant commodity concerning the critical GAP, since forage rape for feeding purposes is harvest at much earlier growth stages.

The field trial data presented here does not involve treatment of genetically modified crops. Therefore only glyphosate and AMPA residues are taken into account for dietary intake purposes. For genetically modified crops, although not cultivated in the EU, please refer to B.7.6.6.

Table B.7.6-4: Glyphosate and AMPA residues after application to rape

Commo-dity	Region [(a)]	Outdoor/ Indoor	Individual trial results (mg/kg)		STMR (mg/kg) [(b)]	HR (mg/kg) [(c)]	Median CF [(d)]
			Enforcement (glyphosate)	Risk assessment (total [(e)])			
Rape seeds	NEU	outdoor	PHI 14: 1.4, 6.4, 9.0 PHI 7: 0.8, 1.3, 1.5, 1.7, 1.8, 14	PHI 14: 1.7, 6.5, 9.0(f) PHI 7: 0.88, 1.4, 1.6, 1.8, 1.9, 14[(f)]	data not sufficient	data not sufficient	data not sufficient
	SEU	outdoor	no data	no data	data not sufficient	data not sufficient	data not sufficient

Underline median value from trial results (based on DoR for risk assessment)
(a): NEU, SEU, EU or Import (country code). In the case of indoor uses there is no necessity to differentiate between NEU and SEU.
(b): Median value of the individual trial results according to the risk assessment residue definition.
(c): Highest value of the individual trial results according to the risk assessment residue definition.
(d): The median conversion factor for enforcement to risk assessment is obtained by calculating the median of the individual conversion factors for each residues trial.
(e): sum of glyphosate and AMPA, expressed as glyphosate (see Table B.7.6-1)
(f): no AMPA analysed

For the application of glyphosate to rapeseed, linseeds and mustard seeds only three supervised field trials of the data submitted match the critical GAP identified for the EU. Five additional field trials are within ± 25 % of the maximum application rate, but with lower PHIs of 7 instead of 14 days. Especially for desiccation this lower GAP is a significant deviation, since the main purposes is the accelerated drying of the crops, resulting in higher residues with longer PHIs.
According to the current data requirement in the EU, at least four supervised field trials are required for the evaluation of a minor crops like linseed and mustard seed and eight trials for a major crop like oilseed rape. The data, even under consideration of possible extrapolation between these crops, is not sufficient for an assessment.

However, the supervised field trial data available for rapeseed and linseed are numerous, involving application rates for desiccation purposes of either 0.72 kg as/ha, 1.0-1.1 kg as/ha, 1.4-1.5 kg as/ha and 2.8-2.9 kg as/ha. For future utilisation of the proportionality principle, which is not yet accepted within the EU, the residues following application rates deviating more than 25 % to the cGAP are also presented. Residues based on these other application rates were:

Table B.7.6-5: Glyphosate and AMPA residues after application to rape and linseed with application rates deviating more than 25 % to the cGAP

Commo-dity	Region [a]	Outdoor/ Indoor	Individual trial results (mg/kg)		STMR (mg/kg) [b]	HR (mg/kg) [c]	Median CF [d]
			Enforcement (glyphosate)	Risk assessment (total [e])			
Rape seeds 0.72 kg as/ha	NEU	outdoor	0.1, 0.14, 0.2(3), 0.28, 0.37, 1.1	0.1[f], 0.14[f], 0.2[f], 0.2[f], 0.2[f], 0.28[f], 0.37[f], 1.4	0.2	1.4	not applicable, AMPA mainly <LOQ
	SEU	outdoor	no data	no data	data not sufficient	data not sufficient	data not sufficient
Rape seeds 1.0-1.1 kg as/ha	NEU	outdoor	0.3, 0.6, 0.96, 1.4, 1.8, 2.6, 3.1, 3.6, 4.4, 4.6, 5.6, 6.8	0.3[f], 0.6[f], 0.96[f], 1.4[f], 2.1, 2.6[f], 3.2, 3.6[f], 4.4[f], 4.7, 5.6[f], 6.95[f]	2.9	6.95	not applicable, AMPA mainly <LOQ
	SEU	outdoor	no data	no data	data not sufficient	data not sufficient	data not sufficient
Rape seeds 1.4-1.5 kg as/ha	NEU	outdoor	<0.05, 0.06, 0.06, 0.12, 0.21, 0.23, 0.28, 0.4(3), 0.48, <0.5, 0.57, 0.6(4), 0.7, 0.8, 0.8, 0.86, 0.9, 0.96, <1.0, 1.0, 1.2, 1.3, 1.5, 2.0, 2.0, 2.8, 4.1, 4.1, 6.7, 8.6, 11.6	<0.05, 0.06[f], 0.135, 0.195, 0.28[f], 0.285, 0.305 0.475, 0.4[f], 0.4[f], 0.48[f], <0.5, 0.57[f], 0.675(3), 0.755, 0.775, 0.875, 0.935, 0.975, <1.0, 1.04, 1.1, 1.1, 1.3, 1.4, 1.8, 2.3, 2.3, 3.1, 4.4, 4.4, 7.0, 8.9, 11.9	0.825	~~30.35~~11.9	not applicable, AMPA mainly <LOQ
	SEU	outdoor	no data	no data	data not sufficient	data not sufficient	data not sufficient
Rape seeds 2.8-2.9 kg as/ha	NEU	outdoor	0.4, 0.8, 0.9, <1.0, 1.3, 1.3, 1.4, 1.4, 1.5, 1.6, 1.8, 2.4, 3.4, 3.5, 3.9, 5.2	0.475, 0.875, 0.975, <1.0, 1.4, 1.4, 1.5, 1.5, 1.6, 1.7, 1.9, 2.5, 3.5, 3.6, 4.2, 5.5	1.55	5.5	not applicable, AMPA mainly <LOQ
	SEU	outdoor	no data	no data	data not sufficient	data not sufficient	data not sufficient

Linseeds	NEU	outdoor	no data	no data	data not sufficient	data not sufficient	data not sufficient
	SEU	outdoor	no data	no data	data not sufficient	data not sufficient	data not sufficient

(a): NEU, SEU, EU or Import (country code). In the case of indoor uses there is no necessity to differentiate between NEU and SEU.
(b): Median value of the individual trial results according to the risk assessment residue definition.
(c): Highest value of the individual trial results according to the risk assessment residue definition.
(d): The median conversion factor for enforcement to risk assessment is obtained by calculating the median of the individual conversion factors for each residues trial.
(e): sum of glyphosate and AMPA, expressed as glyphosate (see Table B.7.6-1)
(f): no AMPA analysed. Since AMPA was normally not found at levels above the LOQ when analysed, the impact of these missing AMPA values is considered minor.

B.7.6.4 Barley and oats

For barley and oats the critical GAP was identified for the desiccation of the crops, involving one pre-harvest treatment with up to 2.16 kg as/ha and a PHI of 7 days. The supervised field trial results were selected according to the following criteria:

- Target application rate 2.16 kg as/ha ± 25 % (1.6 – 2.7 kg as/ha).
- PHI of at least 7 days (longer PHI are also acceptable, since drying of the crops generally results in higher residues)
- one single pre-harvest treatment
- The critical GAP is identical for whole Europe. Data will be selected for Northern and Southern Europe individually.
- The formulation and the active ingredient (sodium salt or isopropylamine salt) are not expected to influence the residue for glyphosate. Side-by-side trials conducted with different formulations but with identical GAPs (location, date, PHI, variety, rate) are considered as replicate plots. For these trials the mean residue is taken account.
- Barley and oats forage are no relevant commodities concerning the critical GAP, since forage for feeding purposes is harvest at much earlier growth stages.

The field trial data presented here does not involve treatment of genetically modified crops. Therefore only glyphosate and AMPA residues are taken into account for dietary intake purposes.

Table B.7.6-6: Glyphosate and AMPA residues after application to barley and oats

Commo-dity	Region [a]	Outdoor/ Indoor	Individual trial results (mg/kg)		STMR (mg/kg) [b]	HR (mg/kg) [c]	Median CF [d]
			Enforcement (glyphosate)	Risk assessment (total [e])			
Barley, grain	NEU	outdoor	1.2, 1.5, 2.0[f], 2.1[f], 2.2, 2.4, 2.5[f], 2.6[f], 2.6[f], 2.8, 3.95[f]; 4.3[f], 4.4[f], 4.5[f], 4.6[f], 4.8[f], 5.1, 5.2, 5.2[f], 5.2[f], 5.5, 5.7[f], 5.9[f], 5.9, 6.2[f], 6.5[f], 6.7, 7.4[f], 7.7[f], 7.8[f], 8.0, 8.1[f], 8.4, 10, 10.3[f], 12.4[f], 12.5[f], 15.5[f], 16.5[f], 17[f], 17.5[f]	1.3, 2.1[f], 1.5[g], 2.2[f], 2.3, 2.5, 2.5[f], 2.7[f], 2.9, 3.2[f], 4.2[f], 4.4[f], 4.6[f], 4.9[f], 5.0[f], 5.1[g], 5.2[f], 5.3, 5.3[f], 5.5[g], 5.8[f], 5.8[f], 5.9[f], 6.2, 6.2[f,g], 6.6[f], 6.9, 7.5[f], 7.9[f], 8.0[f], 8.2[f], 8.3, 8.4[g], 10.3, 10.4[f], 12.4[f,g], 12.8[f], 16[f], 16.6[f], 17.2[f], 17.8[f]	5.8	17.8	1.0
	SEU	outdoor	6.0[f], 7.8[f], 13.5[f], 19[f]	6.0[f], 7.9[f], 13.7[f], 19.3[f]	data not sufficent		
Barley, straw	NEU	outdoor	4.6[f], 6.9, 10.5[f], 11.5[f], 12.8, 12.8[f], 14.5[f], 16, 17[f], 18, 22, 26, 26.3[f], 26.5[f], 27[f], 27.3[f], 28.4, 32.2, 33.3[f], 36.9, 37[f], 41.5[f], 44, 49.7[f], 54, 56, 60.5[f], 69.6, 80.5[f], 86[f], 90.2[f], 109[f], 115[f], 117[f], 136[f], 140[f]	4.7[f], 6.9[g], 10.6[f], 12.1[f], 13.1, 13.2[f], 14.6[f], 16.3, 17.7[f], 18[g], 22[g], 26.7[f], 27.1[f], 27.6[f], 28.6, 28.7[f], 29.3, 29.6, 32.7, 33.9[f], 37.8[f], 38, 42.1[f], 44.4, 51.3[f], 56[g], 60.8, 61.9[f], 70.7, 83.6[f], 89.8[f], 92[f], 109[f,g], 115[f,g], 119[f], 140[f], 142[f]	32.7	142	1.0
	SEU	outdoor	34[f], 49.5[f], 66[f], 102[f]	34.9[f], 51[f], 68.1[f], 105[f]	data not sufficent		
Oats, grain	NEU	outdoor	2.1, 5.3, 5.4, 5.5, 9.8, 14, 18.4, 21, 21.4	2.2, 5.3[g], 5.5, 5.6, 10, 14.4, 18.4[g], 21.4[g], 21.6	10	21.6	1.0
	SEU	outdoor	no data				
Oats, straw	NEU	outdoor	9.6, 11, 24	10, 11.3, 24.5	data not sufficent		
	SEU	outdoor	no data				

Commo-dity	**Region** (a)	**Outdoor/ Indoor**	**Individual trial results (mg/kg)**		**STMR (mg/kg)** (b)	**HR (mg/kg)** (c)	**Median CF** (d)
			Enforcement (glyphosate)	Risk assessment (total (e))			
Barley and oats, grain	NEU	outdoor	1.2, 1.5, 2.0(f), 2.1(f), 2.1, 2.2, 2.4, 2.5(f), 2.6(f), 2.6(f), 2.8, 3.95(f); 4.3(f), 4.4(f), 4.5(f), 4.6(f), 4.8(f), 5.1, 5.2, 5.2(f), 5.2(f), 5.3, 5.4, 5.5, 5.5, 5.7(f), 5.9(f), 5.9, 6.2(f), 6.5(f), 6.7, 7.4(f), 7.7(f), 7.8(f), 8.0, 8.1(f), 8.4, 9.8, 10, 10.3(f), 12.4(f), 12.5(f), 14, 15.5(f), 16.5(f), 17(f), 17.5(f), 18.4, 21, 21.4	1.3, 1.5(g), 2.1(f), 2.2, 2.2(f), 2.3, 2.5, 2.5(f), 2.7(f), 2.9, 3.2(f), 4.2(f), 4.4(f), 4.6(f), 4.9(f), 5.0(f), 5.1(g), 5.2(f), 5.3, 5.3(f), 5.3(g), 5.5, 5.5(g), 5.6, 5.8(f), 5.8(f), 5.9(f), 6.2, 6.2(f,g), 6.6(f), 6.9, 7.5(f), 7.9(f), 8.0(f), 8.2(f), 8.3, 8.4(g), 10, 10.3, 10.4(f), 12.4(f,g), 12.8(f), 14.4, 16(f), 16.6(f), 17.2(f), 17.8(f), 18.4(g), 21.4(g), 21.6	5.85	21.6	1.1
	SEU	outdoor	see barley				
Barley and oats, straw	NEU	outdoor	4.6(f), 6.9, 9.6, 10.5(f), 11, 11.5(f), 12.8, 12.8(f), 14.5(f), 16, 17(f), 18, 22, 24, 26, 26.3(f), 26.5(f), 27(f), 27.3(f), 28.4, 32.2, 33.3(f), 36.9, 37(f), 41.5(f), 44, 49.7(f), 54, 56, 60.5(f), 69.6, 80.5(f), 86(f), 90.2(f), 109(f), 115(f), 117(f), 136(f), 140(f)	4.7(f), 6.9(g), 10, 10.6(f), 11.3, 12.1(f), 13.1, 13.2(f), 14.6(f), 16.3, 17.7(f), 18(g), 22(g), 24.5, 26.7(f), 27.1(f), 27.6(f), 28.6, 28.7(f), 29.3, 29.6, 32.7, 33.9(f), 37.8(f), 38, 42.1(f), 44.4, 51.3(f), 56(g), 60.8, 61.9(f), 70.7, 83.6(f), 89.8(f), 92(f), 109(f,g), 115(f,g), 119(f), 140(f), 142(f)	29.45	142	1.1
	SEU	outdoor	see barley				

(a): NEU, SEU, EU or Import (country code). In the case of indoor uses there is no necessity to differentiate between NEU and SEU.
(b): Median value of the individual trial results according to the risk assessment residue definition.
(c): Highest value of the individual trial results according to the risk assessment residue definition.
(d): The median conversion factor for enforcement to risk assessment is obtained by calculating the median of the individual conversion factors for each residues trial.
(e): sum of glyphosate and AMPA, expressed as glyphosate (see Table B.7.6-1)
(f): mean of replicate samples, trials or plots
(g): no AMPA analysed

Based on the critical GAP for glyphosate in barley and oats (desiccation) residues in the grain in supervised field trials conducted in Northern Europe were between 1.2 to 17.5 mg/kg for barley grain and between 2.1 to 21 mg/kg for oats grain. Corresponding residues for the sum of glyphosate and AMPA, expressed as glyphosate, were 1.3 to 17.8 mg/kg and 2.2 to 21.6 mg/kg, respectively. In barley straw glyphosate residue of 4.6 to 140 mg/kg and residues for the sum of glyphosate and AMPA, expressed as glyphosate, of 4.7 to 142 mg/kg were found.

The data for oats straw for Nothern Europe and for barley and oats for Southern Europe (grain and straw) were not sufficient for an evaluation.

B.7.6.5 Rye, triticale and wheat

For rye, triticale and wheat the critical GAP was identified for the desiccation of the crops, involving one pre-harvest treatment with up to 2.16 kg as/ha and a PHI of 7 days. The supervised field trial results were selected according to the following criteria:

- Target application rate 2.16 kg as/ha ± 25 % (1.6 – 2.7 kg as/ha).
- PHI of at least 7 days (longer PHI are also acceptable, since drying of the crops generally results in higher residues)
- one single pre-harvest treatment
- The critical GAP is identical for whole Europe. Data will be selected for Northern and Southern Europe individually.
- The formulation and the active ingredient (sodium salt or isopropylamine salt) are not expected to influence the residue for glyphosate. Side-by-side trials conducted with different formulations but with identical GAPs (location, date, PHI, variety, rate) are considered as replicate plots. For these trials the mean residue is taken account.
- Barley and oats forage are no relevant commodities concerning the critical GAP, since forage for feeding purposes is harvest at much earlier growth stages.

The field trial data presented here does not involve treatment of genetically modified crops. Therefore only glyphosate and AMPA residues are taken into account for dietary intake purposes.

Table B.7.6-7: Glyphosate and AMPA residues after application to rye, triticale and wheat

Commo-dity	Region [(a)]	Outdoor/ Indoor	Individual trial results (mg/kg)		STMR (mg/kg) [(b)]	HR (mg/kg) [(c)]	Median CF [(d)]
			Enforcement (glyphosate)	Risk assessment (total [(e)])			
Rye, grain	NEU	outdoor	1.6, 2.9, 3.1, 5.4, 17.5[(f)]	1.6[(g)], 2.9[(g)], 3.1[(g)], 5.4[(g)], 18.1[(f)]	data not sufficient for rye only		
	SEU	outdoor	no data				
Rye, straw	NEU	outdoor	24.8[(f)]	25.5[(f)]	data not sufficient for rye only		
	SEU	outdoor	no data				
Triticale	NEU	outdoor	no data				
	SEU	outdoor					
Wheat, grain	NEU	outdoor	0.05, 0.11[(f)], 0.16[(f)], 0.19[(f)], 0.22[(f)], 0.23[(f)], 0.23[(f)], 0.26[(f)], 0.33, 0.5, 0.5[(f)], 0.6, 0.64[(f)], 0.67, 0.7, 0.7, 0.7(3)[(f)], 0.71, 0.74[(f)], 0.75, 0.75, 0.77[(f)], 0.85, 1.3[(f)], 1.4, 1.5[(f)], 1.55[(f)], 1.7, 1.7[(f)], 1.75[(f)], 2.2, 2.4[(f)],	0.125, 0.18[(f)], 0.24[(f)], 0.26[(f)], 0.27[(f)], 0.27[(f)], 0.28[(f)], 0.29[(f)], 0.36, 1.1[(f)], 0.58, 0.64[(f,g)], 0.7, 0.74, 0.74[(f,g)], 0.75, 0.77[(f)], 0.78, 0.78, 0.78[(f)], 0.78[(f)], 0.83, 0.83, 0.84[(f)], 0.93, 1.3[(f,g)], 1.5, 1.6[(f)], 1.6[(f)], 1.7[(g)],	0.83	13.3	1.1

Commo-dity	**Region** [(a)]	**Outdoor/ Indoor**	**Individual trial results (mg/kg)**		**STMR (mg/kg)** [(b)]	**HR (mg/kg)** [(c)]	**Median CF** [(d)]
			Enforcement (glyphosate)	Risk assessment (total [(e)])			
			3.45[(f)], 3.5[(f)], 3.7[(f)], 3.85[(f)], 4.7, 4.8, 4.85[(f)], 9.5[(f)], 12.4[(f)]	1.8[(f)], 1.9[(f)], 2.3, 2.4[(f,g)], 3.5[(f)], 3.6[(f)], 3.8[(f)], 3.9[(f)], 4.9, 5.0, 5.0[(f)], 9.5[(f,g)], 13.3[(f)]			
	SEU	outdoor	0.07, 0.38[(f)], 0.4, 0.4[(f)], 0.47[(f)], 0.6[(f)], 0.95[(f)], 1.2[(f)], 2.8[(f)]	0.15, 0.45[(f)], 0.48, 0.48[(f)], 0.55[(f)], 0.68[(f)], 1.0[(f)], 1.3[(f)], 3.0[(f)]	0.55	3.0	1.1
Wheat, straw	NEU	outdoor	1.4[(f)], 5.3, 8.4[(f)], 9.5[(f)], 10.3[(f)], 10.6[(f)], 11.4[(f)], 14.7[(f)], 14.9[(f)], 17.3, 18.5[(f)], 19.1, 19.7, 21.5[(f)], 26.9, 27.4, 27.5[(f)], 29.6[(f)], 31.4, 34.8, 42, 43.2[(f)], 43.8, 44.5[(f)], 46[(f)], 52.8[(f)], 63.3[(f)], 68, 70.5[(f)], 84.5[(f)], 85[(f)], 95.3[(f)], 95.5[(f)], 95.7[(f)], 96.5[(f)], 99[(f)], 175[(f)]	1.5[(f)], 5.4, 9.3[(f)], 10.5[(f)], 10.9[(f)], 11[(f)], 12.6[(f)], 15.7[(f)], 15.7[(f)], 17.6, 19.2[(f)], 19.4, 19.9, 22.1[(f)], 2.8, 28.2[(f)], 28.9, 29.6[(f,g)], 31.8, 35.9, 42.6, 43.2[(f)], 44.2, 45.4[(f)], 46[(f,g)], 52.8[(f,g)], 64.3[(f)], 68[(g)], 71.4[(f)], 87.5[(f)], 88.5[(f)], 96.5[(f,g)], 97.3[(f)], 97.6[(f)], 98[(f)], 103[(f)], 179[(f)]	31.8	179	1.1
	SEU	outdoor	3.4, 15.5[(f)], 16[(f)], 20[(f)], 22[(f)], 28, 28.5[(f)], 55.5[(f)], 98[(f)]	3.5, 16.9[(f)], 18.6[(f)], 20.9[(f)], 23.2[(f)], 29.6[(f)], 29.7, 56.5[(f)], 99[(f)]	23.2	99	1.1
Rye, wheat and triticale, grain	NEU	outdoor	0.05, 0.11[(f)], 0.16[(f)], 0.19[(f)], 0.22[(f)], 0.23[(f)], 0.23[(f)], 0.26[(f)], 0.33, 0.5, 0.5[(f)], 0.6, 0.64[(f)], 0.67, 0.7, 0.7, 0.7(3)[(f)], 0.71, 0.74[(f)], 0.75, 0.75, 0.77[(f)], 0.85, 1.3[(f)], 1.4, 1.5[(f)], 1.55[(f)], 1.6, 1.7, 1.7[(f)], 1.75[(f)], 2.2, 2.4[(f)], 2.9, 3.1, 3.45[(f)], 3.5[(f)], 3.7[(f)], 3.85[(f)], 4.7, 4.8, 4.85[(f)], 5.4, 9.5[(f)], 12.4[(f)], 17.5	0.125, 0.18[(f)], 0.24[(f)], 0.26[(f)], 0.27[(f)], 0.27[(f)], 0.28[(f)], 0.29[(f)], 0.36, 1.1[(f)], 0.58, 0.64[(f,g)], 0.7, 0.74, 0.74[(f,g)], 0.75, 0.77[(f)], 0.78, 0.78, 0.78[(f)], 0.78[(f)], 0.83, 0.83, 0.84[(f)], 0.93, 1.3[(f,g)], 1.5, 1.6[(f)], 1.6[(f)], 1.6[(g)], 1.7[(g)], 1.8[(f)], 1.9[(f)], 2.3, 2.4[(f,g)], 2.9[(g)], 3.1[(g)], 3.5[(f)], 3.6[(f)], 3.8[(f)], 3.9[(f)], 4.9, 5.0, 5.0[(f)], 5.4[(g)], 9.5[(f,g)], 13.3[(f)], 18.1[(f)]	0.885	18.1	1.1
	SEU	outdoor	see wheat				
Rye, wheat and triticale, straw	NEU	outdoor	1.4[(f)], 5.3, 8.4[(f)], 9.5[(f)], 10.3[(f)], 10.6[(f)], 11.4[(f)], 14.7[(f)], 14.9[(f)],	1.5[(f)], 5.4, 9.3[(f)], 10.5[(f)], 10.9[(f)], 11[(f)], 12.6[(f)], 15.7[(f)], 15.7[(f)], 17.6,	30.7	179	1.1

Commo-dity	Region [(a)]	Outdoor/ Indoor	Individual trial results (mg/kg)		STMR (mg/kg) [(b)]	HR (mg/kg) [(c)]	Median CF [(d)]
			Enforcement (glyphosate)	Risk assessment (total [(e)])			
			17.3, 18.5[(f)], 19.1, 19.7, 21.5[(f)], 24.8[(f)], 26.9, 27.4, 27.5[(f)], 29.6[(f)], 31.4, 34.8, 42, 43.2[(f)], 43.8, 44.5[(f)], 46[(f)], 52.8[(f)], 63.3[(f)], 68, 70.5[(f)], 84.5[(f)], 85[(f)], 95.3[(f)], 95.5[(f)], 95.7[(f)], 96.5[(f)], 99[(f)], 175[(f)]	19.2[(f)], 19.4, 19.9, 22.1[(f)], 25.5[(f)], 28, 28.2[(f)], 28.9, 29.6[(f,g)], 31.8, 35.9, 42.6, 43.2[(f)], 44.2, 45.4[(f)], 46[(f,g)], 52.8[(f,g)], 64.3[(f)], 68[(g)], 71.4[(f)], 87.5[(f)], 88.5[(f)], 96.5[(f,g)], 97.3[(f)], 97.6[(f)], 98[(f)], 103[(f)], 179[(f)]			
	SEU	outdoor	see wheat				

(a): NEU, SEU, EU or Import (country code). In the case of indoor uses there is no necessity to differentiate between NEU and SEU.
(b): Median value of the individual trial results according to the risk assessment residue definition.
(c): Highest value of the individual trial results according to the risk assessment residue definition.
(d): The median conversion factor for enforcement to risk assessment is obtained by calculating the median of the individual conversion factors for each residues trial.
(e): sum of glyphosate and AMPA, expressed as glyphosate (see Table B.7.6-1)
(f): mean of replicate samples, trials or plots
(g): no AMPA analysed

For the selection of supervised field trials data for rye processing trials treated at BBCH 75 (RIP9501327 & RIP9501328) were not considered within GAP, since the point of application was too early to reflect common agricultural practice.

Based on the critical GAP for glyphosate in rye, triticale and wheat (desiccation) residues in supervised field trials conducted in Northern Europe were between 0.05 to 12.4 mg/kg for wheat grain. Corresponding residues for the sum of glyphosate and AMPA, expressed as glyphosate, were 0.125 to 13.3 mg/kg. In wheat straw glyphosate residue of 1.4 to 175 mg/kg and residues for the sum of glyphosate and AMPA, expressed as glyphosate, of 1.5 to 179 mg/kg were found. In Southern Europe residues in wheat were generally lower compared to Northern Europe, ranging up to 2.8 mg/kg for glyphosate in grain (3.0 mg/kg for the sum of glyphosate and AMPA, expressed as glyphosate) and 98 mg/kg in straw (99 mg/kg for the sum of glyphosate and AMPA, expressed as glyphosate).
The data for rye and triticale themselves were not sufficient for an evaluation. However, according to the current Guidance for Extrapolation, data on wheat can be used for extrapolation to rye and triticale.

B.7.6.6 Tolerant crops - supervised field trials conducted in the EU

In the EU the cultivation of crops genetically modified with glyphosate tolerances are not authorised. However, since commodities obtained from glyphosate tolerant crops pose a major share in global trade, supervised field trials on GMOs available are again presented in this document to suppport an appropriate definition of the residue for enforcement and dietary intake purposes. For a detailed description of the following data please refer to the first DAR for glyphosate.

B.7.6.6.1 Cotton

For cotton two supervised field trials on tolerant plants expressing the CP4-EPSPS protein were reported. The plants were treated with five applications (0.36 kg as/ha pre-emergence, 0.36 kg as/ha at BBCH 10-12, 0.45 kg as/ha at BBCH 16, 0.9 kg as/ha at BBCH 51 and 0.9 kg as/ha at BBCH 92) and harvested seven days after the final spraying. In the seeds residues were 0.328 mg/kg and 0.074 mg/kg for glyphosate and below the LOQ of <0.05 mg/kg for AMPA.

B.7.6.6.2 Oilseed rape

Supervised field trials involving glyphosate tolerant oilseed rape grown in Europe were submitted using the line "RT73", which expresses both the CP4-EPSPS and the GOX modification. In the trials submitted the plants received either one application with 1.4 kg as/ha or two applications with 0.72-1.1 kg as/ha followed by 0.72 kg as/ha with a two week interval. All treatment were conducted at early growth stages between BBCH 14 to BBCH 30. Directly after treatment (day zero) parent glyphosate was the major residue in immature plants. Within the first weeks the residue concentration declined strongly while AMPA residues increased. In mature seeds, residues were mostly near the LOQ. Although both glyphosate and AMPA were found frequently, AMPA was the major residue in seeds present at equal or higher levels than parent glyphosate.
The indivual levels in rapeseeds after a PHI of 69 – 163 days ranged from <0.05 – 0.08 mg/kg for glyphosate and <0.05 – 0.4 mg/kg for AMPA. However it has to be noted that the glyphosate applications early in the growing season generally results in a much lower total residue in rapeseeds (glyphosate + AMPA, expressed as glyphosate) than desiccation uses on non-tolerant crops.

B.7.6.6.3 Soya beans

For soya beans several supervised field trials were reported in the first DAR conducted in Southern Europe on plants of line 40-3, containing the CP4-EPSPS mmodification. The plants received three applications (0.72-1.08 kg as/ha each) with the last either up to flowering or close to harvest (desiccation). Samples were collected between 100 – 107 DAT for the early application and 12-14 days for the desiccation.
In these trials residues of glyphosate and AMPA were found at comparable levels in the mature seeds, independent on the point of application. In the seeds residues for glyphosate ranged from <0.05-1.8 mg/kg and for AMPA from <0.05-2.5 mg/kg.

B.7.6.6.4 Maize

For glyphosate tolerant maize supervised field trials were reported in the first DAR from Northern and Southern Europe. The line "MON 802" used in these trials expresses both the CP4-EPSPS and the GOX modification. In all trials the plants received treatments early in the growing season up to BBCH 19. In Northern Europe a pre-emergence application with approx. 1 kg as/ha was followed by two foliar treatment at BBCH 15 and 19 with approx. 0.5 kg as/ha each. Samples were collected 59-95 DAT for forage and 91-120 DAT for grain and fodder. In the grain no residues of glyphosate above the LOQ of 0.05 mg/kg were found. AMPA was present between <0.05 mg/kg to 0.5 mg/kg. In fodder and forage glyphosate was still present, mainly at levels comparable to AMPA. Residues for glyphosate were between <0.05 mg/kg to 1.5 mg/kg in fodder and between <0.05 mg/kg to 0.5 mg/kg in forage. For

AMPA levels between <0.05 mg/kg to 0.5 mg/kg were found in fodder and between <0.05 mg/kg to 0.8 mg/kg in forage.
In Southern Europe also one pre-emergence treatment with approx. 1 kg as/ha was conducted, followed by a single treatment with either 1 kg as/ha or 2.2 kg as/ha. For all trials mature grain and fodder were sampled at 91-140 DAT, while forage was collected early at 76-90 DAT. In the grain both glyphosate and AMPA levels were <0.05 mg/kg to 0.09 mg/kg.. In fodder and forage glyhosate was found in slightly higher amounts with 0.1 mg/kg to 0.6 mg/kg and with <0.05 mg/kg to 0.4 mg/kg, respectively. Corresponding AMPA levels were <0.05 mg/kg to 0.2 mg/kg for both matrices.

B.7.6.6.5 Sugar beets

For sugar beets supervised field trials on glyphosate tolerant crops were submitted for Northern and Southern Europe. In all trials modified plants expressing the CP4 EPSPS enzyme were cultivated. The plants were treated at early growth stages up to BBCH 39 with 2-4 applications of 0.7-1.2 kg as/ha each.
As expected based on the CP4 EPSPS modification, unchanged glyphosate was the major residue in leaves, tops and roots of sugar beets. AMPA was normally not found at levels above the LOQ of 0.05 mg/kg except for very early pre-harvest intervals. In these cases the ratio glyphosate to AMPA was at least 10:1 up to 100:1.
In comparison to the intended uses for unmodified crops, the broadcast spray application to tolerant sugar beets up to BBCH result in glyphosate residue in all commodities above the LOQ. At commercial harvest, glyphosate residue ranged from <0.05 – 1.5 mg/kg for leaves and tops and from <0.05 – 3.1 mg/kg for roots of sugar beets.

B.7.6.6.6 Public literature

A study published by Ando C. et al (2003, ASB2012-12350) investigated the residue situation on typical herbs collected by native Americans in the California National Forest. The results are not related to the representative uses and the situation in the EU.

Arregui et al (2004, ASB2012-12351) reported monitoring results of glyphosate residues in transgenic soya beans from Argentina from 1997-1999. An additional study was provided by Lorenzatti et al (2004, ASB2012-12448). The representative uses evaluated within this document do not involve transgenic plants or import tolerances. The respective study was not taken into account.

In Denmark, the residue levels of glyphosate in cereal grain were monitored 1998 and 1999 by Granby et al (2001, ASB2012-12423). In 1998the average concentration was 0.08 mg/kg (n=49) and increased to 0.11 mg/kg in 1999 (n=46). No MRL violations were identified.

B.7.6.7 Storage stability

The procedual recoveries for glyphosate and its metabolites often gave subpar results between 70-85%. To avoid a systematic bias in the interpretation of the storage stability data near 70% remaining, also recovered residues corrected by the freshly fortified samples were presented to easen the interpretation of the results. However, it should noted that the general basis for the interpretation are the uncorrected residue values and their procedual recoveries.

B.7.6.7.1 Plant matrices

B.7.6.7.1.1 Glyphosate and AMPA

Reference: OECD KIIA 6.1.1

Report: Mueth, M. G.; Allan, J. M.
Storage stability of Glyphosate and AMPA on citrus fruit
01.02.2012, Report No.: MSL0023608, ASB2012-12452

Guidelines: EU Guidance Appendix H: Storage Stability of residue samples (7032/VI/95 rev. 5)

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
The storage stability of glyphosate and AMPA during freezer storage was investigated in whole homogenised samples for e period of up to 24 months. The samples were separately fortified at levels of 0.5 mg/kg for each analyte and stored at -18°C directly after fortification. At each sampling (0, 1, 3, 6, 9, 12, 18 and 24 months) duplicate samples (quintuple at day 0) as well es freshly fortified samples were analyse for residues of glpyhosate and AMPA.

Glyphosate and AMPA were isolated from crop matrices by high speed blender extraction using 0.1% formic acid in water and methylene chloride. Following centifugation, an aliquot of the aqueous phase extract was mixed with isotopically labelled glyphosate and AMPA as internal standard before solid phase extraction for clean-up. The samples were analysed by LC/MS/MS using a cation exchange column. The validated working range of the method was 0.05 to 6.0 mg/kg.

Findings:
In the following table the recovered residues of glyphosate and AMPA in stored orange samples are summarised.

Table B.7.6-8: Recovery of glyphosate and AMAP in oranges after storage at -18°C

Months (days)	Glyphosate (0.5 mg/kg fortification)			AMPA (0.5 mg/kg fortification		
	mg/kg	%[a]	Proc. recovery (%)	mg/kg	%[a]	Proc. recovery (%)
0	0.45, 0.446, 0.443, 0.435, 0.432 (0.441)	90, 89, 89, 87, 86 (88)	-	0.44, 0.428, 0.426, 0.444, 0.435 (0.435)	88, 86, 85, 89, 87 (87)	-
1 (30)	0.477, 0.456 (0.467)	95, 91 (93)	91, 92 (91)	0.452, 0.455 (0.444)	90, 91 (91)	92, 92 (92)
3 (97)	0.458, 0.454 (0.456)	92, 91 (91)	92, 89 (90)	0.453, 0.439 (0.446)	91, 88 (89)	92, 87 (89)
6 (196)	0.463, 0.458 (0.461)	93, 92 (92)	89, 87 (88)	0.448, 0.446 (0.447)	90, 89 (89)	88, 88 (88)
9 (273)	0.434, 0.438 (0.436)	87, 88 (87)	86, 87 (86)	0.436, 0.435 (0.436)	87, 87 (87)	88, 86 (87)
12 (372)	0.471, 0.461	94, 92 (93)	85, 91 (88)	0.46, 0.454	92, 91 (92)	86, 86 (86)

	(0.466)			(0.457)		
18 (546)	0.445, 0.448 (0.447)	89, 90 (89)	89, 89 (89)	0.439, 0.436 (0.438)	88, 87 (88)	86, 85 (85)
24 (727)	0.442, 0.443 (0.443)	88, 89 (89)	87, 84 (86)	0.422, 0.42 (0.421)	84, 84 (84)	92, 92 (92)

Conclusions:
In this study glyphosate and AMPA were proven to be stable in oranges for at least 24 months.

Reference: OECD KIIA 6.1.1

Report: Mueth, M. G.
Storage stability of Glyphosate residues in crop commodities
June 1991, Report No.: MSL-10843, RIP9501332

Guidelines: EPA Guideline 171-4

Deviations: ~~original guideline not submitted~~none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study the storage stability of glyphosate and AMPA under frozen condition was investigated in maize grain, soya bean forage, sorghum stover, clover and tomatoes. Samples of each commodity were fortified at levels of 0.5 mg glyphosate and 0.4845 mg AMPA per kg sample and stored for a period of up to 31 months at -18°C. At each sampling interval duplicate stored samples, duplicate control samples and duplicate freshly fortified samples were analysed for residues of both analytes.
Besides the investigation of exogeneous residues samples from various supervised field trials were frozen and analysed for the endogeneous residues present after several months.

The analytical method used consists of isolation of the glyphosate and AMPA residues by aqueous extraction followed by chelation and ion-exchange chromatography. After elution and concentration, the analytes were derivatized with o-phtalaldehyde and quantified by HPLC-fluorescence detection (455 nm/340nm).

Findings:
In fortified samples recovereed residues after storage were:

Table B.7.6-9: Stability of glyphosate and AMPA in stored plant samples

Commodity	Months (days)	Glyphosate (0.5 mg/kg fortification)				AMPA (0.4845 mg/kg fortification			
		mg/kg	%[a]	Proc. recovery (%)	corrected %[a]	mg/kg	%[a]	Proc. recovery (%)	corrected %[a]
Maize grain	0	0.410	82.0	82.0	100	0.395	81.5	81.5	100
	1 (30)	0.396	79.2	78.4	101	0.351	72.4	73.8	98.2
	3 (95)	0.397	79.4	78.0	102	0.302	62.3	63.1	98.9
	6 (181)	0.312	62.4	60.7	103	0.319	65.8	65.0	101
	9 (271)	0.292	58.4	63.3	92.2	0.313	64.6	69.3	93.3
	12 (376)	0.337	67.4	68.6	98.2	0.297	61.3	71.9	85.2
	18 (528)	0.309	61.8	69.4	89.0	0.336	69.3	72.8	95.4
	24 (742)	0.354	70.8	72.0	98.4	0.358	73.9	81.5	90.6
	31 (944)	0.286	57.2	75.3	76.0	0.270	55.7	70.7	78.8
Soya bean forage	0	0.377	75.5	75.5	99.8	0.340	70.2	70.2	99.9
	1 (27)	0.428	85.6	83.8	102	0.387	79.8	80.4	99.3
	3 (92)	0.372	74.4	77.2	96.4	0.367	75.7	78.8	96.2
	6 (182)	0.382	76.4	80.1	95.4	0.351	72.4	81.0	89.4
	9 (274)	0.379	75.8	77.6	97.6	0.296	61.1	77.1	79.3
	12 (379)	0.406	81.2	74.8	109	0.329	67.9	72.9	93.1
	18 (531)	0.422	84.8	80.2	106	0.348	71.8	79.7	90.2
	24 (743)	0.409	81.8	81.7	100	0.360	74.3	91.0	81.7
	31 (958)	0.290	58.0	80.3	72.2	0.228	47.1	78.6	59.9
Clover	0	0.403	80.7	80.7	99.8	0.401	82.7	82.7	100
	1 (28)	0.430	86.0	85.3	101	0.347	71.6	85.6	83.6
	3 (96)	0.398	79.6	81.0	98.2	0.293	60.5	70.0	86.5
	6 (183)	0.383	76.6	77.5	98.8	0.334	68.9	85.7	80.5
	9 (273)	0.352	70.4	77.9	90.4	0.242	50.2	77.1	64.8
	12 (376)	0.385	77.0	73.2	105	0.242	50.2	73.1	68.5
	18 (530)	0.361	72.2	77.9	92.6	0.242	50.2	78.9	63.6
	24 (741)	0.391	78.2	83.7	93.4	0.230	47.5	84.7	56.1
	31 (944)	0.519	103.8	98.2	106	0.227	46.9	88.6	52.8
Tomatoes	0	0.377	75.4	75.4	100	0.327	67.4	67.4	100
	1 (34)	0.405	81.0	78.4	103	0.340	70.2	66.7	105
	3 (91)	0.375	75.0	78.3	95.8	0.340	70 2	72.7	96.6
	6 (188)	0.422	84.6	81.0	104	0.407	84.0	72.6	116
	9 (272)	0.370	74.0	79.0	93.6	0.328	67.7	74.6	90.8
	12 (379)	0.393	78.6	77.6	101	0.341	70.4	69.1	102
	18 (528)	0.418	83.6	85.4	97.8	0.381	78.6	83.4	94.1
	24 (735)	0.423	84.6	81.8	104	0.361	74.5	81.7	91.2
	31 (938)	0.427	85.4	81.5	105	0.356	73.5	74.1	99.1
Sorghum straw	0	0.461	92.4	92.4	100	0.391	80.6	80.6	100
	1 (29)	0.392	78.4	78.4	100	0.319	65.8	73.3	89.8
	3 (92)	0.422	84.6	84.9	99.6	0.333	68.7	78.5	87.5
	6 /182)	0.381	76.2	78.1	97.6	0.296	61.1	80.9	75.5
	9 (274)	0.398	79.6	74.5	107	0.263	53.4	75.9	71.6
	12 (377)	0.389	77.8	73.7	106	0.221	45.6	69.2	65.8
	18 (531)	0.410	82.0	84.2	97.4	0.237	48.9	81.1	60.3
	24 (743)	0.409	81.8	80.8	101.2	0.278	57.4	80.9	71.0
	31 (958)	0.323	64.6	73.5	87.8	0.124	25.6	69.1	36.9

a: percent recovered based on nominal level (0.5 mg/kg glyphosate, 0.4845 mg/kg AMPA)

For incurred residues the following levels of glyphosate and AMPA were measured after storing:

Table B.7.6-10: Residue of glyphosate and AMPA found in treated field samples after storage at -18°C

Commodity	Months after sampling	Glyphosate		AMPA	
		mg/kg	% procedual recovery	mg/kg	% procedual recovery
Maize grain	13	1.47	83.1	ND	88.2
	23	1.38	82.0	0.19	81.5
	37	1.17	68.6	0.12	71.9
Soya bean forage	32	0.75	112.1	0.02	102.2
	57	0.50	75.5	ND	70.2
	71	0.53	74.8	ND	72.9
Sorghum stover	7	1.46	89.1	0.03	78.6
	45	2.22	92.4	0.11	80.6
	59	2.08	73.7	0.13	69.2
	71	1.61	80.8	0.07	80.9
Clover	17	3.51	96.1	0.01	87.5
	61	2.99	80.7	ND	82.7
	75	2.74	73.2	ND	73.0
Tomatoes	4	0.09	83.3	ND	82.1
	48	0.08	75.4	ND	67.4
	62	0.04	77.6	ND	69.1
Alfalfa seeds	2	15.5	87.0	0.20	77.2
	13	12.7	74.0	0.19	72.8
	25	13.9	99.3	0.23	91.0
Potatoes	1	ND	79.5	0.19	75.1
	2	0.01	102.9	0.23	97.0
	11	ND	85.4	0.23	80.6
	27	ND	92.3	0.16	89.7

Conclusions:
Based on fortified samples glyphosate was stable (>70% remaining) for at least 31 months in tomatoes and clover. In soya bean forage and sorghum stover the last sample collected after 31 months was slightyl below 70% remaining, but under consideration of the procedual recoveries involved within an acceptable range. For maize grain intermediate samples collected after 6, 9, 12, 18 and 31 months gave recoveries below 70% of the fortified level. However under consideration of the procedual recoveries no significant decline was observered. In addition the day zero sample already gave a low recovery of 82%, suggesting a lesser decline of the stored samples compared to the nominal concentration.
For AMPA samples collected after longer storage intervals gave a significant decline. The following intervals were proven to be stable within this study: maize grain (>31 months), soya bean forage (24 months), clover (6 months), tomatoes (>31 months) and sorghum stover (9 months).

Reference: OECD KIIA 6.1.1

Report: Morgenroth, U.
Storage stability of Glyphosate and AMPA in wheat grain and straw and in rye grain and straw
30.11.1995, Report No.: 303614, ASB2010-14764

Guidelines: BBA Richtlinien Teil IV (1983)

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study the stability of glyphosate and AMPA in wheat and rye (grain and straw) during storage was investigated. Samples of all matrices were fortified at levels of 1.0 mgglyphosate and 0.5 mg AMPA per kg and stored at -20°C for a period of up to 3.5 years. For each sampling interval (0 to 45 months) freshly fortified samples were used to measure the procedual recovery of the analytical method.

For analysis the samples were extracted with 0.1N hydrochloric acid and clean-up by solid phase extraction with Chelix coloumn. After concentration glyphosate and AMPA were analysed separately by HPLC. Glyphoate was oxidized with sodium hypochlorite to obtain glycine. Glycine and AMPA were coulped with o-phthaldialdehyde and 2-mercaptoethanol into fluorescent compounds. The method was sufficietly validated and performed within the acceptable limits of 70-110% average recoverey and 20% RSD. The LOQs were 0.03 mg/kg for each analyte.

Findings:
In the following table the recovered residues after storage at -20°C are summarised:

Table B.7.6-11: Recovery of glyphosate and AMAP in wheat and rye matrices after storage at -20°C

Commodity	Months (days)	Glyphosate (1.0 mg/kg fortification)				AMPA (0.5 mg/kg fortification			
		mg/kg	%[a]	Proc. recovery (%)	corrected %[a]	mg/kg	%[a]	Proc. recovery (%)	corrected %[a]
Wheat, grain	0	0.761	76	-	-	0.393	79	-	-
	6 (190)	0.799	80	NP	-	0.413	83	NP	-
	10 (288)	0.823	82	74	111	0.405	81	80	101
	21 (643)	**0.6486 ~~4.8~~**	**65**	57	114	**0.276**	**55**	64	86
	45 (1349)	**0.6886 ~~8.8~~**	**69**	72	96	**0.230**	**46**	76	61
Wheat, straw	0	0.873	87	-	-	0.361	72	-	-
	6 (190)	0.860	86	86	100	0.413	83	86	97
	10 (288)	0.803	80	82	98	**0.316**	**63**	76	83
	21 (643)	0.733	73	75	97	**0.245**	**49**	68	72
	45 (1349)	1.083	108	108	100	**0.286**	**57**	89	64
Rye, grain	0	0.712	71	-	-	0.399	80	-	-
	6 (190)	0.884	88	106	83	0.395	79	89	89
	10 (288)	0.876	88	84	105	0.395	79	79	100
	21 (643)	0.752	75	73	103	**0.328**	**66**	66	100
	45 (1349)	**0.683**	**68**	90	76	**0.266**	**53**	91	58
Rye, straw	0	0.850	85	-	-	0.429	86	-	-
	6 (190)	0.956	96	NP	-	0.395	79	NP	-
	10 (288)	0.777	78	82	95	**0.295**	**59**	71	83
	21 (643)	**0.599**	**60**	82	73	**0.226**	**45**	75	60
	45 (1349)	0.945	95	114	83	**0.195**	**39**	92	42

NP: not performed

Conclusions:
The results of this study indicate that glyphosate is stable in wheat and rye (grain and straw) for a period of at least 45 months. Although samples near the maximum storage interval tested gave residues slightly below the trigger value of 70% remaining, the procedual recoveries and the relatively low recovery at day 0 suggest stability of the residue.
For AMPA a significant decline was observed in all grain samples stored longer than 10 months and in all straw samples stored longer than 6 months.

Reference: OECD KIIA 6.1.1

Report: Schulz, H.
Determination of the storage stability of Glyphosate in beans, oilseed rape and linseed
1997, Report No.: IF-94/13882-00, ASB2010-14803

Guidelines: EPA OPPTS 860.1380 – Storage Stability Data (1996)
EU Guidance Storage Stability (7032/VI/95 Rev. 5, 22.07.1997)

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
This study investigated the freezer storage stability of glyphosate during storage of dry beans, rape seeds and linseeds for a period of up to 18 months at -18°C. Samples of each matrix were fortified at levels of 2.6 mg/kg for beans, 0.6 mg/kg for rape seeds and 5.7 mg/kg for linseeds. On each analysis data (0, 6, 12, 15 and 18 months after fortification) one unfortified control sample, two fortified stored samples and one freshly prepared fortified sample were analysed for residues of glyphosate.

For analysis the samples were extracted with 0.1N hydrochloric acid and clean-up by solid phase extraction with Chelix coloumn. After concentration glyphosate was analysed by HPLC using post-column derivatisation with fluorescence detection. The method was sufficietly validated and performed within the acceptable limits of 70-110% average recoverey and 20% RSD. The LOQs were 0.05 mg/kg for beans and 0.06 mg/kg for rape seeds and linseeds.

Findings:
In the following table the recovered glyphosate residues after storage at -18°C and the procedual recoveries are summarised.

Table B.7.6-12: Recovery of glyphosate after storage of beans, rape seeds and linseeds at -18°C for up to 18 months

Commodity	Months (days)	Glyphosate		
		mg/kg	%[a]	Proc. recovery (%)
Bean, dry seeds (2.6 mg/kg fortification)	0	2.3, 2.34 (2.32)	89, 90 (90)	81
	6 (174)	2.45, 2.33 (2.39)	94, 90 (92)	89
	12 (371)	2.84, 2.76 (2.8)	109, 106 (108)	79
	15 (456)	2.7, 2.75 (2.73)	104, 106 (105)	84
	18 (551)	2.56, 2.51 (2.54)	99, 96 (98)	97
Rape seeds (0.6 mg/kg fortification)	0	0.584, 0.47 (0.527)	96, 77 (88)	78
	6 (174)	0.529, 0.531 (0.53)	87, 88 (88)	85
	12 (371)	0.564, 0.589 (0.577)	93, 97 (95)	68
	15 (456)	0.633, 0.698 (0.666)	104, 115 (111)	83
	18 (551)	0.59, 0.58 (0.585)	97, 95 (96)	102
Linseeds (5.7 mg/kg fortification)	0	5.34, 5.18 (5.26)	94, 91 (93)	86
	6 (182)	5.17, 4.82 (0.50)	91, 85 (88)	96
	12 (371)	4.98, 6.03 (0.55)	88, 106 (97)	74
	15 (456)	6.22, 5.82 (0.602)	109, 103 (106)	87
	18 (551)	5.05, 5.06 (5.06)	89, 89 (89)	87

Conclusions:
In this study glyphosate was proven to be stable in dry beans, rape seeds and linseeds for at least 18 months.

Reference: OECD KIIA 6.1.1

Report:	Weber, H. Storage stability of residues of Glyphosate and AMPA in various plant materials 11.03.2010, Report No.: FSG-0707, ASB2012-12488
Guidelines:	EPA OPPTS 860.1380 – Storage Stability Data (1996) EU Guidance Storage Stability (7032/VI/95 Rev. 5, 22.07.1997)
Deviations:	none
GLP:	yes
Acceptability:	The study is considered to be acceptable.

Material and Methods:
The freezer storage stablity of glyphosate and AMPA was investigated in barley (grain, straw), maize (grain) and sugar beets (roots, leaves) at -18°C for a period of up to 18 months. Each of the samples was fortified at levels of 1 mg/kg for each analyte. After 0, 6, 12 and 18 months triplicate samples were analysed for the remaining residue and compared to freshly fortified samples to take into account the procedual recovery.

For analysis the samples were extracted with hydrochloric acid and clean-up by solid phase extraction with Chelix coloumn. After concentration glyphosate was analysed by HPLCusing post-column derivatisation with fluorescence detection. The method was sufficietly validated and performed within the acceptable limits of 70-110% average recoverey and 20% RSD. The LOQs were 0.05 mg/kg and the LOD 0.01 mg/kg for all matrices.

Findings:
In the following table the recovered residues for glyphosate and AMPA in the various matrices after storage are summarised.

Table B.7.6-13: Recovered residues of glyphosate and AMPA after storage of barley (grain, straw), maize (grain) and sugar beet (roots, leaves) matrices at -18°C for up to 18 months

Commodity	Months	Glyphosate (1.0 mg/kg fortification)				AMPA (0.5 mg/kg fortification)			
		mg/kg	%[a]	Proc. recovery (%)	corrected %[a]	mg/kg	%[a]	Proc. recovery (%)	corrected %[a]
Barley, grain	0	0.773, 0.738, 0.709 (0.740)	77, 74, 71 (74)	-	-	0.952, 1.024, 0.948 (0.975)	95, 102, 95 (98)	-	-
	6	0.795, 0.759, 0.658 (0.737)	80, 76, 66 (74)	73	101	0.812, 0.815, 0.862 (0.83)	81, 82, 86 (83)	75	110
	12	0.731, 0.637, 0.753 (0.707)	73, 64, 75 (71)	70	101	0.721, 0.679, 0.771 (0.724)	72, 68, 77 (72)	82	88
	18	0.686, 0.734, 0.679 (0.70)	69, 73, 68 (70)	71	99	**0.736, 0.661, 0.637 (0.678)**	**74, 66, 64 (68)**	71	96
Barley, straw	0	0.747, 0.723, 0.749 (0.740)	75, 72, 75 (74)	-	-	0.79, 0.722, 0.751 (0.754)	79, 72, 75 (75)	-	-
	6	**0.669, 0.644, 0.665 (0.659)**	**67, 64, 67 (66)**	72	92	**0.512, 0.487, 0.499 (0.499)**	**51, 49, 50 (50)**	71	75
	12	**0.671, 0.70, 0.683 (0.685)**	**67, 70, 68 (69)**	78	87	**0.334, 0.403, 0.362 (0.366)**	**33, 40, 36 (37)**	85	**42**
	18	0.768, 0.632, 0.75 (0.717)	77, 63, 75 (72)	84	86	0.769, 0.736, 0.797 (0.767)	77, 74, 80 (77)	77	100
Maize, grain	0	0.808, 0.774, 0.821 (0.801)	81, 77, 82 (80)	-	-	0.822, 0.954, 1.035 (0.937)	82, 95, 104 (94)	-	-
	6	**0.643, 0.662, 0.675 (0.66)**	**64, 66, 68 (66)**	76	87	0.826, 0.898, 0.731 (0.818)	83, 90, 73 (82)	77	106
	12	0.802, 0.787, 0.781 (0.79)	80, 79, 79 (79)	79	100	0.72, 0.836, 0.801 (0.786)	72, 84, 70 (79)	85	93
	18	0.718, 0.742, 0.734 (0.731)	72, 74, 73 (73)	76	96	0.896, 0.834, 0.837 (0.856)	90, 83, 84 (86)	80	108

Commodity	**Months**	**Glyphosate (1.0 mg/kg fortification)**				**AMPA (0.5 mg/kg fortification)**			
Sugar beet, roots	0	0.823, 0.847, 0.859 (0.843)	82, 85, 86 (84)	-	-	0.94, 0.868, 0.902 (0.903)	94, 87, 90 (90)	-	-
	6	0.91, 0.916, 0.837 (0.888)	91, 92, 84 (89)	94	95	0.88, 0.791, 0.959 (0.877)	88, 79, 96 (88)	80	110
	12	0.763, 0.777, 0.672 (0.737)	76, 78, 67 (74)	79	95	0.711, 0.668, 0.717 (0.699)	71, 67, 72 (70)	79	89
	18	0.795, 0.862, 0.769 (0.809)	80, 86, 77 (81)	71	114	**0.674, 0.67, 0.658 (0.667)**	**67, 67, 66 (67)**	74	91
Sugar beet, leaves	0	0.81, 0.908, 0.808 (0.842)	81, 91, 81 (84)	-	-	0.839, 0.885, 0.891 (0.872)	84, 89, 89 (87)	-	-
	6	0.748, 0.699, 0.706 (0.718)	75, 70, 71 (72)	80	90	0.759, 0.665, 0.665 (0.696)	76, 67, 67 (70)	81	86
	12	**0.663, 0.635, 0.703 (0.667)**	**66, 64, 70 (67)**	70	96	**0.539, 0.591, 0.559 (0.563)**	**54, 59, 56 (56)**	81	69
	18	**0.637, 0.743, 0.657 (0.679)**	**64, 74, 66 (68)**	80	85	0.735, 0.814, 0.674 (0.741)	74, 81, 67 (74)	73	101

Conclusions:
The results of this study are inconsistent. For glyphosate in barley grain and sugar beet roots as well as for AMPA in maize grain no significant degradation was observed within 18 months. In barley straw (glyphosate and AMPA), maize grain (glyphosate) and sugar beet leaves (AMPA) intermediate samples showed a significant decline, while final samples collected after 18 months were stable (>70% remaining). In view of the generally low procedual recoveries it can concluded that these sample are still within the normal variation of residue, especially since day 0 values also gave recoveries between 70-90%.
Barley grain (AMPA), sugar beet roots (AMPA) and sugar beet leaves (glyphosate) showed a decline at the end of the storage interval investigated. However, under consideration of the procedual recoveries, the remaining levels found were above 70% of the fortification level.
In summary both glyphosate and AMPA showed a strong variation in the results, generally tending towards low recovery values between 70-90%. Under consideration of the procedual recoveries and the concentrations measured in day 0 samples an overall stability of both analytes in the matrices investigated seems plausible for a storage interval of 18 months.

Reference: OECD KIIA 6.1.1

Report: Hubbart, N. S.
Determination of Glyphosate in soybean raw agricultural

	commodities (RAC) - Stability report June 1993, Report No.: 91210, ASB2010-14765
Guidelines:	EPA Pesticide Assessment Guideline (171-4)
Deviations:	none
GLP:	yes
Acceptability:	The study is considered to be additional information.

Material and Methods:
In this study samples of soya beans and soya straw were fortified with glyphosate or AMPA at levels of 1 mg/kg and freezer stored at -10°C for a period of up to thirteen months. After selected intervals duplicate samples were taken and analysed for the remaining residues of both analytes. In parallel freshly fortified samples were spiked at 0.1 and 1 mg/kg to take into account the procedual recovery of the analytical method.

The analysis was conducted by HPLC with fluorescence detection. After homogenisation the samples were extracted with chloroform/0.1 N HCl and isolated with a solid phase ion exchange column. The concentrated extracts were oxidized with sodium hypochlorid and coupled with o-phthalaldehyde in presence of mercaptoethanol, to form fluorophors for detection. The validated LOQ of the analytical method was 0.05 mg/kg, although for the storage stability analysis only an LOQ of 0.1 mg/kg was achieved by the respective laboratory.

Findings:
In the following tables the recovered glyphosate and AMPA residues after storage and the procedual recoveries based on freshly fortified samples are summarised.

Table B.7.6-14: Recovered glyphosate residues in soya bean seeds and straw (1 mg/kg fortification)

Month of storage (days)	mg/kg recovered (mean)	% (mean)	proc. recovery (%)	mean proc. recovery (%)	corrected recovery (%)
Soya seeds					
0 (5)	0.762, 0.739 (0.751)	76, 74 (75)	0.1 mg/kg: 64 1 mg/kg: 65	65	115
0.5 (14)	0.74, 0.701 (0.721)	74, 70 (72)	0.1 mg/kg: 73 1 mg/kg: 70	72	100
1.5 (45)	0.526, 0.751 (0.639)	**53, 75 (64)**	0.1 mg/kg: 68 1 mg/kg: 50	**59**	**108**
6 (183)	0.857, 0.797 (0.827)	86, 80 (83)	0.1 mg/kg: 108 1 mg/kg: 64	86	97
Soya straw					
0	0.846, 0.705 (0.776)	85, 71 (78)	0.1 mg/kg: 50 1 mg/kg: 74	62	126
0.5 (15)	0.759, 0.608 (0.684)	**76, 61 (68)**	0.1 mg/kg: 55 1 mg/kg: 80	**67**	**101**
1.5 (44)	0.846, 0.803 (0.825)	85, 80 (83)	0.1 mg/kg: 114 1 mg/kg: 68	91	91
3 (102)	0.709, 0.633 (0.671)	**71, 63 (67)**	0.1 mg/kg: 78 1 mg/kg: 75	**77**	**87**
10 (300)	0.666, 0.765 (0.712)	67, 77 (71)	0.1 mg/kg: 99 1 mg/kg: 91	95	75
13 (398)	0.718, 0.791 (0.755)	72, 79 (76)	0.1 mg/kg: 126 1 mg/kg: 71	98	78

Table B.7.6-15: Recovered AMPA residues in soya bean seeds and straw (1 mg/kg fortification)

Month of storage (days)	mg/kg recovered (mean)	% (mean)	proc. recovery (%)	mean proc. recovery (%)	corrected recovery (%)
Soya seeds					
0 (5)	0.789, 0.779 (0.784)	79, 78 (78)	0.1 mg/kg: 68 1 mg/kg: 69	69	113
0.5 (14)	0.739, 0.805 (0.772)	74, 81 (77)	0.1 mg/kg: 84 1 mg/kg: 86	85	91
1.5 (45)	0.673, 0.769 (0.721)	67, 77 (72)	0.1 mg/kg: 79 1 mg/kg: 52	66	109
6 (183)	0.729, 0.704 (0.717)	73, 70 (72)	0.1 mg/kg: 61 1 mg/kg: 72	66	109
Soya straw					
0	0.802, 0.733 (0.768)	80, 73 (77)	0.1 mg/kg: 66 1 mg/kg: 74	70	110
0.5 (15)	0.625, 0.704 (0.665)	**63, 70 (67)**	0.1 mg/kg: 66 1 mg/kg: 76	**71**	**94**
1.5 (44)	0.687, 0.681 (0.684)	**69, 68 (68)**	0.1 mg/kg: 68 1 mg/kg: 65	**67**	**101**
3 (102)	0.515, 0.552 (0.534)	**52, 55 (53)**	0.1 mg/kg: 62 1 mg/kg: 70	**66**	**80**
10 (300)	0.406, 0.564 (0.485)	**41, 56 (49)**	0.1 mg/kg: 82 1 mg/kg: 88	**85**	**54**
13 (398)	0.605, 0.516 (0.561)	**61, 52 (56)**	0.1 mg/kg: 62 1 mg/kg: 65	**63**	**89**

Conclusions:
In this study the procedual recoveries were generally very low, often not achieving a recovery rate of 70% of the freshly fortified concentrations. Since this is an overall pattern within the study, it can be concluded that the analytical method used had a low precision, resulting in a large variation of the results.
For glyphosate intermediate samples for seeds and straw showed uncorrected recovery values below the significance trigger of 70% remaining. However samples collected after longer storage intervals were above this trigger, suggesting the overall stabililty of glyphosate in both matrices for up to 6 months in soya bean seeds and up to 13 months in soya bean straw.
Uncorrected residues recovered for AMPA from soya bean seeds were between 72-78% after up to 6 months. In soya bean straw all samples except at fortification gave recovered residues below 70% of the nominal concentration. Taking into account the low procedual recoveries, most samples lay between 70-100% remaining, however one single corrected value after 10 month storage was 54 %. In summary a slight degradation was observed for AMPA in soya bean straw but the poor procedual recoveries do not allow the estimation of reliable storage intervals based on this study.

Reference:	OECD KIIA 6.1.1
Report:	Hubbart, N. S. Determination of Glyphosate in pasture grasses - Stability report June 1993, Report No.: 91212, ASB2010-14766
Guidelines:	EPA Pesticide Assessment Guideline (171-4)
Deviations:	none
GLP:	yes
Acceptability:	The study is considered to be additional information.

Material and Methods:
In this study pasture samples were fortified with glyphosate or AMPA at levels of 1 mg/kg and freezer stored at -10°C for a period of up to twelve months. After selected intervals duplicate samples were taken and analysed for the remaining residues of both analytes. In parallel freshly fortified samples were spiked at 0.1 and 1 mg/kg to take into account the procedual recovery of the analytical method.

The analysis was conducted by HPLC with fluorescence detection. After homogenisation the samples were extracted with chloroform/0.1 N HCl and isolated with a solid phase ion exchange column. The concentrated extracts were oxidized with sodium hypochlorid and coupled with o-phthalaldehyde in presence of mercaptoethanol, to form fluorophors for detection. The validated LOQ of the analytical method was 0.05 mg/kg, although for the storage stability analysis only an LOQ of 0.1 mg/kg was achieved by the respective laboratory.

Findings:
In the following tables the recovered glyphosate and AMPA residues after storage and the procedual recoveries based on freshly fortified samples are summarised.

Table B.7.6-16: Recovered residues of glyphosate and AMPA from samples fortified at levels of 1 mg/kg after freezer storage

Month of storage (days)	mg/kg recovered (mean)	% (mean)	proc. recovery (%)	mean proc. recovery (%)	corrected recovery (%)
Glyphosate					
0 (6)	0.781, 0.929 (0.855)	78, 93 (86)	0.1 mg/kg: 110 1 mg/kg: 86	98	88
0 (10)	1.02, 1.09 (1.06)	102, 109 (106)	0.1 mg/kg: 122 1 mg/kg: 104	113	94
1 (19)	0.922, 0.911 (0.917)	92, 91 (92)	0.1 mg/kg: 140 1 mg/kg: 91	115	80
2 (51)	0.831, 0.697 (0.764)	83, 70 (76)	0.1 mg/kg: 244 1 mg/kg: 98	98 (based on 1 mg/kg fort. only)	78
3 (95)	0.755, 0.637 (0.692)	**76, 64 (69)**	0.1 mg/kg: 74 1 mg/kg: 83	**79**	**87**
6 (187)	0.706, 0.772 (0.739)	71, 77 (74)	0.1 mg/kg: 98 1 mg/kg: 79	89	83
12 (362)	0.849, 0.758 (0.804)	85, 76 (80)	0.1 mg/kg: 102 1 mg/kg: 77	90	89
AMPA			0.1 mg/kg: 55 1 mg/kg: 80		
0 (6)	0.626, 0.553 (0.59)	**63, 55 (59)**	0.1 mg/kg: 67 1 mg/kg: 81	**74**	**80**
0 (10)	0.706, 0.731 (0.719)	71, 73 (72)	0.1 mg/kg: 77 1 mg/kg: 91	84	86
1 (19)	0.688, 0.686 (0.687)	**69, 69 (69)**	0.1 mg/kg: 75 1 mg/kg: 80	**77**	**90**
2 (51)	0.69, 0.638 (0.664)	**69, 64 (66)**	0.1 mg/kg: 70 1 mg/kg: 74	**72**	**92**
3 (95)	0.54, 0.634 (0.587)	**54, 63 (59)**	0.1 mg/kg: 73 1 mg/kg: 80	**77**	**77**
6 (187)	0.554, 0.654 (0.604)	**55, 65 (60)**	0.1 mg/kg: 80 1 mg/kg: 74	**77**	**78**
12 (362)	0.756, 0.729 (0.743)	76, 73 (74)	0.1 mg/kg: 84 1 mg/kg: 78	81	91

Conclusions:

In this study the results of the procedual recoveries based on freshly fortied samples ranged from 55-244% for glyphosate and 67-91% for AMPA, suggesting a relative low precision of the analyical method.

For glyphosate all uncorrected recoveries, except one sample after 3 months, were above the trigger value for a significant degradation of 70% remaining.

For AMPA samples collected after 6, 19, 51, 95 and 187 days gave unsatifactory recoveries of 59-69% remaining. However corresponding procedual recoveries were also relatively low, suggesting corrected recoveries well above 70% remaining. The samples collected after 10 and 362 days gave 72 % and 74 % remaining.

In summary it can be concluded that both glyphosate and AMPA are stable during the 12 month storage interval investigated. However, the high amplitude in the procedual recoveries and the low initial residues directly after fortification lead to a strong variation in the results, suggesting to consider the study as additional information.

B.7.6.7.1.2 Glyphosate-trimesium and AMPA

Reference: OECD KIIA 6.1.1

Report: McKay, J. C.
Storage stability validation for ICIA0224 in raw agricultural commodities
30.04.1989, Report No.: WRC 89-22, RIP9500028

Guidelines: EPA Guideline 171-4

Deviations: ~~original guideline not submitted~~none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study the storage stability of glyphosate-trimesium and AMPA was investigated in sorghum grain, soya bean seeds & straw and wheat grain. For this purposes duplicate samples (triplicate samples at the final time point of 4 years) of each material were macerated and fortified at 1.0 to 5.0 mg/kg for the glyphosate anion and AMPA and at 0.46 to 2.3 mg/kg for the trimethylsulfonium cation (TMS). After the fortification the samples were stored at -20°C ± 10°C for periods up to 2 years (4 years for sorghum grain).
No samples for measuring the procedual recovery were freshly fortified.

For the analyses of freshly fortified and stored samples acidic water extraction and cation exchange columns were used. The cleaned extract was analysed by GC (TMS, LOQ: 0.05 mg/kg) or HPLC following derivatisation with 9-fluorenylmethyl (glyphosate and AMPA, LOQ: 0.1-0.2 mg/kg). Both analytical methods were validated against a broad variery of matrices (water, acidic, starch, oil, difficult) with acceptable average recoveries between 70-110% and a RSD of <20%.

Findings:
In the following table the recovered residues after storage, based on the nominal level, are summarised:

Table B.7.6-17: Percent recovered after storage of sorghum grain, soya bean seeds & straw and wheat grain

Matrix/Analyte	Fortification in mg/kg	Percent of nominal fortification recovered (% of day 0)[a]				
		0 months (0 d)	6 months (191 d)	12 months (285-394 d)	24 months (633-786 d)	48 months (1462 d)
Sorghum grain	TMS: 2.3	89 (100)	NA	85 (96)	84 (94)	90 (101)
	Glyphosate: 5.0	93 (100)	NA	90 (97)	94 (101)	100 (108)
	AMPA: 5.0	83 (100)	NA	84 (101)	69 (83)	82 (99)
Soya bean seeds	TMS: 0.46	86 (100)	69 (80)	90 (105)	102 (119)	NA
	Glyphosate: 1.0	100 (100)	108 (108)	75 (75)	104 (104)	NA
	AMPA: 1.0	90 (100)	86 (96)	106 (118)	81 (90)	NA
Soya bean straw	TMS: 0.46	66 (100)	74 (112)	76 (115)	64 (97)	NA
	Glyphosate: 1.0	100 (100)	102 (102)	84 (84)	106 (106)	NA
	AMPA: 1.0	74 (100)	70 (95)	93 (126)	82 (111)	NA
Wheat grain	TMS: 0.46	93 (100)	75 (81)	74 (80)	90 (97)	NA
	Glyphosate: 1.0	92 (100)	88 (96)	82 (89)	76 (83)	NA
	AMPA: 1.0	98 (100)	87 (89)	71 (72)	70 (71)	NA

a mean of duplicate/triplicate samples

Conclusions:
This study demonstrates that glyphosate, AMPA and the trimethylsulfonium-cation are stable in soya bean seeds & straw and in wheat grain for at least 24 months and in sorghum grain for at least 48 month. Although single values <70% of the nominal concentration were found, freshly fortified samples also showed such values suggesting analytical variation or matrix effect during fortification. The residues levels itself over the whole storage period remained above 70 % of the day zero values, no suggesting a significant decline.

B.7.6.7.1.3 Glyphosate, N-acetyl-glyphosate, AMPA and N-acetyl-AMPA

Reference: OECD KIIA 6.1.1

Report: Schwartz, N. L.
Stability of Glyphosate, N-Acetylglyphosate and Aminomethyl phosphonic acid in GAT corn forage, grain, and stover, stored frozen 15.10.2007, Report No.: DuPont-17379, ASB2008-2655

Guidelines: EPA OPPTS 860.1380 – Storage Stability Data (1996)
EU Guidance Storage Stability (7032/VI/95 Rev. 5, 22.07.1997)

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study the storage stability of N-acety-glyphosate, glyphosate and AMPA in maize (forage, grain and stover) was investigated. The raw agricultural commodities were fortified with 0.5 mg/kg of each analyte and stored at -20 °C for a period of 12 months.

After specified time intervals duplicate samples were extracted using 0.1% formic acid/methanol (96/4 v/v), cleaned by SPE and analyzed using LC/MS/MS (Method DuPont

15444). Validated LOD and LOQ levels were 0.02 and 0.05 mg/kg, respectively, with mean recoveries between 70-110% and a RSD of <20%.

Findings:
In the following table the recovered residues and procedual recoveries for the three analytes after storage are summarised:

Table B.7.6-18: Recovery of glyphosate, N-acetyl-glyphosate and AMPA in stored maize matrices

Commodity	Months	Glyphosate				N-acetyl-glyphosate				AMPA			
		mg/kg (mean)	%[a] (mean)	mean proc. recovery (%)	mean corrected %[a]	mg/kg (mean)	%[a] (mean)	mean proc. recovery (%)	mean corrected %[a]	mg/kg (mean)	%[a] (mean)	mean proc. recovery (%)	mean corrected %[a]
Maize, forage	0	0.52, 0.5 (0.51)	104, 100 (102)	-	-	0.45, 0.46 (0.46)	90, 92 (91)	-	-	0.47, 0.49 (0.48)	94, 98 (96)	-	-
	1	0.51, 0.5 (0.51)	102, 100 (101)	106	97	0.36, 0.35 (0.36)	72, 70 (71)	72	99	0.45, 0.45 (0.45)	90, 0 (90)	96	95
	3	0.48, 0.45 (0.47)	96, 90 (93)	87	107	0.41, 0.40 (0.41)	82, 80 (81)	79	104	0.5, 0.46 (0.48)	100, 92 (96)	97	91
	6	0.4, 0.41 (0.41)	80, 82 (81)	83	99	0.37, 0.38 (0.38)	74, 76 (75)	81	94	0.37, 0.37 (0.37)	74, 74 (74)	82	91
	12	0.49, 0.48 (0.49)	98, 96 (97)	99	99	0.43, 0.44 (0.44)	86, 88 (87)	99	89	0.38, 0.35 (0.37)	76, 70 (73)	95	78
Maize, grain	0	0.52, 0.5 (0.51)	104, 100 (102)	-	-	0.49, 0.49 (0.49)	98, 98 (98)	-	-	0.5, 0.48 (0.49)	100, 96 (98)	-	-
	1	0.54, 0.57 (0.56)	108, 114 (111)	106	106	0.37, 0.47 (0.42)	74, 94 (84)	81	105	0.49, 0.47 (0.48)	98, 94 (96)	105	92
	3	0.41, 0.44 (0.43)	82, 88 (86)	88	97	0.5, 0.51 (0.51)	100, 102 (101)	80	102	0.46, 0.41 (0.44)	92, 82 (87)	92	111
	6	0.40, 4.2 (0.41)	80, 84 (82)	80	103	0.37, 0.37 (0.37)	74, 74 (74)	79	94	0.42, 0.41 (0.42)	84, 82 (83)	86	96
	12	0.44, 0.53 (0.49)	88, 106 (97)	91	107	0.40, 0.41 (0.41)	80, 82 (81)	79	102	0.44, 0.47 (0.46)	88, 94 (91)	85	103
Maize, stover	0	0.53, 0.53 (0.53)	106, 106 (106)	-	-	0.49, 0.45 (0.47)	98, 90 (94)	-	-	0.47, 0.47 (0.47)	94, 94 (94)	-	-
	1	0.5, 0.5 (0.5)	100, 100 (100)	96	104	0.43, 0.43 (0.43)	86, 86 (86)	87	98	0.45, 0.47 (0.46)	90, 94 (92)	93	101
	3	0.44, 0.39 (0.42)	88, 78 (83)	90	93	0.41, 0.38 (0.4)	82, 76 (79)	77	102	0.41, 0.43 (0.42)	82, 86 (84)	96	89
	6	0.44, 0.43 (0.44)	88, 86 (87)	100	87	0.46, 0.48 (0.47)	92, 96 (94)	101	94	0.35, 0.39 (0.37)	70, 78 (74)	92	81

Commodity	**Months**	**Glyphosate**				**N-acetyl-glyphosate**				**AMPA**			
		mg/kg (mean)	%[a] (mean)	mean proc. recovery (%)	mean corrected %[a]	mg/kg (mean)	%[a] (mean)	mean proc. recovery (%)	mean corrected %[a]	mg/kg (mean)	%[a] (mean)	mean proc. recovery (%)	mean corrected %[a]
	12	0.52, 0.5 (0.51)	104, 100 (102)	93	97	**0.32, 0.32 (0.32)**	**64, 64 (64)**	101	101	0.46, 0.46 (0.46)	92, 92 (92)	86	75

a: percent recovered based on nominal level (0.5 mg/kg)

Conclusions:
This study demonstrates the storage stability of glyphosate, N-acetyl-glyphosate and AMPA in maize matrices (grain, forage and stover) for a period of at least 12 months. For N-acetyl-glyphosate in maize stover the final sample collected after 12 months gave residues <70% of the nominal level. However the concurrent recoveries were exceptionally low (64%), suggesting an underreporting of the true residue remaining.

Reference:	OECD KIIA 6.1.1
Report:	Schwartz, N. L. Stability of Glyphosate and metabolites in corn green plant, forage, grain, and stover containing the GAT and ZM-HRA genes during frozen storage: Interim report 29.10.2007, Report No.: DuPont-20094, ASB2008-2656
Guidelines:	EPA OPPTS 860.1380 – Storage Stability Data (1996) EU Guidance Storage Stability (7032/VI/95 Rev. 5, 22.07.1997)
Deviations:	none
GLP:	yes
Acceptability:	The study is considered to be acceptable.

Material and Methods:
In this study the storage stability of N-acety-glyphosate, glyphosate, N-acetyl-AMPA and AMPA in maize (green plant, forage, grain and stover) was investigated. The raw agricultural commodities were fortified with 0.5 mg/kg of each analyte and stored at -20°C for a period of up to 9 months (N-acetyl-AMPA only one month).

After specified time intervals duplicate samples were extracted using 0.1% formic acid/methanol (96/4 v/v), cleaned by SPE and analyzed using LC/MS/MS (Method DuPont 15444). Validated LOD and LOQ levels were 0.02 and 0.05 mg/kg, respectively, with mean recoveries between 70-110% and a RSD of <20%.

Findings:
In the following table the recovered residues and procedual recoveries for the four analytes after storage are summarised:

Table B.7.6-19: Recovered residues of glyphosate, N-acetyl-glyphosate, AMPA and N-acetyl-AMPA in maize matrices after storage at intervals of up to nine months

Commodity	Months	Glyphosate			N-acetyl-glyphosate			AMPA			N-acetyl- AMPA		
		mg/kg (mean)	%[a] (mean)	mean proc. recovery (%)	mg/kg (mean)	%[a] (mean)	mean proc. recovery (%)	mg/kg (mean)	%[a] (mean)	mean proc. recovery (%)	mg/kg (mean)	%[a] (mean)	mean proc. recovery (%)
Maize, green plant	0	0.43, 0.42 (0.425)	86, 84 (85)	-	0.4, 0.43 (0.415)	80, 86 (83)	-	0.44, 0.42 (0.43)	88, 84 (86)	-	0.48, 0.41 (0.445)	96, 98 (97)	-
	1	0.43, 0.44 (0.435)	86, 88 (87)	104, 92 (98)	0.41, 0.42 (0.415)	82, 84 (83)	96, 84 (90)	0.35, 0.38 (0.365)	70, 76 (73)	92, 86 (89)	0.41, 0.41 (0.41)	82, 82 (82)	84, 84 (84)
	3	0.49, 0.48 (0.485)	98, 96 (97)	98, 98 (98)	0.45, 0.45 (0.45)	90, 90 (90)	94, 96 (95)	0.37, 0.41 (0.39)	74, 82 (78)	92, 94 (93)	NA	-	-
	6	0.45, 0.44 (0.445)	90, 88 (89)	90, 86 (88)	0.48, 0.47 (0.475)	96, 94 (95)	88, 92 (90)	0.37, 0.36 (0.365)	74, 72 (73)	88, 88 (88)	NA	-	-
	9	0.43, 0.42 (0.425)	86, 84 (85)	84, 90 (87)	0.42, 0.47 (0.445)	84, 94 (89)	100, 80 (90)	**0.32, 0.32 (0.32)**	**64, 64 (64)**	80, 74 (77)	NA	-	-
Maize, forage	0	0.47, 0.48 (0.475)	94, 96 (95)	-	0.47, 0.47 (0.47)	94, 94 (94)	-	0.45, 0.46 (0.455)	90, 92 (91)	-	0.48, 0.48 (0.48)	96, 96 (96)	-
	1	0.44, 0.43 (0.435)	88, 86 (87)	86, 76 (81)	0.35, 0.35 (0.35)	70, 70 (70)	86, 70 (78)	0.36, 0.36 (0.36)	72, 72 (72)	74, 76 (75)	0.46, 0.45 (0.455)	92, 90 (91)	88, 86 (87)
	3	0.46, 0.49 (0.475)	98, 92 (95)	104, 98 (101)	0.47, 0.44 (0.455)	94, 88 (91)	94, 90 (92)	0.42, 0.43 (0.425)	84, 86 (85)	94, 90 (92)	NA	-	-
	6	0.47, 0.46 (0.465)	94, 92 (93)	88, 92 (90)	0.43, 0.44 (0.435)	86, 88 (87)	88, 84 (86)	0.37, 0.43 (0.40)	74, 86 (80)	88, 90 (89)	NA	-	-
	9	0.45, 0.45 (0.45)	90, 90 (90)	80, 86 (83)	0.45, 0.49 (0.47)	90, 98 (94)	84, 78 (81)	0.38, 0.36 (0.37)	76, 72 (74)	90, 90 (90)	NA	-	-
Maize, grain	0	0.45, 0.42 (0.435)	90, 84 (87)	-	0.46, 0.42 (0.44)	92, 84 (88)	-	0.48, 0.41 (0.445)	96, 82 (89)	-	0.46, 0.45 (0.455)	92, 90 (91)	-
	1	0.47, 0.47 (0.47)	94, 94 (94)	94, 92 (93)	0.47, 0.49 (0.48)	94, 98 (96)	88, 92 (90)	0.36, 0.36 (0.36)	72, 72 (72)	82, 74 (78)	0.43, 0.42 (0.425)	86, 84 (85)	78, 84 (81)
	3	0.41, 0.44 (0.425)	82, 88 (85)	88, 100 (94)	0.44, 0.51 (0.475)	88, 102 (95)	90, 106 (98)	0.43, 0.46 (0.445)	86, 92 (89)	88, 90 (89)	NA	-	-
	6	0.45, 0.48	96, 90	84, 96	0.42, 0.42	84, 84	84, 82	0.48, 0.48	96, 96	96, 96	NA	-	-

Commodity	Months	Glyphosate			N-acetyl-glyphosate			AMPA			N-acetyl- AMPA		
		(0.465)	(93)	(90)	(0.42)	(84)	(83)	(0.48)	(96)	(96)			
	9	0.41, 0.42 (0.415)	82, 84 (83)	78, 86 (82)	0.38, 0.41 (0.395)	76, 82 (79)	80, 74 (77)	0.42, 0.43 (0.425)	84, 86 (85)	80, 90 (85)	NA	-	-
Maize, stover	0	0.45, 0.54 (0.495)	90, 108 (99)	-	0.45, 0.48 (0.465)	90, 96 (93)	-	0.42, 0.45 (0.435)	84, 90 (87)	-	0.44, 0.43 (0.435)	88, 86 (87)	-
	1	0.53, 0.53 (0.53)	106, 106 (106)	94, 92 (93)	0.53, 0.56 (0.545)	106, 112 (109)	88, 92 (90)	0.43, 0.44 (0.435)	86, 88 (87)	82, 74 (78)	0.48, 0.45 (0.455)	96, 90 (93)	78, 84 (81)

NA: not analysed

Conclusions:
Int his study glyphosate and N-acetyl-glyphosate proved to be stable in maize (green plant, forage and grain) for at least 9 months.
AMPA was also stable in maize forage and grain for at least 9 months. In green plants only 64% of the applied concentration were recovered. However the procedual recovery was also very low for these samples (77%). In addition in maize forage, which is a closely related matrix to green maize plants, no significant degradation was observed. In summary it can be concluded that AMPA is also stable for at least 9 months in green maize plants.
For N-acetly-AMPA only samples stored for 1 months were analysed, no showing a significant degradation.
In maize stover all analytes were stored for one month without significant degradation.

Reference:	OECD KIIA 6.1.1
Report:	Schwartz, N. L. Stability of Glyphosate, N-Acetylglyphosate, Aminomethyl phosphonic acid and N-Acetyl AMPA in GAT soybean forage, seed, and hay stored frozen: Second interim report 15.10.2007, Report No.: DuPont-17573, ASB2008-2654
Guidelines:	EPA OPPTS 860.1380 – Storage Stability Data (1996) EU Guidance Storage Stability (7032/VI/95 Rev. 5, 22.07.1997)
Deviations:	none
GLP:	yes
Acceptability:	The study is considered to be acceptable.

Material and Methods:
In this study the storage stability of N-acety-glyphosate, glyphosate, N-acetyl-AMPA and AMPA in soya beans (forage, seeds and hay) was investigated. The raw agricultural commodities were fortified with 0.5 mg/kg of each analyte and stored at -20°C for a period of up to 12 months (N-acetyl-AMPA only one month).

After specified time intervals duplicate samples were extracted using 0.1% formic acid/methanol (96/4 v/v), cleaned by SPE and analyzed using LC/MS/MS (Method DuPont 15444). Validated LOD and LOQ levels were 0.02 and 0.05 mg/kg, respectively, with mean recoveries between 70-110% and a RSD of <20%.

Findings:
In the following table the recovered residues and procedual recoveries for the four analytes after storage are summarised:

Table B.7.6-20: Recovered residues of glyphosate, N-acetyl-glyphosate, AMPA and N-acetyl-AMPA in soya bean matrices after storage at intervals of up to twelve months

Commodity	Months	Glyphosate			N-acetyl-glyphosate			AMPA			N-acetyl- AMPA		
		mg/kg (mean)	%[a] (mean)	mean proc. recovery (%)	mg/kg (mean)	%[a] (mean)	mean proc. recovery (%)	mg/kg (mean)	%[a] (mean)	mean proc. recovery (%)	mg/kg (mean)	%[a] (mean)	mean proc. recovery (%)
Soya bean, forage	0	0.48, 0.49 (0.485)	96, 98 (97)	-	0.53, 0.53 (0.53)	106, 106 (106)	-	0.45, 0.45 (0.45)	90, 90 (90)	-	0.41, 0.41 (0.41)	82, 82 (82)	-
	1	0.43, 0.46 (0.445)	86, 92 (89)	92, 94 (93)	0.48, 0.46 (0.47)	96, 92 (94)	90, 92 (91)	0.43, 0.41 (0.42)	86, 82 (84)	84, 78 (81)	0.41, 0.38 (0.395)	82, 76 (79)	86, 86 (86)
	3	0.38, 0.39 (0.385)	76, 78 (77)	66, 76 (71)	0.53, 0.52 (0.525)	106, 104 (105)	106, 108 (107)	0.41, 0.40 (0.405)	82, 80 (81)	92, 86 (89)	NA	-	-
	6	0.43, 0.44 (0.435)	86, 88 (87)	88, 92 (90)	0.47, 0.53 (0.50)	94, 106 (100)	98, 100 (99)	0.51, 0.47 (0.49)	102, 94 (98)	112, 114 (113)	NA	-	-
	9	0.39, 0.43 (0.41)	78, 86 (82)	84, 88 (86)	0.46, 0.46 (0.46)	92, 92 (92)	84, 94 (89)	0.39, 0.38 (0.385)	78, 76 (77)	86, 78 (82)	NA	-	-
	12	0.45, 0.45 (0.45)	90, 90 (90)	84, 84 (84)	0.53, 0.53 (0.53)	106, 106 (106)	104, 106 (105)	0.37, 0.37 (0.37)	74, 74 (74)	78, 82 (80)	NA	-	-
Soya bean, seeds	0	0.37, 0.38 (0.375)	76, 74 (75)	-	0.47, 0.44 (0.455)	94, 88 (91)	-	0.36, 0.40 (0.38)	72, 80 (76)	-	0.40, 0.40 (0.40)	80, 80 (80)	-
	1	0.39, 0.41 (0.40)	78, 82 (80)	80, 78 (79)	0.43, 0.40 (0.415)	86, 80 (83)	80, 88 (84)	0.39, 0.36 (0.375)	78, 72 (75)	70, 80 (75)	0.34, 0.38 (0.36)	68, 76 (72)	70, 70 (70)
	3	0.37, 0.38 (0.375)	74, 76 (75)	76, 80 (78)	0.39, 0.39 (0.39)	78, 78 (78)	78, 78 (78)	0.42, 0.39 (0.405)	84, 78 (81)	88, 80 (84)	NA	-	-
	6	0.40, 0.39 (0.395)	80, 78 (79)	78, 84 (81)	0.40, 0.39 (0.395)	80, 78 (79)	76, 82 (79)	0.51, 0.53 (0.52)	102, 106 (104)	106, 110 (108)	NA	-	-
	9	0.35, 0.37 (0.36)	70, 74 (72)	72, 72 (72)	0.38, 0.37 (0.375)	76, 74 (75)	76, 74 (75)	0.37, 0.39 (0.38)	74, 78 (76)	82, 78 (80)	NA	-	-
	12	0.38, 0.38 (0.38)	76, 76 (76)	76, 76 (76)	0.42, 0.42 (0.42)	84, 84 (84)	82, 82 (82)	0.38, 0.39 (0.385)	76, 78 (77)	78, 76 (77)	NA	-	-
Soya bean, hay	0	0.36, 0.46 (0.41)	72, 92 (82)	-	0.51, 0.52 (0.515)	102, 104 (103)	-	0.37, 0.44 (0.405)	74, 88 (81)	-	0.41, 0.41 (0.41)	82, 82 (82)	-
	1	0.37, 0.36	74, 72	64, 74	0.41, 0.42	82, 84	82, 80	0.53, 0.48	106, 96	96, 98	0.31, 0.41	62, 82	82, 74

Commodity	Months	Glyphosate			N-acetyl-glyphosate			AMPA			N-acetyl- AMPA		
		(0365)	(73)	(69)	(0.415)	(83)	(81)	(0.505)	(101)	(97)	(0.36)	(72)	(78)
	3	0.38, 0.39 (0.385)	76, 78 (77)	70, 72 (71)	0.58, 0.51 (0.545)	116, 102 (109)	80, 98 (89)	0.45, 0.42 (0.435)	90, 84 (87)	74, 86 (80)	NA	-	-
	6	0.38, 0.37 (0.375)	76, 74 (75)	68, 74 (71)	0.42, 0.40 (0.41)	84, 80 (82)	74, 80 (77)	0.43, 0.37 (0.40)	86, 74 (80)	74, 88 (81)	NA	-	-
	9	0.38, 0.40 (0.39)	76, 80 (78)	76, 82 (79)	0.36, 0.43 (0.395)	72, 86 (79)	86, 76 (81)	0.37, 0.39 (0.38)	74, 78 (76)	78, 80 (79)	NA	-	-
	12	**0.34, 0.34 (0.34)**	**68, 68 (68)**	74, 72 (73)	0.47, 0.44 (0.455)	94, 88 (91)	98, 96 (97)	**0.33, 0.33 (0.33)**	**66, 66 (66)**	80, 72 (76)	NA	-	-

NA: not analysed

Conclusions:
The residues of glyphosate, N-acetyl-glyphosate and AMPA showed no significant degradation in soya bean matrices (forage, seeds and hay) within 12 months. For soya bean hay glyphosate and AMPA were slightly below the trigger value of 70% remaining for a significant degradation, however the procedual recoveries were 73% and 76%, respectively, suggesting a higher true residue concentration.
For N-acetyl-AMPA only one month storage interval was investigated for all matrices. No significant decline was observed within this time frame.

B.7.6.7.2 Animal matrices

Reference: OECD KIIA 6.1.1

Report: Manning, M. J.; Mueth, M. G.
Storage stability of Glyphosate and AMPA in swine tissues, dairy cow tissues and milk laying hen tissues and eggs
February 1988, Report No.: MSL-7515, RIP9501253

Guidelines: not stated

Deviations: not applicable

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study samples of animal tissues were stored to investigate the decline of residues. The samples were fortified with both glyphosate and AMPA approximately corresponding to levels found in livestock animal feeding studies. To account of the procedual recovery freshly fortified samples were analysed spiked at levels comparable to the levels found in the various tissues.

All samples were analysed using validated analytical methods. The residue was extracted with water and chloroform. After concentration of the extract, a clean-up was performed with a Chelex column an ion-exchange chromatography. After concentration glyphosate and AMPA were analysed by HPLC using post-column derivatisation (o-phthalaldehyde) with fluorescence detection.

Findings:

In the following tables the recovered residues in swine, cattle and chicken matrices fortified with glyphosate or AMPA at various levels are summarised.

Table B.7.6-21: Recovered glyphosate residues after storage of animal matrices

Commodity (fortification level)	Months (days)	mg/kg recovered	%	Proc. recovery (%)	average corrected %
Pig fat (0.2 mg/kg glyphosate)	0	0.164, 0.145, 0.154 (0.154)	82, 73, 77 ()	-	-
	7 (210)	0.182, 0.190 (0.186)	91, 95 (93)	98	95
	15 (437)	0.161, 0.159 (0.160)	81, 80 (81)	96	84
	17 (524)	0.169, 0.165 (0.167)	85, 83 (84)	101	83
	26 (794)	0.172, 0.178 (0.175)	86, 86 (86)	85	101
Pig muscle (0.2 mg/kg glyphosate)	0	0.207, 0.187, 0.18 (0.191)	104, 94, 90 (96)	-	-
	7 (213)	0.167, 0.165 (0.166)	84, 83 (83)	91	91
	13 (382)	0.201, 0.205 (0.203)	101, 103 (102)	106	96
	16 (467)	0.18, 0.188 (0.184)	90, 94 (92)	101	91
	26 (794)	0.172, 0.166 (0.169)	86, 83 (85)	103	83
Pig liver (0.8 mg/kg glyphosate)	0	0.678, 0.639, 0.663 (0.66)	85, 80, 83 (83)	-	-
	14 (217)	0.609, 0.601 (0.605)	76, 75 (76)	84	90
	16 (468)	0.65, 0.646 (0.648)	81, 81 (81)	93	87
	17 (521)	0.564, 0.562 (0.563)	71, 70 (70)	79	89
	26 (790)	0.559, 0.612 (0.586)	70, 77 (73)	90	81
Pig kidney (4.0 mg/kg glyphosate)	0	4.027, 4.093, 3.942 (4.021)	101, 102, 99 (101)	-	-
	8 (241)	3.848, 3.615 (3.732)	96, 90 (93)	97	96
	13 (377)	3.448, 3.65 (3.549)	86, 91 (89)	90	99
	16 (469)	3.556, 3.132 (3.344)	89, 78 (84)	97	87
	26 (790)	3.158, 3.602 (3.38)	79, 90 (85)	83	102
Cow fat (0.2 mg/kg glyphosate)	0	0.172, 0.165, 0.177 (0.171)	86, 83, 89 (86)	-	-
	6 (175)	0.175, 0.165 (0.17)	88, 83 (85)	95	89
	12 (349)	0.168, 0.17 (0.169)	84, 85 (85)	99	86
	15 (436)	0.163, 0.164 (0.164)	82, 82 (82)	96	85
	24 (715)	0.168, 0.199 (0.184)	84, 100 (92)	105	88
Cow muscle (0.2 mg/kg glyphosate)	0	0.176, 0.163, 0.178 (0.172)	88, 82, 89 (86)	-	-
	6 (177)	0.151, 0.145 (0.148)	76, 73 (74)	83	89
	10 (300)	0.174, 0.178 (0.176)	87, 89 (88)	95	93
	13 (373)	0.175, 0.18 (0.178)	88, 90 (89)	98	91
	24 (721)	0.184, 0.207 (0.196)	92, 104 (98)	96	102
Cow liver (4.9 mg/kg glyphosate)	0	3.936, 3.755, 3.785 (3.825)	98, 94, 95 (96)	-	-
	10 (288)	3.447, 3.265 (3.356)	86, 82 (84)	92	91
	13 (380)	3.51, 3.417 (3.464)	88, 85 (87)	96	91
	15 (433)	3.248, 3.275 (3.262)	81, 82 (82)	89	92
	24 (717)	3.638, 3.726 (3.682)	91, 93 (92)	93	99
Cow kidney (6 mg/kg glyphosate)	0	5.666, 5.451, 5.566 (5.561)	94, 91, 93 (93)	-	-
	6 (181)	5.221, 5.187 (5.204)	87, 87 (87)	93	94
	10 (296)	5.358, 5.345 (5.352)	89, 89 (89)	90	99
	13 (377)	5.395, 5.377 (5.386)	90, 90 (90)	95	95
	24 (717)	5.459, 5.426 (5.443)	91, 90 (91)	89	102
Cow milk (0.2 mg/kg glyphosate)	0	0.17, 0.167 (0.169)	85, 84 (84)	-	-
	5 (137)	0.162, 0.159 (0.161)	81, 80 (80)	95	84
	7 (200)	0.158, 0.158 (0.158)	79, 79 (79)	93	85
	16 (473)	0.192, 0.181 (0.187)	96, 91 (93)	110	85
Chicken fat (0.2 mg/kg glyphosate)	0	0.159, 0.183, 0.188 (0.177)	80, 92, 94 (88)	-	-
	12 (368)	0.162, 0.164 (0.163)	81, 82 (82)	97	85
	14 (420)	0.163, 0.162 (0.163)	82, 81 (81)	95	85
	16 (474)	0.16, 0.159 (0.16)	80, 80 (80)	96	83
	25 (753)	0.156, 0.142 (0.149)	78, 71 (75)	85	88

Commodity (fortification level)	**Months (days)**	**mg/kg recovered**	**%**	**Proc. recovery (%)**	**average corrected %**
Chicken muscle (0.2 mg/kg glyphosate)	0	0.176, 0.173, 0.174 (0.174)	88, 87, 87 (87)	-	-
	12 (361)	0.176, 0.179 (0.178)	88, 90 (89)	93	96
	14 (426)	0.181, 0.179 (0.18)	91, 88 (89)	102	87
	16 (483)	0.173, 0.173 (0.173)	87, 87 (87)	96	91
	25 (753)	0.173, 0.149 (0.161)	87, 75 (81)	88	92
Chicken liver (2 mg/kg glyphosate)	0	1.766, 1.618, 1.639 (1.674)	88, 81, 82 (84)	-	-
	13 (384)	1.63, 1.557 (1.594)	82, 78 (80)	114	70
	14 (426)	1.641, 1.654 (1.648)	82, 83 (82)	93	88
	16 (474)	1.621, 1.616 (1.619)	81, 81 (81)	103	79
	25 (747)	1.904, 1.919 (1.912)	95, 96 (96)	111	86
Chicken kidney (4 mg/kg glyphoste)	0	3.697, 3.685, 3.648 (3.677)	92, 92, 91 (92)	-	-
	1 (19)	3.894, 3.875 (3.885)	97, 97 (97)	98	99
	4 (132)	3.658, 3.514 (3.586)	91, 88 (90)	92	98
	13 (390)	4.107, 4.343 (4.225)	103, 109 (106)	105	101
Chicken eggs (0.2 mg/kg glyphosate)	0	0.182, 0.169, 0.179 (0.177)	91, 85, 90 (88)	-	-
	12 (368)	0.158, 0.157 (0.158)	79, 79 (79)	90	88
	14 (431)	0.159, 0.16 (0.16)	80, 80 (80)	91	88
	25 (755)	0.0729, 0.0819 (0.0774)	**36, 41 (39)**	91	**43**
	28 (852)	0.063,0 064 (0.0635)	**32, 32 (32)**	66	**48**

Table B.7.6-22: Recovered AMPA residues after storage of animal matrices

Commodity (fortification level)	Months (days)	mg/kg recovered	%	Proc. recovery (%)	average corrected %
Pig fat (0.05 mg/kg AMPA)	0	0.0416, 0.0376, 0.0392 (0.0395)	83, 75, 78 (79)	-	-
	7 (210)	0.0422, 0.043 (0.0426)	84, 86 (85)	93	91
	15 (437)	0.0376, 0.0355 (0.0366)	75, 67 (71)	96	74
	17 (524)	0.0336, 0.0331 (0.0334)	**67, 66 (67)**	91	**74**
	26 (794)	0.0320, 0.0318 (0.0319)	**64, 64 (64)**	84	**76**
Pig muscle (0.05 mg/kg AMPA)	0	0.0501, 0.0505, 0.0469 (0.492)	100, 101, 94 (98)	-	-
	7 (213)	0.0419, 0.0411 (0.0415)	84, 82 (83)	93	89
	13 (382)	0.0403, 0.0412 (0.0408)	81, 82 (82)	96	85
	16 (467)	0.0372, 0.0415 (0.0394)	74, 83 (79)	94	84
	26 (794)	0.0351, 0.0354 (0.353)	70, 71 (71)	94	76
Pig liver (0.1 mg/kg AMPA)	0	0.0927, 0.0854, 0.0883 (0.0888)	93, 85, 88 (89)	-	-
	14 (217)	0.0625, 0.0606 (0.0616)	**63, 61 (62)**	78	**79**
	16 (468)	0.0758, 0.0788 (0.0773)	76, 79 (77)	93	83
	17 (521)	0.0811, 0.0786 (0.0799)	81, 79 (80)	95	84
	26 (790)	0.076, 0.0808 (0.0784)	76, 81 (78)	98	80
Pig kidney (0.5 mg/kg AMPA)	0	0.505, 0.505, 0.504 (0.505)	101, 101, 101 (101)	-	-
	8 (241)	0.51, 0.456 (0.483)	102, 91 (97)	104	93
	13 (377)	0.474, 0.498 (0.486)	95, 100 (97)	97	100
	16 (469)	0.465, 0.403 (0.434)	93, 81 (87)	102	85
	26 (790)	0.389, 0.486 (0.438)	78, 97 (88)	89	99
Cow fat (0.05 mg/kg AMPA)	0	0.0423, 0.0386, 0.0418 (0.0409)	85, 77, 84 (82)	-	-
	6 (175)	0.042, 0.0385 (0.0403)	84, 77 (81)	96	84
	12 (349)	0.0386, 0.0387 (0.0387)	77, 77 (77)	94	82
	15 (436)	0.0323, 0.0327 (0.0325)	**65, 65 (65)**	82	**79**
	24 (715)	0.0345, 0.0406 (0.0376)	69, 81 (75)	93	81
Cow muscle (0.05 mg/kg AMPA)	0	0.0422, 0.0428, 0.0439 (0.043)	84, 86, 88 (86)	-	-
	6 (177)	0.0416, 0.0414 (0.0415)	83, 83 (83)	98	85
	10 (300)	0.0379, 0.0381 (0.038)	76, 76 (76)	89	85
	13 (373)	0.0334, 0.0357 (0.0346)	**67, 71 (69)**	97	**71**
	24 (721)	0.0358, 0.041 (0.0384)	72, 82 (77)	84	92
Cow liver (0.5 mg/kg AMPA)	0	0.466, 0.463, 0.636 (0.522)	93, 93, 127 (104)	-	-
	10 (288)	0.433, 0.414 (0.424)	87, 83 (85)	94	90
	13 (380)	0.438, 0.427 (0.433)	88, 85 (87)	98	89
	15 (433)	0.437, 0.427 (0.432)	87, 85 (86)	100	86
	24 (717)	0.448, 0.429 (0.439)	90, 86 (88)	95	93
Cow kidney (1.5 mg/kg AMPA)	0	1.389, 1.328, 1.355 (1.357)	93, 89, 90 (90)	-	-
	6 (181)	1.218, 1.228 (1.223)	81, 82 (82)	90	91
	10 (296)	1.198, 1.2 (1.199)	80, 80 (80)	84	95
	13 (377)	1.229, 1.211 (1.22)	82, 81 (81)	91	89
	24 (717)	1.299, 1.313 (1.306)	87, 88 (87)	78	112
Cow milk (0.05 mg/kg AMPA)	0	0.0409, 0.0431 (0.042)	82, 86 (84)	-	-
	5 (137)	0.0369, 0.0365 (0.0367)	74, 73 (73)	89	82
	7 (200)	0.0398, 0.0387 (0.0393)	80, 77 (79)	103	77
	16 (473)	0.0374, 0.0338 (0.0356)	75, 68 (71)	82	87

Commodity (fortification level)	**Months (days)**	**mg/kg recovered**	**%**	**Proc. recovery (%)**	**average corrected %**
Chicken fat (0.05 mg/kg AMPA)	0	0.0406, 0.0464, 0.0472 (0.0447)	81, 93, 94 (89)	-	-
	12 (368)	0.0382, 0.0387 (0.0385)	76, 77 (77)	89	87
	14 (420)	0.035, 0.0363 (0.0357)	70, 73 (71)	83	86
	16 (474)	0.038, 0.039 (0.0385)	76, 78 (77)	93	83
	25 (753)	0.033, 0.029 (0.031)	**71, 64 (68)**	76	**89**
Chicken muscle (0.05 mg/kg AMPA)	0	0.0484, 0.0478, 0.0485 (0.0482)	97, 96, 97 (96)	-	-
	12 (361)	0.0444, 0.0445 (0.0445)	89, 89 (89)	95	94
	14 (426)	0.0467, 0.0472 (0.047)	93, 94 (94)	105	90
	16 (483)	0.0445, 0.0469 (0.0457)	89, 94 (91)	105	87
	25 (753)	0.039, 0.035 (0.037)	78, 70 (74)	76	97
Chicken liver (0.25 mg/kg AMPA)	0	0.196, 0.178, 0.187 (0.187)	78, 71, 75 (75)	-	-
	13 (384)	0.195, 0.184 (0.19)	**78, 74 (76)**	116	**66**
	14 (426)	0.205, 0.2 (0.203)	82, 80 (81)	97	84
	16 (474)	0.213, 0.216 (0.215)	85, 86 (86)	109	79
	25 (747)	0.214, 0.213 (0.214)	86, 85 (86)	95	91
Chicken kidney (0.5 mg/kg AMPA)	0	0.508, 0.515, 0.506 (0.51)	102, 103, 101 (102)	-	-
	1 (19)	0.486, 0.498 (0.492)	97, 100 (98)	96	102
	4 (132)	0.476, 0.435 (0.456)	95, 87 (91)	99	92
	13 (390)	0.447, 0.461 (0.454)	89, 92 (91)	91	100
Chicken eggs (0.05 mg/kg AMPA)	0	0.0494, 0.0448, 0.0479 (0.474)	99, 90, 96 (95)	-	-
	12 (368)	0.0401, 0.0395 (0.0398)	80, 79 (80)	89	90
	14 (431)	0.0418, 0.0418 (0.0418)	84, 84 (84)	94	89
	25 (755)	0.0165, 0.018 (0.0173)	**33, 36 (35)**	78	**45**
	28 (852)	0.0174, 0.0169 (0.0172)	**35, 34 (34)**	71	**48**

Conclusions:

The storage stability for glyphosate and AMPA in animal matrices was investigated in swine, cattle and chicken samples.

For glyphosate no significant degradation during storage was observed for all matrices investigated except for chicken eggs. In eggs 14 months was the maximum storage period without a signnificant degradation of the residue. For all other matrices the maximum storage intervals were: 26 month for pig far, muscle, liver and kidney; 16 months for cattle milk, 24 months for cattle fat, muscle, liver and kidney; 13 months for chicken kidney and 25 months for chicken fat, muscle and liver.

For AMPA the fortification levels and the corresponding recoveries after storage were generally lower compared to glyphosate. For swine fat and liver, for cattle fat and muscle and for chicken fat and liver single recoveries below 70% were observed. However, either the low corresponding procedual recoveries or other samples stored for longer intervals suggest no true degradation of the residue. Under consideration of the overall samples for each commodity and the initial concentrations directly after fortification these single results do not indicate a significant degradation of the residue. For chicken eggs, corresponding to the results for glyphosate, the degradation AMPA was signficant during storage, indicating stable residues of AMPA after storage for only 14 months. Samples of chicken eggs stored for 25 and 28 months gave a significant decline of the AMPA residue. For all other animal commodities maximum storage intervals were: 26 month for pig far, muscle, liver and kidney;

16 months for cattle milk, 24 months for cattle fat, muscle, liver and kidney; 13 months for chicken kidney and 25 months for chicken fat, muscle and liver.

B.7.7 Effects of industrial processing and/or household preparation (Annex IIA 6.5; Annex IIIA 8.5)

B.7.7.1 Nature of the residue during processing

Reference: OECD KIIA 6.5.1

Report: Hiler, T.
Nature of [^{14}C]Glyphosate residues in processed commodities - High temperature hydrolysis
07.10.2010, Report No.: MSL0023072, ASB2012-12432

Guidelines: OECD Guideline 507

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study the hydrolysis of ^{14}C-glyphosate was investigated under simulated processing conditions. The hydrolysis tests were conducted with glyphosate at a target rate of 1 mg/L in sterile 0.05 M aqueous biphthalate solutions buffered at pH 4, 5 and 6. The samples were prepared in duplicate for each buffer system. Duplicate samples were analysed immediately for time zero. Two additional samples were placed in an oven under the following conditions: pH4 at 90°C for 20 min, pH5 at 100°C for 60 min and pH6 at 120°C for 20 min. After incubation the heated samples were also analysed for the composition of the residue.

The test solutions were analysed by cation-exchange HPLC with LSC analysis of the collected eluent fractions. As a confirmatory method strong anion exchange chromatography was was used.

Findings:
In the following table the recovered radioactivity from the sterile buffer solutions and the identified amount of glyphosate are summarised.

Table B.7.7-1: Recovered radioactivity after hydrolysis of ^{14}C-glyphosate in sterile buffer solutions

Hydrolysis conditions	Sample	Mass balance (% AR)	% recovered as glyphosate
pH4, 90°C, 20 min	Time 0, Rep. A	96.1	99.7
	Time 0, Rep. B	95.7	99.6
	Time 20, Rep. A	95.6	99.5
	Time 20, Rep. B	95.9	99.5
pH5, 100°C, 60 min	Time 0, Rep. A	96.7	99.8
	Time 0, Rep. B	96.6	99.8
	Time 60, Rep. A	98.9	99.5
	Time 60, Rep. B	99.3	99.5
pH6, 120°C, 20 min	Time 0, Rep. A	98.3	99.5
	Time 0, Rep. B	97.6	99.7
	Time 20, Rep. A	98.3	98.4
	Time 20, Rep. B	99.4	98.5

Conclusions:
In this study no hydrolysis of glyphosate under simulated processing conditions was observed.

Reference: OECD KIIA 6.5.1

Report: Umstätter, S.; Peterson, B.
High temperature hydrolysis of [^{14}C]IN-MCX20 in buffered aqueous solution at pH 4, 5, and 6
10.08.2006, Report No.: DuPont-19797, ASB2008-2675

Guidelines: EU: Document 1607/VI/97 rev.2 Appendix E (Processing Studies, 7035/VI/95 Rev. 5 July 1997)

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study the stability of ^{14}C-N-acetyl-glyphosate during simulated processing was investigated. The hydrolysis tests were conducted with N-acetyl-glyphosate at a target rate of 1 mg/L in sterile 0.01 M citrate buffer solutions at pH 4, 5 and 6. The samples were prepared in triplicate for each buffer system. The samples were placed in an oven under the following conditions: pH4 at 90°C for 20 min, pH5 at 100°C for 60 min and pH6 at 120°C for 20 min. After incubation the samples were also analysed for the composition of the residue.

All samples were analysed using a HPLC system with radiodetection. The confirmation of the identified structures was conducted by LC-MS.

Findings:
In the following table the recovered radioactivity from the sterile buffer solutions and the identified amount of N-acetyl-glyphosate are summarised.

Table B.7.7-2: Recovered radioactivity after hydrolysis of ^{14}C-N-acetyl-glyphosate in sterile buffer solutions

Hydrolysis conditions	Sample	Mass balance (% AR)	% AR recovered as N-acetyl-glyphosate	% AR in other peaks [a]
pH4, 90°C, 20 min	Time 0, Rep. A, B, C (mean)	99.7, 87.0, 99.3 (95.3)	96.8, 83.6, 93.8 (91.4)	2.9, 3.4, 5.5 (3.9)
	Time 20, Rep. A, B, C (mean)	92.0, 99.9, 98.1 (96.7)	89.5, 96.9, 93.2 (93.2)	2.5, 2.9, 4.9 (3.4)
pH5, 100°C, 60 min	Time 0, Rep. A, B, C (mean)	86.5, 77.7, 98.8 (87.7)	80.9, 74.8, 96.3 (84.0)	5.6, 2.9, 2.5 (3.7)
	Time 60, Rep. A, B, C (mean)	98.7, 99.3, 92.5 (96.8)	94.5, 91.7, 90.2 (92.1)	4.2, 7.5, 2.2 (4.7)
pH6, 120°C, 20 min	Time 0, Rep. A, B, C (mean)	98.4, 99.1, 98.4 (98.6)	91.7, 92.6, 95.0 (93.1)	6.7, 6.6, 3.4 (5.5)
	Time 20, Rep. A, B, C (mean)	97.9, 98.0, 98.2 (98.0)	94.2, 91.2, 96.3 (93.9)	3.7, 6.8, 1.9 (4.1)

a: sum of minor peaks each <5% AR

Conclusions:
In this study no hydrolysis of N-acetyl-glyphosate under simulated processing conditions was observed.

B.7.7.2 Magnitude of the residue during processing

For glyphosate and AMPA numerous food processing studies were submitted involving citrus fruits, . Based on the hydrolysis data, no change in the composition of the residue during porcessing are expected.
In the current evaluation the representative uses do no involve treatment of genetically modified plants or an assessment of import tolerances. For processing studies on glyphosate tolerant crops please refer to the previous DAR for glyphosate (RIP9800121, RIP9800122, RIP9800149, RIP9800148)

Previously described processing studies on tea (RIP9501333) and sugar cane (RIP9501262) do not relate to the representative uses of this evaluation. Please refer to the previous DAR for further information.

B.7.7.2.1 Citrus fruit

Reference: OECD KIIA 6.5.3

Report: Beasley, R. K.
CP 57573, Residue and metabolism part 27: Determination of CP 67573 and CP 50435 residues in citrus process fractions
18.06.1975, Report No.: 377, RIP9501260

Guidelines: none

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be acceptable.

Reference: OECD KIIA 6.5.3

Report: Cowell, J. E.
Determination of Glyphosate and Aminometh-ylphosphonic acid residues in citrus fruit and process fractions following post-directed treatment with Roundup herbicide
November 1986, Report No.: MSL-6194, RIP9501261

Guidelines: none

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study the residues of glyphosate and AMPA during processing following ground directed spraying in citrus orchards was investiged. In the USA supervised field trials on various citrus crops were conducted at four locations involving foliar treatment of ground weeds at rates of 3 x 4.48 kg as/ha or 3 x 8.97 kg as/ha. Fruits were samples after 0 to 21 days and processed into juice, peel, press liqour, feed meal and further products following commercial techniques.

The analysis of glyphosate and AMPA in the samples obtained was conducted by aqueous extraction, followed by ion exchange chromatography for clean-up and finally by GC-NPD after derivatisation to the N-trifluoroacetyl-methyl-esters. Validated LOQs were 0.05 mg/kg for most matrices except for wash-water samples (0.025 mg/kg).

Findings:
In the following table the results for treated citrus fruits and their processed products are summarised. Control sample were all below the LOQ of the respective matrix and therefore not reported.

Table B.7.7-3: Residues and corresponding processing factors in citrus fruits

Crop/ Location/ Variety	GAP	PHI (d)	Commodity	Glyphosate in mg/kg	PF	AMPA in mg/kg	PF
Orange USA (CA), Riverside Navel – upper level	3 x 4.48 kg as/ha	1	whole fruit[a]	<0.05	-	<0.05	-
			juice	<0.05	-	<0.05	-
			peel	<0.05	-	<0.05	-
			feed meal	<0.05	-	<0.05	-
			press liquor	<0.05	-	<0.05	-
		7	whole fruit[a]	<0.05	-	<0.05	-
			juice	<0.05	-	<0.05	-
			peel	<0.05	-	<0.05	-
			feed meal	0.06	-	<0.05	-
			press liquor	<0.05	-	<0.05	-
		21	whole fruit[a]	<0.05	-	<0.05	-
			juice	<0.05	-	<0.05	-
			peel	<0.05	-	<0.05	-
			feed meal	<0.05	-	<0.05	-
			press liquor	<0.05	-	<0.05	-
	3 x 8.97 kg as/ha	1	whole fruit[a]	<0.05	-	<0.05	-
			juice	<0.05	-	<0.05	-
			peel	0.05	-	<0.05	-
			feed meal	0.07	-	<0.05	-
			press liquor	<0.05	-	<0.05	-
		7	whole fruit[a]	<0.05	-	<0.05	-
			juice	<0.05	-	<0.05	-
			peel	<0.05	-	<0.05	-
			feed meal	<0.05	-	<0.05	-
			press liquor	<0.05	-	<0.05	-
		21	whole fruit[a]	<0.05	-	<0.05	-
			juice	<0.05	-	<0.05	-
			peel	<0.05	-	<0.05	-
			feed meal	<0.05	-	<0.05	-
			press liquor	<0.05	-	<0.05	-
Orange USA (CA), Riverside Navel – lower level	3 x 4.48 kg as/ha	1	whole fruit[a]	0.05	-	<0.05	-
			juice	<0.05	<1	<0.05	-
			peel	0.14	2.8	<0.05	-
			feed meal	0.265	5.3	<0.05	-
			press liquor	0.115	2.3	<0.05	-
		7	whole fruit[a]	0.06	-	<0.05	-
			juice	<0.05	<0.83	<0.05	-
			peel	0.185	3.1	<0.05	-
			feed meal	0.105	1.8	<0.05	-
			press liquor	0.115	1.9	<0.05	-
		21	whole fruit[a]	<0.05	-	<0.05	-
			juice	<0.05	-	<0.05	-
			peel	0.07	-	<0.05	-
	3 x 8.97 kg as/ha	1	whole fruit[a]	0.22	-	<0.05	-
			juice	0.1	0.45	<0.05	-
			peel	0.69	3.1	<0.05	-
			feed meal	0.385	1.8	<0.05	-
			press liquor	0.365	1.7	<0.05	-
		7	whole fruit[a]	0.07	-	<0.05	-
			juice	<0.05	<0.71	<0.05	-
			peel	0.16	2.3	<0.05	-
			feed meal	0.345	4.9	<0.05	-
			press liquor	0.15	2.1	<0.05	-

Crop/ Location/ Variety	GAP	PHI (d)	Commodity	Glyphosate in mg/kg	PF	AMPA in mg/kg	PF
		21	whole fruit[a]	0.09	-	<0.05	-
			juice	0.075	0.83	<0.05	-
			peel	0.45	5.0	<0.05	-
			feed meal	0.295	3.3	<0.05	-
			press liquor	0.245	2.7	<0.05	-
Orange, USA (FL), Lake Alfred Pineapple	3 x 8.97 kg as/ha	1	whole fruit[a]	<0.05	-	<0.05	-
			washed	<0.05	-	<0.05	-
			juice	<0.05	-	<0.05	-
			peel	<0.05	-	<0.05	-
			pulp	<0.05	-	<0.05	-
			dried pulp	0.05	-	<0.05	-
			oil	<0.05	-	<0.05	-
			molasses	0.05	-	<0.05	-
		21	whole fruit[a]	<0.05	-	<0.05	-
			washed	<0.05	-	<0.05	-
			juice	<0.05	-	<0.05	-
			peel	<0.05	-	<0.05	-
Orange, USA (FL), Lake Alfred Valencia	3 x 8.97 kg as/ha	1	whole fruit[a]	<0.05	-	<0.05	-
			washed	<0.05	-	<0.05	-
			juice	<0.05	-	<0.05	-
			peel	<0.05	-	<0.05	-
		21	whole fruit[a]	<0.05	-	<0.05	-
			washed	<0.05	-	<0.05	-
			juice	<0.05	-	<0.05	-
			peel	<0.05	-	<0.05	-
			pulp	<0.05	-	<0.05	-
			dried pulp	<0.05	-	<0.05	-
			oil	<0.05	-	<0.05	-
			molasses	<0.05	-	<0.05	-
Lemon, USA (CA), Riverside variety not specified	3 x 4.48 kg as/ha	1	whole fruit[a]	<0.05	-	<0.05	-
			juice	<0.05	-	<0.05	-
			peel	0.06	-	<0.05	-
			feed meal	0.15	-	<0.05	-
			press liquor	0.055	-	<0.05	-
		7	whole fruit[a]	<0.05	-	<0.05	-
			juice	<0.05	-	<0.05	-
			peel	<0.05	-	<0.05	-
			feed meal	0.05	-	<0.05	-
			press liquor	<0.05	-	<0.05	-
		21	whole fruit[a]	<0.05	-	<0.05	-
			juice	<0.05	-	<0.05	-
			peel	<0.05	-	<0.05	-
			feed meal	<0.05	-	<0.05	-
			press liquor	<0.05	-	<0.05	-
	3 x 8.97 kg as/ha	1	whole fruit[a]	<0.05	-	<0.05	-
			juice	<0.05	-	<0.05	-
			peel	0.06	-	<0.05	-
			feed meal	0.08	-	<0.05	-
			press liquor	<0.05	-	<0.05	-
		7	whole fruit[a]	0.06	-	<0.05	-
			juice	<0.05	<0.83	<0.05	-
			peel	<0.05	<0.83	<0.05	-
			feed meal	0.085	1.4	<0.05	-
			press liquor	<0.05	<0.83	<0.05	-

Crop/ Location/ Variety	GAP	PHI (d)	Commodity	Glyphosate in mg/kg	PF	AMPA in mg/kg	PF
		21	whole fruit[a] juice peel feed meal press liquor	<0.05 <0.05 <0.05 0.06 <0.05	- - - - -	<0.05 <0.05 <0.05 <0.05 <0.05	- - - - -
Grapefruit, USA (TX), Weslaco Ruby Red	3 x 8.97 kg as/ha	1	whole fruit[a] juice peel feed meal pulp, rag, seeds	<0.05 <0.05 <0.05 <0.05 <0.05	- - - - -	<0.05 <0.05 <0.05 <0.05 <0.05	- - - - -
		21	whole fruit[a] juice peel feed meal pulp, rag, seeds	<0.05 <0.05 <0.05 <0.05 <0.05	- - - - -	<0.05 <0.05 <0.05 <0.05 <0.05	- - - - -
Grapefruit, USA (FL), Lake Alfred Marsh	3 x 8.97 kg as/ha	1	whole fruit[a] washed juice peel pulp dried pulp oil	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05	- - - - - - -	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05	- - - - - - -
		21	whole fruit[a] washed juice peel	<0.05 <0.05 <0.05 <0.05	- - - -	<0.05 <0.05 <0.05 <0.05	- - - -

upper level: higher part of the fruit tree (>90cm)
lower level: lower part of the fruit tree (0 - 90cm)
a: calculated value based on juice yield x residue in juice + peel yield x residue in peel

Conclusions:
In most of the supervised field trials conducted in the USA no residues above the LOQ were present in the raw agricultural commodity, thus not allowing the calculation of processing factors. When residues above the LOQ were present, the following median processing factors were derived for glyphosate: citrus juice 0.83 (0.45, <0.71, <0.83, <0.83, 0.83, <1); citrus peel 3.0 (<0.83, 2.3, 2.8, 3.1, 3.1, 5.0); citrus feed meal 2.6 (1.4, 1.8, 1.8, 3.3, 4.9, 5.3) and citrus press liquor 2 (<0.83, 1.7, 1.9, 2.1, 2.3, 2.7). In dried pulp and peel an accumulation of the residue was observed, however since no residue could be measured in the RAC, calculation of processing factors was not possible.
For AMPA no residues above the LOQ were found.

B.7.7.2.2 Potatoes

Reference: OECD KIIA 6.5.3

Report: Mueth, M. G.
Glyphosate residues in potatoes and processed fractions of potatoes after treatment with Roundup herbicide
July 1988, MSL-7877, RIP9501263

Guidelines: EPA Guideline 171-4

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In two locations in the USA potatoes were treated with glyphosate at application rates of either 21 or 42 kg as/ha. Mature tubers were harvested 97-104 days after the treatment and processed into chips, flakes and granules following industrial processing. In addition the peel was collected in the chips and flakes processing.

The analysis was conducted by aqueous extraction, ion-exchange column clean-up and HPLC with post-column reaction using o-phthalaldehyde. Validation data of the analytical method used support a LOQ of 0.05 mg/kg for both glyhosate and AMPA.

Findings:
In the following table the residues of AMPA in processed potatoes and the resulting processing factors are summarised.
For glyphosate no residues above the LOQ were found in the tubers (RAC) and in most processed products except two peel samples (0.066 mg/kg and 0.09 mg/kg). The residue data for glyphosate is not presented in the following table.

Table B.7.7-4: Processing of potatoes

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
Tuber	21	AMPA: 0.07	97	chips	0.11	1.6	RIP9501263, USA, Madera (CA)
				wet peel (chips)	<0.05	<0.71	
				flakes	0.12	1.7	
				wet peel (flakes)	<0.05	<0.71	
				dry peel (flakes)	0.19	2.7	
				granules	0.17	2.4	
	42	AMPA: <0.05	97	chips	0.12	-	
				wet peel (chips)	<0.05	-	
				flakes	0.094	-	
				wet peel (flakes)	<0.05	-	
				dry peel (flakes)	0.15	-	
				granules	0.20	-	
Tuber	21	AMPA: 0.16	104	chips	0.19	1.2	RIP9501263, USA, Madera (CA)
				wet peel (chips)	<0.05	<0.31	
				flakes	0.19	1.2	
				wet peel (flakes)	<0.05	<0.31	
				dry peel (flakes)	0.24	1.5	
				granules	0.23	1.4	
	42	AMPA: 0.26	104	chips	0.35	1.3	
				wet peel (chips)	0.056	0.22	
				flakes	0.40	1.5	
				wet peel (flakes)	0.077	0.3	
				dry peel (flakes)	0.32	1.2	
				granules	0.59	2.3	

Conclusions:
In the study provided for potato processing no residue above the LOQ were found for glyphosate in the tuber, thus not allowing calculation of processing factors.
For AMPA, which was frequently found in most samples, processing factors were: 1.3 for chips (1.2, 1.3, 1.6), 1.5 for flakes (1.2 1.5, 1.7), 0.31 for wet peel (0.26, <0.31, <0.71), 1.5 for dry peel (1.2, 1.5, 2.7) and 2.3 for granules (1.4, 2.3, 2.4).

B.7.7.2.3 Olives

For olives the behaviour of glyphosate and AMPA were investigated in several supervised field trials conducted in Southern Europe. For a detailed description of these trials please refer to the Appendix of the residue section.
In trials RIP9501289 and RIP9501290 the processing of olives was conducted according to the common industrial practice for unrefined olive oil ("extra vergine"). The fruit were washed for 60 seconds and mashed with an olive grindstone mill. The material obtained was centrifugated to separate the oil fraction.
For RIP9700184 the processing of unrefined oil was conducted by crushing of the ground picked fruits and pressing through clothes to obtain the virgin (or "crude") oil. Subsequently a simulated refining of the oil was achieved by "addition of alkali".

In some additional trials only the vergin olive oil was analysed for residues of glyphosate and AMPA following ground directed treatment at rates between 2.16 and 4.32 kg as/ha, In these trials no residue above the LOQ of 0.05 mg/kg for both analytes were found.

The the following table the processing information for glyphosate from supervised field trials on olives with measured results from olives (RAC) and the obtained olive oil are summarised. For AMPA no residues above the LOQ of 0.05 mg/kg were found for raw olives or olive oil in any of the field trials submitted.

Table B.7.7-5: Processing of olive fruits into vergin and refined olive oil

RAC	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
Olive	<0.05	6	Olive oil (unrefined)	<0.05	not applicable	RIP9501290, IT-Francavilla, Puglia
Olive	0.08	13	Olive oil (unrefined)	<0.05	<0.63	
Olive	<0.05	7	Olive oil (unrefined)	<0.05	not applicable	RIP9501290, IT-San Casciano, Toscana
Olive	<0.05	14	Olive oil (unrefined)	<0.05	not applicable	
Olive	0.9	1	Olive oil (unrefined)	<0.05	<0.06	RIP9501289, ES-Pedrera
Olive	1.1	7	Olive oil (unrefined)	<0.05	<0.05	
Olive	1.1	1	Olive oil (unrefined)	<0.05	<0.05	RIP9501289, ES-Puebla de Cazalla
Olive	1.2	7	Olive oil (unrefined)	<0.05	<0.04	
Olive	1.8	0	Olive oil (unrefined)	<0.05	<0.03	RIP9501289, ES-Torreblasco-pedro
Olive	0.8	32	Olive oil (unrefined)	<0.05	<0.06	
Olive	0.3	0	Olive oil (unrefined)	<0.05	<0.17	RIP9501289, ES-Sierra Yegua
Olive	0.4	24	Olive oil (unrefined)	<0.05	<0.13	
Olive	0.2	0	Olive oil (unrefined)	<0.05	<0.25	RIP9501289, ES-Linares
Olive	2	30	Olive oil (unrefined)	<0.05	<0.03	
Olive	0.08	0	Olive oil (unrefined)	<0.05	<0.63	RIP9501289, ES-Córdoba
Olive	0.4	41	Olive oil (unrefined)	<0.05	<0.13	
Olive	0.14	7	Olive oil (crude) Olive oil (refined)	<0.05 <0.05	<0.35 <0.35	RIP9700184, ES-Chataojal
Olive	0.12	14	Olive oil (crude) Olive oil (refined)	<0.05 <0.05	<0.42 <0.42	
Olive	<0.05	28	Olive oil (crude) Olive oil (refined)	<0.05 <0.05	not applicable not applicable	
Olive	0.53	7	Olive oil (crude) Olive oil (refined)	<0.05 <0.05	<0.09 <0.09	RIP9700184, ES-Villa del Rio
Olive	0.13	14	Olive oil (crude) Olive oil (refined)	<0.05 <0.05	<0.38 <0.38	
Olive	<0.05	28	Olive oil (crude) Olive oil (refined)	<0.05 <0.05	not applicable not applicable	
Olive	0.93	7	Olive oil (crude) Olive oil (refined)	<0.05 <0.05	<0.05 <0.05	RIP9700184, ES-Ubeda
Olive	0.93	14	Olive oil (crude) Olive oil (refined)	<0.05 <0.05	<0.05 <0.05	
Olive	<0.05	28	Olive oil (crude) Olive oil (refined)	<0.05 <0.05	not applicable not applicable	

Conclusions:

The transfer of glyphosate and AMPA into olive oil is very limited. For AMPA no residues above the LOQ were found in olive fruits and oil. Glyphosate was found in unprocessed olives at levels up to 2 mg/kg, but not in crude or refined oil. The corresponding processing factors were <0.09 for crude olive oil (<0.03, <0.03, <0.04, <0.05(4), <0.06, <0.06, <0.09, <0.13, <0.13, <0.17, <0.25, <0.35, <0.38, <0.42, <0.63, <0.63) and <0.22 for refined olive oil (<0.05, <0.05, <0.09, <0.35, <0.38, <0.42).

B.7.7.2.4 Linseed (Flax)

Reference: OECD KIIA 6.3

Report: Mestdagh, P.
Residue analysis for Glyphosate and AMPA in flax and processed fractions following preharvest Roundup herbicide treatments. UK and Ireland 1982 trials, 1983, MLL 30106, RIP9501266

Guidelines: none

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study containing supervised field trials on flax conducted in the UK in 1982 the behaviour of glyphosate and AMPA during processing of the seeds was investigated (also refer to list of supervised field trials at the end of the residue chapter). The flax plants were treated with glyphosate for desiccation 5 days before harvest at rates of 1.44 kg as/ha or 2.9 kg as/ha.
The collected seeds were milled and afterwards extracted with hexane in a Soxhlet apparatus in reflux. The remainings were defined as press cake while the extracted lipids, after removal of the solvent, were considered as oil.

The analysis was conducted by HPLC with post-column reaction with o-phthalaldehyde and ninhydrin. The validated LOQs reached were for glyphosate 1 mg/kg in seeds, 2 mg/kg in press cake and 0.4 mg/kg in oil. For AMPA the LOQs were 0.2 mg/kg in seeds, 0.2 mg/kg in press cake and 0.08 mg/kg in oil.

Findings:
In the following table the residues of glyphosate and AMPA in processed flax fractions and the resulting processing factors are summarised.

Table B.7.7-6: Processing of flax

RAC	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
Seed	Glyphosate: 1.3 AMPA: <0.2	5	Glyphosate: crude oil press cake AMPA: crude oil press cake	 <0.4 2.1 <0.08 <0.2	 <0.31 1.6 not applicable not applicable	RIP9501266, UK-Arboath, Plot 2C
Seed	Glyphosate: 2.2 AMPA: <0.2	5	Glyphosate: crude oil press cake AMPA: crude oil press cake	 <0.4 2.4 <0.08 <0.2	 <0.18 1.1 not applicable not applicable	RIP9501266, UK-Arboath, Plot 2D
Seed	Glyphosate: 1.3 AMPA: <0.2	5	Glyphosate: crude oil press cake AMPA: crude oil press cake	 <0.4 <2.0 <0.08 <0.2	 <0.31 <1.5 not applicable not applicable	RIP9501266, UK-Backboath, Plot 3C
Seed	Glyphosate: 4.0 AMPA: <0.2	5	Glyphosate: crude oil press cake AMPA: crude oil press cake	 <0.4 6.3 <0.08 <0.2	 <0.1 1.6 not applicable not applicable	RIP9501266, UK-Backboath, Plot 3D

Conclusions:
In four supervised field trials involving processing of linseeds into oil (by extraction) and press cake only the press cake contained glyphosate residues above the LOQ. In the extracted oil no glyphosate residues were detected. For glyphosate the corresponding processing factors were <0.25 for linseed oil (<0.1, <0.18, <0.31, <0.31) and 1.6 for linseed press cake (1.1, <1.5, 1.6, 1.6).
For AMPA all residues (seeds, oil and press cake) were below the respective LOQs, not allowing the calculation of processing factors.

B.7.7.2.5 Oilseed rape

Reference: OECD KIIA 6.3

Report: Mestdagh, P.,
Residue analysis for Glyphosate and AMPA in brassica seedcrops and processed fractions following preharvest Roundup herbicide treatments. UK and Scandinavian trials 1980-1982
18.04.1983, MLL 30.104, RIP9501265

Guidelines: none

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study the behaviour of glyphosate and AMPA during processing of rapeseeds was investigated in four supervised field trials in the UK and Denmark. The plants were treated with glyphosate for desiccation 14 to 21 days before harvest at rates of 1.4 kg as/ha or 2.8 kg as/ha.
The collected seeds were processed following commercial practice with large-scale equiment. After cleaning and breaking the seeds were steamed. The crude oils was removed by an expeller. The remaining presscake was additionally extracted by solvent, leaving the spent press cake. The combined oil fractions, after removal of the solvent, were refined, including neutralisation, bleaching and deodorisation.

The analysis was conducted by HPLC with post-column reaction with o-phthalaldehyde and ninhydrin. The validated LOQs reached were for glyphosate 1 mg/kg in seeds, 0.4 mg/kg in press cake and 0.4 mg/kg in oil. For AMPA the LOQs were 0.2 mg/kg in seeds, 0.2 mg/kg in press cake and 0.08 mg/kg in oil.

Findings:
In the following table the residues of glyphosate and AMPA in processed linseed fractions and the resulting processing factors are summarised.

Table B.7.7-7: Processing of oilseed rape

RAC	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
Seeds	Glyphosate: 8.6 AMPA: <0.2	21	Glyphosate: press cake press cake, roasted refined oil AMPA: press cake press cake, roasted refined oil	 11.8 11.4 <0.4 <0.2 <0.2 <0.2	 1.4 1.4 <0.05 not applicable not applicable not applicable	RIP9501265, DK-Hasley
Seeds	Glyphosate: 1.5 AMPA: <0.2	14	Glyphosate: press cake crude oil refined oil AMPA: press cake crude oil refined oil	 <2.0 <0.4 <0.4 <0.2 <0.2 <0.2	 <1.3 <0.27 <0.27 not applicable not applicable not applicable	RIP9501265, UK-Easton, Plot 100 A
Seeds	Glyphosate: 3.2 AMPA: <0.2	14	Glyphosate: press cake crude oil refined oil AMPA: press cake crude oil refined oil	 4.9 <0.4 <0.4 <0.2 <0.2 <0.2	 1.5 <0.13 <0.13 not applicable not applicable not applicable	RIP9501265, UK-Easton Plot 100 B
Seeds	Glyphosate: 2.7 AMPA: <0.2	14	Glyphosate: press cake crude oil refined oil AMPA: press cake crude oil refined oil	 3.1 <0.4 <0.4 <0.2 <0.2 <0.2	 1.2 <0.15 <0.15 not applicable not applicable not applicable	RIP9501265, UK-Easton Plot 101 A
Seeds	Glyphosate: 4.0 AMPA: <0.2	14	Glyphosate: press cake crude oil refined oil AMPA: press cake crude oil refined oil	 8.9 <0.4 <0.4 <0.2 <0.2 <0.2	 2.2 <0.1 <0.1 not applicable not applicable not applicable	RIP9501265, UK-Easton Plot 101 B

Conclusions:

In five supervised field trials involving processing of oilseed rape into crude and refined oil and into press cake only the press cake contained glyphosate residues above the LOQ. In the extracted oil no glyphosate residues were detected. For glyphosate the corresponding processing factors were <0.14 for crude rapeseed oil (<0.1, <0.13, <0.15, <0.27), <0.13 for refined rapeseed oil (<0.05, <0.1, <0.13, <0.15, <0.27) and 1.4 for rapeseed press cake (1.2, <1.3, 1.4, 1.5, 2.2).

For AMPA all residues (seeds, oil and press cake) were below the respective LOQs, not allowing the calculation of processing factors.

B.7.7.2.6 Soya beans

Reference: OECD KIIA 6.5.3

Report: Kunstman, J. L.; Steinmetz, J. R.; Farmer, P. S.; Blount, L. M. Glyphosate residues in soybeans and soybean fractions following recirculating sprayer and preharvest topical treatment with Roundup herbicide
November 1983, MSL-3259, RIP9501259

Guidelines: no

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study involving the transfer of glyphosate and AMPA into processed soya beans plants grown in the USA (Banks, Mississippi) were treated with 0.84 kg as/ha or 3.36 kg as/ha 15 days before harvest. The sampled seeds were processed into fat-free meal, hulls, crude oil and soapstock. In the first step the soya beans were mechanically dehulled. Hulls and seeds were finely ground up along with dry ice in a blender. After dissipation of the dry ice, the meal was fractionated. Oil was extracted with hexane (→ crude oil, fat-free meal). The oil recovered was mixed with 10% NaOH solution to obtain the soapstock.

For the analysis of the residue the samples were extracted using 50 ml of chloroform and 150 ml of 0.1N HCl. The aqueous phase eluted via a Chelix ion exchange column for clean-up. The concentrated elute was analysed with a two column HPLC including post-column reaction with Ninhydrin. The detection was achieved with a photometric detector at 546 nm wavelength. The validated LOQs reached were for glyphosate and AMPA 0.05 mg/kg in seeds, 0.1 mg/kg oil and 0.5 mg/kg in fat-free meal, hulls and soapstock.

Findings:
In the following table the residues of glyphosate and AMPA in processed soya beans and the resulting processing factors are summarised.

Table B.7.7-8: Processing of soya beans

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
Seed	0	Glyphosate: 0.05 AMPA: 0.05	15	Glyphosate fat-free meal hulls crude oil soapstock AMPA: fat-free meal hulls crude oil soapstock	 <0.5 <0.5 <0.1 <0.5 <0.5 <0.5 <0.1 <0.5	 - - - - - - - -	RIP9501259, USA, Banks (MS)
	0.84	Glyphosate: 9.43 AMPA: 1.56	15	Glyphosate fat-free meal hulls crude oil soapstock AMPA: fat-free meal hulls crude oil soapstock	 8.99 41.8 <0.1 <0.5 1.54 3.81 <0.1 <0.5	 0.95 4.4 <0.01 <0.05 0.99 2.4 <0.06 <0.32	
	3.36	Glyphosate: 11.8 AMPA: 1.94	15	Glyphosate fat-free meal hulls crude oil soapstock AMPA: fat-free meal hulls crude oil soapstock	 12.0 60.8 0.11 <0.5 1.77 4.76 <0.1 <0.5	 1.0 5.2 0.01 <0.04 0.91 2.5 0.05 0.26	

Conclusions:
Based on the submitted processing data the folowing median processing factors were derived for glyphosate and AMPA in soya bean products: fat-free meal 0.98 and 0.95, hulls 4.8 and 2.45, crude oil 0.01 and 0.055 and soapstock <0.045 and 0.29.

B.7.7.2.7 Barley

Reference: OECD KIIA 6.3 & 6.5.3

Report: Mestdagh, P.
Residual Glyphosate in processed barley grains following a preharvest application of Roundup herbicide in the United Kingdom 28.10.1981, MLL.30.070, RIP9501238

Guidelines: no

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be additional information.

Material and Methods:
In supervised field trials on barley the crop was treatd with glyphosate for desiccation either with 1.44 kg as/ha or 2.88 kg as/ha. Within one week barley grain samples were collected and processed into malt following commercial practice.

The analysis of the residue in grain and malt was conducted by GC-FPD following clean-up via ion exchange resin and subsequent derivatization. Validation data was not provided.

Findings:
In the following table the residues of glyphosate in processed barley and the resulting processing factors are summarised. AMPA residue levels were not reported.

Table B.7.7-9: Processing of barley

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
Grain	1.44	Glyphosate: 2.8	7	Glyphosate: malt	0.09	0.03	RIP9501238, UK, Fulbourn
	2.88	Glyphosate: 6.6	4	Glyphosate: malt	0.20	0.03	
	1.44	Glyphosate: 4.5	7	Glyphosate: malt	0.07	0.02	
	2.88	Glyphosate: 9.5	7	Glyphosate: malt	0.18	0.02	
	2.88	Glyphosate: 8.2	4	Glyphosate: malt	0.21	0.03	
Grain	1.44	Glyphosate: 2.6	6	Glyphosate: malt	0.1	0.04	RIP9501238, UK, Debden
	2.88	Glyphosate: 6.0	6	Glyphosate: malt	0.19	0.03	
	2.88	Glyphosate: 6.3	3	Glyphosate: malt	0.15	0.02	
Grain	1.44	Glyphosate: 6.2	7	Glyphosate: malt	0.06	0.01	RIP9501238, UK, Cottenham
	2.88	Glyphosate: 11.2	7	Glyphosate: malt	0.20	0.02	
	2.88	Glyphosate: 10.7	3	Glyphosate: malt	0.17	0.02	
Grain	1.44	Glyphosate: 12.8	8	Glyphosate: malt	0.10	0.01	RIP9501238, UK, Balsham
	2.88	Glyphosate: 13.7	8	Glyphosate: malt	0.15	0.01	
	2.88	Glyphosate: 15.4	4	Glyphosate: malt	0.19	0.01	
Grain	1.44	Glyphosate: 12.5	7	Glyphosate: malt	0.22	0.02	RIP9501238, UK, Kelso Borders
	2.88	Glyphosate: 32.8	7	Glyphosate: malt	0.40	0.01	
	2.88	Glyphosate: 25.7	4	Glyphosate: malt	0.28	0.01	

Conclusions:
Based on five supervised field trials for the processing of barley into malt it can be concluded that the transfer of residues into the malt is very low. Obviously the initial watering step during the typical malting process removes the major part of the good watersoluble glyphosate from the grain. Based on the PFs calculated an overall processing factor of 0.02 for barley malt can be derived.

Reference: OECD KIIA 6.3 & 6.5.3

Report: Mestdagh, P.
Residual Glyphosate in beer, wort and malt obtained from barley grains following a pre-harvest application of Roundup herbicide in France
24.09.1982, MLL 30.084, RIP9501248

Guidelines: no

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be not acceptable.

Material and Methods:
In supervised field trials on barley the crop was treatd with glyphosate for desiccation either with 2.15 kg as/ha, 4.3 kg as/ha or 8.6 kg as/ha. Within three weeks barley grain samples were collected and processed into malt and wort following commercial practice.
For the malting the dried grain was water for several hours. Afterwards the grains was kept at 45% moisture for 5 days for germination. Germinated grains were kilned following a temperature regiment from 60°C to 80°C.
The cleaned malt was milled and mixed with water in a 1:8 ratio (m:m). The mixture was heated and filtered.

The analysis of the residue in grain and malt was conducted by GC-FPD following clean-up via ion exchange resin and subsequent derivatization. Validation data was not provided.

Findings:
In the following table the residues of glyphosate in processed barley and the resulting processing factors are summarised. AMPA residue levels were generally below the LOQ of 0.05 mg/kg, unless reported.

Table B.7.7-10: Processing of barley

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
Grain	0	Glyphosate: 0.5	8	Glyphosate:			RIP9501248, France, Sermaize (Trial 41295)
				malt	0.09	(0.18)	
				wort	<0.05	(<0.1)	
	2.15	Glyphosate: 6.4	8	Glyphosate:			
				malt	1.23	(0.19)	
				wort	0.11	(0.02)	
	4.3	Glyphosate: 17.3	8	Glyphosate:			
				malt	3.95	(0.23)	
		AMPA: 0.07		wort	0.35	(0.02)	
				AMPA:			
				malt	<0.05	(<0.71)	

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
				wort	<0.05	(<0.71)	
	8.6	Glyphosate: 32.8 AMPA: 0.13	8	Glyphosate: malt wort AMPA: malt wort	 4.63 0.64 <0.05 <0.05	 (0.14) (0.02) (<0.38) <0.38)	
Grain	0	Glyphosate: 3.8	9	Glyphosate: malt wort	 0.36 0.08	 (0.1) (0.02)	RIP9501248, France, Averd (Trial 41293)
	2.15	Glyphosate: 4.0	9	Glyphosate: malt wort	 0.87 0.20	 (0.22) (0.05)	
	4.3	Glyphosate: 6.4 AMPA: 0.06	9	Glyphosate: malt wort AMPA: malt wort	 1.43 0.16 <0.05 <0.05	 (0.22) (0.03) (<0.83) <0.83	
	8.6	Glyphosate: 15.7 AMPA: 0.08	9	Glyphosate: malt wort AMPA: malt wort	 1.76 0.42 0.05 <0.05	 (0.11) (0.03) (0.63) (<0.63)	
Grain	0	Glyphosate: n.s.	9	Glyphosate: malt wort	 n.s. <0.05	 (-) (-)	RIP9501248, France, Charest (Trial 41294)
	2.15	Glyphosate: 11.8 AMPA: 0.16	9	Glyphosate: malt wort AMPA: malt wort	 3.35 0.50 0.05 <0.05	 (0.28) (0.04) (0.31) (<0.31)	
	4.3	Glyphosate: 9.6 AMPA: 0.17	9	Glyphosate: malt wort AMPA: malt wort	 3.14 0.60 0.18 <0.05	 (0.33) (0.06) (1.1) (<0.29)	
	8.6	Glyphosate: 49.5 AMPA: 0.36	9	Glyphosate: malt wort AMPA: malt wort	 n.s. 1.6 n.s. 0.06	 (-) (0.03) (-) (0.17)	
Grain	0	Glyphosate: 1.2	12	Glyphosate: malt wort	 0.27 <0.05	 (0.23) (<0.04)	RIP9501248, France, Hoursault (Trial 41240)

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
	2.15	Glyphosate: 2.9 AMPA: 0.08	12	Glyphosate: malt wort AMPA: malt wort	 n.s. 0.13 n.s. <0.05	 (-) (0.04) (-) (<0.63)	
	4.3	Glyphosate: 6.8 AMPA: 0.08	12	Glyphosate: malt wort AMPA: malt wort	 2.7 0.17 <0.05 <0.05	 (0.40) (0.03) (<0.63) (<0.63)	
	8.6	Glyphosate: 12.1 AMPA: 0.18	12	Glyphosate: malt wort AMPA: malt wort	 2.1 0.33 <0.05 <0.05	 (0.17) (0.03) (<0.28) (<0.28)	
Grain	0	Glyphosate: 3.1	13	Glyphosate: malt wort	 n.s. 0.08	 (-) (0.03)	RIP9501248, France, Vendeuil (Trial 41241)
	2.15	Glyphosate: 2.7	13	Glyphosate: malt wort	 0.84 0.07	 (0.31) (0.03)	
	4.3	Glyphosate: 3.4 AMPA: 0.08	13	Glyphosate: malt wort AMPA: malt wort	 0.96 0.13 0.09 <0.05	 (0.28) (0.04) (1.1) (<0.63)	
	8.6	Glyphosate: 14.5 AMPA: 0.19	13	Glyphosate: malt wort AMPA: malt wort	 2.41 0.27 0.11 <0.05	 (0.17) (0.02) (0.58) (<0.26)	
Grain	0	Glyphosate: 2.0	15	Glyphosate: malt wort	 0.97 0.13	 (0.49) (0.07)	RIP9501248, France, Neuville sous bois (Trial 41291)
	2.15	Glyphosate: 2.6 AMPA: 0.10	15	Glyphosate: malt wort AMPA: malt wort	 1.06 0.15 <0.05 <0.05	 (0.41) (0.06) (<0.5) (<0.5)	
	4.3	Glyphosate: 9.2 AMPA: 0.09	15	Glyphosate: malt wort AMPA: malt	 n.s. 0.3 n.s.	 (-) (0.03) (-)	

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
				wort	<0.05	(<0.56)	
	8.6	Glyphosate: 11.4 AMPA: 0.06	15	Glyphosate: malt wort AMPA: malt wort	 4.71 0.6 <0.05 <0.05	 (0.41) (0.05) (<0.83) (<0.83)	
Grain	0	Glyphosate: 1.2	16	Glyphosate: malt wort	 0.4 <0.05	 (0.33) (<0.04)	RIP9501248, France, Bovilly (Trial 41246)
	2.15	Glyphosate: 1.9	16	Glyphosate: malt wort	 0.85 0.07	 (0.45) (0.04)	
	4.3	Glyphosate: 3.9	16	Glyphosate: malt wort	 n.s. 0.14	 (-) (0.04)	
	8.6	Glyphosate: 4.6 AMPA: 0.08	16	Glyphosate: malt wort AMPA: malt wort	 2.06 0.23 0.07 <0.05	 (0.45) (0.05) (0.88) (<0.63)	
Grain	0	Glyphosate: 5.0	16	Glyphosate: malt wort	 0.59 0.11	 (0.12) (0.02)	RIP9501248, France, Bailly (Trial 41244)
	2.15	Glyphosate: 11.0	16	Glyphosate: malt wort	 2.23 0.25	 (0.2) (0.02)	
	4.3	Glyphosate: 16.1 AMPA: 0.12	16	Glyphosate: malt wort AMPA: malt wort	 1.49 0.35 <0.05 <0.05	 (0.09) (0.02) (<0.42) (<0.42)	
	8.6	Glyphosate: 23.3 AMPA: 0.20	16	Glyphosate: malt wort AMPA: malt wort	 3.16 0.39 <0.05 <0.05	 (0.14) (0.02) (<0.25) (<0.25)	
Grain	0	Glyphosate: 0.2	18	Glyphosate: malt wort	 0.05 <0.05	 (0.25) (<0.25)	RIP9501248, France, Saint Satur (Trial 41297)
	2.15	Glyphosate: 1.7	18	Glyphosate: malt wort	 0.46 0.06	 (0.27) (0.04)	
	4.3	Glyphosate: 2.2	18	Glyphosate: malt	 0.86	 (0.39)	

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
				wort	0.11	(0.05)	
Grain	0	Glyphosate: 2.1	19	Glyphosate: malt wort	 0.42 <0.05	 (0.2) (0.02)	RIP9501248, France, Moyviller (Trial 41242)
	2.15	Glyphosate: 1.1	19	Glyphosate: malt wort	 0.24 <0.05	 (0.22) (<0.05)	
	4.3	Glyphosate: 5.9	19	Glyphosate: malt wort	 0.48 0.13	 (0.08) (0.02)	
	8.6	Glyphosate: 5.7 AMPA: 0.12	19	Glyphosate: malt wort AMPA: malt wort	 1.8 0.14 <0.05 <0.05	 (0.32) (0.02) (<0.42) (<0.42)	
Grain	0	Glyphosate: 0.1	19	Glyphosate: malt wort	 n.s. <0.05	 (-) (<0.5)	RIP9501248, France, Bailly (Trial 41292)
	2.15	Glyphosate: 4.7 AMPA: 0.13	19	Glyphosate: malt wort AMPA: malt wort	 n.s. 0.4 n.s. <0.05	 (-) (0.09) (-) (<0.38)	
	4.3	Glyphosate: 9.9 AMPA: 0.34	19	Glyphosate: malt wort AMPA: malt wort	 n.s. 0.41 n.s. <0.05	 (-) (0.04) (-) (<0.15)	
	8.6	Glyphosate: 12.3 AMPA: 0.23	19	Glyphosate: malt wort AMPA: malt wort	 5.73 0.70 0.15 <0.05	 (0.47) (0.06) (0.65) (<0.22)	
Grain	0	Glyphosate: 0.7	20	Glyphosate: malt wort	 0.12 <0.05	 (0.17) (<0.07)	RIP9501248, France, Estrees Saint Denis (Trial 41245)
	2.15	Glyphosate: 7.0	20	Glyphosate: malt wort	 1.29 0.16	 (0.18) (0.02)	
	4.3	Glyphosate: 7.1	20	Glyphosate: malt wort	 1.26 0.17	 (0.18) (0.02)	
	8.6	Glyphosate: 9.7	20	Glyphosate: malt	 2.16	 (0.22)	

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
				wort	0.40	(0.04)	
Grain	0	Glyphosate: 0.1	21	Glyphosate:			RIP9501248, France, Saint Satur (Trial 41296)
				malt	<0.05	(<0.5)	
				wort	<0.05	(<0.5)	
	2.15	Glyphosate: 1.9	21	Glyphosate:			
				malt	0.57	(0.3)	
				wort	0.08	(0.04)	
	4.3	Glyphosate: 3.0	21	Glyphosate:			
				malt	1.16	(0.39)	
				wort	0.14	(0.05)	
	8.6	Glyphosate: 8.4	21	Glyphosate:			
				malt	3.02	(0.36)	
		AMPA: 0.06		wort	0.40	(0.48)	
				AMPA:			
				malt	<0.05	(<0.83)	
				wort	<0.05	(<0.83)	
Grain	0	Glyphosate: 4.7	22	Glyphosate:			RIP9501248, France, Baslieux/Chatillon (Trial 41239)
				malt	2.58	(0.55)	
				wort	0.35	(0.07)	
	2.15	Glyphosate: 5.5	22	Glyphosate:			
				malt	1.33	(0.24)	
				wort	0.17	(0.03)	
	4.3	Glyphosate: 5.3	22	Glyphosate:			
				malt	1.25	(0.24)	
		AMPA: 0.08		wort	0.18	(0.03)	
				AMPA:			
				malt	0.09	(1.1)	
				wort	<0.05	(<0.63)	
	8.6	Glyphosate: 9.7	22	Glyphosate:			
				malt	1.44	(0.15)	
		AMPA: 0.09		wort	0.24	(0.02)	
				AMPA:			
				malt	0.1	(1.1)	
				wort	<0.05	(<0.56)	
Grain	0	Glyphosate: 0.5	25	Glyphosate:			RIP9501248, France, Olizy (Trial 41243)
				malt	0.1	(0.2)	
				wort	<0.05	(<0.1)	
	2.15	Glyphosate: 1.3	25	Glyphosate:			
				malt	0.5	(0.38)	
				wort	0.07	(0.05)	
	4.3	Glyphosate: 4.1	25	Glyphosate:			
				malt	1.67	(0.41)	
		AMPA: 0.08		wort	0.28	(0.07)	
				AMPA:			
				malt	0.06	(0.75)	
				wort	<0.05	(<0.63)	
	8.6	Glyphosate: 10	25	Glyphosate:			
				malt	3.38	(0.34)	

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
		AMPA: 0.20		wort AMPA: malt wort	0.52 0.18 <0.05	(0.05) (0.9) (<0.25)	

n.s. not sampled

Conclusions:
In all supervised field trials the untreated control plots contained finite glyphosate residues at the same order of magnitude as treated trials. However, processing of grain was conducted on sample level, which makes the distinction between control and treated samples irrelevant. In summary the processing information seems acceptable, but the overall positive residues in control samples and missing validation data make the study not acceptable.

B.7.7.2.8 Maize

Reference: OECD KIIA 6.5.3

Report: Kunstman, J. L.
Glyphosate residues in corn grain fractions following preharvest applications to corn with Roundup herbicide
September 1987, MSL-6917, RIP9501257

Guidelines: EPA Guideline 171-4

Deviations: ~~original guideline not submitted~~none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
Maize samples treated with glyphosate for desiccation purposes in the USA were processing into fat-free meal, crude oil, refined oil and the remaining soapstock. The crop was treated with application rates of 0.84 kg as/ha and harvested after one to two weeks. The processing of the samples was conducted on laboratory scale. After drying the grain was milled and subsequently extracted with hexane (→ fat-free meal and crude oil). After removal of the solvent the crude oil was mixed with a solution of sodium hydroxide (→ refined oil and soapstock).

Analysis of all samples was performed by aqueous extraction, followed by ion-exchange chromatography for clean-up. The separation of analytes was conducted by HPLC-fluorescence detection with post-column derivatisation using OPA. The analytical method was successfully validated for glyphosate and AMPA with LOQs of 0.05 mg/kg for grain and meal and with LOQs of 0.1 for oil and soapstock.

Findings:

In the following table the residues of glyphosate in processed maize and the resulting processing factors are summarised. In all samples AMPA residues did not exceed the LOQ of the analytical method.

Table B.7.7-11: Processing of maize

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
Grain	0	Glyphosate: <0.05	-	Glyphosate:			RIP9501257, USA, Wahoo (NE)
				fat-free meal	<0.05	-	
				crude oil	<0.1	-	
				refined oil	n.a.	-	
				soapstock	n.a.	-	
	0	Glyphosate: <0.05	-	Glyphosate:			
				fat-free meal	<0.05	-	
				crude oil	<0.1	-	
				refined oil	n.a.	-	
				soapstock	n.a.	-	
	0.84	Glyphosate: 0.94	7	Glyphosate:			
				fat-free meal	1.1	1.2	
				crude oil	<0.1	<0.11	
				refined oil	n.a.	-	
				soapstock	n.a.	-	
	0.84	Glyphosate: 1.19	14	Glyphosate:			
				fat-free meal	1.22	1.0	
				crude oil	<0.1	<0.08	
				refined oil	n.a.	-	
				soapstock	n.a.	-	
Grain	0	Glyphosate: <0.05	-	Glyphosate:			RIP9501257, USA, Milton (WI)
				fat-free meal	<0.05	-	
				crude oil	<0.1	-	
				refined oil	<0.1	-	
				soapstock	<0.1	-	
	0.84	Glyphosate: 0.7	7	Glyphosate:			
				fat-free meal	0.82	1.2	
				crude oil	<0.1	<0.14	
				refined oil	<0.1	<0.14	
				soapstock	<0.1	<0.14	
Grain	0	Glyphosate: <0.05	-	Glyphosate:			RIP9501257, USA, Olanta (SC)
				fat-free meal	<0.05	-	
				crude oil	<0.1	-	
				refined oil	<0.1	-	
				soapstock	<0.1	-	
	0.84	Glyphosate: 2.04	14	Glyphosate:			
				fat-free meal	2.07	1.0	
				crude oil	<0.1	<0.05	
				refined oil	<0.1	<0.05	
				soapstock	<0.1	<0.05	

Conclusions:

The study is considered acceptable. For glyphosate median processing factors of 1.1 for fat-free meal (1.0, 1.0, 1.2, 1.2) and of <0.1 for crude and refined oil and soapstock (each <0.05, <0.08, <0.11, <0.14)..

Reference: OECD KIIA 6.5.3

Report: Kunda, U. S.
Glyphosate residues in or on corn grits and flour following preharvest applications of Roundup herbicide to corn
January 1990, MSL-9797, RIP9501258

Guidelines: EPA Guideline 171-4

Deviations: ~~original guideline not submitted~~none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
Maize samples treated with glyphosate for desiccation purposes in the USA were processing into large, medium and small grits and flour. The crop was treated with application rates of 0.84 kg as/ha and harvested after 14 days. The processing of the samples was conducted on small scale. After drying the grain was first cleaned in a Gravity separator to remove the germ and large grits. The remainings were milled and separated using different sieves (>2mm → medium grits; <2mm & >1.4mm → small grits; <0.25 mm → flour).

Analysis of all samples was performed by aqueous extraction, followed by ion-exchange chromatography for clean-up. The separation of analytes was conducted by HPLC-fluorescence detection with post-column derivatisation using OPA. The analytical method was successfully validated for glyphosate and AMPA with LOQs of 0.05 mg/kg.

Findings:
In the following table the residues of glyphosate in processed maize and the resulting processing factors are summarised. In all samples AMPA residues did not exceed the LOQ of the analytical method.

Table B.7.7-12: Processing of maize

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
Grain	0.84	Glyphosate: 0.65	14	Glyphosate large grits medium grits small grits flour	 0.71 0.57 0.56 0.56	 1.1 0.9 0.9 0.9	RIP9501258, USA, Millsboro (DE)
Grain	0.84	Glyphosate: 0.87	14	Glyphosate large grits medium grits small grits flour	 0.64 0.51 0.52 0.81	 0.7 0.6 0.6 0.9	RIP9501258, USA, Boling (TX)

Conclusions:
The study is considered acceptable. For glyphosate median processing factors of 0.9 (0.7, 1.1), 0.75 (0.6, 0.9) and 0.75 (0.6, 0.9) for large, medium and small grits and of 0.9 for maize flour (0.9, 0.9).

Reference: OECD KIIA 6.5.3

Report: Oppenhuizen, M. E.
Magnitude of Glyphosate residues in corn processed commodities following preharvest application of Roundup herbicide
May 1995, MSL-13655, RIP9800120

Guidelines: EPA Guideline 171-4

Deviations: ~~original guideline not submitted~~none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study maize grown in two locations in the USA was treated with glyphosate at application rates of 2.52 kg as/ha for desiccation. The grain was harvested six to seven days after the treatment and processed into clean grain, crude oil (wet & dry milled), refined oil (wet and dry milled), flour, grits, meal, starch and the grain dust.
After sieving (→ clean grain) the grain was milled. The milled material was again sieved (3mm), leaving the top material (grits, germ). Using dry milling, the crude and refined oil were extracted from the germ. The bottom material was again sieved separated into meal, grain dust and flour.

Analysis of all samples was performed by extraction with diluted HCl, followed by ion-exchange chromatography for clean-up. The separation of analytes was conducted by HPLC-fluorescence detection with post-column derivatisation using OPA. The analytical method was successfully validated for glyphosate and AMPA with LOQs of 0.05 mg/kg.

Findings:
In the following table the residues of glyphosate and AMPA in processed maize and the resulting processing factors are summarised.

Table B.7.7-13: Processing of maize

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
Grain	2.52	Glyphosate: <0.05 (0.021) AMPA: 0.084	6	Glyphosate: clean grain crude oil (dry) refined oil (dry) flour grain dust grits meal starch crude oil (wet) refined oil (wet) AMPA: clean grain crude oil (dry) refined oil (dry) flour grain dust grits meal starch crude oil (wet) refined oil (wet)	 0.05 <0.05 <0.05 <0.05 (0.036) 8.296 <0.05 <0.05 (0.028) <0.05 <0.05 <0.05 0.099 <0.05 <0.05 0.066 1.619 <0.05 (0.016) 0.073 <0.05 <0.05 <0.05	 - (2.4) - - - (1.7) - (395) - - (1.3) - - - 1.2 <0.6 <0.6 0.79 19.3 <0.6 (0.19) 0.87 <0.6 <0.6 <0.6	RIP9800120, USA, Mon-mouth (IL)
Grain	2.52	Glyphosate: <0.05 (0.045) AMPA: 0.116	7	Glyphosate: clean grain crude oil (dry) refined oil (dry) flour grain dust grits meal starch crude oil (wet) refined oil (wet) AMPA: clean grain crude oil (dry) refined oil (dry) flour grain dust grits meal starch crude oil (wet) refined oil (wet)	 <0.05 (0.004) <0.05 <0.05 <0.05 (0.023) 0.64 <0.05 (0.004) <0.05 (0.046) <0.05 <0.05 <0.05 0.92 <0.05 <0.05 <0.05 (0.021) <0.05 <0.05 (0.049) <0.05 (0.026) <0.05 (0.009) <0.05 <0.05	 - (0.09) - - - (0.51) - (14.2) - (0.09) - (1.0) - - - 7.9 <0.4 <0.4 <0.4 (0.18) <0.4 <0.4 (0.42) <0.4 (0.22) <0.4 (0.08) <0.4 <0.4	RIP9800120, USA, West Burlington (IA)

values in brackets were extrapolated below the validated LOQ

Conclusions:

In this study no residues of glyphosate above the validated LOQ were found in the RAC, denying the calculation of reliable processing factors for maize products. A general accumulation of the residue was observed in clean grain and grain dust, while crude oil, refined oil and starch showed no residues above the LOQ.

For AMPA median processing factors were 4.55 for clean grain, <0.5 for crude oil, refined oil, grits and starch, 0.59 for flour and 0.64 for meal.

B.7.7.2.9 Oats

Reference: OECD KIIA 6.3 & 6.5.3

Report: Mestdagh, P.
Residual Glyphosate in processed oat grains following a preharvest application of Roundup herbicide in the United Kingdom
28.10.1981, MLL 30.071, RIP9501239

Guidelines: no

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be not acceptable.

Material and Methods:
In this study oats treated in the UK for desiccation purposes with application rates of 0.72 kg as/ha to 2.88 kg as/ha was processed into groats and hulls following common industrial practice.

No information on the processing conditions, storage intervals or analytical methods and their validation was presented.

Findings:
In the following table the residues of glyphosate an AMPA in processed oats and the resulting processing factors are summarised.

Table B.7.7-14: Processing of oats

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
Grain	0	Glyphosate: n.r. AMPA: n.r	-	Glyphosate: groats hulls AMPA: groats hulls	 <0.1 0.5 <0.05 <0.05	 (-) (-) (-) (-)	RIP9501239, UK, Winch-burgh/Lothian
	0	Glyphosate: 0.5 AMPA: <0.05	-	Glyphosate: groats hulls AMPA: groats hulls	 0.2 1.7 <0.05 <0.05	 (0.4) (3.4) (-) (-)	
	0.72	Glyphosate: 1.8 AMPA:	36	Glyphosate: groats hulls	 0.9 2.0	 (0.5) (1.1)	

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
		0.08		AMPA:			
				groats	<0.05	(<0.63)	
				hulls	<0.05	(<0.63)	
	0.72	Glyphosate: 0.8	28	Glyphosate:			
				groats	0.2	(0.25)	
				hulls	6.6	(8.3)	
		AMPA: <0.05		AMPA:			
				groats	<0.05	(-)	
				hulls	<0.05	(-)	
	1.44	Glyphosate: 4.3	36	Glyphosate:			
				groats	3.0	(0.7)	
				hulls	3.9	(0.91)	
		AMPA: 0.19		AMPA:			
				groats	0.13	(0.68)	
				hulls	0.1	(0.53)	
	1.44	Glyphosate: 1.4	28	Glyphosate:			
				groats	0.8	(0.57)	
				hulls	6.3	(4.5)	
		AMPA: <0.05		AMPA:			
				groats	<0.05	(-)	
				hulls	<0.05	(-)	
	2.88	Glyphosate: 6.0	36	Glyphosate:			
				groats	3.3	(0.555)	
				hulls	11.1	(1.9)	
		AMPA: 0.18		AMPA:			
				groats	0.13	(0.72)	
				hulls	0.2	(1.1)	
	2.88	Glyphosate: 1.9	25	Glyphosate:			
				groats	0.5	(0.26)	
				hulls	3.5	(1.8)	
		AMPA: <0.05		AMPA:			
				groats	<0.05	(-)	
				hulls	0.1	(>2)	
Grain	0	Glyphosate: <0.1	-	Glyphosate:			RIP9501239, UK, Wintringham
				groats	<0.1	(-)	
				hulls	0.1	(<1)	
		AMPA: <0.05		AMPA:			
				groats	<0.05	(-)	
				hulls	<0.05	(-)	
	1.44	Glyphosate: 4.3	7	Glyphosate:			
				groats	1.4	(0.33)	
				hulls	5.6	(1.3)	
		AMPA: 0.11		AMPA:			
				groats	0.05	(0.45)	
				hulls	0.15	(1.4)	
	1.44	Glyphosate: 5.2	7	Glyphosate:			
				groats	4.0	(0.77)	
				hulls	17.8	(3.4)	
		AMPA: 0.09		AMPA:			
				groats	0.13	(1.4)	

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
				hulls	0.40	(4.4)	
	2.88	Glyphosate: 7.0 AMPA: 0.18	7	Glyphosate: groats hulls AMPA: groats hulls	 1.1 14.2 0.05 0.34	 (0.16) (2.0) (0.28) (1.9)	
	2.88	Glyphosate: 11.3 AMPA: 0.22	7	Glyphosate: groats hulls AMPA: groats hulls	 1.1 9.1 0.10 0.25	 (0.1) (0.81) (0.45) (1.1)	

n.r. not reported

Conclusions:
In the study report crucial information on the processing conditions, storage intervals or analytical methods and their validation are missing. The study is not considered acceptable.

B.7.7.2.10 Rye

Reference: OECD KIIA 6.3 & 6.5.3

Report: Schulz, H.
Determination of residues of Glyphosate and AMPA in cereals - (CHE 03690H)
10.11.1992, 275848, RIP9501327

Guidelines: BBA Guideline Pat IV, 3-3(9)

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Reference: OECD KIIA 6.3 & 6.5.3

Report: Schulz, H.
Determination of residues of Glyphosate and AMPA in cereals - (SAG 539 00)
10.11.1992, 275837, RIP9501328

Guidelines: BBA Guideline Pat IV, 3-3(9)

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In supervised field trials involving application of glyphosate to rye for desiccation grain samples were processed into whole meal bread, bran, flour, middlings and whole meal flour. The sample were milled using a household milling machine capable of processing 6 kg grain per hour. The obtained matrices were directly analysed or, in case of whole meal flour, baked into whole meal bread.

The analysis of all samples for glyphosate and AMPA residues was performed according to the validated method DFG 405, involving aqueous extraction, ion-exchange clean-up and HPLC determination with post-column derivatisation and flourescence detection. Validated LOQs were 0.05 mg/kg for all matrices and analytes.

Findings:
In the following table the residues of glyphosate and AMPA in processed rye and the resulting processing factors are summarised.

Table B.7.7-15: Processing of rye

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
grain	1.8	Glyphosate: 36 AMPA: 0.27	21	Glyphosate whole meal bread bran flour middlings whole-flour meal AMPA[a] whole meal bread bran flour middlings whole-flour meal	 2.4 6.0 12 2.5 0.37 0.16 0.18 0.42 0.12 0.05	 0.07 0.17 0.33 0.07 0.01 0.59 0.67 1.6 0.44 0.19	RIP9501327 & RIP9501328, Germany, Frank-furt-Bergen/Enkheim
grain	1.8	Glyphosate: 18 AMPA: 0.68	21	Glyphosate whole meal bread bran flour middlings whole-flour meal AMPA[a] whole meal bread bran flour middlings whole-flour meal	 14 24 2.0 21 16 0.42 0.34 0.12 0.5 0.27	 0.78 1.3 0.11 1.2 0.89 0.62 0.5 0.18 0.74 0.4	RIP9501327 & RIP9501328, Germany, Haslangbreit
grain	1.8	Glyphosate: 4.0 AMPA: 0.25	21	Glyphosate whole meal bread bran flour	 1.9 6.7 2.2	 0.48 1.7 0.55	RIP9501327 & RIP9501328, Germany, Frank-furt-

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
				middlings whole-flour meal AMPA[a] whole meal bread bran flour middlings whole-flour meal	5.9 4.5 0.12 0.21 0.25 0.21 0.22	1.5 1.1 0.48 0.84 1 0.84 0.88	Bergen/Enkheim
grain	1.8	Glyphosate: 5.0 AMPA: 0.05	21	Glyphosate whole meal bread bran flour middlings whole-flour meal AMPA[a] whole meal bread bran flour middlings whole-flour meal	 13 24 7.5 39 22 0.34 0.4 0.2 0.65 0.69	 2.6 4.8 1.5 7.8 4.4 6.8 8 4 13 13.8	RIP9501327 & RIP9501328, Germany, Frankfurt-Bergen/Enkheim

a: calulated as glyphosate (factor 1.523)

Conclusions:
Median processing factors for glyphosate in processed rye were: 1.5 for bran (0.17, 1.3, 1.7, 4.8), for 0.44 flour (0.11, 0.33, 0.55, 1.5), 1 for whole meal flour (0.01, 0.89, 1.1, 4.4), 0.63 for whole meal bread (0.07, 0.48, 0.78, 2.6) and 1.35 for middlings (0.07, 1.2, 1.5, 7.8).
For AMPA the median processing factors were 0.76 for bran (0.5, 0.67, 0.84, 8), 1.3 for flour (0.18, 1, 1.6, 4), 0.31 for whole meal flour (0.19, 0.22, 0.4, 13.8), 0.61 for whole meal bread (0.48, 0.59, 0.62, 6.8) and 0.79 for middlings (0.44, 0.74, 0.84, 13).

B.7.7.2.11 Wheat

Reference: OECD KIIA 6.3 & 6.5.3

Report: Steinmetz, J. R.
Glyphosate residues in wheat and wheat grain milling/fractionation products following pre-harvest applications with Roundup herbicide
July 1984, MSL-3677, RIP9501254

Guidelines: no

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be acceptable.

Reference: OECD KIIA 6.3 & 6.5.3

Report: Steinmetz, J. R.; Cowell, J. E.
Addendum to MSL-3612: Glyphosate residues in wheat grain milling/fractionation products
October 1984, MSL-4005, RIP9501255

Guidelines: no

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study conducted in the USA wheat grain treated before harvest either with 0.43 kg as/ha or 3.36 kg as/ha was processed into bran, break flour, flour and shorts. The pre-harvest interval for the raw agricultural commodity was 7 days. The samples were processed in a commercial mill, following common practice for wheat processing.

For the analysis of the residue the samples were extraced with water. After clean-up using ion-exchange chromatography the analytes were separated by HPLC with post-column derivatisation (OPA or ninhydrin. The detection was conducted either via fluorescence detected (OPA, 340 nm → 455 nm) or UV-VIS (ninhydrin, 546 nm). The analytical method was validated within an acceptable range (n≥5, 70-110% recovery, <20% RSD) between 0.05 to 300 mg/kg for grain (RAC and processed).

Findings:
In the following table the residues of glyphosate and AMPA in processed wheat and the resulting processing factors are summarised.

Table B.7.7-16: Processing of wheat

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
Grain	0.43	Glyphosate: 0.71 AMPA: <0.05	7	Glyphosate bran break flour flour shorts AMPA: bran break flour flour shorts	 1.66 0.14 0.12 1.2 0.07 <0.05 <0.05 <0.05	 2.3 0.2 0.17 1.7 >1.4 - - -	RIP9501254, USA, Saltillo (TN)
Grain	3.36	Glyphosate: 78 AMPA: 2.07	7	Glyphosate bran break flour flour shorts AMPA:	 121 22.8 22.8 94.7	 1.6 0.29 0.29 1.2	RIP9501254, USA, Mayview (MO)

				bran break flour flour shorts	2.58 1.77 1.68 2.51	1.2 0.86 0.81 1.2	

Conclusions:
Based on the two trials presented median processing factors for glyphsate of 1.95 for bran (1.6, 2.3), 0.245 for break flour (0.2, 0.29), 0.23 for flour (0.17, 0.29) and 1.45 for shorts (1.2, 1.7) were estimated. For AMPA only the second trials gave acceptable residues to calculated processing factors: 1.2 for bran, 0.86 for break flour, 0.81 for flour and 1.2 for shorts.,

Reference: OECD KIIA 6.3 & 6.5.3

Report: Schulz, H.
Determination of residues of Glyphosate and AMPA in cereals - (CHE 03690H)
10.11.1992, 275848, RIP9501327

Guidelines: BBA Guideline Pat IV, 3-3(9)

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Reference: OECD KIIA 6.3 & 6.5.3

Report: Schulz, H.
Determination of residues of Glyphosate and AMPA in cereals - (SAG 539 00)
10.11.1992, 275837, RIP9501328

Guidelines: BBA Guideline Pat IV, 3-3(9)

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Reference: OECD KIIA 6.3 & 6.5.3

Report: Anon.
Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln – Weizen
FRG-0065/R192-90 &
FRG-0066/R-196-90, ASB2009-6582, ASB2009-6583

Guidelines: BBA Guideline Pat IV, 3-3(9)

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In supervised field trials involving application of glyphosate to wheat for desiccation grain samples were processed into whole meal bread, bran, flour, middlings, whole meal flour semolina and semolina bran. The sample were milled using a household milling machine capable of processing 6 kg grain per hour. The obtained matrices were directly analysed or, in case of whole meal flour, baked into whole meal bread.

The analysis of all samples for glyphosate and AMPA residues was performed according to the validated method DFG 405, involving aqueous extraction, ion-exchange clean-up and HPLC determination with post-column derivatisation and flourescence detection. Validated LOQs were 0.05 mg/kg for all matrices and analytes.

Findings:
In the following table the residues of glyphosate and AMPA in processed wheat and the resulting processing factors are summarised.

Table B.7.7-17: Processing of wheat

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
grain	1.8	Glyphosate: 0.64 AMPA: <0.05	21	Glyphosate whole meal bread bran flour middlings whole-flour meal semolina semolina bran AMPA[a] whole meal bread bran flour middlings whole-flour meal semolina semolina bran	 0.25 0.83 0.05 0.57 1.1 0.09 1.4 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05	 0.39 1.3 0.08 0.89 1.7 0.14 2.2 - - - - - - -	RIP9501327, RIP9501328 & ASB2009-6582 Germany, Solms-Oberbiel/Wetzlar
grain	1.8	Glyphosate: 0.74 AMPA: <0.05	21	Glyphosate whole meal bread bran flour middlings whole-flour meal semolina semolina bran AMPA[a]	 0.25 0.71 0.09 0.24 0.4 0.12 1.0	 0.34 0.96 0.12 0.32 0.54 0.16 1.4	RIP9501327, RIP9501328 & ASB2009-6583 Germany, Solms-Oberbiel/Wetzlar

				whole meal bread bran flour middlings whole-flour meal semolina semolina bran	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05	- - - - - - -	

Conclusions:
Median processing factors for glyphosate in processed wheat were: 1.1 for bran (0.96, 1.3), for 0.1 flour (0.08, 0.12), 1.1 for whole meal flour (0.54, 1.7), 0.37 for whole meal bread (0.34, 0.39), 0.61 for middlings (0.32, 0.89), 0.15 for semolina (0.14, 0.16) and 1.8 for semolina bran (1.4, 2.2).
For AMPA no residues above the LOQ of 0.05 mg/kg were found in the grain or its processed products. Calculation of processing factors is not possible based on this study.

Reference: OECD KIIA 6.3 & 6.5.3

Report: Mestdagh, P.
Glyphosate residues in cereals following preharvest application of Roundup in France
25.01.1980, MLL 30046, RIP9501231

Guidelines: no

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be acceptable.

Material and Methods:
In supervised field trials on wheat conducted in France grain samples were processed into bran and flour by using a cutter to reduce the particle size below 1mm. The flour and bran were defined by sifting on 0.3 mm (flour) and 1 mm (bran) screens.

The analysis of the samples was performed with an in-house method involving aqueous extraction, ion-exchange chromatorgraphy clean-up and detection with GC-NPD after derivatisation into the N-trifluoroacetylmethyl-esters. The validated LOQs for glyphosate and AMPA in all matrices was 0.05 mg/kg (n>5, 70-110% recovery, RSD: <20%).

Findings:
In the following table the residues of glyphosate and AMPA in processed wheat and the resulting processing factors are summarised.

Table B.7.7-18: Processing of wheat

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
grain	2.15	Glyphosate: 0.67	8	Glyphosate flour	 0.48	 0.72	RIP9501231, France, Remy

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
		AMPA:		bran	1.11	1.7	
		<0.05		AMPA			
				flour	<0.05	-	
				bran	<0.05	-	
	4.3	Glyphosate:	8	Glyphosate			
		1.56		flour	0.9	0.58	
		AMPA:		bran	2.74	1.8	
		<0.05		AMPA			
				flour	<0.05	-	
				bran	<0.05	-	
	8.6	Glyphosate:	8	Glyphosate			
		2.66		flour	2.46	0.92	
		AMPA:		bran	7.44	2.8	
		<0.05		AMPA			
				flour	<0.05	-	
				bran	<0.05	-	
grain	2.15	Glyphosate:	8	Glyphosate			RIP9501231,
		0.5		flour	0.36	0.72	France, Plessis
		AMPA:		bran	0.59	1.2	Belleville
		<0.05		AMPA			
				flour	<0.05	-	
				bran	<0.05	-	
	4.3	Glyphosate:	8	Glyphosate			
		1.24		flour	0.64	0.52	
		AMPA:		bran	2.39	1.9	
		<0.05		AMPA			
				flour	<0.05	-	
				bran	<0.05	-	
	8.6	Glyphosate:	8	Glyphosate			
		2.16		flour	1.19	0.55	
		AMPA:		bran	4.4	2.0	
		<0.05		AMPA			
				flour	<0.05	-	
				bran	<0.05	-	
grain	2.15	Glyphosate:	8	Glyphosate			RIP9501231,
		0.75		flour	0.47	0.63	France, Le Plessier
		AMPA:		bran	1.37	1.8	St. Juste
		<0.05		AMPA			
				flour	<0.05	-	
				bran	<0.05	-	
	4.3	Glyphosate:	8	Glyphosate			
		1.32		flour	1.02	0.77	
		AMPA:		bran	2.4	1.8	
		<0.05		AMPA			
				flour	<0.05	-	
				bran	<0.05	-	
	8.6	Glyphosate:	8	Glyphosate			
		3.44		flour	1.96	0.57	
		AMPA:		bran	4.35	1.3	
		<0.05		AMPA			
				flour	0.05	-	

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
				bran	0.07	-	

Conclusions:
Based on this study median processing factors for glyphosate in wheat matrices were 0.63 for flour (0.52, 0.55, 0.57, 0.58, 0.63, 0.72, 0.72, 0.77, 0.92) and 1.8 for bran (1.2, 1.3, 1.7, 1.8, 1.8, 1.8, 1.9, 2.0, 2.8).
For AMPA no residues above the LOQ were found in the RAC, no allowing the calculation of processing factors.

Reference: OECD KIIA 6.3 & 6.5.3

Report: Zietz, E.
Determination of residues of Glyphosate in cereals and processing products - Treatment with GLYFOS - Germany season 1992
06.04.1993, IF-92/11567-01, RIP9501329, RIP9500134, ASB2011-9181

Guidelines: BBA Guideline IV, 3-1 and 3-3

Deviations: none

GLP: yes

Acceptability: The study is considered to be additional information.

Material and Methods:
In two locations in Germany side-by-side plots of wheat plants were treated with two different formulations of glyphosate for desiccation purposes. The plants were treated three weeks before harvest with application rates of 1.8 kg as/ha for each formulation. Harvested grain was processed into bran, whloe-meal flour, whole-meal bread and flour type 550 (white flour). Detailed information of the processing conditions were not reported.

The analysis of all samples for glyphosate and AMPA residues was performed according to the validated method DFG 405, involving aqueous extraction, ion-exchange clean-up and HPLC determination with post-column derivatisation and flourescence detection. Validated LOQs were 0.03 mg/kg for grain and 0.05 mg/kg for all processed matrices.

Findings:
In the following table the residues of glyphosate and AMPA in processed wheat and the resulting processing factors are summarised.

Table B.7.7-19: Processing of wheat

RAC	kg as/ha	Residue in RAC (mg/kg)	PHI (d)	Processed commodity	Residue (mg/kg)	Processing factor	Reference
grain	1.8	Glyphosate:	21	Glyphosate			RIP9501329,

	(Glyfos)	0.04 AMPA: <0.03		whole-meal whole-meal bread flour bran AMPA whole-meal whole-meal bread flour bran	0.13 0.2 <0.05 0.36 <0.05 0.08 <0.05 <0.05	(3.3) (5) - (9) - - - -	RIP9500134, ASB2011-9181, Germany, Goch-Nierswalde
	1.8 (Roundup)	Glyphosate: 0.05 AMPA: <0.03	21	Glyphosate whole-meal whole-meal bread flour bran AMPA whole-meal whole-meal bread flour bran	 0.16 0.12 <0.05 0.43 <0.05 0.08 <0.05 <0.05	 (3.2) (2.4) (<1) (8.6) - - - -	
grain	1.8 (Glyfos)	Glyphosate: 0.05 AMPA: <0.03	21	Glyphosate whole-meal whole-meal bread flour bran AMPA whole-meal whole-meal bread flour bran	 0.16 0.11 <0.05 0.25 <0.05 <0.05 <0.05 <0.05	 (3.2) (2.2) (<1) (5) - - - -	RIP9501329, RIP9500134, ASB2011-9181, Germany, Angermuende
	1.8 (Roundup)	Glyphosate: 0.09 AMPA: <0.03	21	Glyphosate whole-meal whole-meal bread flour bran AMPA whole-meal whole-meal bread flour bran	 0.16 0.14 <0.05 0.45 <0.05 <0.05 <0.05 <0.05	 (1.8) (1.6) (<0.56) (5) - - - -	

values in brackets are not considered reliable PFs

Conclusions:
In this study the effect of wheat processing on glyphosate and AMPA residues was investigated. However, the results are very unexpected showing a strong concentration in whole-meal fractions without separation or removal of grain part compared to the RAC. The RMS is of the opinion, that the low residues in the RAC induce an overestimation of the true PF. This study is therefore considered as additional information only.
For AMPA no residues above the LOQ were found in the grain, not allowing prognosis on the behaviour of the metabolite during wheat processing.

Reference: OECD KIIA 6.3 & 6.5.3

Report: Mestdagh, P.
Appendix D: Analytical residues methods - Glyphosate residues in cereal grain and straw following preharvest treatment with Roundup herbicide in the United Kingdom

02.12.1982, MLL 30087, ASB2009-5491

Guidelines: no

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be not acceptable.

Conclusions:
In several supervised field trials conducted in the United Kingdom wheat grain was processed into flour and bread. However, the analytical method involved were validated at a very high LOQ of 0.4 mg/kg for glyphosate and 0.2 mg/kg for AMPA (see list of supervised field studies). For the calcuation of processing factors for wheat this study is not considered acceptable.

B.7.7.2.12 Cereal grains, general

Reference: OECD KIIA 6.3 & 6.5.3

Report: Mestdagh, P.; Dirks, R. C.
Glyphosate residues in processed fractions obtained from Roundup herbicide treated wheat, barley, and oat. European field trials
Febuary 1987, MLL 30.179, RIP9501256

Guidelines: no

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be not acceptable.

Reference: OECD KIIA 6.3 & 6.5.3

Report: Mestdagh, P.
Residual Glyphosate in processed wheat grains following a preharvest application of Roundup herbicide in the United Kingdom
28.10.1981, MLL 30069, RIP9501237

Guidelines: no

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be not acceptable.

In these study already described in the first DAR for glyphosate summary information on residues in barley, oats and wheat in the EU are presented. However, apart from studies listed above under the respective commodities, no field parts, analytical methods used or processing condition were reported. Detailed information on the supervised field trials are reported in separate studies for the respective crops. This summary report is not considered an acceptable reference.

B.7.7.3 Open literature

The influence of glyphosate residues during malting after desiccation of barley was investigated by Caierao et al (2007, ASB2012-12382). The residue concentration of glyphosate showed no effect to the malting of barley.

Low et al (2005, ASB2012-12449) investigated the inpact of *Saccharomyces cerevisiae* on the stability of glyphosate during bread leavening. It was shown that the approximately 20% of the initial glyphosate concentration was degraded within 1 hour. However, no analysis on the metabolites formed was conducted.

B.7.8 Livestock feeding studies (Annex IIA 6.4; Annex IIIA 8.4)

Livestock feeding studies are available investigating the behaviour of glyphosate, AMPA, N-acetyl-glyphosate in ruminants (all analytes), poultry (all analytes) and swine (glyphosate and AMPA).

B.7.8.1 Ruminants

Reference: OECD KIIA 6.4.2

Report: Cowell, J. E.
Residue determination of Glyphosate and AMPA in dairy cow tissues and milk following a 28 day feeding study
September 1987, Report No.: MSL-6729, RIP9501250

Guidelines: not stated

Deviations: not appplicable

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study lactating cows (Holstein) were administered a 9:1 mixture of glyphosate and AMPA at total rates equivalent to 0, 40, 120 and 400 mg/kg in the diet. The active substances were adminsited via a contentrate milking ration fortified with 4x the target dose (160, 480 and 1600 ppm in the concentrate). The milking ration was fed at a rate 25% of the individual animals feed consumption recorded. In addition the animals had ad libitum access to roughage and water. Actual feed level per kg bw varied between the animals.
Actual dose levels were:

Table B.7.8-1: Actual dose rates of glyphosate and AMPA (9:1) administered to lactating cows for 28 days

Nominal dose level	Animal	Average contentrate milking ration per day (kg)	Average bodyweight during dosing (kg)	Glyphosate dose per day		AMPA dose per day	
				mg per animal	mg/kg bw	mg per animal	mg/kg bw
40 mg/kg feed (glyphosate : AMPA, 9:1; 160 ppm in concentrate)	079	5	562	720	1.28	80	0.144
	055	6.75	617	972	1.56	108	0.176
	097	5.1	613	736	1.2	81.6	0.132
	053	4.8	487	692	1.44	76.8	0.156
	086	5.1	485	736	1.52	81.6	0.168
	Mean	5.35	553	772	1.4	85.6	0.156
120 mg/kg feed (glyphosate : AMPA, 9:1, 480 ppm in concentrate)	059	5.5	571	2376	4.16	264	0.48
	102	5.9	634	2548	4	283.2	0.44
	105	6.4	609	2764	4.4	307.2	0.52
	080	4.6	511	1988	3.88	220.8	0.44
	106	5.6	589	2420	4	268.8	0.44
	Mean	5.6	583	2420	4	268.8	0.48
400 mg/kg feed (glyphosate : AMPA, 9:1, 1600 ppm in concentrate)	090	4.8	554	6912	12.4	768	1.4
	098	5.6	637	8064	12.8	896	1.4
	100	6.0	632	8640	13.6	960	1.52
	107	6.0	629	8640	13.6	960	1.52
	093	4.2	540	6048	11.2	672	1.24
	Mean	5.3	598	7632	12.8	848	1.4

Each dose goup consisted of 3 animals, from which three were slaughtered at the end of dosing after 28 days, one after 7 days depuration (day 35) and one after 28 days depuration (day 28).

Milk was collected over the whole dosing study period and analysed together with tissues samples for residues of glyphosate and AMPA. Samples were extracted with water, cleaned by a cation exchange column and finally analysed by a validated HPLC method using fluorescence detection after derivatisation.

Findings:
The analysis of milk and tissues for residues of glyphosate and AMPA showed no detectable residues above the LOQs (0.025 mg/kg for milk, 0.05 mg/kg for tissues) in milk, muscle and fat for all dose groups.

Concerning animal health during the dosing period nodules (apparent hepatic abscesses) were observed in one cow, and kidney lesions in another. Both were retained in the study. No observations were considered treatment related.

The results for liver and kidney were:

Table B.7.8-2: Residues of glyphosate and AMPA found in liver and kidney of lactating cows following administration of 9:1 mixtures dose at 0 to 400 mg/kg feed for 28 days

Actual feed level in mg/kg bw	Animal (days)	Residue in liver in mg/kg[a]		Residue in kidney in mg/kg[a]	
		Glyphosate	AMPA	Glyphosate	AMPA
Nominal: 36 mg glyphosate + 4 mg AMPA per kg feed					
Glyphosate: 1.28 AMPA: 0.144	079 (28)	0.06, 0.08 (mean: 0.07)	<0.05, <0.05	0.25, 0.26 (0.255)	0.08, 0.08 (mean: 0.08)
Glyphosate: 1.56 AMPA: 0.176	055 (28)	<0.05, 0.06 (mean: 0.055)	<0.05, <0.05	0.3, 0.38 (mea: 0.34)	0.07, 0.09 (mean: 0.08)
Glyphosate: 0.2 AMPA: 0.132	097 (28)	<0.05, 0.05 (mean: 0.05)	<0.05, <0.05	0.17, 0.17 (mean: 0.17)	0.05, 0.05 (mean: 0.05)
Glyphosate: 1.44 AMPA: 0.156	053 (35)	<0.05, <0.05	<0.05, <0.05	<0.05, <0.05	<0.05, <0.05
Glyphosate: 1.52 AMPA: 0.168	086 (56)	<0.05, <0.05	<0.05, <0.05	<0.05, <0.05	<0.05, <0.05
Nominal: 108 mg glyphosate + 12 mg AMPA per kg feed					
Glyphosate: 4.16 AMPA: 0.48	059 (28)	0.08, 0.08 (mean: 0.08)	<0.05, <0.05	0.79, 0.76 (mean: 0.775)	0.15, 0.15 (mean: 0.15)
Glyphosate: 4.0 AMPA: 0.44	102 (28)	0.05, 0.07 (mean: 0.06)	0.06, 0.06 (mean: 0.06)	0.86, 0.88 (mean: 0.87)	0.27, 0.27 (mean: 0.27)
Glyphosate: 4.4 AMPA: 0.52	105 (28)	0.07, 0.07 (mean: 0.07)	0.05, 0.05 (mean: 0.05)	0.73, 0.73 (mean: 0.73)	0.21, 0.22 (mean: 0.215)
Glyphosate: 3.88 AMPA: 0.44	080 (35)	<0.05, <0.05	<0.05, <0.05	<0.05, <0.05	<0.05, <0.05
Glyphosate: 4.0 AMPA: 0.44	106 (56)	<0.05, <0.05	<0.05, <0.05	<0.05, <0.05	<0.05, <0.05
Nominal: 360 mg glyphosate + 40 mg AMPA per kg feed					
Glyphosate: 12.4 AMPA: 1.4	090 (28)	0.26, 0.23 (mean: 0.245)	0.13, 0.14 (mean: 0.135)	2.84, 2.91 (mean: 2.86)	1.01, 1.0 (mean: 1.01)
Glyphosate: 12.8 AMPA: 1.4	098 (28)	no results reported			
Glyphosate: 13.6 AMPA: 1.52	100 (28)	0.24, 0.24 (mean: 0.24)	0.2, 0.2 (mean: 0.2)	3.43, 3.46 (mean: 3.45)	0.87, 0.87 (mean: 0.87)
Glyphosate: 13.6 AMPA: 1.52	107 (35)	0.12, 0.13 (mean: 0.125)	0.08, 0.08 (mean: 0.08)	0.05, 0.06 (mean: 0.055)	0.07, 0.08 (mean: 0.075)
Glyphosate: 11.2 AMPA: 1.24	093 (56)	<0.05, <0.05	<0.05, <0.05	<0.05, <0.05	<0.05, <0.05

a: duplicate analysis

Conclusions:

Following adiminstration of glyphosate and AMPA to lactating cows at a 9:1 mixture, no detectable transfer of residues into milk, muscle and fat was observed. Liver and kidney were the only tissues with detectable residues.

In liver glpyhosate was found in nearly all samples for the 40 and 120 mg/kg feed animals, but at levels between the LOQ of 0.05 mg/kg up to 0.08 mg/kg. Elevated residues were only found for the highest dose group, ranging from 0.12 to 0.26 mg/kg. AMPA residues in liver were in the same order of magnitude as glyphosate, confirming the findings in animal metabolism studies on goats.

In kidney detectable residues of glyphosate and AMPA were found for all dose groups, scaling with the dose administered. Ratios found for glyphosate to AMPA were approximated 3:1 to 4:1.

Reference: OECD KIIA 6.4.2

Report: Graham, D. G.
Magnitude of SC-0224 residues in meat and milk
31.07.1987, Report No.: RRC 87-44, RIP9500024

Guidelines: EPA: Guideline §161-2

Deviations: ~~original guideline not submitted~~none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study lactating cows were fed nominal dose levels of 0, 0.5, 5, 50, 300 and 1000 mg/kg glyphosate-trimesium in the diet for 28 consecutive days. Expressed as glyphosate equivalents the nominal dose levels were 0, 0.345, 3.45, 34.5, 206.7 and 689 mg/kg glyphosate in the diet (0, 0.012, 0.13, 1.44, 7.38 and 19.4 mg glyphosate eq/kg bw and day). Each dose group consisted of three animal of which two were sacrificed within 24h after the final administration and one animal was kept for additional 7 days depuration.

Milk was collected over the whole dosing study period and analysed together with tissues samples for residues of glyphosate, AMPA and the trimesium-cation (TMS). Samples were extracted with water, cleaned by a cation exchange column and finally analysed by HPLC with fluorescence detection after derivatisation. For milk lacial acetic acid was added to precipate proteins.

Findings:
Results in all samples obtained from the control group (0 ppm dosing) were generally below the LOQ of the analytical method used except for two values of 0.03 mg/kg in milk from one animal and traces of TMS. These values are not presented in detail.

No apparent treatment-related effects on the general condition of the animals, their behaviours, body weights, feed consumption or egg/milk production were reported.

In the following table the residues of glyphosate (Gly), AMPA and TMS in milk are summarised:

Table B.7.8-3: Residues of glyphosate, AMPA and TMS in milk of cattle dosed with glyphosate-trimesium at levels between 0 and 1000 mg/kg in the diet

Day	Mean residues found per dose group in mg/kg				
	0.012 mg glyphosate eq/kg bw	0.130~~053~~ mg glyphosate eq /kg bw	1.44~~0.13~~ mg glyphosate eq /kg bw	7.38~~3.78~~ mg glyphosate eq /kg bw	19.4 mg glyphosate eq /kg bw
-1	Gly: NA AMPA: NA TMS: <0.02	Gly: NA AMPA: NA TMS: <0.02	Gly: NA AMPA: NA TMS: <0.02	Gly: <0.02 AMPA: <0.02 TMS: <0.02	Gly: <0.02 AMPA: <0.02 TMS: <0.02
1	Gly: <0.02[a] AMPA: <0.02[a] TMS: <0.02	Gly: NA AMPA: NA TMS: 0.02	Gly: <0.02[a] AMPA: <0.02[a] TMS: 0.09	Gly: 0.02 AMPA: <0.02 TMS: 0.44	Gly: <0.02 AMPA: <0.02 TMS: 1.5
2	Gly: <0.02[a] AMPA: <0.02[a] TMS: 0.02	Gly: NA AMPA: NA TMS: 0.02	Gly: <0.02[a] AMPA: <0.02[a] TMS: 0.1	Gly: <0.02 AMPA: <0.02 TMS: 0.54	Gly: 0.02 AMPA: <0.02 TMS: 2.2
4	Gly: <0.02[a] AMPA: <0.02[a] TMS: 0.02	Gly: NA AMPA: NA TMS: 0.02	Gly: <0.02[a] AMPA: <0.02[a] TMS: 0.11	Gly: 0.02 AMPA: <0.02 TMS: 0.64	Gly: 0.03 AMPA: <0.02 TMS: 2.2
7	Gly: <0.02[a] AMPA: <0.02[a] TMS: <0.02	Gly: NA AMPA: NA TMS: 0.02	Gly: <0.02[a] AMPA: <0.02[a] TMS: 0.11	Gly: <0.02 AMPA: <0.02 TMS: 0.58	Gly: 0.03 AMPA: <0.02 TMS: 1.6
14	Gly: NA AMPA: NA TMS: 0.02	Gly: NA AMPA: NA TMS: 0.02	Gly: <0.02 AMPA: <0.02 TMS: 0.13	Gly: NA AMPA: NA TMS: 0.76	Gly: 0.02 AMPA: <0.02 TMS: 2.0
21	Gly: NA AMPA: NA TMS: <0.02	Gly: NA AMPA: NA TMS: 0.02	Gly: <0.02 AMPA: <0.02 TMS: 0.11	Gly: NA AMPA: NA TMS: 0.53	Gly: 0.02 AMPA: <0.02 TMS: 1.9
28	Gly: NA AMPA: NA TMS: <0.02	Gly: <0.02 AMPA: <0.02 TMS: 0.02	Gly: <0.02[b] AMPA: <0.02[b] TMS: 0.1	Gly: <0.02 AMPA: <0.02 TMS: 0.45	Gly: 0.02 AMPA: <0.02 TMS: 1.4
35 (depuration)[a]	Gly: NA AMPA: NA TMS: 0.02	Gly: NA AMPA: NA TMS: 0.02	Gly: <0.02 AMPA: <0.02 TMS: 0.04	Gly: <0.02 AMPA: <0.02 TMS: 0.04	Gly: <0.02 AMPA: <0.02 TMS: 0.48

a: based on one animal
b: based on two animals

In tissues sampled after sacrifice residues found were:

Table B.7.8-4: Residues of glyphosate, AMPA and TMS in tissues of cattle dosed with glyphosate-trimesium (glyphosate equivalents) at levels between 0 and 1000 ppm in the diet

Sample	Residues found per animal in mg/kg				
	0.012 mg glyphosate eq /kg bw	0.13~~0053~~ mg glyphosate eq /kg bw	1.44~~0.13~~ mg glyphosate eq /kg bw	7.38~~3.78~~ mg glyphosate eq /kg bw	19.4 mg glyphosate eq /kg bw
Kidney	Gly: <0.05[a] AMPA: <0.05[a] TMS: <0.02, 0.02	Gly: <0.05[a] AMPA: <0.05[a] TMS: 0.03, 0.03	Gly: 0.33, 0.44 AMPA: 0.06, 0.08 TMS: 0.17, 0.18	Gly: 1.8, 2.6 AMPA: 0.47, 0.58 TMS: 0.76, 1.9	Gly: 4.1, 7.6 AMPA: 1.6, 1.7 TMS: 2.5, 4.4
Kidney (depuration)	Gly: <0.05 AMPA: <0.05 TMS: <0.02	Gly: <0.05 AMPA: <0.05 TMS: <0.02	Gly: <0.05 AMPA: <0.05 TMS: 0.02	Gly: 0.12 AMPA: <0.05 TMS: 0.04	Gly: 0.18 AMPA: 0.24 TMS: 0.05
Liver	Gly: <0.2[a] AMPA: <0.2[a] TMS: <0.08, <0.08	Gly: <0.2[a] AMPA: <0.2[a] TMS: <0.08, <0.08	Gly: <0.2[a] AMPA: <0.2[a] TMS: 0.17, 0.32	Gly: <0.2, <0.2 AMPA: <0.2, <0.2 TMS: 0.28, 0.69	Gly: 0.22, 0.51 AMPA: <0.2, <0.2 TMS: 1.1, 2.0
Liver (depuration)	Gly: <0.2 AMPA: <0.2 TMS: <0.08	Gly: <0.2 AMPA: <0.2 TMS: <0.08	Gly: <0.2 AMPA: <0.2 TMS: 0.12	Gly: <0.2 AMPA: <0.2 TMS: 0.15	Gly: <0.2 AMPA: <0.2 TMS: 0.16
Fat	Gly: <0.05[a] AMPA: <0.05[a] TMS: <0.01, <0.01	Gly: <0.05, <0.05 AMPA: <0.05, <0.05 TMS: <0.01, <0.01	Gly: 0.06, <0.05 AMPA: <0.05, <0.05 TMS: 0.01, 0.01	Gly: 0.06, 0.06 AMPA: <0.05, <0.05 TMS: 0.02, 0.13	Gly: 0.06, 0.1 AMPA: <0.05, <0.05 TMS: 0.07, 0.07
Fat (depuration)	Gly: <0.05 AMPA: <0.05 TMS: 0.01	Gly: NA AMPA: NA TMS: <0.01	Gly: <0.05 AMPA: <0.05 TMS: 0.01	Gly: 0.06 AMPA: <0.05 TMS: <0.01	Gly: 0.08 AMPA: <0.05 TMS: 0.06
Muscle	Gly: <0.05[a] AMPA: <0.05[a] TMS: <0.04, <0.04	Gly: <0.05[a] AMPA: <0.05[a] TMS: 0.04, 0.04	Gly: <0.05, <0.05 AMPA: <0.05, <0.05 TMS: 0.07, 0.11	Gly: <0.05, <0.05 AMPA: <0.05, <0.05 TMS: 0.48, 0.63	Gly: 0.08, 0.08 AMPA: <0.05, <0.05 TMS:1.4, 1.5
Muscle (depuration)	Gly: <0.05 AMPA: <0.05 TMS: 0.04	Gly: NA AMPA: NA TMS: 0.05	Gly: <0.05 AMPA: <0.05 TMS: 0.05	Gly: <0.05 AMPA: <0.05 TMS: 0.05	Gly: <0.05 AMPA: <0.05 TMS: 0.05

a: only samples from one animal analysed
NA not analysed

Conclusions:
The findings in this livestock feeding study confirm the results from animal metabolism studies on ruminants concerning glyphosate. The overall transfer of residues into milk, muscle and fat is very limited. Highest residues were found in kidney, followed by liver. AMPA was only relevant in kidney, but it was normally present at lower levels.

Reference: OECD KIIA 6.4.2

Report: McLellan, G.; Bramble, F. Q.
Magnitude of residues of N-Acetylglyphosate and degradates in dairy cow tissues and milk
15.11.2007, Report No.: DuPont-20087, ASB2008-2653

Guidelines: EPA: OPPTS 860.1480, Meat/Milk/Poultry/Eggs
EU: Document 1607/VI97 rev.2
OECD: 505 – Residues in Livestock

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study the magnitude of residues of N-acetyl-glyphosate in dairy cow tissues and milk was investigated. N-acetyl-glyphosate was administered orally as an aqueous solution via drench gun to four groups of lactating Holstein/Friesian cows (3 cows/group) twice daily for 28 consecutive days. Dosing was conducted at treatment levels of 1.25, 3.75, 12.5 and 37.5 mg/kg bw. Two additional cows were dosed at 37.5 mg/kg bw followed by a 7-day depuration period. Milk samples were collected over the whole period of dosing. After sacrifice, the tissues and milk were analyzed for residues of N-acetyl-glyphosate and glyphosate.

The samples were extracted using 0.1% formic acid/methanol (96/4, v/v). Milk samples were portioned with methylene chloride and hexane. Tissue and milk samples were cleaned by SPE against C18 and polymeric anion exchange phases. Stable isotopes were used as internal standard for the analysis of the residues with LC/MS/MS.

Findings:

In the following tables the results for milk, milk after depuration and skim milk/cream are summarised. In all samples no residues above the limit of quantification were found. N-acetyl-glyphosate gave the most frequent rate of residues above the limit of detection. AMPA and N-acetyl-AMPA could only be detected in traces and are not summarised below.

Concerning animal health during the study post-mortem histology showed pyometra in one animal and moderate multifocal hepatic abscesses and a mild bile duct hyperplasia in another animal. Both effects were not related to the dose rates administered.

Table B.7.8-5: N-acetyl-glyphosate residues in whole milk

Dose level in mg/kg bw	**Animal**	**Residues in mg/kg (glyphosat equivalents)**								
		Day -1	Day 1	Day 3	Day 5	Day 7	Day 10	Day 14	Day 21	Day 28
Control	015F	ND	ND	ND	ND	ND	ND	ND	ND	ND
1.25	001F	ND	ND	ND	<0.025	ND	ND	NA	NA	NA
	002F	ND	ND	ND	ND	ND	ND	NA	NA	NA
	003F	ND	ND	ND	ND	ND	ND	NA	NA	NA
	Mean	ND	ND	ND	<0.025	ND	ND	NA	NA	NA
3.75	004F	ND	<0.025	ND	ND	<0.025	ND	ND	NA	NA
	005F	ND	ND	ND	ND	<0.025	ND	ND	NA	NA
	006F	ND	<0.025	ND	ND	<0.025	ND	ND	NA	NA
	Mean	ND	<0.025	ND	ND	<0.025	ND	ND	NA	NA
12.5	007F	ND	ND	<0.025	ND	<0.025	ND	ND	<0.025	<0.025
	008F	ND	ND	ND	<0.025	<0.025	ND	<0.025	<0.025	<0.025
	009F	ND	<0.025	ND	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
	Mean	ND	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
37.5	010F	ND	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
	011F	ND	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
	012F	ND	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
	013F	ND	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
	014F	ND	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
	Mean	ND	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025

ND not detected (LOD: 0.004 mg/kg)
NA not analysed

Table B.7.8-6: Glyphosate residues in whole milk

Dose level in mg/kg bw	**Animal**	**Residues in mg/kg (glyphosat equivalents)**								
		Day -1	Day 1	Day 3	Day 5	Day 7	Day 10	Day 14	Day 21	Day 28
Control	015F	ND	ND	ND	ND	ND	ND	ND	ND	ND
1.25	001F	ND	ND	ND	ND	ND	ND	NA	NA	NA
	002F	ND	ND	ND	<0.025	ND	ND	NA	NA	NA
	003F	ND	ND	ND	<0.025	ND	ND	NA	NA	NA
	Mean	ND	ND	ND	<0.025	ND	ND	NA	NA	NA
3.75	004F	ND	ND	ND	<0.025	ND	ND	ND	NA	NA
	005F	ND	ND	ND	ND	ND	ND	ND	NA	NA
	006F	ND	ND	ND	<0.025	ND	ND	ND	NA	NA
	Mean	ND	ND	ND	<0.025	ND	ND	ND	NA	NA
12.5	007F	ND	ND	ND	<0.025	ND	<0.025	ND	<0.025	ND
	008F	ND	ND	ND	ND	ND	ND	ND	<0.025	ND
	009F	ND	ND	ND	<0.025	ND	<0.025	ND	<0.025	ND
	Mean	ND	ND	ND	<0.025	ND	<0.025	ND	<0.025	ND
37.5	010F	ND	ND	ND	<0.025	ND	ND	ND	ND	ND
	011F	ND	ND	ND	<0.025	ND	<0.025	ND	<0.025	ND
	012F	ND	<0.025	ND	ND	ND	ND	ND	<0.025	ND
	013F	ND	ND	ND	<0.025	<0.025	ND	ND	<0.025	ND
	014F	ND	ND	ND	<0.025	ND	ND	ND	<0.025	ND
	Mean	ND	<0.025	ND	<0.025	<0.025	<0.025	ND	<0.025	ND

ND not detected (LOD: 0.004 mg/kg)
NA not analysed

Table B.7.8-7: Residues in whole milk after 7 days depuration

Dose level in mg/kg bw	Days after last dose	Animal	Residue in mg/kg (glyphosat equivalents)	
			N-acetyl-glyphosate	Glyphosate
37.5	0	013F	<0.025	ND
		014F	<0.025	ND
		Mean	<0.025	ND
	1	013F	<0.025	ND
		014F	<0.025	ND
		Mean	<0.025	ND
	3	013F	<0.025	ND
		014F	<0.025	ND
		Mean	<0.025	ND
	5	014F	<0.025	ND
	7	014F	<0.025	ND

ND not detected (LOD: 0.004 mg/kg)

Table B.7.8-8: Distribution of N-acetyl-glyphosate and glyphosate residues in skim milk and cream

Dose level in mg/kg bw	Collection day	Animal	Skim Milk - residue levels in mg/kg (glyphosat equivalents)		Cream – residue levels in mg/kg (glyphosat equivalents)	
			N-acetyl-glyphosate	Glyphosate	N-acetyl-glyphosate	Glyphosate
3.75	14	004F	ND	ND	<0.025	<0.025
		005F	ND	ND	ND	ND
		006F	ND	ND	ND	ND
		Mean	ND	ND	<0.025	<0.025
12.5	14	007F	<0.025	ND	ND	ND
		008F	ND	ND	ND	ND
		009F	ND	ND	<0.025	ND
		Mean	<0.025	ND	<0.025	ND
	28	007F	ND	ND	<0.025	ND
		008F	<0.025	ND	ND	ND
		009F	<0.025	ND	<0.025	ND
		Mean	<0.025	ND	<0.025	ND
37.5	14	010F	<0.025	<0.025	<0.025	ND
		011F	<0.025	<0.025	<0.025	ND
		012F	<0.025	<0.025	<0.025	ND
		Mean	<0.025	<0.025	<0.025	ND
	28	010F	<0.025	<0.025	<0.025	ND
		011F	<0.025	ND	<0.025	ND
		012F	<0.025	ND	<0.025	ND
		Mean	<0.025	<0.025	<0.025	ND

ND not detected (LOD: 0.004 mg/kg)

In liver, kidney, fat and muscle unchanged N-acetly-glyphosate was the major residues. Findings for N-acetyl-glyphosate, glyphosate, N-acetyl-AMPA and AMPA were:

Table B.7.8-9: N-acetyl-glyphosate, glyphosate, AMPA and N-acetyl-AMPA residues in cow liver

Dose level in mg/kg bw	Animal	Residues in mg/kg (Glyphosate equivalents)			
		N-acetyl-glyphosate	Glyphosate	AMPA	N-acetyl-AMPA
0	015F	ND	ND	ND	ND
1.25	001F	<0.05	ND	ND	ND
	002F	<0.05	ND	ND	ND
	003F	ND	ND	ND	ND
	Mean	0.02	ND	ND	ND
3.75	004F	<0.05	ND	ND	ND
	005F	<0.05	ND	ND	ND
	006F	<0.05	ND	ND	ND
	Mean	<0.05	ND	ND	ND
12.5	007F	0.1	ND	ND	ND
	008F	0.1	ND	ND	ND
	009F	<0.05	ND	<0.05	ND
	Mean	0.07	ND	<0.05	ND
37.5	010F	0.37	<0.05	<0.05	ND
	011F	0.52	<0.05	ND	ND
	012F	0.38	<0.05	<0.05	ND
	Mean	0.43	<0.05	<0.05	ND
37.5 (4-day depuration)	013F	0.1	<0.05	<0.05	ND
37.5 (8-day depuration)	014F	<0.05	<0.05	0.028	ND

ND not detected (LODs in liver: N-acetyl-glyphosate: 0.018 mg/kg, glyphosate: 0.009 mg/kg, AMPA: 0.019 mg/kg and N-acetyl-AMPA: 0.008 mg/kg)

Table B.7.8-10: N-acetyl-glyphosate, glyphosate, AMPA and N-acetyl-AMPA residues in cow kidney

Dose level in mg/kg bw	Animal	Residues in mg/kg (Glyphosate equivalents)			
		N-acetyl-glyphosate	Glyphosate	AMPA	N-acetyl-AMPA
0	015F	ND	<0.05	ND	ND
1.25	001F	0.079	<0.05	ND	<0.05
	002F	0.11	<0.05	ND	<0.05
	003F	0.06	<0.05	ND	ND
	Mean	0.082	<0.05	ND	<0.05
3.75	004F	0.16	<0.05	ND	ND
	005F	0.24	<0.05	ND	<0.05
	006F	0.11	<0.05	ND	<0.05
	Mean	0.17	<0.05	ND	<0.05
12.5	007F	0.62	0.072	<0.05	<0.05
	008F	0.69	0.071	<0.05	<0.05
	009F	0.71	0.078	<0.05	<0.05
	Mean	0.67	0.074	<0.05	<0.05
37.5	010F	2.0	0.19	<0.05	0.069
	011F	3.2	0.23	0.089	0.083
	012F	3.2	0.20	<0.05	0.078
	Mean	2.8	0.21	0.063	0.077
37.5 (4-day depuration)	013F	0.087	<0.05	<0.05	ND
37.5 (8-day depuration)	014F	<0.05	<0.05	<0.05	ND

ND not detected (LODs in liver: N-acetyl-glyphosate: 0.014 mg/kg, glyphosate: 0.004 mg/kg, AMPA: 0.009 mg/kg and N-acetyl-AMPA: 0.008 mg/kg)

Table B.7.8-11: N-acetyl-glyphosate, glyphosate, AMPA and N-acetyl-AMPA residues in cow fat

Dose level in mg/kg bw	Animal	Residues in mg/kg (Glyphosate equivalents)			
		N-acetyl-glyphosate	Glyphosate	AMPA	N-acetyl-AMPA
0	015F	ND	ND	ND	ND
1.25	001F	<0.05	ND	NA	ND
	002F	<0.05	ND	NA	ND
	003F	<0.05	<0.05	NA	ND
	Mean	<0.05	<0.05	NA	ND
3.75	004F	<0.05	ND	NA	ND
	005F	0.17	<0.05	NA	ND
	006F	ND	<0.05	NA	ND
	Mean	0.11	<0.05	NA	ND
12.5	007F	0.054	<0.05	NA	<0.05
	008F	0.051	<0.05	NA	<0.05
	009F	<0.05	ND	NA	<0.05
	Mean	0.052	<0.05	NA	<0.05
37.5	010F	0.22	<0.05	ND	<0.05
	011F	0.055	<0.05	ND	<0.05
	012F	0.075	ND	ND	<0.05
	Mean	0.12	<0.05	ND	<0.05
37.5 (4-day depuration)	013F	0.058	<0.05	NA	<0.05
37.5 (8-day depuration)	014F	0.14	<0.05	NA	<0.05

ND not detected (LODs in liver: N-acetyl-glyphosate: 0.015 mg/kg, glyphosate: 0.004 mg/kg, AMPA: 0.015 mg/kg and N-acetyl-AMPA: 0.009 mg/kg)

NA not analysed

Table B.7.8-12: N-acetyl-glyphosate, glyphosate, AMPA and N-acetyl-AMPA residues in cow muscle

Dose level in mg/kg bw	Animal	Residues in mg/kg (Glyphosate equivalents)			
		N-acetyl-glyphosate	Glyphosate	AMPA	N-acetyl-AMPA
0	015F	ND	ND	NA	NA
1.25	001F	ND	ND	NA	NA
	002F	ND	ND	NA	NA
	003F	ND	<0.025	NA	NA
	Mean	ND	<0.025	NA	NA
3.75	004F	ND	<0.025	NA	NA
	005F	<0.025	<0.025	NA	NA
	006F	ND	<0.025	NA	NA
	Mean	<0.025	<0.025	NA	NA
12.5	007F	<0.025	ND	NA	ND
	008F	<0.025	ND	NA	ND
	009F	ND	ND	NA	ND
	Mean	<0.025	ND	NA	ND
37.5	010F	<0.025	ND	ND	ND
	011F	<0.025	ND	ND	ND
	012F	0.053	ND	ND	ND
	Mean	0.034	ND	ND	ND
37.5 (4-day depuration)	013F	<0.025	ND	NA	NA
37.5 (8-day depuration)	014F	<0.025	0.009	NA	NA

ND not detected (LODs in liver: N-acetyl-glyphosate: 0.006 mg/kg, glyphosate: 0.004 mg/kg, AMPA: 0.008 mg/kg and N-acetyl-AMPA: 0.006 mg/kg)
NA not analysed

Conclusions:
Following administration of N-acetyl-glyphosate to lactating cows the unchanged analyte was the major residue in all matrices investigated. The transfer of residues into milk and muscle was low, showing no quantifiable residues in milk and residues up to 0.053 mg/kg in muscle from the highest dose group.
In fat measurable residues of N-acetyl-glyphosate up to 0.17 mg/kg were found in all dose groups from 3.75 to 37.5 mg/kg bw, however no dependency on the dose administered was observed.
Residues found in liver and kidney scaled with the dose administered to the animals. In liver measurable residues of N-acetyl-glyphosate were observed in the 12.5 mg/kg bw group (0.1 mg/kg) and in the 37.5 mg/kg bw group (0.52 mg/kg). Significant amounts of other metabolites could not detected. Kidney showed the highest residues of all samples with residues ranging from 0.11 mg/kg for the lowest up to 3.2 mg/kg for the highest dose group. In kidney minor amounts of glyphosate (up to 0.23 mg/kg), AMPA (up to 0.089 mg/kg) and N-acetyl-AMPA (up to 0.083 mg/kg) were found in the two highest dose groups.

B.7.8.2 Poultry

Reference: OECD KIIA 6.4.1

Report: Manning, M. J.; Wilson, G. R.
Residue determination of Glyphosate and AMPA in laying hen tissues and eggs follow-ing a 28 day feeding study
November 1987, Report No.: MSL-6676, RIP9501252

Guidelines: not stated

Deviations: not appplicable

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study laying hens (White Leghorn) were administered a 9:1 mixture of glyphosate and AMPA at total rates equivalent to 0, 40, 120 and 400 mg/kg in the diet. The active substances were adminsited via fortified feed. The actual dose rates based on the feed consuption and bodyweight were very homogeneous. Average daily feed consumption was 120 g (117 to 121 g) with a corresponding mean bodyweights of 1.7 kg per animal (1.6-1.8 kg). Based on these data the following dose levels were estimated:

Table B.7.8-13: Actual feed levels of glyphosate and AMPA to laying hens for 28 consecutive days

Nominal feed levels	Glyphosate dose per day		AMPA dose per day	
	mg per animal	mg/kg bw	mg per animal	mg/kg bw
40 mg/kg feed (glyphosate : AMPA, 9:1)	4.32	2.54	0.48	0.28
120 mg/kg feed (glyphosate : AMPA, 9:1)	12.96	7.62	1.44	0.85
400 mg/kg feed (glyphosate : AMPA, 9:1)	43.2	25.4	4.8	2.8

The animals were separated into four subsets per dose levels consisting of 5 animals each. After 28 days dosing two subsets wer sacrificed, one subset after 7 additional days for depuration (day 35) and the last subset after 28 days depuration (day 56).

Eggs were collected over the whole dosing study period and analysed together with tissues samples for residues of glyphosate and AMPA. Samples were extracted with water, cleaned by a cation exchange column and finally analysed by a validated HPLC method using fluorescence detection after derivatisation.

Findings:
No apparent treatment-related effects on the general condition of the animals, their behaviours, body weights, feed consumption or egg/milk production were reported.

In the following table the residuse found in eggs are summarised.

Table B.7.8-14: Residues of glyphosate and AMPA found in eggs following administration of 9:1 mixtures dose at 0 to 400 mg/kg feed for 28 days

Days	Glyphosate: 2.54 mg/kg bw AMPA: 0.28 mg/kg bw (40 mg/kg feed)		Glyphosate: 7.62 mg/kg bw AMPA: 0.85 mg/kg bw (120 mg/kg feed)		Glyphosate: 25.4 mg/kg bw AMPA: 2.8 mg/kg bw (400 mg/kg feed)	
	Glyphosate (mg/kg)	AMPA (mg/kg)	Glyphosate (mg/kg)	AMPA (mg/kg)	Glyphosate (mg/kg)	AMPA (mg/kg)
1	<0.025(4)	<0.025(4)	<0.025(4)	<0.025(4)	<0.025(4)	<0.025(4)
2	<0.025(4)	<0.025(4)	<0.025(4)	<0.025(4)	<0.025(4)	<0.025(4)
4	<0.025(4)	<0.025(4)	<0.025(4)	<0.025(4)	<0.025(2), 0.026, 0.026 [0.026]	<0.025(4)
7	<0.025(4)	<0.025(4)	<0.025(2), 0.027, 0.028 [0.026]	<0.025(4)	0.07, 0.071, 0.076, 0.087 [0.074]	<0.025(4)
14	<0.025(4)	<0.025(4)	0.029(3), 0.027 [0.028]	<0.025(4)	0.071, 0.086, 0.102, 0.109 [0.092]	<0.025(4)
21	<0.025(4)	<0.025(4)	0.026, 0.028, 0.029, 0.03 [0.028]	<0.025(4)	0.09, 0.095, 0.096, 0.131 [0.103]	<0.025(3), 0.031 [0.027]
28	<0.025(4)	<0.025(4)	0.025, 0.026, 0.029, 0.03 [0.028]	<0.025(4)	0.083, 0.087, 0.096, 0.107 [0.093]	<0.025(4)
29[a]	<0.025(2)	<0.025(2)	0.027, 0.032 [0.03]	<0.025(2)	0.088, 0.089 [0.089]	<0.025(2)
30[a]	<0.025(2)	<0.025(2)	0.029, 0.031 [0.03]	<0.025(2)	0.091, 0.094 [0.093]	<0.025(2)
32[a]	<0.025(2)	<0.025(2)	<0.025, 0.025 [0.025]	<0.025(2)	0.063, 0.071 [0.067]	<0.025(2)
35[a]	<0.025(2)	<0.025(2)	<0.025(4)	<0.025(2)	<0.025, 0.027 [0.026]	<0.025(2)
42[a]	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
49[a]	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
56[a]	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02

a: depuration
[] mean value

For muscle, fat, liver and kidney residues were:

Table B.7.8-15: Residues of glyphosate and AMPA found in fat, muscle, liver and kidney of laying hens following administration of 9:1 mixtures dose at 0 to 400 mg/kg feed for 28 days

Tissue	Days	Glyphosate: 2.54 mg/kg bw AMPA: 0.28 mg/kg bw (40 mg/kg feed)		Glyphosate: 7.62 mg/kg bw AMPA: 0.85 mg/kg bw (120 mg/kg feed)		Glyphosate: 25.4 mg/kg bw AMPA: 2.8 mg/kg bw (400 mg/kg feed)	
		Glyphosate (mg/kg)	AMPA (mg/kg)	Glyphosate (mg/kg)	AMPA (mg/kg)	Glyphosate (mg/kg)	AMPA (mg/kg)
Fat	28	<0.05(2)	<0.05(2)	<0.05(2)	<0.05(2)	0.06, 0.06 [0.06]	<0.05(2)
	35[a]	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
	56[a]	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Muscle	28	<0.05(2)	<0.05(2)	<0.05(2)	<0.05(2)	<0.05, 0.05 [0.05]	<0.05(2)
	35[a]	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
	56[a]	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Liver	28	0.06, 0.08 [0.07]	<0.05(2)	0.18, 0.22 [0.2]	0.09, 0.11 [0.1]	0.76, 0.79 [0.78]	0.34, 0.42 [0.38]
	35[a]	<0.05	<0.05	<0.05	<0.05	0.15	0.15
	56[a]	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Kidney	28	0.33, 0.43 [0.38]	<0.05(2)	0.87, 1.48 [1.18]	0.05, 0.07 [0.06]	3.95, 5.14 [4.54]	0.31, 0.35 [0.33]
	35[a]	0.06	<0.05	0.23	<0.05	0.35	<0.05
	56[a]	<0.05	<0.05	0.17	<0.05	0.08	<0.05

a: depuration
[] mean value

Conclusions:
Following administration of glyphosate and AMPA (9:1 mixture) highest residues in poultry matrices were found in liver and kidney. In liver glyphosate residues increased from 0.07 mg/kg (40 mg/kg feed) up to 0.78 mg/kg (400 mg/kg feed). In kidney a range of 0.38-4.54 mg/kg was found. AMPA was more present in liver with approximately half of the levels found for glyphosate (<0.05-0.38 mg/kg). In kidney AMPA was detect in the second and highest dose group, but at levels <10% compared to glyphosate. In fat and muscle only glyphosate (0.05 mg/kg in muscle, 0.06 mg/kg in fat) was detected in samples obtained from the 400 mg/kg feed dose group.
In eggs glyphosate was only found in the second and highest dose group, being present at levels up to 0.03 mg/kg and 0.103 mg/kg, respectively. AMPA was detected in only one sample from the 400 mg/kg feed group at day 21, amounting 0.031 mg/kg. A plateau of the residue was observed after appoximately 14 days for glyphosate.

Reference: OECD KIIA 6.4.1

Report: Graham, D. G.
Magnitude of SC-0224 residues in eggs and poultry
31.07.1987, Report No.: RRC 87-43, RIP9500025

Guidelines: EPA: Guideline §161-2

Deviations: ~~original guideline not submitted~~none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study layign hens (White Leghorn) were fed nominal dose levels of 0, 0.5, 5 and 50 mg/kg glyphosate-trimesium in the diet for 28 consecutive days (actual levels: 0, 0.057, 0.57 and 5.7 mg per animal and day or 0, 0.037, 0.35 and 3.2 mg/kg bw and day). Expressed as glyphosate equivalents the nominal dose levels were 0, 0.345, 3.45 and 34.5 mg/kg glyphosate in the diet (actual levels: 0, 0.039, 0.39 and 3.9 mg glyphosate per animal and day or 0, 0.025, 0.24 and 2.2 mg glyphosate per kg bw and day). Each dose group consisted of ten animal of which seven were sacrificed within 24h after the final administration and three animal was kept for additional 7 days depuration.

Eggs were collected over the whole dosing study period and analysed together with pooled tissues samples for residues of glyphosate, AMPA and the trimesium-cation (TMS). Samples were extracted with water, cleaned by a cation exchange column and finally analysed by HPLC with fluorescence detection after derivatisation.

Findings:
Except for one result (kidney, 28 d, glyphosate: 0.08 mg/kg) all samples obtained from the control group (0 ppm dosing) were below the LOQ of the analytical method used. These values are not presented in detail.

No apparent treatment-related effects on the general condition of the animals, their behaviours, body weights, feed consumption or egg/milk production were reported.

In the following table the residues of glyphosate (Gly), AMPA and TMS in eggs are summarised:

Table B.7.8-16: Residues of glyphosate, AMPA and TMS in eggs of laying hens dosed with glyphosate-trimesium at levels between 0 and 50 mg/kg in the diet

Days	**Mean residues found per dose group in mg/kg**		
	0.025 mg glyphosate eq/kg bw	0.24 mg glyphosate eq /kg bw	2.2 mg glyphosate eq /kg bw
-1	Gly: <0.01 AMPA: <0.02 TMS: <0.02	Gly: <0.01 AMPA: <0.02 TMS: <0.02	Gly: <0.01 AMPA: <0.02 TMS: <0.02
1	Gly: <0.01 AMPA: <0.02 TMS: <0.02	Gly: <0.01 AMPA: <0.02 TMS: <0.02	Gly: <0.01 AMPA: <0.02 TMS: <0.02
2	Gly: <0.01 AMPA: <0.02 TMS: <0.02	Gly: <0.01 AMPA: <0.02 TMS: <0.02	Gly: <0.01 AMPA: <0.02 TMS: <0.02
4	Gly: <0.01 AMPA: <0.02 TMS: <0.02	Gly: <0.01 AMPA: <0.02 TMS: <0.02	Gly: <0.01 AMPA: <0.02 TMS: <0.02
7	Gly: <0.01 AMPA: <0.02 TMS: <0.02	Gly: <0.01 AMPA: <0.02 TMS: <0.02	Gly: 0.01 AMPA: <0.02 TMS: <0.02
14	Gly: <0.01 AMPA: <0.02 TMS: <0.02	Gly: <0.01 AMPA: <0.02 TMS: <0.02	Gly: 0.011 AMPA: <0.02 TMS: <0.02
21	Gly: <0.01 AMPA: <0.02 TMS: <0.02	Gly: <0.01 AMPA: <0.02 TMS: <0.02	Gly: 0.015 AMPA: <0.02 TMS: <0.02
28	Gly: <0.01 AMPA: <0.02 TMS: <0.02	Gly: <0.01 AMPA: <0.02 TMS: <0.02	Gly: 0.014 AMPA: <0.02 TMS: <0.02
35 (depuration)	Gly: <0.01 AMPA: <0.02 TMS: <0.02	Gly: <0.01 AMPA: <0.02 TMS: <0.02	Gly: <0.01 AMPA: <0.02 TMS: <0.02

In tissues sampled after sacrifice residues found were:

Table B.7.8-17: Residues of glyphosate, AMPA and TMS in tissues of laying hens dosed with glyphosate-trimesium at levels between 0 and 50 ppm in the diet

Sample	Residues found per dose group in mg/kg		
	0.025 mg glyphosate eq/kg bw	0.24 mg glyphosate eq /kg bw	2.2 mg glyphosate eq /kg bw
Kidney	Gly: <0.05 AMPA: <0.05 TMS: <0.05	Gly: 0.072 AMPA: <0.05 TMS: <0.05	Gly: 0.31 AMPA: <0.05 TMS: 0.18
Kidney (depuration)	Gly: <0.05 AMPA: <0.05 TMS: <0.05	Gly: <0.05 AMPA: <0.05 TMS: <0.05	Gly: 0.11 AMPA: <0.05 TMS: <0.05
Liver	Gly: <0.05 AMPA: <0.05 TMS: <0.05	Gly: <0.05 AMPA: <0.05 TMS: <0.05	Gly: <0.05 AMPA: <0.05 TMS: 0.13
Liver (depuration)	Gly: <0.05 AMPA: <0.05 TMS: <0.05	Gly: <0.05 AMPA: <0.05 TMS: <0.05	Gly: <0.05 AMPA: <0.05 TMS: 0.065
Fat	Gly: <0.05 AMPA: <0.05 TMS: <0.05	Gly: <0.05 AMPA: <0.05 TMS: <0.05	Gly: <0.05 AMPA: <0.05 TMS: <0.05
Fat (depuration)	Gly: <0.05 AMPA: <0.05 TMS: <0.05	Gly: <0.05 AMPA: <0.05 TMS: <0.05	Gly: <0.05 AMPA: <0.05 TMS: <0.05
Muscle	Gly: <0.05 AMPA: <0.05 TMS: <0.05	Gly: <0.05 AMPA: <0.05 TMS: <0.05	Gly: <0.05 AMPA: <0.05 TMS: <0.05
Muscle (depuration)	Gly: <0.05 AMPA: <0.05 TMS: <0.05	Gly: <0.05 AMPA: <0.05 TMS: <0.05	Gly: <0.05 AMPA: <0.05 TMS: <0.05

Conclusions:
The transfer of residues following administration of glyphosate-trimesium into poultry tissues and eggs was very limited. The glyphosate anion was soley found in eggs (0.011-0.015 mg/kg) after administration of 50 mg/kg in the diet and in kidney after 5 or 50 mg/kg in the diet (0.072 mg/kg and 0.31 mg/kg, respectively). In none of the samples detectable residues of AMPA were found. Liver, fat and muscle did not contain detectable residues of glyphosate and AMPA for any dose group.

Reference: OECD KIIA 6.4.1

Report: Dibb-Fuller, M.; Bramble, F. Q.
Magnitude of residues of N-Acetylglyphosate and degradates in laying hen tissues and eggs
03.12.2007, Report No.: DuPont-20088, ASB2008-2652

Guidelines: EPA: OPPTS 860.1480, Meat/Milk/Poultry/Eggs
EU: Document 1607/VI97 rev.2
OECD: 505 – Residues in Livestock

Deviations: none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
For laying hens the transfer of N-acetyl-glyphosate into animal products was investigated by Dibb-Fuller, M. (2007). Groups of 10 hens each were dosed with 0, 1.5, 5, 15 and 50 mg N-acetyl-glyphosate per kg bw for 35 consecutive days. From the highest dose group some animals were kept for depuration for additional 19 days. Eggs were collected over the whole period of time. After sacrifice the tissues and eggs were analysed for residues.

The samples from each dose group were pooled into three subgroups and extracted using 0.1% formic acid/methanol (96/4, v/v). Egg samples (including separate analysis of white and yolk) were portioned with methylene chloride and hexane. Tissue and egg samples were cleaned by SPE against C18 and polymeric anion exchange phases. Stable isotopes were used as internal standard for the analysis of the residues with LC/MS/MS.

Findings:
In control samples of eggs and tissues not detectable residues were found. The results are not presented in the following tables.

No apparent treatment-related effects on the general condition of the animals, their behaviours, body weights, feed consumption or egg/milk production were reported.

For eggs, egg white and egg yolk no residues of AMPA or N-acetyl-AMPA were detected (estimated LODs: AMPA 0.006 mg/kg, N-acetyl-AMPA 0.009 mg/kg). The findings for N-acetyl-glyphosate and glyphosate were:

Table B.7.8-18: N-acetyl-glyphosate and glyphosate residues in whole eggs

Day	1.5 mg/kg bw Residues in mg/kg (glyphosat equivalents)		5 mg/kg bw Residues in mg/kg (glyphosat equivalents)		15 mg/kg bw Residues in mg/kg (glyphosat equivalents)		50 mg/kg bw Residues in mg/kg (glyphosat equivalents)	
	N-acetyl-glyphosate (mean)	Glyphosate (mean)	N-acetyl-glyphosate (mean)	Glyphosate (mean)	N-acetyl-glyphosate (mean)	Glyphosate (mean)	N-acetyl-glyphosate (mean)	Glyphosate (mean)
1	ND (3)	ND (3)	ND (3)	ND (3)	ND (3)	ND (3)	ND (3)	ND (3)
3	ND (3)	ND (3)	<0.025 ND, <0.025 (<0.025)	ND (3)	0.044, 0.041, 0.025 (0.036)	ND (3)	0.12, 0.15, 0.12 (0.13)	ND (3)
5	ND, ND, <0.025 (<0.025)	ND (3)	0.044, 0.030, 0.046 (0.040)	ND (3)	0.10, 0.089, 0.051 (0.080)	ND (3)	0.29, 0.23, 0.26 (0.26)	ND (3)
7	<0.025(3) (<0.025)	ND (3)	0.045, 0.064, 0.051 (0.053)	ND (3)	0.13, 0.10, 0.07 (0.10)	ND (3)	0.48, 0.50, 0.30 (0.43)	ND (3)
10	<0.025, <0.025, 0.025 (0.025)	ND (3)	0.066, 0.060, 0.069 (0.065)	ND (3)	0.11, 0.15, 0.092 (0.12)	ND (3)	0.56, 0.59, 0.34 (0.50)	<0.025, <0.025, ND (<0.025)
14	<0.025, <0.025, 0.028 (0.025)	ND (3)	0.066, 0.076, 0.079 (0.074)	ND (3)	0.12, 0.30, 0.11 (0.18)	ND (3)	0.84, 0.71, 0.42 (0.66)	<0.025, <0.025, ND (<0.025)
17	0.028, <0.025, 0.030 (0.028)	ND (3)	0.081, 0.079, 0.078 (0.079)	ND (3)	0.16, 0.19, 0.12 (0.16)	ND (3)	0.69, 0.53, 0.43 (0.55)	ND (3)
24	0.045, 0.026, 0.030 (0.034)	<0.025, ND, ND (<0.025)	0.094, 0.087, 0.091 (0.091)	ND (3)	0.18, 0.17, 0.10 (0.15)	ND (3)	0.72, 0.75, 0.23 (0.57)	<0.025, ND, ND (<0.025)
28	0.050, <0.025, 0.025 (0.033)	ND (3)	0.093, 0.072, 0.080 (0.081)	ND (3)	0.18, 0.13, 0.10 (0.14)	ND (3)	0.71, 0.56, 0.30 (0.53)	<0.025, ND, ND (<0.025)
31	0.050, 0.025, 0.035 (0.037)	ND (3)	0.087, 0.090, 0.064 (0.080)	ND (3)	0.18, 0.16, 0.86 (0.14)	ND (3)	0.69, 0.70, 0.43 (0.61)	<0.025(3) (<0.025)
34	0.044, 0.030, 0.029 (0.035)	0.030, ND, <0.025 (0.027)	0.102, 0.093, 0.065 (0.087)	<0.025, ND, ND (<0.025)	0.17, 0.15, 0.097 (0.14)	ND (3)	0.65, 0.83, 0.41 (0.63)	0.033, 0.037, <0.025 (0.036)

ND not detected (estimated LODs: N-acetyl-glyphosate 0.007 mg/kg and glyphosate 0.006 mg/kg)

Table B.7.8-19: N-acetyl-glyphosate and glyphosate residues in whole eggs after 19 days of depuration

Dose level in mg/kg bw	**Days after last dose**	**Residues in mg/kg (glyphosat equivalents)** N-acetyl-glyphosate (mean)	Glyphosate (mean)
50	-1	0.63, 0.78, 0.78 (0.73)	0.044, 0.035, <0.025 (0.035)
	0	0.51, 0.74, 0.80 (0.69)	<0.025(3), (<0.025)
	1	0.59, 0.73, 0.55 (0.62)	<0.025(3), (<0.025)
	3	0.37, 0.71, 0.52 (0.53)	0.034, <0.025, <0.025 (0.028)
	5	0.10, 0.23, 0.23 (0.19)	<0.025(3), (<0.025)
	7	0.064, 0.032 (0.048)	<0.025, <0.025 (<0.025)
	10	<0.025, <0.025 (<0.025)	n.d., n.d.
	13	<0.025, ND (<0.025)	n.d., n.d.
	16	ND	n.d.
	19	ND	n.d.

ND not detected (estimated LODs: N-acetyl-glyphosate 0.007 mg/kg and glyphosate 0.006 mg/kg)

Table B.7.8-20: N-acetyl-glyphosate and glyphosate residues in egg white and yolk at collection day 21

Dose level in mg/kg bw	**Residues in mg/kg (glyphosat equivalents)**			
	Egg yolk		Egg white	
	N-acetyl-glyphosate (mean)	Glyphosate (mean)	N-acetyl-glyphosate (mean)	Glyphosate (mean)
1.5	0.074, 0.049, 0.11 (0.076)	ND	<0.025, ND, ND (<0.025)	<0.025, ND, ND (<0.025)
5	0.20, 0.22, 0.42 (0.28)	<0.025, ND, ND (<0.025)	<0.025, <0.025, ND (<0.025)	ND
15	0.17, 0.23, 0.28 (0.23)	<0.025, ND, ND (<0.025)	<0.025(3) (<0.025)	0.03, <0.025, ND (0.028)
50	1.7, 1.6, 0.85 (1.4)	<0.025(3) (<0.025)	0.057, 0.054, <0.025 (0.044)	0.025, <0.025, 0.047 (0.032)

ND not detected (estimated LODs: N-acetyl-glyphosate 0.007 mg/kg and glyphosate 0.006 mg/kg)

The residues found for N-acetyl-glyphosate, glyphosate, N-acetyl-AMPA and AMPA in liver, muscle and fat were:

Table B.7.8-21: N-acety-glyphosate, glyphosate, AMPA and N-acetyl-AMPA residues in poultry liver

Dose level in mg/kg bw	**Residues in mg/kg (glyphosat equivalents)**			
	N-acetyl-glyphosate	Glyphosate	AMPA	N-acetyl-AMPA
0	ND	ND	ND	ND
1.5	0.21, 0.20, 0.16 (0.19)	ND	<0.05(3) (<0.05)	ND
5	0.76, 0.67, 0.43 (0.62)	<0.05, ND, ND (<0.05)	<0.05(3) (<0.05)	ND
15	0.84, 0.94, 0.59 (0.79)	<0.05, <0.05, ND (<0.05)	<0.05(3) (<0.05)	ND
50	4.3, 5.2, 3.4 (4.3)	<0.05(3) (<0.05)	<0.05(3) (<0.05)	ND
50 (6 day depuration)	0.053	<0.05	<0.05	ND
50 (14 day depuration)	ND	ND	<0.05	ND
50 (20 day depuration)	ND	<0.05	<0.05	ND

ND not detected (estimated LODs: N-acetyl-glyphosate 0.011 mg/kg, glyphosate 0.005 mg/kg, AMPA: 0.009 mg/kg and N-acetyl-AMPA: 0.006 mg/kg)

Table B.7.8-22: N-acety-glyphosate, glyphosate, AMPA and N-acetyl-AMPA residues in poultry fat

Dose level in mg/kg bw	**Residues in mg/kg (glyphosat equivalents)**			
	N-acetyl-glyphosate	Glyphosate	AMPA	N-acetyl-AMPA
0	<0.05	<0.05	ND	ND
1.5	0.13, 0.089, 0.11 (0.11)	<0.05(3) (<0.05)	NA	ND
5	0.48, 0.60, 0.38 (0.49)	<0.05(3) (<0.05)	NA	ND
15	0.22, 0.39, 0.15 (0.25)	<0.05(3) (<0.05)	NA	ND
50	1.2, 1.9, 0.88 (1.3)	<0.05(3) (<0.05)	ND	ND
50 (6 day depuration)	0.071	<0.05	NA	ND
50 (14 day depuration)	0.051	<0.05	NA	ND
50 (20 day depuration)	ND	<0.05	NA	ND

NA not analysed
ND not detected (estimated LODs: N-acetyl-glyphosate 0.014 mg/kg, glyphosate 0.009 mg/kg, AMPA: 0.014 mg/kg and N-acetyl-AMPA: 0.007 mg/kg)

Table B.7.8-23: N-acety-glyphosate, glyphosate, AMPA and N-acetyl-AMPA residues in poultry muscle

Dose level in mg/kg bw	Residues in mg/kg (glyphosat equivalents)			
	N-acetyl-glyphosate	Glyphosate	AMPA	N-acetyl-AMPA
0	ND	ND	<0.025	ND
1.5	0.036, <0.025, 0.032 (0.031)	<0.025(3) (<0.025)	NA	ND
5	0.14, 0.16, 0.10 (0.13)	<0.025(3) (<0.025)	NA	ND
15	0.078, 0.13, 0.042 (0.082)	<0.025(3) (<0.025)	<0.025(3) (<0.025)	ND
50	0.39, 0.58, 0.26 (0.41)	<0.025(3) (<0.025)	<0.025(3) (<0.025)	ND
50 (6 day depuration)	<0.025	ND	NA	ND
50 (14 day depuration)	<0.025	ND	NA	ND
50 (20 day depuration)	ND	ND	NA	ND

NA not analysed
ND not detected (estimated LODs: N-acetyl-glyphosate 0.003 mg/kg, glyphosate 0.002 mg/kg, AMPA: 0.007 mg/kg and N-acetyl-AMPA: 0.002 mg/kg)

Conclusions:
In all poultry matrices investigated unchanged N-acetyl-glyphosate was the major residue found. AMPA and N-acetyl-AMPA as potential metabolites were seldomly detected, but their levels were always below the limit for quantification.
Glyphosate was quantified in the highest dose group at levels near the LOQ in eggs. Separation of egg white and egg yolk revealed the major part of the glyphosate was present in the egg white.
N-acetyl-glyphosate was present in all tissues and dose rates, ranging from 0.031-0.41 mg/kg in muscle, 0.11-1.3 mg/kg in fat and 0.19-4.3 mg/kg in liver. In eggs a plateau of the residue was reached after 14 days at levels of 0.037-0.66 mg/kg, depending on the dose administered.

B.7.8.3 Swine

Reference: OECD KIIA 6.4.1

Report: Manning, M. J.; Wilson, G. R.
Residue determination of Glyphosate and AMPA in swine tissues following a 28-day feeding study
September 1987, Report-No.: MSL-6627, RIP9501251

Guidelines: not stated

Deviations: not appplicable

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study cross-bred swine were administered a 9:1 mixture of glyphosate and AMPA at total rates equivalent to 0, 40, 120 and 400 mg/kg in the diet. The active substances were adminsited via to the feed and animals were free to eat ad libitum. Each dose group consisted of four animals (2 male, 2 female) dosed for 28 days. One animal of each sex was slaughterd after 28 days, the other were kept for additional 28 days of depuration. Actual amounts of feed consumed per animal were measured on a daily basis:

Table B.7.8-24: Actual dose rates of glyphosate and AMPA (9:1) administered to swine for 28 days

Nominal dose level	Animal	Average feed consumption per day (kg)	Average bodyweight during dosing (kg)	Glyphosate dose per day		AMPA dose per day	
				mg per animal	mg/kg bw	mg per animal	mg/kg bw
40 mg/kg feed (glyphosate : AMPA, 9:1)	M00140	3.1	96.5	112	1.16	12.4	0.13
	M00142	1.3	82	46.8	0.57	5.2	0.06
	M00138	3.5	97	126	1.30	14.0	0.14
	M00144	2.8	80	101	1.26	11.2	0.14
	Mean	2.7	89	96.5	1.08	10.7	0.12
120 mg/kg feed (glyphosate : AMPA, 9:1)	M00141	3.2	93	346	3.72	38.4	0.41
	M00135	2.4	90	259	2.88	28.8	0.32
	M00130	3.4	99	367	3.71	40.8	0.41
	M00137	2.3	82	248	3.02	27.6	0.34
	Mean	2.8	91	305	3.35	33.9	0.37
400 mg/kg feed (glyphosate : AMPA, 9:1)	M00145	3.5	96	1260	13.1	140	1.46
	M00146	2.7	88	972	11.0	108	1.23
	M00132	3.1	94	1116	11.9	124	1.32
	M00129	2.8	89	1008	11.3	112	1.26
	Mean	3.0	92	1089	11.8	121	1.32

Tissues were collected after sacrifice and analysed for residues of glyphosate and AMPA. Samples were extracted with water, cleaned by a cation exchange column and finally analysed by a validated HPLC method using fluorescence detection after derivatisation.

Findings:
In pig fat and muscle no AMPA residues were detected in any of the samples. For glyphosate only the samples from the highest dose group showed residues near the LOQ with 0.06 mg/kg for each tissue after 28 days. Samples after depuration gave no detectable residues for both analytes.

Concerning animal health during the dosing period one animal (lowest treatment group) was sacrificed early due to gastric ulcers. Examinations of all other animals were normal.

The results for liver and kidney were:

Table B.7.8-25: Residues of glyphosate and AMPA found in liver and kidney of swine following administration of 9:1 mixtures dose at 0 to 400 mg/kg feed for 28 days

Actual feed level in mg/kg bw	Animal (days)	Residue in liver in mg/kg[a]		Residue in kidney in mg/kg[a]	
		Glyphosate	AMPA	Glyphosate	AMPA
Nominal: 36 mg glyphosate + 4 mg AMPA per kg feed					
Glyphosate: 1.26 AMPA: 0.14	M00144 (28)	<0.05, <0.05	<0.05, <0.05	0.62, 0.60 (mean: 0.61)	0.08, 0.08 (mean: 0.08)
Glyphosate: 0.57 AMPA: 0.06	M00142 (28)	<0.05, <0.05	<0.05, <0.05	0.13, 0.14 (mean: 0.135)	<0.05, <0.05
Glyphosate: 1.3 AMPA: 0.14	M00138 (56)	<0.05, <0.05	<0.05, <0.05	<0.05, <0.05	<0.05, <0.05
Glyphosate: 1.16 AMPA: 0.13	M00140 (56)	<0.05, <0.05	<0.05, <0.05	<0.05, <0.05	<0.05, <0.05
Nominal: 108 mg glyphosate + 12 mg AMPA per kg feed					
Glyphosate: 3.02 AMPA: 0.34	M00137 (28)	0.19, 0.19 (mean: 0.19)	0.10, 0.11 (mean: 0.105)	2.19, 2.2 (mean: 2.2)	0.24, 0.24 (mean: 0.24)
Glyphosate: 2.88 AMPA: 0.32	M00135 (28)	0.22, 0.22 (mean: 0.22)	0.13, 0.13 (mean: 0.13)	2.94, 3.01 (mean: 2.98)	0.33, 0.35 (mean: 0.34)
Glyphosate: 3.71 AMPA: 0.41	M00130 (56)	<0.05, <0.05	<0.05, <0.05	0.05, 0.05 (mean: 0.05)	<0.05, <0.05
Glyphosate: 3.72 AMPA: 0.41	M00141 (56)	<0.05, <0.05	<0.05, <0.05	0.1, 0.1 (mean: 0.1)	<0.05, <0.05
Nominal: 360 mg glyphosate + 40 mg AMPA per kg feed					
Glyphosate: 11.3 AMPA: 1.26	M00129 (28)	0.59, 0.61 (mean: 0.6)	0.26, 0.26 (mean: 0.26)	6.29, 6.28 (mean: 6.28)	0.85, 0.83 (mean: 0.84)
Glyphosate: 11.0 AMPA: 1.23	M00146 (28)	0.89, 0.92 (mean: 0.905)	0.52, 0.53 (mean: 0.525)	9.47, 9.19 (mean: 9.33)	1.11, 1.03 (mean: 1.07)
Glyphosate: 11.9 AMPA: 1.32	M00132 (56)	<0.05, <0.05	<0.05, <0.05	0.2, 0.2 (mean: 0.2)	<0.05, <0.05
Glyphosate: 13.1 AMPA: 1.46	M00145 (56)	<0.05, <0.05	<0.05, <0.05	0.17, 0.16 (mean: 0.165)	<0.05, <0.05

a: duplicate analysis

Conclusions:
After feeding of glyphosate and AMPA (9:1 mixture) to swine residues in tissues were comparable to ruminants. In the lowest and middle dose group, which corresponded to the highest dose group in ruminants on a mg/kg bw basis, results in kidney and liver were at the same magnitude. In both tissues residues in swine scaled with the administered dose. For muscle and fat residues near the LOQ with 0.06 mg/kg were detected. Compared to ruminants the highest dosing in swine was approximately three times higher, explaining the non-detects in cows.
The radio of glyphosate to AMPA in liver and kidney matched the 9:1 ratio administered.

B.7.8.4 Public literature

In paper primarily dealing with determinations of glyphosate in the urine of humans and cattle, Krüger *et al.* (2014, ASB2014-5024) reported data from Danmark also including findings in various tissues. The samples obtained from cows on "conventional husbandry" (compared to "organic husbandry") were analysed by means of a not further specified ELISA (Abraxis, USA) but an LOD or LOQ were not mentioned. No numeric values such as mean and standard deviation are given in this very brief paper but only figures. Based on these

figures, maximum glyphosate concentrations of up to 0.06 mg/kg in kidney (n = 26), 0.04 mg/kg in liver (n = 41) and 0.02 mg/kg in muscle (n = 6) can be estimated.

The study confirms the findings from livestock animal feeding studies that liver and kidney are the target tissues for glyphosate residues. However, since no linkage to a potentical dietary burden can be made, the study is of limited value for the estimation of maximum residue levels in animal commodities or the dietary intake assessment.

B.7.9 Residues in succeeding or rotational crops (Annex IIA 6.6; Annex IIIA 8.6)

B.7.9.1 Confined rotational crop studies

Reference: OECD 6.6.2

Report: Nicholls, R. G.
Confined rotational crops study of Glyphosate - Part I: In-field portion
22.06.1990, Report No.: MSL 9810, RIP9501201
&
McMullan, P. C.; Honegger, J. L.; Logusch, E. W.
Confined rotational crops study of Glyphosate - Part II: Quantitation, characterization and identification of Glyphosate and its metabolites in rotational crops
22.06.1990, Report No.: MSL 9811, RIP9501202

Guidelines: EPA 165-1

Deviations: ~~original guideline not submitted~~none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
For the investigation of the potential of glyphosate residues to be present in rotational crops one trial site was devided into three test plots. ^{14}C-glyphosate was applied to approximately 15cm high rye grass at rates equivalent to 4.16 kg as/ha. One week after the treatment the weeds were tilled and soya beans were plants as primary crop on all plots. After 30, 120 and 365 days the soya beans present were harvested, the soil was tillered to a depth of 10cm and carrots, lettuce or barley were grown as rotational crops. In addition to plant samples from soya beans and the rotational crops at various growth stages soil samples were collected from a depth of up to 30cm.

Total radioactive residues in all samples collected were measured by combustion in combination with LSC. For the idenfication of radioactive residues soil samples wer extracted using 0.5N NH_4OH. Plant matrices were extracted with water and partitioned against chloroform. After concentration all extracts were analysed by HPLC with a cation-exchange column and radiodetection. Unextracted radioactivity was first extracted with 5 N NH_4OH. The remaining tissue was refluxed with ammonium hydroxide for 16h. In parallel DMSO extraction was performed, followed by enzymatic hydrolysis with amyloglucosidase or cellulase. The identification was achieved by comparison of retention times with standard reference substances and after purification with NMR.

Findings:

In the following table the TRR found in soil samples is summarised. In addition for the samples from 0-15 cm soil depth the extractability and the composition of radioactivity are presented.

Table B.7.9-1: TRR levels, extractablility and composition of the residue in soil treated at rates equivalent to 4.16 kg as/ha

Sample	**0-15 cm soil depth in mg eq/kg (% TRR)**					**15-30 cm soil depth in mg eq/kg**
	TRR	Extracted	Metabolite A	Glyphosate	AMPA	TRR
0 DAT	0.711	98 %	0.0202 (2.8)	0.6431 (90.5)	0.0334 (4.7)	0.0453
7 DAT	0.738	-	-	-	-	0.0088
30 day subplot						
30 DAT	0.518	76 %	0.0315 (6.1)	0.1453 (29.3)	0.2020 (39.0)	0.0017
76 DAT	0.518	76 %	0.0465 (9.0)	0.1189 (23.0)	0.2224 (42.9)	0.0036
90 DAT	0.354	63 %	0.0087 (2.5)	0.0268 (7.6)	0.1831 (51.7)	0.0016
105 DAT	0.526	59 %	0.0196 (3.7)	0.0456 (8.7)	0.2442 (46.4)	0.0021
125 DAT	0.625	56 %	0.0210 (3.4)	0.0266 (4.3)	0.3014 (48.2)	0.0009
154 DAT	0.250	55 %	0.0111 (4.5)	0.0081 (3.2)	0.1170 (46.8)	0.0010
120 day subplot						
119 DAT	0.142	67 %	0.0066 (4.6)	0.0449 (31.6)	0.0389 (27.4)	0.0011
147 DAT	0.589	54 %	0.0483 (8.2)	0.0083 (1.4)	0.2287 (38.8)	0.0001
167 DAT	0.372	45 %	0.0080 (2.2)	ND	0.1590 (42.8)	0.0006
181 DAT	0.203	56 %	0.0107 (5.3)	0.0017 (0.8)	0.0983 (48.4)	0.0009
210 DAT	0.277	69 %	0.0176 (6.3)	0.0029 (1.0)	0.1565 (56.5)	0.0006
314 DAT	0.250	57 %	0.0171 (6.8)	0.0026 (1.0)	0.1194 (47.8)	ND
365 day subplot						
364 DAT	0.184	72 %	0.0111 (6.0)	0.0327 (17.8)	0.0839 (45.6)	0.0009
399 DAT	0.297	49 %	0.0141 (4.8)	0.0413 (13.9)	0.0853 (28.7)	ND
412 DAT	0.211	63 %	0.0122 (5.8)	0.0072 (3.4)	0.0957 (45.4)	0.0032
425 DAT	0.248	64 %	0.0124 (5.0)	ND	0.1449 (58.4)	0.0011
455 DAT	0.172	59 %	0.0057 (4.0)	0.0010 (0.7)	0.0757 (52.9)	0.0021
482 DAT	0.179	63 %	0.0127 (7.1)	ND	0.1012 (56.5)	ND

ND: not detected

For plant matrices collected from rotational crops the TRR levels were presented below. In addition the extractability with water and the identification of the radioactivity are summarised.

Table B.7.9-2: Total radioactive residues, extractability and compositon of the residue in rotational crops following application of ^{14}C-glyphosate to soil at rates of 4.16 kg as/ha

Sample	mg eq/kg (% TRR)					
	TRR	Extracted[a]	Metabolite 1[b]	Glyphosate	AMPA	Unextracted[a]
30 day subplot						
Lettuce, 70 DAT	0.108	(49.1)	0.0309 (28.6)	0.0041 (3.8)	0.0158 (14.6)	(48.9)
Lettuce, 90 DAT	0.048	(45.7)	0.0162 (33.8)	ND	0.0039 (8.1)	(59.9)
Lettuce, 105 DAT	0.097	(44.6)	0.0327 (33.7)	0.0028 (2.9)	0.0137 (28.5)	(5.6)
Barley, straw 125 DAT	0.175	(25.9)	0.0294 (16.8)	0.0018 (1.0)	0.0065 (3.7)	(74.3)
Barley, grain 125 DAT	0.188	(15.3)	0.0144 (7.7)	0.0184 (9.8)	0.0336 (17.9)	(82.8)
Carrot, tops 154 DAT	0.051	(23.7)	0.0136 (26.7)	ND	0.0007 (1.4)	(70.7)
Carrot, root 154 DAT	0.037	(54.4)	0.0151 (40.8)	ND	0.0041 (11.1)	(49.7)
120 day subplot						
Lettuce, 147 DAT	0.059	(35.3)	0.0147 (24.9)	ND	0.0027 (4.6)	(70.3)
Lettuce, 167 DAT	0.055	(34.3)	0.0083 (15.1)	0.0009 (1.6)	0.0050 (9.1)	(57.0)
Lettuce, 181 DAT	0.037	(40.1)	0.0087 (23.5)	ND	0.0046 (12.4)	(62.4)
Barley, straw 314 DAT	0.056	(19.2)	0.0046 (8.2)	ND	0.0054 (9.6)	(78.3)
Barley, grain 314 DAT	0.078	(25.2)	0.0049 (6.3)	ND	0.0111 (14.2)	(61.7)
Carrot, tops 210 DAT	0.028	(24.5)	0.0049 (17.5)	ND	0.0003 (1.1)	(83.2)
Carrot, root 210 DAT	0.017	(32.1)	0.0039 (22.9)	ND	0.0014 (8.2)	(61.9)
365 day subplot						
Lettuce, 399 DAT	0.057	(49.0)	0.0182 (31.9)	ND	0.0076 (13.3)	(54.5)
Lettuce, 425 DAT	0.043	(37.2)	0.0080 (18.6)	ND	0.0045 (10.5)	(62.2)
Lettuce, 455 DAT	0.028	(56.4)	0.0079 (28.2)	ND	0.0056 (20.0)	(45.3)
Barley, forage 412 DAT	0.056	(31.1)	0.0082 (14.6)	ND	0.0093 (16.6)	(67.3)
Barley, straw 482 DAT	0.061	(22.7)	0.0068 (11.1)	ND	0.0047 (7.7)	(80.9)
Barley, grain 482 DAT	0.047	(24.8)	0.0031 (6.6)	ND	0.0074 (15.7)	(69.6)
Carrot, tops 482 DAT	0.018	(22.7)	-	-	-	(67.0)
Carrot, root 482 DAT	0.0096	(39.9)	-	-	-	(64.3)

a: measured by combustion
b: after purification identical to glucose/fructose with NMR

For lettuce (105 DAT), barley straw (125 DAT) and barley grain (125 DAT) further characterisation using different solvents and hydrolysis were performed:

Table B.7.9-3: Characterisation of the unextracted residue in lettuce, barley straw and barley grain

Extraction method (characterised as)	% TRR		
	Lettuce (105 DAT)	Barley straw (125 DAT)	Barley grain (125 DAT)
Water	45.2	17.4	26.2
NH_4OH	11.3	8.9	22.5
DMSO (starch and lignin)	14.8	17.0	20.4
Cellulase (cellulose)	-	36.0	-
Total	71.3	79.3	69.1

Conclusions:
In this confined rotational crops study involving application of 4.16 kg as/ha 14C-glyphosate to soil most of the radioactivity extracted was incorperated into fractions identical to glucose and fructose. Unchanged parent substance was mostly undetected, ranging up to a maximum of 9.8 % of the TRR in barley grain in the 1st rotation. AMPA, which was identified as the major residue in treated soil, was frequently found at levels higher 10% of the TRR, showing

a highest level 28.5% of the TRR in lettuce. However, absolute levels of radioactivity were very low, not exceeding 0.05 mg eq/kg for all metabolites. Highest TRR levels were found in lettuce (0.108 mg eq/kg), barley straw (0.175 mg eq/kg) and barley grain (0.188 mg eq/kg). Characterisation of the residue in these commodities revealed some incorperation into natural products.

Reference: OECD KIIA 6.6.2

Report: Hatterman, D. R.
LX1146-02 (Glyphosate technical) confined rotational crop study on lettuce, radish, and wheat in California
20.04.1998, Report No.: 1651-91-146-01-09B-17, RIP2003-1112

Guidelines: EPA Guideline 165-1

Deviations: ~~original guideline not submitted~~none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study conductedn in California (USA) ^{14}C-glyphosate was applied to bare soil in pots at rates equivalent to 6.5 kg as/ha. The treated soil wa aged for 30, 120 or 365 days, simulating crop failure, second crop planting and yearly rotation. The test crops for this study were radish, lettuce and wheat. All crops were harvested at an immature stage of development and at full maturity. Soil samples were also taken at strategic points throughout the study.

The determination of total radioactivity in soil and plant samples was conducted by LSC. For plant matrices the homogenised samples were extracted with chloroform/water. After partitioning, clean-up via solid phase extraction and ion-exchange, analysis was performed with HPLC-fluorescence detection after derivatisation.

Findings:
In the following table the TRR levels found in wet and dry soil for the different crop rotation intervals are summarised:

Table B.7.9-4: TRR levels found in soil treated with ^{14}C-glyphosate at rates equivalent to 6.5 kg as/ha

Rotational crop	Rotation	Days after treatment	TRR in mg eq/kg (0-7.5 cm / 7.5-15 cm)	
			wet soil	dry soil
Soil	0 (preplanting)	0	6.4 / 0.1	7.9 / 0.1
Radish	30 days	55 (immature)	2.2 / 0.1	2.5 / 0.1
		75 (mature)	2.1 / 0.04	2.3 / 0.04
	120 days	145 (immature)	1.0 / 0.9	1.1 / 1.0
		165 (mature)	2.0 / 0.09	2.2 / 0.1
	365 days	390 (immature)	0.9 / 0.03	1.0 / 0.03
		410 (mature)	0.7 / 0.08	0.8 / 0.09
Wheat forage	30 days	60 (immature)	1.6 / 0.03	1.8 / 0.03
		120 (mature)	2.2 / 0.1	2.6 / 0.1
	120 days	150 (immature)	1.8 / 0.2	2.4 / 0.3
		210 (mature)	1.9 / 0.1	2.1 / 0.1
	365 days	395 (immature)	0.7 / 0.04	0.8 / 0.04
		455 (mature)	0.6 / 0.09	0.6 / 0.1
Lettuce	30 days	55 (immature)	2.5 / 0.04	3.0 /.0.05
		75 (mature)	2.5 / 0.02	2.8 /.0.02
	120 days	145 (immature)	3.2 / 0.05	3.6 / 0.06
		165 (mature)	1.5 / 0.1	1.8 / 0.1
	365 days	390 (immature)	1.1 / 0.04	1.2 / 0.04
		410 (mature)	0.7 / 0.06	0.8 / 0.07

Immature and mature plant samples of rotational crops were investigated for their total radioactive residues and the composition of the residue (mature samples only). Further efforts to identify/characterise unextracted radioactivity was not conducted.

Table B.7.9-5: TRR and identified residues in rotational crops grown in soil treated with ^{14}C-glyphosate at application rates equivalent to 6.5 kg as/ha

Crop	Radioactive residues in mg eq/kg					
	Immature		Mature			
	Days after seeding	TRR	Days after seeding	TRR	Glyphosate	AMPA
30 day rotation						
Radish, leaf	25	2.2	45	4.8	<0.05	<0.05
Radish, root	25	0.38	45	0.24	<0.05	<0.05
Lettuce, leaf	25	0.46	45	0.34	<0.05	<0.05
Wheat, forage	30	0.46	90	1.3	<0.05	0.2
Wheat, chaff	NA	NA	90	1.6	<0.05	0.4
Wheat, grain	NA	NA	90	2.0	<0.05	0.3
120 day rotation						
Radish, leaf	25	0.33	45	0.17	<0.05	<0.05
Radish, root	25	0.71	45	0.15	<0.05	<0.05
Lettuce, leaf	25	0.68	45	0.25	<0.05	<0.05
Wheat, forage	30	0.45	90	1.4	0.4	0.1
Wheat, chaff	NA	NA	90	1.0	0.3	0.2
Wheat, grain	NA	NA	90	0.7	<0.05	0.2
365 day rotation						
Radish, leaf	25	0.01	45	0.02	<0.05	<0.05
Radish, root	25	0.06	45	0.05	<0.05	<0.05
Lettuce, leaf	25	0.02	45	0.02	<0.05	<0.05
Wheat, forage	30	0.01	90	0.08	<0.05	<0.05
Wheat, chaff	NA	NA	90	0.19	0.06	<0.05
Wheat, grain	NA	NA	90	0.16	<0.05	<0.05

NA: not applicable

Conclusions:
This confined study provides limited information on the metabolism of glyphosate in rotational crops. Glyphosate were the only metabolites looked for. Both analytes were mainly present in wheat matrices, ranging up to 0.4 mg eq/kg in forage and chaff. In grain only AMPA was found with 0.3 mg eq/kg in the 30 day rotation sample and 0.2 mg/kg in the 120 day rotation sample. In radish and lettuce not residues of glyphosate or AMPA were detected.

Reference: OECD KIIA 6.6.2

Report: Suba, L. A.
Metabolism of CP 67573 in representative vegetables and rotation crops
April 1976, Report No.: 406, RIP9501199

Guidelines: none

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be acceptable.

Material and Methods:
In this study the uptake of ^{14}C-glyphosate and its metabolites from soil by rotational crops was investigated in the USA. For this purpose a primary crops were grown in containers treated by application of radiolabelled active substance to the soil 16-60 days after planting (4-11 weeks before harvest). The application rates were equivalent to 4.48 kg as/ha. After harvesting the main crops, rotational crops were planted with a 1-23 day interval. Untreated containers were kept as control plots to account for $^{14}CO_2$ fixation from soil metabolism.
The actual main and rotational crops planted were peas → carrots and cabbage, string beans → sweet corn, carrots → cabbage and string beans and cabbage → carrots and peas.

Soil samples and samples from mature plants were collected and analysed for radioactive residues and their composition. For the total radioactivity residues were measured by LSC and combustion. After aqueous (soil) or 0.5N NH_4OH (plants) extraction identification of metabolites was conducted by TLC, electrophoresis and GC/FID after derivatisation against reference substances.

Findings:

The rcovered radioactivity from treated soil was:

Table B.7.9-6: Recovered radioactivity, extractability and composition of ^{14}C-glyphosate residues in soil

Soil sample	Duration	% AR recovered	% extracted	glyphosate (% of extracted)	AMPA (% of extracted)
Primary crops					
Plot 1 – after harvest	4 weeks	81.9	NP	NP	NP
Plot 2 – after harvest	6.5 weeks	71.7	91.8	92.5	4.4
Plot 3 – after harvest	7 weeks	67.8	96.5	92.9	5.0
Plot 4 – after harvest	11 weeks	52.6	98.9	84.5	13.0
Rotaional crops					
Plot 1 – after harvest	18 weeks	60.9	97.2	90.8	6.7
Plot 2 – Maize forage	16.5 weeks	40.4	124.4	78.9	11.2
Plot 2 Maize forage	22 weeks	53.2	90.8	66.5	15.1
Plot 3 – cabbage	27 weeks	28.7	81.1	67.5	15.3
Plot 3 – string beans	13.5 weeks	61.8	90.7	79.5	8.0
Plot 4 – carrots	31	27.1	94.0	68.0	15.9
Plot 4 – peas	17.5	57.4	100.5	67.8	22.5

NP: not performed

In the following table the recovered radioactivity and its composition in primary and rotational crops planted is summarised. Intervals between harvest of the primary crop and planting of the rotational crops were not reported in detail, but the range was between 1-23 days.

Table B.7.9-7: Recovered radioactivity and its composition in primary and rotational crops treated with ^{14}C-glyphosate

Crop	Type	Weeks	TRR in mg eq/kg	% TRR (mg eq/kg)					
				Extracted	Neutrals	Glyphosate	AMPA	other	indeter-minate
Plot 1									
Peas	primary	4[a]	0.22	92.2 (0.20)	6.2 (0.014)	62.0 (0.137)	5.9 (0.013)	4.7 (0.01)	13.4 (0.029)
Carrot roots	rotational	14[b]	0.094	41.4 (0.039)	12.5 (0.012)	19.6 (0.018)	4.1 (0.004)	3.8 (0.004)	-
Carrot leaves	rotational	14[b]	0.086	30.6 (0.026)	14.0 (0.012)	21.5 (0.018)	2.3 (0.002)	4.6 (0.004)	3.3 (0.003)
Cabbage	rotational	14[b]	0.056	17.3 (0.01)	15.9 (0.009)	46.4 (0.026)	4.5 (0.003)	8.4 (0.005)	16.7 (0.009)
Plot 2									
String beans	primary	6.5[a]	0.13	87.5 (0.11)	12.4 (0.016)	54.2 (0.07)	6.0 (0.008)	7.2 (0.009)	7.8 (0.01)
Maize, forage	rotational	10[b]	0.04	48.0 (0.019)	12.8 (0.005)	12.0 (0.005)	11.1 (0.004)	12.0 (0.005)	-
Maize, forage	rotational	15.5[b]	0.09	59.4 (0.053)	23.3 (0.021)	5.6 (0.005)	8.9 (0.008)	7.6 (0.007)	14.0 (0.013)
Maize, kernel	rotational	15.5[b]	0.06	39.9 (0.024)	NP				
Maize, cob	rotational	15.5[b]	0.05	51.5 (0.026)	26.6 (0.013)	3.3 (0.002)	9.0 (0.005)	10.8 (0.005)	1.9 (0.001)
Plot 3									
Carrot roots	primary	7[a]	0.11	77.2 (0.085)	17.8 (0.02)	34.5 (0.038)	5.0 (0.006)	5.0 (0.006)	14.8 (0.016)
Carrot leaves	primary	7[a]	0.17	57.0 (0.097)	12.5 (0.021)	30.2 (0.051)	3.2 (0.005)	3.6 (0.006)	7.6 (0.013)
String bean leaves	rotational	6.5[b]	0.08	66.2 (0.053)	14.4 (0.012)	23.8 (0.019)	3.5 (0.003)	5.0 (0.004)	19.6 (0.016)
String bean pod	rotational	6.5[b]	0.04	90.3 (0.036)	20.0 (0.008)	40.1 (0.016)	7.9 (0.003)	8.9 (0.004)	13.4 (0.005)
Cabbage	rotational	17.5[b]	0.045	57.5 (0.026)	24.0 (0.011)	8.3 (0.004)	6.7 (0.003)	9.3 (0.004)	9.3 (0.004)
Plot 4									
Cabbage, leaves	primary	11[a]	0.08	69.1 (0.055)	17.0 (0.014)	32.1 (0.026)	7.0 (0.006)	4.2 (0.003)	8.8 (0.007)
Cabbage, head	primary	11[a]	NQ	65.9	18.5	39.2	3.3	2.0	2.9
Pea leaves	rotational	6.5[b]	0.19	88.7 (0.17)	13.6 (0.026)	40.4 (0.077)	11.1 (0.021)	5.5 (0.01)	18.2 (0.035)
Pea pods	rotational	6.5[b]	0.28	88.5 (0.25)	9.0 (0.025)	45.8 (0.13)	15.6 (0.044)	4.8 (0.013)	13.3 (0.037)
Carrot roots	rotational	17.5[b]	0.05	73.4 (0.037)	43.2 (0.022)	6.9 (0.003)	7.6 (0.004)	8.5 (0.004)	9.8 (0.005)
Carrot leaves	rotational	17.5[b]	0.08	51.6 (0.041)	17.9 (0.014)	7.7 (0.006)	2.9 (0.002)	8.6 (0.007)	14.5 (0.012)

NP: not performed, NQ: not quantified, a: after treatment, b: after planting

Conclusions:
This study provides limited information on the degradation of glyphosate in soil and the uptake of residues into rotational crops. Glyphosate and AMPA were the only metabolites identified. In contrast to plant metabolism studies, most of the residue was identified as glyphosate instead of AMPA.

Reference: OECD KIIA 6.6.2

Report: Brightwell, B.; Cooper, B. J.
Uptake and metabolism of Glyphosate in root, leaf and cereal type rotation crops
September 1978, Report No.: MSL 0882, RIP9501200

Guidelines: none

Deviations: not applicable

GLP: no

Acceptability: The study is considered to be acceptable.

Material and Methods:
The uptake and metabolism of ^{14}C-glyphosate into rotational crops was investigated using radiolabelled active substance in the phosphonomethyl-moiety of the molecule. The active substance was applied to planting pot at rates equivalent to 4.48 kg as/ha. In parallel unlabelled active substance was applied to identical pots for control purposes and kept in the same greenhouse to account for $^{14}CO_2$ fixiation from degradation in soil. After three days the primary crops (see below) were planted. After 4 months and one year plants were harvested, the soil surface was lightly tilled and the next rotation was planted.

To simulate crop failure, some of the pots containing the primary crop received a second treatment of 4.48 kg as/ha after 30 days and were replanted with the same crops as before (except for soya beans where beets were replanted. Samples of these emergency crops were harvested after 90 days.

Table B.7.9-8: Overview of the different crop rotation sequences

Primary crop	Four month rotation	One year rotation	Emergency crop
Soya beans	Beets	Cabbage	Beets
Cabbage	Wheat	Beets	Cabbage
Wheat	Beets	Cabbage	Wheat
Beets	Cabbage	Wheat	Beets

Total radioactive residues in the plant samples were analysed by combustion and LSC. For further analyses the lyophilized plant samples were extracted with water for 2 hours. The extract was separated by an ion-exchange column into fractions representing glyphosate and AMPA and again analysed by LSC.

Findings:
In the following table the TRR levels found in different plant matrices are summarised:

Table B.7.9-9: TRR and % AR found in plant matrices following application of ^{14}C-glyphosate to bare soil at rates equivalent to 4.48 kg as/ha

Plot	Primary crop		Four month rotation		One year rotation		Emergency crop (PBI 30)[c]	
1	Soya bean foliage[a]		Beet, foliage[a]		Cabbage[a]		Beet, foliage[b]	
	Control: 0.15 mg eq/kg (0.07 % AR)	Treated: 0.21 mg eq/kg (0.08 % AR)	Control: 0.02 mg eq/kg (0.01 % AR)	Treated: 0.05 mg eq/kg (0.01 % AR)	Control: 0.01 mg eq/kg (0.01 % AR)	Treated: 0.03 mg eq/kg (0.02 % AR)	Control: 0.05 mg eq/kg (0.01 % AR)	Treated: 0.32 mg eq/kg 0.04 (% AR)
	Soya bean pods[a]		Beet, root[a]				Beet, roots[b]	
	Control: 0.1 mg eq/kg (0.06 % AR)	Treated: 0.14 mg eq/kg (0.06 % AR)	Control: 0.02 mg eq/kg (0.01 % AR)	Treated: 0.08 mg eq/kg (0.03 % AR)			Control: 0.08 mg eq/kg (0.01 % AR)	Treated: 0.49 mg eq/kg (0.03 % AR)
2	Cabbage[a]		Wheat, forage[a]		Beet, foliage[a]		Cabbage[b]	
	Control: 0.03 mg eq/kg (0.02 % AR)	Treated: 0.13 mg eq/kg (0.09 % AR)	Control: 0.27 mg eq/kg (0.01 % AR)	Treated: 1.1 mg eq/kg (0.04 % AR)	Control: 0.01 mg eq/kg (<0.01% AR)	Treated: 0.05 mg eq/kg (0.01 % AR)	Control: 0.07 mg eq/kg (0.02 % AR)	Treated: 0.18 mg eq/kg (0.05 % AR)
					Beet, root[a]			
					Control: 0.01mg eq/kg (<0.01 % AR)	Treated: 0.05 mg eq/kg (0.01 % AR)		
3	Wheat, forage[a]		Beet, foliage[a]		Cabbage[a]		Wheat, forage[b]	
	Control: 0.26 mg eq/kg (0.02 % AR)	Treated: 3.7mg eq/kg (0.29 % AR)	Control: 0.03 mg eq/kg (0.01 % AR)	Treated: 0.08 mg eq/kg (0.01 % AR)	Control: 0.02 mg eq/kg (0.01 % AR)	Treated: 0.06 mg eq/kg (0.01 % AR)	Control: 0.23 mg eq/kg (0.01 % AR)	Treated: 1.3 mg eq/kg (0.07 % AR)
			Beet, root[a]					
			Control: 0.02 mg eq/kg (0.01 % AR)	Treated: 0.1 mg eq/kg (0.01 % AR)				
4	Beet, foliage[a]		Cabbage[a]		Wheat, forage[a]		Beet, foliage[b]	
	Control: 0.1 mg eq/kg (0.01 % AR)	Treated: 0.46 mg eq/kg (0.05 % AR)	Control: 0.01 mg eq/kg (0.01 % AR)	Treated: 0.08 mg eq/kg (0.03 % AR)	Control: 0.05 mg eq/kg (<0.01% AR)	Treated: 0.19 mg eq/kg (0.02 % AR)	Control: 0.05 mg eq/kg (0.01 % AR)	Treated: 0.2 mg eq/kg (0.02 % AR)
	Beet, root[a]						Beet, roots[b]	
	Control: 0.06 mg eq/kg (0.01 % AR)	Treated: 0.31mg eq/kg (0.08 % AR)					Control: 0.07 mg eq/kg (0.02 % AR)	Treated: 0.37 mg eq/kg (0.04 % AR)

PBI: plant back interval in days, a: mean of two samples, b: mean of four samples, c: emergency crop after additional application of 4.48 kg as/ha, no further crop rotation

The investigation of the extracted radioactivity by ion-exchange chromatography in selected samples characterised the following amounts of glyphosate and AMPA (no structure analysing methods or TLC used).

Table B.7.9-10: Characterised radioactivity in selected plant matrices obtained from rotational crops

Crop	Rotation	TRR in mg eq/kg	% extracted	Glyphosate mg eq/kg (% TRR)	AMPA mg eq/kg (% TRR)
Cabbage	Primary	0.10	51	0.005 (4.8)	0.008 (8.0)
	Emergency	0.11	48	0.003 (3.1)	0.007 (6.7)
	4 month	0.07	58	0.005 (7.6)	0.008 (11.7)
	1 year[a]	0.03	67	0.002 (6.7)	0.003 (9.5)
Wheat	Primary	3.39	57	0.116 (4.9)	0.362 (10.7)
	Emergency	1.08	48	0.128 (11.9)	0.046 (4.3)
	4 month	0.86	64	0.003 (3.6)	0.014 (16.5)
	1 year	0.14	61	<0.001 (4.0)	<0.001 (4.8)
Beet, foliage	Primary	0.36	47	0.021 (5.9)	0.008 (2.3)
	Emergency[a]	0.21	37	0.005 (2.7)	0.0035 (1.6)
	4 months[a]	0.04	45	0.001 (3.0)	<0.001 (0.9)
	1 year	0.04	52	0.002 (4.2)	<0.001 (2.2)
Beet, root	Primary	0.24	56	0.030 (12.4)	0.022 (9.0)
	Emergency[a]	0.33	50	0.039 (12.2)	0.015 (4.7)
	4 months[a]	0.08	50	0.009 (10.9)	0.002 (2.3)
	1 year	0.04	58	0.004 (10.4)	0.002 (3.8)

a: mean of two samples

Conclusions:
The information provided by this study are limited for the identification of relevant metabolites of glyphosate in rotational crops. Highest TRR levels following soil treatment were found in wheat forage and beets (foliage and roots), mainly in the primary or the emergency crop. The characterisation using separation by ion-exchange columns showed both glyphosate and AMPA being present at approximately 2-12 % of the TRRs measured.

Reference: OECD KIIA 6.6.2

Report: Spillner, C. J.; Bowler, D. T.
[^{14}C-Anion] Glyphosate-trimesium: Confined accumulation studies on rotational crops
20.05.1993, Report No.:RR92-096B, RIP9500018

Guidelines: EPA Guideline 165-1

Deviations: ~~original guideline not submitted~~none

GLP: yes

Acceptability: The study is considered to be acceptable.

Material and Methods:

In this study the behaviour of ^{14}C-glyphosate-trimesium radiolabelled in the methylene position of the glyphosate anion was investigated in rotational crops. The test system included five pots (appoximately 1 square meter size), treated differently with the active substance:

Pot 1 & 2 received one application equivalent to 5.62 kg glyphosate-trimesium per ha (3.87 kg glyphosate per ha). Radiolabelled active substance was applied to pot 1, pot 2 received unlabelled glyphosate-trimesium as a control plot.

Pot 5 received three applications at monthly interval with 6.36, 2.08 and 1.07 kg ^{14}C-glyphosate-trimesium per ha (4.38, 1.4 and 0.74 kg glyphosate per ha). Pot four received a comparable treatment of unlabelled active substance as control plot.

Finally pot 3 was treated with an exaggerated rate equivalent to 33.6 kg ^{14}C-glyphosate-trimesium per ha (23.2 kg glyphosate per ha) for identification of residues. However no analysis of the samples was conducted.

In all pots soya beans were planted prior to treatment. After 35 days (DAT 35) the immature crop was removed and lettuce, wheat and radish were planted as rotational crops. Pots 4 & 5 received the last two treatments after removal of the soya bean plants and before planting of the rotational crops. All rotational crops were harvested when mature (DAT 35 for pot 1 and DAT 63 & 308 for pot 5). In addition, wheat forage was harvested when the grain was at the milk stage.

Since glyphosate is quickly degraded in soil under formation of CO_2, soil samples were analysed by combusion and HPLC to take into account the degradation of the active substance.

The analysis of the total radioactivity in soil and plant samples was conducted by combustion and LSC.

For the characterisation and identification of residues samples were extracted using a mixture of 0.1N HCl and chloroform, followed by column fractioning using different solvents to separate residues and natural products.To characterise incorperation into natural products post-extraction solids were additionally hydrolysed with 0.5N HCl.

Extracts were analysed using TLC, HPLC and GC-MS methods agains standard reference substances.

Findings:

In the following table the TRRs found in soil samples collected from pot 1 and 5, which received treatment with ^{14}C-radiolabelled active substance, are summarised:

Table B.7.9-11: TRR and identification of the residue found in soil samples of pot treated with ^{14}C-glyphosate-trimesium

Days after first treatment	TRR in mg glyphosate eq/kg (metabolites as % TRR, if analysed))		
	Soil depth 0-10 cm	Soil depth 10-25 cm	Soil depth 25-40 cm
Pot 1 (1 x 3.87 kg glyphosate anion per ha)			
-1	<0.0005	<0.0005	<0.0005
0	3.55 (gly: 59.5 % AMPA: 0%)	0.003	0.001
34	1.44 (gly: 12% AMPA: 58.6%	0.001	<0.0005
132	0.806	0.010	0.001
148	0.710	0.012	<0.0005
188	0.590 (gly: 3.5% AMPA: 54.9%)	0.007	<0.0005
Pot 5 (4.38, 1.4 and 0.74 kg glyphosate anion per ha, monthly interval)			
-1	<0.0005	<0.0005	<0.0005
0	3.78 (gly: 31.9% AMPA: 6.3%)	0.013	0.004
34 (before 2nd treatment)	1.48	0.014	0.002
34 (after 2nd treatment)	2.97	0.005	0.001
62 (before 3rd treatment)	1.87	0.004	0.003
63 (after 3rd treatment)	2.60	0.006	0.001
125 (62 DAT)	1.65 (gly: 7.1% AMPA: 34.1%)	0.016	<0.0005
223 (160 DAT)	1.41 (gly: 4.09% AMPA: 27.5%)	0.009	0.002
239 (176 DAT)	1.52 (gly: 2.98% AMPA: 27.4%)	0.014	0.001
279 (216 DAT)	0.557 (gly: 8.85%) AMPA: 24.1%)	0.008	0.001
370 (307 DAT)	1.07	0.015	<0.0005
413 (350 DAT)	0.744	0.041	0.001
436 (373 DAT)	0.271	0.019	0.001
517 (454 DAT)	0.345	0.019	0.001

DAT days after final treatment
gly glyphosate

In plant matrices no significant residues above the natural background levels were detected in samples receiving treatment with unlabelled active substance (pot 2 & 4). Results for these samples are not presented in the following tables. For the pots 1 and 5 TRR levels found were:

Table B.7.9-12: TRR levels found in rotational crops

Sample	TRR found in mg glyphosate eq/kg		
	Pot 1 (1 x 3.87 kg glyphosate/ha) 35 DAT	Pot 5 (4.38, 1.4 and 0.74 kg glyphosate anion per ha, monthly interval) DAT 63	Pot 5 (4.38, 1.4 and 0.74 kg glyphosate anion per ha, monthly interval) DAT 308
Lettuce	0.072	0.127	0.017
Radish, roots	0.02	0.022	0.01
Radish, tops	0.02	0.021	0.016
Wheat, forage	0.024	0.033	0.017
Wheat, straw	0.05	0.063	0.031
Wheat, grain	0.076	0.092	0.038

The characterisation/identification of radioactive residues found in plant samples from rotational crops are summarised below.

Table B.7.9-13: Characterisation and identification of radioactive residues found in lettuce grown as rotational crop

Fraction	35 DAT		63 DAT		308 DAT	
	% TRR	mg eq/kg[a]	% TRR	mg eq/kg[a]	% TRR	mg eq/kg[a]
TRR[b]	100	0.072	100	0.127	100	0.017
Non-polar ($CHCl_3$)	2.8	0.002	1.6	0.002	ND	<0.001
Polar (aqueous)	51.4	0.037	48.7	0.062	44.7	0.008
glyphosate	0.7	0.001	0.9	0.001	19.6	0.003
AMPA	20.4	0.015	18.5	0.024		
Metabolite 1	5.1	0.004	6.5	0.008		
Unknown	0.6	0.0004	0.2	0.0003		
Carbohydrates					25.1	0.004
Glu	10.0	0.007	9.3	0.012	NP	NP
Fru	7.5	0.005	6.8	0.009	NP	NP
Malic acid	7.2	0.005	6.5	0.008	NP	NP
Post-extraction solids	45.8	0.003	49.6	0.063	55.3	0.009
Acid hydrolysate					NP	NP
Gly+AMPA	4.4	0.003	4.9	0.006		
Carbohydrates	22.4	0.016				
Glu	NP	NP	16.7	0.021		
Fru	NP	NP	7.0	0.009		
Acid hydrolysed solids	19.0	0.014			NP	NP
Lignin	NP	NP	4.8	0.006		
Aminoacids	NP	NP	10.2	0.013		
Cellulose	NP	NP	6.0	0.008		
Total	100.1	0.071	99.9	0.127	100	0.016

a: expressed as glyphosate equivalents, b: measured by combustion/LSC, ND: not detected, NP: not performed, Glu: glucose, Fru: fructose, gly: glyphosate, Metabolite 1: single, unidentified stucture

Table B.7.9-14: Characterisation and identification of radioactive residues found in wheat (grain) grown as rotational crop

Fraction	35 DAT		63 DAT		308 DAT	
	% TRR	mg eq/kg[a]	% TRR	mg eq/kg[a]	% TRR	mg eq/kg[a]
TRR[b]	100	0.076	100	0.092	100	0.038
Non-polar ($CHCl_3$)	3.1	0.002	7.1	0.007	0.8	<0.01
Polar (aqueous)	46.9	0.036	40.6	0.037	50.0	0.019
glyphosate	ND	<0.001	2.3	0.002	41.7	0.016
AMPA	34.0	0.026	25.8	0.024		
Metabolite 1	3.0	0.002	2.5	0.002		
Unknown	ND	<0.001	ND	<0.001		
Carbohydrates	9.8	0.007	9.8	0.009	8.4	0.003
Post-extraction solids	50.1	0.038	51.9	0.048	49.2	0.019
Acid hydrolysate					36.1	0.014
Gly+AMPA	3.7	0.003	2.3	0.003	NP	NP
Carbohydrates						
Glu	32.3	0.025	39.8	0.037		
Fru	ND	<0.001	ND	<0.001		
Acid hydrolysed solids	14.1	0.011	10.2	0.009	13.1	0.005
Total	100	0.076	99.8	0.093	99.3	0.038

a: expressed as glyphosate equivalents, b: measured by combustion/LSC, ND: not detected, NP: not performed, Glu: glucose, Fru: fructose, gly: glyphosate

Table B.7.9-15: Characterisation and identification of radioactive residues found in wheat (straw) grown as rotational crop

Fraction	35 DAT		63 DAT		308 DAT	
	% TRR	mg eq/kg[a]	% TRR	mg eq/kg[a]	% TRR	mg eq/kg[a]
TRR[b]	100	0.050	100	0.063	100	0.031
Non-polar ($CHCl_3$)	1.4	0.001	0.9	0.001	0.7	0.0002
Polar (aqueous)	39.3	0.020	37.2	0.024	23.1	0.007
glyphosate	0.4	0.0002	0.3	0.0002	8.5	0.003
AMPA	11.7	0.006	12.7	0.008		
Metabolite 1	4.1	0.002	4.2	0.003		
Unknown	0.1	0.0001	0.1	0.0001		
Carbohydrates	22.9	0.012			15.0	0.004
Glu	NP	NP	3.5	0.002	NP	NP
Fru			3.7	0.002		
Malic acid			4.6	0.003		
Metabolite 2			8.3	0.005		
Post-extraction solids	59.3	0.030	61.5	0.039	76.1	0.024
Acid hydrolysate	16.0	0.008	19.1	0.012	34.5	0.011
Acid hydrolysed solids					41.7	0.013
Lignin	included with cellulose		12.8	0.008	NP	NP
Aminoacids	20.7	0.010	15.6	0.010		
Cellulose	22.6	0.011	13.9	0.009		
Total	99.9	0.050	99.6	0.063	99.9	0.031

a: expressed as glyphosate equivalents, b: measured by combustion/LSC, ND: not detected, NP: not performed, Glu: glucose, Fru: fructose, Metabolite 1 & 2: single, unidentified stuctures

Table B.7.9-16: Characterisation and identification of radioactive residues found in wheat (forage) grown as rotational crop

Fraction	35 DAT		63 DAT		308 DAT	
	% TRR	mg eq/kg[a]	% TRR	mg eq/kg[a]	% TRR	mg eq/kg[a]
TRR[b]	100	0.024	100	0.033	100	0.017
Non-polar ($CHCl_3$)	3.1	0.001	2.0	0.001	NP	NP
Polar (aqueous)	46.2	0.011	47.3	0.016		
glyphosate	0.5	0.0001	ND	<0.001		
AMPA	20.5	0.005	20.5	0.007		
Metabolite 1	1.9	0.0003	2.0	0.001		
Unknown	2.2	0.001	3.3	0.001		
Carbohydrates	17.9	0.004	21.6	0.007		
Post-extraction solids	53.8	0.013	50.7	0.016		
Acid hydrolysate	NP	NP				
Carbohydrates			23.1	0.008		
Acid hydrolysed solids						
Lignin			4.4	0.001		
Aminoacids			10.5	0.003		
Cellulose			12.7	0.004		
Total	99.9	0.024	100.1	0.033		

a: expressed as glyphosate equivalents, b: measured by combustion/LSC, ND: not detected, NP: not performed, Metabolite 1: single, unidentified stucture

Table B.7.9-17: Characterisation and identification of radioactive residues found in radish roots grown as rotational crop

Fraction	35 DAT		63 DAT		308 DAT	
	% TRR	mg eq/kg[a]	% TRR	mg eq/kg[a]	% TRR	mg eq/kg[a]
TRR[b]	100	0.020	100	0.022	100	0.010
Non-polar ($CHCl_3$)	0.4	0.0001	1.4	0.0003	0.8	0.0001
Polar (aqueous)	46.3	0.010	47.1	0.010	50.6	0.005
glyphosate	1.8	0.0004	1.7	0.0004	7.4	0.001
AMPA	8.7	0.002	11.0	0.002		
Metabolite 1	1.0	0.0002	1.2	0.0003		
Unknown	2.1	0.0004	1.6	0.0004		
Carbohydrates	32.7	0.007	31.8	0.007	43.2	0.004
Post-extraction solids	53.3	0.011	51.5	0.011	48.6	0.005
Total	100	0.021	100	0.021	100	0.010

a: expressed as glyphosate equivalents, b: measured by combustion/LSC, ND: not detected, NP: not performed, Metabolite 1: single, unidentified stucture

Table B.7.9-18: Characterisation and identification of radioactive residues found in radish tops grown as rotational crop

Fraction	35 DAT		63 DAT		308 DAT	
	% TRR	mg eq/kg[a]	% TRR	mg eq/kg[a]	% TRR	mg eq/kg[a]
TRR[b]	100	0.020	100	0.021	100	0.016
Non-polar ($CHCl_3$)	4.8	0.001	5.5	0.001	3.1	0.001
Polar (aqueous)	36.9	0.007	39.5	0.008	37.3	0.006
glyphosate	0.9	0.0002	1.1	0.0002	10.0	0.002
AMPA	12.3	0.002	9.5	0.002		
Metabolite 1	1.0	0.0002	1.4	0.0003		
Unknown	1.8	0.0004	1.8	0.0004		
Carbohydrates	21.0	0.004	25.7	0.005	27.4	0.004
Post-extraction solids	58.2	0.012	54.9	0.012	59.4	0.01
Total	99.9	0.020	99.9	0.022	99.9	0.017

a: expressed as glyphosate equivalents, b: measured by combustion/LSC, ND: not detected, NP: not performed, Metabolite 1: single, unidentified stucture

Conclusions:

Following application of ^{14}C-glyphosate-trimesium to soil, a steady decline of total radioactivity (probably after degradation as CO_2) was observed. In matrices obtained from rotational crops, TRR levels were relatively low, not exceeding 0.1 mg eq/kg except for lettuce (0.127 mg eq/kg). The identification of the residues primarily revealed AMPA, being found at levels of 8.7-34% of the TRR. Glyphosate was also found in most samples, however its levels were <2.3% of the TRR. Most of the radioactivity was present in natural products like carbohydrates, amino acids and lignin/cellulose.

B.7.9.2 Field rotational crop studies

Confined rotational crop metabolism studies indicate a very minor uptake of residue from soil into plants. The actice substance glyphosate is quickly degraded into AMPA and within few weeks into CO_2. The uptake of AMPA, as demonstrated in the confined rotational crop studies as well as in plant metabolism studies involving soil treatment, is also very low, not resulting in expected residues above 0.01 mg/kg event at exaggerated rates compared to the intended uses.

In summary it can be concluded that neither glyphosate nor AMPA show a potential uptake into rotational crops. Further studies involving the uptake under field conditions are not considered necessary.

B.7.10 Proposed pre-harvest intervals for envisaged uses, or withholding periods, in the case of post-harvest uses (Annex IIA 6.8; Annex IIIA 8.8)

For all uses involving application of glyphosate before sowing or planting no pre-harvest intervals are required, since the active substance and its main metabolite in soil (AMPA) are not translocated into plants via the roots.

Pre-harvest uses on cereal grains and oilseeds for desiccation purposes were defined with PHIs of 7 to 14 days (see Table B.7.4-1).

For the ground treatment of orchards and vineyards for weed control the target crops are not intentionally sprayed. If the application is conducted according to good agricultural practice, no residues in harvested commodities have to be expected, making the proposal of a pre-harvest interval unnecessary.

B.7.11 Community MRLs and MRLs in EU Member States (Annex IIIA 11.2)

Under Regulation (EC) No. 396/2005 (last amended by Reg. (EU) No. 441/2012) the following MRLs are established for glyphosate in the EU:

Table B.7.11-1: List of MRLs estabished for glyphosate in the EU

Code number	Groups and examples of individual products to which the MRLs apply (a)	Glyphosate
100000	1. FRUIT FRESH OR FROZEN; NUTS	
110000	(i) Citrus fruit	
110010	Grapefruit (Shaddocks, pomelos, sweeties, tangelo (except mineola), ugli and other hybrids)	0,1*
110020	Oranges (Bergamot, bitter orange, chinotto and other hybrids)	0,5
110030	Lemons (Citron, lemon)	0,1*
110040	Limes	0,1*
110050	Mandarins (Clementine, tangerine, mineola and other hybrids)	0,5
110990	Others	0,1*
120000	(ii) Tree nuts (shelled or unshelled)	0,1*
120010	Almonds	0,1*
120020	Brazil nuts	0,1*
120030	Cashew nuts	0,1*
120040	Chestnuts	0,1*
120050	Coconuts	0,1*
120060	Hazelnuts (Filbert)	0,1*
120070	Macadamia	0,1*
120080	Pecans	0,1*
120090	Pine nuts	0,1*
120100	Pistachios	0,1*
120110	Walnuts	0,1*
120990	Others	0,1*
130000	(iii) Pome fruit	0,1*
130010	Apples (Crab apple)	0,1*
130020	Pears (Oriental pear)	0,1*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Glyphosate
130030	Quinces	0,1*
130040	Medlar	0,1*
130050	Loquat	0,1*
130990	Others	0,1*
140000	(iv) Stone fruit	0,1*
140010	Apricots	0,1*
140020	Cherries (sweet cherries, sour cherries)	0,1*
140030	Peaches (Nectarines and similar hybrids)	0,1*
140040	Plums (Damson, greengage, mirabelle, sloe)	0,1*
140990	Others	0,1*
150000	(v) Berries & small fruit	
151000	(a) Table and wine grapes	0,5
151010	Table grapes	0,5
151020	Wine grapes	0,5
152000	(b) Strawberries	0,1*
153000	(c) Cane fruit	0,1*
153010	Blackberries	0,1*
153020	Dewberries (Loganberries, boysenberries, and cloudberries)	0,1*
153030	Raspberries (Wineberries, arctic bramble/raspberry, (*Rubus arcticus*), nectar *raspberries (Rubus arcticus x idaeus)*)	0,1*
153990	Others	0,1*
154000	(d) Other small fruit & berries	0,1*
154010	Blueberries (Bilberries)	0,1*
154020	Cranberries (Cowberries (red bilberries))	0,1*
154030	Currants (red, black and white)	0,1*
154040	Gooseberries (Including hybrids with other ribes species)	0,1*
154050	Rose hips	0,1*
154060	Mulberries (arbutus berry)	0,1*
154070	Azarole (mediteranean medlar) (Kiwiberry *(Actinidia arguta)*)	0,1*
154080	Elderberries (Black chokeberry (appleberry), mountain ash, buckthorn (sea sallowthorn), hawthorn, service berries, and other treeberries)	0,1*
154990	Others	0,1*
160000	(vi) Miscellaneous fruit	
161000	(a) Edible peel	
161010	Dates	0,1*
161020	Figs	0,1*
161030	Table olives	1
161040	Kumquats (Marumi kumquats, nagami kumquats, limequats (*Citrus aurantifolia x Fortunella spp*.))	0,1*
161050	Carambola (Bilimbi)	0,1*
161060	Persimmon	0,1*
161070	Jambolan (java plum) (Java apple (water apple), pomerac, rose apple, Brazilean cherry Surinam cherry (grumichama *Eugenia uniflora*),)	0,1*
161990	Others	0,1*
162000	(b) Inedible peel, small	0,1*
162010	Kiwi	0,1*
162020	Lychee (Litchi) (Pulasan, rambutan (hairy litchi), mangosteen)	0,1*
162030	Passion fruit	0,1*
162040	Prickly pear (cactus fruit)	0,1*
162050	Star apple	0,1*
162060	American persimmon (Virginia kaki) (Black sapote, white sapote, green sapote, canistel (yellow sapote), and mammey sapote)	0,1*
162990	Others	0,1*
163000	(c) Inedible peel, large	0,1*
163010	Avocados	0,1*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Glyphosate
163020	Bananas (Dwarf banana, plantain, apple banana)	0,1*
163030	Mangoes	0,1*
163040	Papaya	0,1*
163050	Pomegranate	0,1*
163060	Cherimoya (Custard apple, sugar apple (sweetsop), llama and other medium sized Annonaceae)	0,1*
163070	Guava (Red pitaya or dragon fruit (*Hylocereus undatus)*)	0,1*
163080	Pineapples	0,1*
163090	Bread fruit (Jackfruit)	0,1*
163100	Durian	0,1*
163110	Soursop (guanabana)	0,1*
163990	Others	0,1*
200000	2. VEGETABLES FRESH OR FROZEN	
210000	(i) Root and tuber vegetables	
211000	(a) Potatoes	0,5
212000	(b) Tropical root and tuber vegetables	0,1*
212010	Cassava (Dasheen, eddoe (Japanese taro), tannia)	0,1*
212020	Sweet potatoes	0,1*
212030	Yams (Potato bean (yam bean), Mexican yam bean)	0,1*
212040	Arrowroot	0,1*
212990	Others	0,1*
213000	(c) Other root and tuber vegetables except sugar beet	0,1*
213010	Beetroot	0,1*
213020	Carrots	0,1*
213030	Celeriac	0,1*
213040	Horseradish (Angelica roots, lovage roots, gentiana roots,)	0,1*
213050	Jerusalem artichokes	0,1*
213060	Parsnips	0,1*
213070	Parsley root	0,1*
213080	Radishes (Black radish, Japanese radish, small radish and similar varieties, tiger nut (*Cyperus esculentus)*)	0,1*
213090	Salsify (Scorzonera, Spanish salsify (Spanish oysterplant))	0,1*
213100	Swedes	0,1*
213110	Turnips	0,1*
213990	Others	0,1*
220000	(ii) Bulb vegetables	0,1*
220010	Garlic	0,1*
220020	Onions (Silverskin onions)	0,1*
220030	Shallots	0,1*
220040	Spring onions (Welsh onion and similar varieties)	0,1*
220990	Others	0,1*
230000	(iii) Fruiting vegetables	0,1*
231000	(a) Solanacea	0,1*
231010	Tomatoes (Cherry tomatoes, tree tomato, *Physalis*, gojiberry, wolfberry (*Lycium barbarum and L. chinense*))	0,1*
231020	Peppers (Chilli peppers)	0,1*
231030	Aubergines (egg plants) (Pepino)	0,1*
231040	Okra, lady's fingers	0,1*
231990	Others	0,1*
232000	(b) Cucurbits - edible peel	0,1*
232010	Cucumbers	0,1*
232020	Gherkins	0,1*
232030	Courgettes (Summer squash, marrow (patisson))	0,1*
232990	Others	0,1*
233000	(c) Cucurbits-inedible peel	0,1*
233010	Melons (Kiwano)	0,1*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Glyphosate
233020	Pumpkins (Winter squash)	0,1*
233030	Watermelons	0,1*
233990	Others	0,1*
234000	(d) Sweet corn	0,1*
239000	(e) Other fruiting vegetables	0,1*
240000	(iv) Brassica vegetables	0,1*
241000	(a) Flowering brassica	0,1*
241010	Broccoli (Calabrese, Chinese broccoli, broccoli raab)	0,1*
241020	Cauliflower	0,1*
241990	Others	0,1*
242000	(b) Head brassica	0,1*
242010	Brussels sprouts	0,1*
242020	Head cabbage (Pointed head cabbage, red cabbage, savoy cabbage, white cabbage)	0,1*
242990	Others	0,1*
243000	(c) Leafy brassica	0,1*
243010	Chinese cabbage (Indian (Chinese) mustard, pak choi, Chinese flat cabbage (tai goo choi), choi sum, peking cabbage (pe-tsai),)	0,1*
243020	Kale (Borecole (curly kale), collards, Portuguese Kale, Portuguese cabbage, cow cabbage)	0,1*
243990	Others	0,1*
244000	(d) Kohlrabi	0,1*
250000	(v) Leaf vegetables & fresh herbs	0,1*
251000	(a) Lettuce and other salad plants including Brassicacea	0,1*
251010	Lamb´s lettuce (Italian cornsalad)	0,1*
251020	Lettuce (Head lettuce, lollo rosso (cutting lettuce), iceberg lettuce, romaine (cos) lettuce)	0,1*
251030	Scarole (broad-leaf endive) (Wild chicory, red-leaved chicory, radicchio, curld leave endive, sugar loaf)	0,1*
251040	Cress	0,1*
251050	Land cress	0,1*
251060	Rocket, Rucola (Wild rocket)	0,1*
251070	Red mustard	0,1*
251080	Leaves and sprouts of Brassica spp (Mizuna, leaves of peas and radish and other babyleaf brassica crops (crops harvested up to 8 true leaf stage))	0,1*
251990	Others	0,1*
252000	(b) Spinach & similar (leaves)	0,1*
252010	Spinach (New Zealand spinach, amaranthus spinach)	0,1*
252020	Purslane (Winter purslane (miner’s lettuce), garden purslane, common purslane, sorrel, glassworth, Agretti (*Salsola soda*))	0,1*
252030	Beet leaves (chard) (Leaves of beetroot)	0,1*
252990	Others	0,1*
253000	(c) Vine leaves (grape leaves)	0,1*
254000	(d) Water cress	0,1*
255000	(e) Witloof	0,1*
256000	(f) Herbs	0,1*
256010	Chervil	0,1*
256020	Chives	0,1*
256030	Celery leaves (Fennel leaves, Coriander leaves, dill leaves, Caraway leaves, lovage, angelica, sweet cisely and other *Apiacea* leaves)	0,1*
256040	Parsley	0,1*
256050	Sage (Winter savory, summer savory,)	0,1*
256060	Rosemary	0,1*
256070	Thyme (Marjoram, oregano)	0,1*
256080	Basil (Balm leaves, mint, peppermint)	0,1*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Glyphosate
256090	Bay leaves (laurel)	0,1*
256100	Tarragon (Hyssop)	0,1*
256990	Others (Edible flowers)	0,1*
260000	(vi) Legume vegetables (fresh)	0,1*
260010	Beans (with pods) (Green bean (french beans, snap beans), scarlet runner bean, slicing bean, yardlong beans)	0,1*
260020	Beans (without pods) (Broad beans, Flageolets, jack bean, lima bean, cowpea)	0,1*
260030	Peas (with pods) (Mangetout (sugar peas, snow peas))	0,1*
260040	Peas (without pods) (Garden pea, green pea, chickpea)	0,1*
260050	Lentils	0,1*
260990	Others	0,1*
270000	(vii) Stem vegetables (fresh)	0,1*
270010	Asparagus	0,1*
270020	Cardoons	0,1*
270030	Celery	0,1*
270040	Fennel	0,1*
270050	Globe artichokes	0,1*
270060	Leek	0,1*
270070	Rhubarb	0,1*
270080	Bamboo shoots	0,1*
270090	Palm hearts	0,1*
270990	Others	0,1*
280000	(viii) Fungi	
280010	Cultivated (Common mushroom, Oyster mushroom, Shi-take)	0,1*
280020	Wild (Chanterelle, Truffle, Morel, Cep)	50
280990	Others	0,1*
290000	(ix) Sea weeds	
300000	3. PULSES, DRY	
300010	Beans (Broad beans, navy beans, flageolets, jack beans, lima beans, field beans, cowpeas)	2
300020	Lentils	10
300030	Peas (Chickpeas, field peas, chickling vetch)	10
300040	Lupins	10
300990	Others	0,1*
400000	4. OILSEEDS AND OILFRUITS	
401000	(i) Oilseeds	
401010	Linseed	10
401020	Peanuts	0,1*
401030	Poppy seed	0,1*
401040	Sesame seed	0,1*
401050	Sunflower seed	20
401060	Rape seed (Bird rapeseed, turnip rape)	10
401070	Soya bean	20
401080	Mustard seed	10
401090	Cotton seed	10
401100	Pumpkin seeds (Other seeds of *cucurbitacea*)	0,1*
401110	Safflower	0,1*
401120	Borage	0,1
401130	Gold of pleasure	0,1
401140	Hempseed	0,1*
401150	Castor bean	0,1
401990	Others	0,1*
402000	(ii) Oilfruits	
402010	Olives for oil production	1
402020	Palm nuts (palmoil kernels)	0,1

Code number	Groups and examples of individual products to which the MRLs apply (a)	Glyphosate
402030	Palmfruit	0,1
402040	Kapok	0,1
402990	Others	0,1*
500000	5. CEREALS	
500010	Barley	20
500020	Buckwheat (Amaranthus, quinoa)	0,1*
500030	Maize	1
500040	Millet (Foxtail millet, teff)	0,1*
500050	Oats	20
500060	Rice	0,1*
500070	Rye	10
500080	Sorghum	20
500090	Wheat (Spelt, triticale)	10
500990	Others	0,1*
600000	6. TEA, COFFEE, HERBAL INFUSIONS AND COCOA	
610000	(i) Tea (dried leaves and stalks, fermented or otherwise of Camellia sinensis)	2
620000	(ii) Coffee beans	0,1
630000	(iii) Herbal infusions (dried)	2*
631000	(a) Flowers	2*
631010	Camomille flowers	2*
631020	Hybiscus flowers	2*
631030	Rose petals	2*
631040	Jasmine flowers (Elderflowers *(Sambucus nigra)*)	2*
631050	Lime (linden)	2*
631990	Others	2*
632000	(b) Leaves	2*
632010	Strawberry leaves	2*
632020	Rooibos leaves (Ginkgo leaves)	2*
632030	Maté	2*
632990	Others	2*
633000	(c) Roots	2*
633010	Valerian root	2*
633020	Ginseng root	2*
633990	Others	2*
639000	(d) Other herbal infusions	2*
640000	(iv) Cocoa (fermented beans)	0,1*
650000	(v) Carob (st johns bread)	0,1*
700000	7. HOPS (dried) , including hop pellets and unconcentrated powder	0,1*
800000	8. SPICES	0,1*
810000	(i) Seeds	0,1*
810010	Anise	0,1*
810020	Black caraway	0,1*
810030	Celery seed (Lovage seed)	0,1*
810040	Coriander seed	0,1*
810050	Cumin seed	0,1*
810060	Dill seed	0,1*
810070	Fennel seed	0,1*
810080	Fenugreek	0,1*
810090	Nutmeg	0,1*
810990	Others	0,1*
820000	(ii) Fruits and berries	0,1*
820010	Allspice	0,1*
820020	Anise pepper (Japan pepper)	0,1*
820030	Caraway	0,1*
820040	Cardamom	0,1*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Glyphosate
820050	Juniper berries	0,1*
820060	Pepper, black and white (Long pepper, pink pepper)	0,1*
820070	Vanilla pods	0,1*
820080	Tamarind	0,1*
820990	Others	0,1*
830000	(iii) Bark	0,1*
830010	Cinnamon (Cassia)	0,1*
830990	Others	0,1*
840000	(iv) Roots or rhizome	0,1*
840010	Liquorice	0,1*
840020	Ginger	0,1*
840030	Turmeric (Curcuma)	0,1*
840040	Horseradish	0,1*
840990	Others	0,1*
850000	(v) Buds	0,1*
850010	Cloves	0,1*
850020	Capers	0,1*
850990	Others	0,1*
860000	(vi) Flower stigma	0,1*
860010	Saffron	0,1*
860990	Others	0,1*
870000	(vii) Aril	0,1*
870010	Mace	0,1*
870990	Others	0,1*
900000	9. SUGAR PLANTS	
900010	Sugar beet (root)	1*
900020	Sugar cane	0,1*
900030	Chicory roots	0,1*
900990	Others	0,1*
1000000	10. PRODUCTS OF ANIMAL ORIGIN-TERRESTRIAL ANIMALS	
1010000	(i) Meat, preparations of meat, offals, blood, animal fats fresh chilled or frozen, salted, in brine, dried or smoked or processed as flours or meals other processed products such as sausages and food preparations based on these	
1011000	(a) Swine	
1011010	Meat	0,05*
1011020	Fat free of lean meat	0,05*
1011030	Liver	0,05*
1011040	Kidney	0,5
1011050	Edible offal	0,05*
1011990	Others	0,05*
1012000	(b) Bovine	
1012010	Meat	0,05*
1012020	Fat	0,05*
1012030	Liver	0,2
1012040	Kidney	2
1012050	Edible offal	0,05*
1012990	Others	0,05*
1013000	(c) Sheep	0,05*
1013010	Meat	0,05*
1013020	Fat	0,05*
1013030	Liver	0,05*
1013040	Kidney	0,05*
1013050	Edible offal	0,05*
1013990	Others	0,05*
1014000	(d) Goat	0,05*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Glyphosate
1014010	Meat	0,05*
1014020	Fat	0,05*
1014030	Liver	0,05*
1014040	Kidney	0,05*
1014050	Edible offal	0,05*
1014990	Others	0,05*
1015000	(e) Horses, asses, mules or hinnies	0,05*
1015010	Meat	0,05*
1015020	Fat	0,05*
1015030	Liver	0,05*
1015040	Kidney	0,05*
1015050	Edible offal	0,05*
1015990	Others	0,05*
1016000	(f) Poultry -chicken, geese, duck, turkey and Guinea fowl-, ostrich, pigeon	
1016010	Meat	0,05*
1016020	Fat	0,05*
1016030	Liver	0,05*
1016040	Kidney	0,1*
1016050	Edible offal	0,05*
1016990	Others	0,05*
1017000	(g) Other farm animals (Rabbit, Kangaroo)	0,05*
1017010	Meat	0,05*
1017020	Fat	0,05*
1017030	Liver	0,05*
1017040	Kidney	0,05*
1017050	Edible offal	0,05*
1017990	Others	0,05*
1020000	(ii) Milk and cream, not concentrated, nor containing added sugar or sweetening matter, butter and other fats derived from milk, cheese and curd	0,05*
1020010	Cattle	0,05*
1020020	Sheep	0,05*
1020030	Goat	0,05*
1020040	Horse	0,05*
1020990	Others	0,05*
1030000	(iii) Birds' eggs, fresh preserved or cooked Shelled eggs and egg yolks fresh, dried, cooked by steaming or boiling in water, moulded, frozen or otherwise preserved whether or not containing added sugar or sweetening matter	0,05*
1030010	Chicken	0,05*
1030020	Duck	0,05*
1030030	Goose	0,05*
1030040	Quail	0,05*
1030990	Others	0,05*
1040000	(iv) Honey (Royal jelly, pollen)	0,05*
1050000	(v) Amphibians and reptiles (Frog legs, crocodiles)	0,05*
1060000	(vi) Snails	0,05*
1070000	(vii) Other terrestrial animal products	0,05*

B.7.12 Proposed EU MRLs and justification for the acceptability of those residues (Annex IIA 6.7; Annex IIIA 8.7)

For the estimation of maximum residue levels for treated commodities the OECD-Calculator was used.
(http://www.oecd.org/env/ehs/pesticides-biocides/oecdmaximumresiduelimitcalculator.htm)

B.7.12.1 Tree crops, except olives

In supervised field trials on tree fruits other than olives conducted in Northern and Southern Europe no residues above the LOQs of 0.02 mg/kg to 0.05 mg/kg were found in the fruits:

Northern Europe:

Apples	<0.02, <0.05(3) mg/kg
Cherries	<0.05, <0.05 mg/kg

Southern Europe:

Treenuts	<0.05(4) mg/kg
Apples	<0.05(13) mg/kg
Pears	<0.05(4) mg/kg
Peaches	<0.05, <0.05 mg/kg

Since no detectable residue were found, the OECD MRL-Calculator is not applicable. For tree fruits, except olives, a MRL at the LOQ of the analytical method of 0.05* mg/kg is proposed.

B.7.12.2 Grapes

For grapes only data from Northern Europe was provided. In these supervised field trials low hanging fruits contained detectable residues, since the fruits were exposed to the ground directed application to the weeds:

Northern Europe:

Grapes	<0.05(6), 0.07, 0.3 mg/kg

Based on the OECD MRL-Calculator a MRL of 0.5 mg/kg was estimated. However, due to the high amount of values below the LOQ, a high uncertainty is involved in this estimation.

Table B.7.12-1: Summary of the OECD MRL-Calculator for grapes

Grapes	
Glyphosate	
EU-North	
GAP	
Total number of data (n)	8
Percentage of censored data	75%
Number of non-censored data	2
Lowest residue	0.050
Highest residue	0.300
Median residue	0.050
Mean	0.084
Standard deviation (SD)	0.088
Correction factor for censoring (CF)	0.500
Proposed MRL estimate	
- Highest residue	0.300
- Mean + 4 SD	0.434
- CF x 3 Mean	0.126
Unrounded MRL	0.434
Rounded MRL	0.5

B.7.12.3 Olives

The common agricultural practice for harvesting olives may involve picking of ground laying fruits. While tree picked olives did not contain detectable residues of glyphosate after ground treatment, substantial residues were found in the ground picked olives. To considered this agricultural practice, only ground picked fruits are taken into account to the MRL calculation, leaving four supervised field trials. In SEU, olives for oil production are a major crop, requiring a set of eight trials for estimating a MRL. Therefore the current proposal is limited to table olives, which are a minor crop in the EU:

Southern Europe:
Olives 0.11, 0.14, 0.53, 0.93~~<0.05(4), 0.11, 0.11, 0.12, 0.13, 0.14, 0.53, 0.93, 0.93~~ mg/kg

Based on the OECD MRL-Calculator a MRL of 2 mg/kg was estimated for table olives.

Table B.7.12-2: Summary of the OECD MRL-Calculator for table olives (ground picked)

Olives (ground picked)	
Glyphosate	
EU-South	
GAP	
Total number of data (n)	4~~12~~
Percentage of censored data	0~~33~~%
Number of non-censored data	4~~8~~
Lowest residue	0.11~~050~~
Highest residue	0.930
Median residue	0.335~~115~~
Mean	0.428~~267~~
Standard deviation (SD)	0.386~~36~~
Correction factor for censoring (CF)	1~~0.778~~
Proposed MRL estimate	
- Highest residue	0.930
- Mean + 4 SD	1.971~~611~~
- CF x 3 Mean	1.283~~0.622~~
Unrounded MRL	1.971~~611~~
Rounded MRL	2

B.7.12.4 Linseed, mustard seed, rape seed

For the desiccation of linseed, mustard seed or rape seed only three supervised field trials according to the representative use were submitted. For a minor crop (linseed, mustard seed) at least four supervised field trials and for a major crop (rape seed) at least eight supervised field trials are required to estimate a MRL for the respective commodities.

For pre-emergence treatment please refer to the MRL proposal for other annual crops.

B.7.12.5 Barley and oats

According to the current guidance for extrapolation (SANCO 7525/VI/95 Rev.9) data on barley grain treated before harvest can be also be extrapolated to oats grain for the estimation of MRLs. In comparison to pre-emergence application the desiccation seven days before harvest is considered as critical use for glyphosate.

Northern Europe:

Barley and oats grain: 1.2, 1.5, 2.0, 2.1, 2.1, 2.2, 2.4, 2.5, 2.6, 2.6, 2.8, 3.95, 4.3, 4.4, 4.5, 4.6, 4.8, 5.1, 5.2, 5.2, 5.2, 5.3, 5.4, 5.5, 5.5, 5.7, 5.9, 5.9, 6.2, 6.5, 6.7, 7.4, 7.7, 7.8, 8.0, 8.1, 8.4, 9.8, 10, 10.3, 12.4, 12.5, 14, 15.5, 16.5, 17, 17.5, 18.4, 21, 21.4 mg/kg

Based on the OECD MRL-Calculator a MRL of 30 mg/kg was estimated.

Table B.7.12-3: Summary of the OECD MRL-Calculator for barley and oats grain

Barley & oats, grain	
Glyphosate	
EU-North	
GAP	
Total number of data (n)	51
Percentage of censored data	0%
Number of non-censored data	51
Lowest residue	1.200
Highest residue	21.400
Median residue	5.500
Mean	7.395
Standard deviation (SD)	5.236
Correction factor for censoring (CF)	1.000
Proposed MRL estimate	
- Highest residue	21.400
- Mean + 4 SD	28.340
- CF x 3 Mean	22.185
Unrounded MRL	28.340
Rounded MRL	30

For Southern Europe the data submitted was not sufficient for an evaluation of glyphosate residues in grain.

Straw of cereals for feeding purposes are current no subject of Reg. (EC) 396/2005.

B.7.12.6 Rye, triticale and wheat

According to the current guidance for extrapolation (SANCO 7525/VI/95 Rev.9) data on wheat grain treated before harvest can be also be extrapolated to rye grain for the estimation of MRLs. In comparison to pre-emergence application the desiccation seven days before harvest is considered as critical use for glyphosate.

Northern Europe:

Wheat and rye grain	0.05, 0.11, 0.16, 0.19, 0.22, 0.23, 0.23, 0.26, 0.33, 0.5, 0.5, 0.6, 0.64, 0.67, 0.7(5), 0.71, 0.74, 0.75, 0.75, 0.77, 0.85, 1.3, 1.4, 1.5, 1.55, 1.6, 1.7, 1.7, 1.75, 2.2, 2.4, 2.9, 3.1, 3.45, 3.5, 3.7, 3.85, 4.7, 4.8, 4.85, 5.4, 9.5, 12.4, 17.5 mg/kg

Based on the OECD MRL-Calculator a MRL of 20 mg/kg was estimated.

Table B.7.12-4: Summary of the OECD MRL-Calculator for wheat and rye grain (Northern Europe)

Wheat & rye, grain	
Glyphosate	
EU-North	
GAP	
Total number of data (n)	48
Percentage of censored data	0%
Number of non-censored data	48
Lowest residue	0.050
Highest residue	17.500
Median residue	0.810
Mean	2.281
Standard deviation (SD)	3.285
Correction factor for censoring (CF)	1.000
Proposed MRL estimate	
- Highest residue	17.500
- Mean + 4 SD	15.423
- CF x 3 Mean	6.844
Unrounded MRL	17.500
Rounded MRL	20

Southern Europe:
Wheat grain 0.07, 0.38, 0.4, 0.4, 0.47, 0.6, 0.95, 1.2, 2.8 mg/kg

Based on the OECD MRL-Calculator a MRL of 4 mg/kg was estimated.

Table B.7.12-5: Summary of the OECD MRL-Calculator for wheat and rye grain (Southern Europe)

Wheat, grain	
Glyphosate	
EU-South	
GAP	
Total number of data (n)	9
Percentage of censored data	0%
Number of non-censored data	9
Lowest residue	0.070
Highest residue	2.800
Median residue	0.470
Mean	0.808
Standard deviation (SD)	0.819
Correction factor for censoring (CF)	1.000
Proposed MRL estimate	
- Highest residue	2.800
- Mean + 4 SD	4.082
- CF x 3 Mean	2.423
Unrounded MRL	4.082
Rounded MRL	4

For wheat, rye and triticale the data available according to the representative use from Northern Europe pose the higher residue situation, resulting in a MRL of 20 mg/kg for glyphosate.

Straw of cereals for feeding purposes are current no subject of Reg. (EC) 396/2005.

B.7.12.7 Other annual crops

Glyphosate may be use for weed control before emergence/sowing in all annual crops. Apart from single detects in cereal straw, none of the crops investigated gave residues above the LOQ of the analytical methods. These results are in line with plant metabolism studies and confined rotational crops studies, suggesting a negligable uptake of the residue via the roots. It is proposed to establish MRLs on the LOQ of 0.05 mg/kg for all commodities obtained from annual crops, that are not covered by other uses:

Strawberries	0.05* mg/kg
Root and tuber vegetables	0.05* mg/kg
Bulb vegetables	0.05* mg/kg
Fruiting vegetables, except sweet corn	0.05* mg/kg
Sweet corn	0.1* mg/kg (potential tolerant crop, higher LOQ)
Brassica vegetables	0.05* mg/kg
Leaf vegetables & fresh herbs	0.05* mg/kg
Legume vegetables	0.05* mg/kg
Stem vegetables	0.05* mg/kg

Pulses, except lentils	0.05* mg/kg
Oilseeds, except rape seed and soya beans	0.05* mg/kg
Maize, rape seed, lentils, soya beans	0.1* mg/kg (potential tolerant crop, higher LOQ)
Buckwheat, millet, rice, sorghum, others	0.05* mg/kg
Herbal infusions	0.05* mg/kg
Sugar plants	0.05* mg/kg

B.7.12.8 Animal commodities

For the estimation of potential residues in animal commodities the livestock animals dietary burden will calculated according the current guidance for the generation of livestock feeding studies (SANCO 7031/VI/95 rev.4).
For the estimation of maximum residue levels only glyphosate will be taken into account, since no conversion from AMPA to glyphosate is expected in animals. N-acetyl-glyphosate can also no be taken into account, since no information in residues in imported feed items were evaluated within this document. For the purpose of MRL estimation only the maximum dietary burden is of interest.
For dietary intake purposes both glyphosate and AMPA residues will be taken into account. ~~Glyphosate and AMPA are both of low acute toxicity, no making an ARfD necessary. Therefore only the mean dietary burden will be required to estimate STMR-values for animal commodities.~~

The following residues are taken into account for the estimation of the dietary burdens:

Table B.7.12-6: Summary of residue data for the calculation of the maximum ~~and mean~~ dietary burden for MRL setting purposes (glyphosate only)

~~Commodity~~	~~Maximum dietary burden for MRL setting (glyphosate)~~		~~Mean dietary burden for dietary intake purposes (Sum of glyphosate and AMPA, expressed as glyphosate~~	
	~~mg/kg~~	~~Comment~~	~~mg/kg~~	~~Comment~~
~~Green forage (all annual commodities)~~	~~0.05~~	~~pre-emergence application, LOQ of analytical method~~	~~0.05~~	~~pre-emergence application, LOQ of analytical method~~
~~Grains, except maize~~	~~5.5~~	~~STMR barley (NEU)~~	~~5.85~~	~~STMR barley (NEU)~~
~~Maize grain~~	~~0.05~~	~~pre-emergence application, LOQ of analytical method~~	~~0.05~~	~~pre-emergence application, LOQ of analytical method~~
~~Bran, wheat and rye~~	~~1.458~~	~~STMR wheat (NEU) x PF 1.8~~	~~1.494~~	~~STMR wheat (NEU) x PF 1.8~~
~~Cereal straw~~	~~175~~	~~HR wheat (NEU)~~	~~30.7~~	~~STMR wheat (NEU)~~
~~Pulses~~	~~0.05~~	~~pre-emergence application, LOQ of analytical method~~	~~0.05~~	~~pre-emergence application, LOQ of analytical method~~
~~Roots and tubers~~	~~0.05~~	~~pre-emergence application, LOQ of analytical method~~	~~0.05~~	~~pre-emergence application, LOQ of analytical method~~
~~Oilseed meals~~	~~0.05~~	~~pre-emergence application, LOQ of analytical method~~	~~0.05~~	~~pre-emergence application, LOQ of analytical method~~

Commodity	**Maximum dietary burden for MRL setting (glyphosate)**	
	mg/kg	Comment
Green forage (all annual commodities)	0.05	pre-emergence application, LOQ of analytical method
Grains, except maize	5.5	~~STMR~~ Median barley (NEU)
Maize grain	0.05	pre-emergence application, LOQ of analytical method
Bran, wheat and rye	1.458	~~STMR~~ Median wheat (NEU) x PF 1.8

Cereal straw	175	~~HR~~Highest residue wheat (NEU)
Pulses	0.05	pre-emergence application, LOQ of analytical method
Roots and tubers	0.05	pre-emergence application, LOQ of analytical method
Oilseed meals	0.05	pre-emergence application, LOQ of analytical method

Table B.7.12-7: Summary of residue data for the calculation of the maximum and mean dietary burden for dietary intake purposes

Commodity	**Maximum dietary burden for ~~MRL setting~~ dietary intake purposes (~~glyphosate~~ Sum of glyphosate and AMPA, expressed as glyphosate)**		**Mean dietary burden for dietary intake purposes (Sum of glyphosate and AMPA, expressed as glyphosate)**	
	mg/kg	Comment	mg/kg	Comment
Green forage (all annual commodities)	0.05	pre-emergence application, LOQ of analytical method	0.05	pre-emergence application, LOQ of analytical method
Grains, except maize	5.85	STMR barley (NEU)	5.85	STMR barley (NEU)
Maize grain	0.05	pre-emergence application, LOQ of analytical method	0.05	pre-emergence application, LOQ of analytical method
Bran, wheat and rye	1.494~~58~~	STMR wheat (NEU) x PF 1.8	1.494	STMR wheat (NEU) x PF 1.8
Cereal straw	179~~5~~	HR wheat (NEU)	30.7	STMR wheat (NEU)
Pulses	0.05	pre-emergence application, LOQ of analytical method	0.05	pre-emergence application, LOQ of analytical method
Roots and tubers	0.05	pre-emergence application, LOQ of analytical method	0.05	pre-emergence application, LOQ of analytical method
Oilseed meals	0.05	pre-emergence application, LOQ of analytical method	0.05	pre-emergence application, LOQ of analytical method

In the following tables the maximum dietary burden for MRL setting (parent only) and the maximum and mean dietary burden for dietary intake (sum of glyphosate and AMPA, expressed as glyphosate) are presented:

Table B.7.12-8: Maximum dietary burden for glyphosate (parent) in livestock animals for MRL setting purposes

Feedstuff	**% DM**	**Percent of daily livestock diet (dry feed basis)**				**Residue (mg/kg)**	**Intake (mg/kg, dry feed basis)**			
		Chicken 1,9 kg bw daily maximum feed (DM) 120 g	Dairy cattle 550 kg bw daily maximum feed (DM) 20 kg	Beef cattle 350 kg bw daily maximum feed (DM) 15 kg	Pig 75 kg bw daily maximum feed (DM) 3 kg		Chicken	Dairy cattle	Beef cattle	Pig
Kale/ Cabbage	14	5	10	–	–	0.05	0.018	0.036	–	–
Grains except Maize	86	70	40	50	80	5.5	4.477	2.558	3.198	5.116
Straw (Cereals)	86	–	20	50	–	175	–	40.698	101.744	–
Pulses	86	5	–	–	–	0.05	0.003	–	–	–
Swede/ Turnip	10	20	30	–	20	0.05	0.100	0.150	–	0.100
Intake (mg/kg dry weight feed)							**4.598**	**43.442**	**104.942**	**5.216**
Intake (mg/kg bw/d)							**0.290**	**1.580**	**4.498**	**0.209**
Intake (mg/animal/d)							**0.552**	**868.831**	**1574.12**	**15.649**

Table B.7.12-9: Maximum dietary burden for glyphosate (sum of glyphosate and AMPA, expressed as glyphosate) in livestock animals for dietary intake purposes

Feedstuff	**% DM**	**Percent of daily livestock diet (dry feed basis)**				**Residue (mg/kg)**	**Intake (mg/kg, dry feed basis)**			
		Chicken 1,9 kg bw daily maximum feed (DM) 120 g	Dairy cattle 550 kg bw daily maximum feed (DM) 20 kg	Beef cattle 350 kg bw daily maximum feed (DM) 15 kg	Pig 75 kg bw daily maximum feed (DM) 3 kg		Chicken	Dairy cattle	Beef cattle	Pig
Kale/ Cabbage	14	5	10	–	-	0.05	0.018	0.036	–	-
Grains except Maize	86	70	40	50	80	5.85	4.762	2.721	3.401	5.442
Straw (Cereals)	86	–	20	50	–	179	–	41.628	104.07	–
Pulses	86	5	–	–	–	0.05	0.003	–	–	–
Swede/ Turnip	10	20	30	–	20	0.05	0.1	0.15	–	0.1
Intake (mg/kg dry weight feed)							**4.882**	**44.535**	**107.471**	**5.542**
Intake (mg/kg bw/d)							**0.308**	**1.619**	**4.606**	**0.222**
Intake (mg/animal/d)							**0.586**	**890.691**	**1612.06**	**16.626**

Table B.7.12-10: Mean dietary burden for glyphosate (sum of glyphosate and AMPA, expressed as glyphosate) in livestock animals for dietary intake purposes

Feedstuff	**% DM**	**Percent of daily livestock diet (dry feed basis)**				**Residue (mg/kg)**	**Intake (mg/kg, dry feed basis)**			
		Chicken 1,9 kg bw daily maximum feed (DM) 120 g	Dairy cattle 550 kg bw daily maximum feed (DM) 20 kg	Beef cattle 350 kg bw daily maximum feed (DM) 15 kg	Pig 75 kg bw daily maximum feed (DM) 3 kg		Chicken	Dairy cattle	Beef cattle	Pig
Kale/ Cabbage	14	5	10	–	–	0.05	0.018	0.036	–	–
Grains except Maize	86	70	40	50	80	5.85	4.762	2.721	3.401	5.442
Straw (Cereals)	86	–	20	50	–	30.7	–	7.140	17.849	–
Pulses	86	5	–	–	–	0.05	0.003	–	–	–
Swede/ Turnip	10	20	30	–	20	0.05	0.100	0.150	–	0.100
Intake (mg/kg dry weight feed)							**4.882**	**10.046**	**21.250**	**5.542**
Intake (mg/kg bw/d)							**0.308**	**0.365**	**0.911**	**0.222**
Intake (mg/animal/d)							**0.586**	**200.924**	**318.750**	**16.626**

Based on the calculations presented above the following livestock animals dietary burdens were calculated:

Table B.7.12-11: Maximum and mean livestock animal sdietary burden for glyphosate

Animal	**Maximum dietary burden for MRL setting (glyphosate)~~Maximum dietary burden~~**	**Maximum dietary burden for MRL setting dietary intake purposes (sum of glyphosate and AMPA, expressed as glyphosate)**	**Mean dietary burden for dietary intake purposes (Sum of glyphosate and AMPA, expressed as glyphosate)~~Mean dietary burden~~**
Chicken	4.598 mg per kg feed DM (0.29 mg/kg bw and d)	4.882 mg per kg feed DM (0.306 mg/kg bw and d)	4.882 mg per kg feed DM (0.308 mg/kg bw and d)
Dairy cattle	43.442 mg per kg feed DM (1.58 mg/kg bw and d)	44.535 mg per kg feed DM (1.619 mg/kg bw and d)	10.046 mg per kg feed DM (0.365 mg/kg bw and d)
Beef cattle	104.942 mg per kg feed DM (4.498 mg/kg bw and d)	107.471 mg per kg feed DM (4.606 mg/kg bw and d)	21.25 mg per kg feed DM (0.911 mg/kg bw and d)
Pig	5.216 mg per kg feed DM (0.209 mg/kg bw and d)	5.542 mg per kg feed DM (0.222 mg/kg bw and d)	5.542 mg per kg feed DM (0.222 mg/kg bw and d)

Ruminants

For the estimation of residues in ruminant commodities three livestock feeding studies on cattle are available involving administration of glyphosate/AMPA (9:1 mixture), glyphosate trimesium or N-acetyl-glyphosate. The feeding study involving administration of N-acetyl-glyphosate is also not applicable, since imported feed commodities from genetically modified plants are not within the scoop of this evaluation.

The maximum dietary burden for beef cattle (4.498 mg/kg bw) is in line with dose levels reported in the study by Cowell, J.E. (1987, RIP9501250) using glyphosate/AMPA (9:1) and with the feeding study conducted with glyphosate-trimesium (Graham, D. G., 1987, RIP9500024).

In the following table the estimated residues in ruminant commodities and the corresponding MRLs are summarised:

Table B.7.12-12: Glyphosate residues in ruminants based on Graham, D. G. (1987, RIP9500024)

Commodity	dietary burden	Lower Dose group [b]	Upper Dose group [b]	Interpolated residue [a]
MRL setting (glyphosate only)				
Milk	MAX dairy cattle: 1.58 mg/kg bw	*Cowell:* *1.4 mg/kg bw* Gly: <0.025 mg/kg	*Cowell:* *4 mg/kg bw* Gly: <0.025 mg/kg	Gly: <0.02 mg/kg MRL: 0.05* mg/kg
		Graham: *1.44 mg/kg bw* Gly: <0.02 mg/kg	*Graham:* *7.38 mg/kg bw* Gly: <0.02 mg/kg	
Muscle	MAX beef cattle: 4.498 mg/kg bw	*Cowell:* *4 mg/kg bw* Gly: <0.05 mg/kg	*Cowell:* *12.8 mg/kg bw* Gly: <0.05 mg/kg	Gly: <0.05 mg/kg MRL: 0.05* mg/kg
		Graham: *1 44 mg/kg bw* Gly: <0.05 mg/kg	*Graham:* *7.38 mg/kg bw* Gly: <0.05 mg/kg	
Fat	MAX beef cattle: 4.498 mg/kg bw	*Cowell:* *4 mg/kg bw* Gly: <0.05 mg/kg	*Cowell:* *12.8 mg/kg bw* Gly: <0.05 mg/kg	Gly: 0.06 mg/kg MRL: 0.1 mg/kg
		Graham: *1 44 mg/kg bw* Gly: 0.06 mg/kg	*Graham:* *7.38mg/kg bw* Gly: 0.06 mg/kg	
Liver	MAX beef cattle: 4.498 mg/kg bw	*Cowell:* *4 mg/kg bw* Gly: 0.07 mg/kg	*Cowell:* *7.38 mg/kg bw* Gly: 0.08 mg/kg	Gly: 0.07 mg/kg MRL: 0.1 mg/kg
		Graham: *1 44 mg/kg bw* Gly: <0.2 mg/kg	*Graham:* *7.38 mg/kg bw* Gly: <0.2 mg/kg	
Kidney	MAX beef cattle: 4.498 mg/kg bw	*Cowell:* *4 mg/kg bw* Gly: 0.34 mg/kg	*Cowell:* *12.8 mg/kg bw* Gly: 0.87 mg/kg	Gly: 1.6 mg/kg MRL: 2 mg/kg
		Graham: *1.44 mg/kg bw* Gly: 0.44 mg/kg	*Graham:* *7.38 mg/kg bw* Gly: 2.6 mg/kg	
Dietary intake (sum of glyphosate and AMPA, expressed as AMPA)				
Milk	MEAN dairy cattle: 0.365 mg/kg bw	*Cowell:* *not applicable, dietary burden below lowest dose group*	*Cowell:* *1.4 mg/kg bw* Gly: <0.025 mg/kg	Gly: <0.02 mg/kg AMPA: <0.02 mg/kg STMR: <0.05 mg/kg
		Graham: *0.13 mg/kg bw* Gly: <0.02 mg/kg AMPA: <0.02 mg/kg	*Graham:* *1.44 mg/kg bw* Gly: <0.02 mg/kg AMPA: <0.02 mg/kg	
Muscle	MAX beef cattle: 4.606 mg/kg bw	*Cowell:* *4 mg/kg bw* Gly: <0.05 mg/kg AMPA: <0.05 mg/kg	*Cowell:* *12.8 mg/kg bw* Gly: <0.05 mg/kg AMPA: <0.05 mg/kg	Gly: <0.05 mg/kg AMPA: <0.05 mg/kg HR: <0.125 mg/kg
		Graham: *1 44 mg/kg bw* Gly: <0.05 mg/kg AMPA: <0.05 mg/kg	*Graham:* *7.38 mg/kg bw* Gly: <0.05 mg/kg AMPA: <0.05 mg/kg	
	MEAN beef cattle: 0.911 mg/kg bw	*Cowell:* *not applicable, dietary burden below lowest dose group*	*Cowell:* *1.4 mg/kg bw* Gly: <0.05 mg/kg	Gly: <0.05 mg/kg AMPA: <0.05 mg/kg STMR: <0.125 mg/kg
		Graham: *0.13 mg/kg bw*	*Graham:* *1.44 mg/kg bw*	

Commodity	dietary burden	Lower Dose group [b]	Upper Dose group [b]	Interpolated residue [a]
		Gly: <0.05 mg/kg AMPA: <0.05 mg/kg	Gly: <0.05 mg/kg AMPA: <0.05 mg/kg	
Fat	MAX beef cattle: 4.606 mg/kg bw	*Cowell:* *4 mg/kg bw* Gly: <0.05 mg/kg AMPA: <0.05 mg/kg	*Cowell:* *12.8 mg/kg bw* Gly: <0.05 mg/kg AMPA: <0.05 mg/kg	Gly: 0.06 mg/kg AMPA: <0.05 mg/kg HR: 0.135 mg/kg
		Graham: *1 44 mg/kg bw* Gly: 0.06 mg/kg AMPA: <0.05 mg/kg	*Graham:* *7.38 mg/kg bw* Gly: 0.06 mg/kg AMPA: <0.05 mg/kg	
	MEAN beef cattle: 0.911 mg/kg bw	*Cowell:* *not applicable, dietary burden below lowest dose group*	*Cowell:* *1.4 mg/kg bw* Gly: <0.05 mg/kg	Gly: 0.056 mg/kg AMPA: <0.05 mg/kg STMR: 0.131 mg/kg
		Graham: *0.13 mg/kg bw* Gly: <0.05 mg/kg AMPA: <0.05 mg/kg	*Graham:* *1.44 mg/kg bw* Gly: 0.06 mg/kg AMPA: <0.05 mg/kg	
Liver	MAX beef cattle: 4.606 mg/kg bw	*Cowell:* *4 mg/kg bw* Gly: 0.08 mg/kg AMPA: 0.06 mg/kg	*Cowell:* *12.8 mg/kg bw* Gly: 0.245 mg/kg AMPA: 0.2 mg/kg	Gly: 0.086 mg/kg AMPA: 0.073 mg/kg HR: 0.2 mg/kg
		Graham: *1 44 mg/kg bw* Gly: <0.2 mg/kg AMPA: <0.2 mg/kg	*Graham:* *7.38 mg/kg bw* Gly: <0.2 mg/kg AMPA: <0.2 mg/kg	
	MEAN beef cattle: 0.911 mg/kg bw	*Cowell:* *not applicable, dietary burden below lowest dose group*	*Cowell:* *1.4 mg/kg bw* Gly: 0.055 mg/kg AMPA: <0.05 mg/kg	Gly: 0.035 mg/kg AMPA: <0.05 mg/kg STMR: 0.11 mg/kg
		Graham: *0.13 mg/kg bw* Gly: <0.2 mg/kg AMPA: <0.2 mg/kg	*Graham:* *1.44 mg/kg bw* Gly: <0.2 mg/kg AMPA: <0.2 mg/kg	
Kidney	MAX beef cattle: 4.606 mg/kg bw	*Cowell:* *4 mg/kg bw* Gly: 0.87 mg/kg AMPA: 0.27 mg/kg	*Cowell:* *12.8 mg/kg bw* Gly: 3.45 mg/kg AMPA: 0.87 mg/kg	Gly: 1.35 mg/kg AMPA: 0.31 mg/kg HR: 1.82 mg/kg
		Graham: *1 44 mg/kg bw* Gly: 0.44 mg/kg AMPA: 0.08 mg/kg	*Graham:* *7.38 mg/kg bw* Gly: 2.6 mg/kg AMPA: 0.58 mg/kg	
	MEAN beef cattle: 0.911 mg/kg bw	*Cowell:* *not applicable, dietary burden below lowest dose group*	*Cowell:* *1.4 mg/kg bw* Gly: 0.22 mg/kg AMPA: 0.08 mg/kg	Gly: 0.25 mg/kg AMPA: 0.06 mg/kg STMR: 0.31 mg/kg
		Graham: *0.13 mg/kg bw* Gly: <0.05 mg/kg AMPA: <0.05 mg/kg	*Graham:* *1.44 mg/kg bw* Gly: 0.385 mg/kg AMPA: 0.07 mg/kg	

STMR: glyphosate + 1.5 x AMPA

a Interpolated residue based on highest measured residue estimated from the available studies (underlined). In case of all residues being below the LOQ, the lowest LOQ was selected.

b For MRL setting purposes and the interpretation of the HR in animal tissues the highest single value from the dose group was selected. For milk (MRL setting and dietary intake) and for the interpretation of the STMR in animal tissues the mean of the respective dose group was taken into account.

Chicken/Poultry

For chicken the situation is comparable to ruminants. Three livestock feeding studies are available involving administration of either glyphosate/AMPA (9:1), glyphosate-trimesium or N-acetyl-glyphosate. In the study conducted with glyphosate/AMPA (9:1) the lowest dose group received a dose of 2.54 mg glyphosate/kg bw, which is approximately ten times higher than the calculated dietary burden. N-acetyl-glyphosate is irrelevant in view of the representative uses.

Therefore the estimation will be based on the glyphosate-trimesium study on laying hens, involving dose group of 0.025, 0.24 and 2.2 mg/kg bw.

In the following table the estimated residues in chicken/poultry commodities and the corresponding MRLs are summarised:

Table B.7.12-13: Glyphosate residues in chicken/poultry based on Graham, D. G. (1987, RIP9500025)

Commodity	dietary burden	Lower Dose group	Upper Dose group	Interpolated residue
MRL setting (glyphosate only)				
Eggs	MAX chicken: 0.29 mg/kg bw	*0.24 mg/kg bw* Gly: <0.01 mg/kg	*2.2 mg/kg bw* Gly: 0.015 mg/kg	Gly: <0.01 mg/kg MRL: 0.05* mg/kg
Muscle	MAX chicken: 0.29 mg/kg bw	*0.24 mg/kg bw* Gly: <0.05 mg/kg	*2.2 mg/kg bw* Gly: <0.05 mg/kg	Gly: <0.05 mg/kg MRL: 0.05* mg/kg
Fat	MAX chicken: 0.29 mg/kg bw	*0.24 mg/kg bw* Gly: <0.05 mg/kg	*2.2 mg/kg bw* Gly: <0.05 mg/kg	Gly: <0.05 mg/kg MRL: 0.05* mg/kg
Liver	MAX chicken: 0.29 mg/kg bw	*0.24 mg/kg bw* Gly: <0.05 mg/kg	*2.2 mg/kg bw* Gly: <0.05 mg/kg	Gly: <0.05 mg/kg MRL: 0.05* mg/kg
Kidney	MAX chicken: 0.29 mg/kg bw	*0.24 mg/kg bw* Gly: 0.072 mg/kg	*2.2 mg/kg bw* Gly: 0.31 mg/kg	Gly: 0.078 mg/kg MRL: 0.1 mg/kg
Dietary intake (sum of glyphosate and AMPA, expressed as AMPA)				
Eggs	MAX and MEAN chicken: 0.308 mg/kg bw	*0.24 mg/kg bw* Gly: <0.01 mg/kg AMPA: <0.02 mg/kg	*2.2 mg/kg bw* Gly: <0.01 mg/kg AMPA: <0.02 mg/kg	Gly: <0.01 mg/kg AMPA: <0.02 mg/kg HR and STMR: <0.04 mg/kg
Muscle	MAX and MEAN chicken: 0.308 mg/kg bw	*0.24 mg/kg bw* Gly: <0.05 mg/kg AMPA: <0.05 mg/kg	*2.2 mg/kg bw* Gly: <0.05 mg/kg AMPA: <0.05 mg/kg	Gly: <0.05 mg/kg AMPA: <0.05 mg/kg HR and STMR: <0.125 mg/kg
Fat	MAX and MEAN chicken: 0.308 mg/kg bw	*0.24 mg/kg bw* Gly: <0.05 mg/kg AMPA: <0.05 mg/kg	*2.2 mg/kg bw* Gly: <0.05 mg/kg AMPA: <0.05 mg/kg	Gly: <0.05 mg/kg AMPA: <0.05 mg/kg HR and STMR: <0.125 mg/kg
Liver	MAX and MEAN chicken: 0.308 mg/kg bw	*0.24 mg/kg bw* Gly: <0.05 mg/kg AMPA: <0.05 mg/kg	*2.2 mg/kg bw* Gly: <0.05 mg/kg AMPA: <0.05 mg/kg	Gly: <0.05 mg/kg AMPA: <0.05 mg/kg HR and STMR: <0.125 mg/kg
Kidney	MAX and MEAN chicken: 0.308 mg/kg bw	*0.24 mg/kg bw* Gly: 0.072 mg/kg AMPA: <0.05 mg/kg	*2.2 mg/kg bw* Gly: 0.31 mg/kg AMPA: <0.05 mg/kg	Gly: 0.08 mg/kg AMPA: <0.05 mg/kg HR and STMR: 0.155 mg/kg

STMR: glyphosate + 1.5 x AMPA

Swine

For the estimation of glyphosate residues in swine only one study is available involving administration of glyphosate/AMPA (9:1) at dose levels of 1.08/0.12 mg/kg bw, 3.35/0.37 mg/kg bw and 11.8/1.32 mg/kg bw, respectively. However, even the lowest dose group is approximately five times higher than the maximum and mean dietary burdens calculated for

swine of 0.209 mg/kg bw and 0.222 mg/kg bw. Since no other feeding study is available, the calculation for residues in swine commodities will be conducted by extrapolation from the lowest dose group of the swine feeding study.

Table B.7.12-14: Glyphosate residues in swine

Commodity	dietary burden	Lowest Dose group	Interpolated residue
MRL setting (glyphosate only)			
Muscle	MAX swine: 0.209 mg/kg bw	*1.08 mg/kg bw* Gly: <0.05 mg/kg	Gly: <0.05 mg/kg MRL: 0.05* mg/kg
Fat	MAX swine: 0.209 mg/kg bw	*1.08 mg/kg bw* Gly: <0.05 mg/kg	Gly: <0.05 mg/kg MRL: 0.05* mg/kg
Liver	MAX swine: 0.209 mg/kg bw	*1.08 mg/kg bw* Gly: <0.05 mg/kg	Gly: <0.05 mg/kg MRL: 0.05* mg/kg
Kidney	MAX swine: 0.209 mg/kg bw	*1.08 mg/kg bw* Gly: 0.61 mg/kg	Gly: 0.12 mg/kg MRL: 0.2 mg/kg
Dietary intake (sum of glyphosate and AMPA, expressed as AMPA)			
Muscle	MAX and MEAN swine: 0.222 mg/kg bw	*1.08 mg/kg bw* Gly: <0.05 mg/kg AMPA: <0.05 mg/kg	Gly: <0.05 mg/kg AMPA: <0.05 mg/kg HR and STMR: <0.125 mg/kg
Fat	MAX and MEAN swine: 0.222 mg/kg bw	*1.08 mg/kg bw* Gly: <0.05 mg/kg AMPA: <0.05 mg/kg	Gly: <0.05 mg/kg AMPA: <0.05 mg/kg HR and STMR: <0.125 mg/kg
Liver	MAX and MEAN swine: 0.222 mg/kg bw	*1.08 mg/kg bw* Gly: <0.05 mg/kg AMPA: <0.05 mg/kg	Gly: <0.05 mg/kg AMPA: <0.05 mg/kg HR and STMR: <0.125 mg/kg
Kidney	MAX and MEAN swine: 0.222 mg/kg bw	*1.08 mg/kg bw* Gly: 0.21 mg/kg AMPA: 0.06 mg/kg	Gly: 0.041 mg/kg AMPA: 0.012 mg/kg HR and STMR: 0.059 mg/kg

STMR: glyphosate + 1.5 x AMPA

In summary, the following MRLs were estimated for animal commodities. MRLs estimated for bovine products based on cow feeding studies are extrapolated to goat and sheep commodities also. For swine and poultry (fat and liver) a higher MRL of 0.1* mg/kg was proposed based on the analytical methods for enforcement purposes available:

Swine, muscle~~, fat and liver~~	0.05* mg/kg
Swine, fat and liver	0.1* mg/kg
Swine, kidney	0.2 mg/kg
Bovine, goat and sheep, muscle	0.05* mg/kg
Bovine, goat and sheep, fat	0.1 mg/kg
Bovine, goat and sheep, liver	0.1 mg/kg
Bovine, goat and sheep, kidney	2 mg/kg
Milk	0.05* mg/kg
Poultry, muscle~~, fat and liver~~	0.05* mg/kg
Poultry, fat and liver	0.1* mg/kg
Poultry, kidney	0.1 mg/kg
Eggs	0.05* mg/kg

B.7.13 Proposed EU Import tolerances and justification for the acceptability of those residues

For glyphosate imported commodities are normally treated with comparable GAPs as in the EU, especially as pre-harvest applications for desiccation purposes. However on international level the cultivation of glyphosate tolerant crops is common practice, resulting in a deviating composition of the overall residue.

The re-evaluation of import tolerances for glyphosate was not supported by the applicant Task Force. No information on GAPs for glyphosate authorised in third countries were submitted. However, since glyphosate residues in commodities obtained from tolerant crops provide necessary information to establish global residue definitions for plant and animal commodities, a brief summary of the information already presented in European level is presented (for evaluations of glyphosate on CODEX level please refer to B.7.14):

CP4-EPSPS and GOX modified crops

In the first DAR for glyphosate import tolerances have been evaluated for modified cotton, sugar beets, soya beans and maize. Residues in sugar beet and cotton were comparable to non-tolerant crops, not demanding specific consideration. For soya beans and maize summarised residues based on US GAP for the different commodities were:

Table B.7.13-1: Summary of import tolerances on CP4-EPSPS and GOX modified crops from the US presented in the first DAR for glyphosate

Crop	Portion analysed	DAT	glyphosate in mg/kg	AMPA in mg/kg
Soya beans	Seeds	10 - 108	<0.05 – 5.6	<0.05 – 7.6
Maize	Grain	6 – 8	<0.05 – 0.1	<0.05 – 1.4
	Fodder	6 - 8	1.8 - 41	<0.05 – 4.7
	Forage	25 - 72	<0.05 – 0.8	0.05 – 2.6

Based on the results it was concluded that European uses for desiccation purposes result in higher residues and pose the critical case for MRL setting and the dietary intake.

GAT modified crops

For GAT modified crops import tolerances have be evaluated on European levels by EFSA in 2009 (EFSA Journal 2009; 7(9):1310, ASB2012-3480) for maize, soya beans and animal products.

Besides plant and animal metabolism studies, storage stability studies and studies on industrial processing also presented in detail within this document, summary information on supervised field trials were presented for different scenarios of the residue definition for enforcement purposes:

Option 1 - Residue definition for enforcement purposes: glyphosate

Table B.7.13-2: Residue in imported commodities based (Option 1)

Commo-dity	Region [(a)]	Outdoor/ Indoor	Individual trial results (mg/kg)		STMR (mg/kg) [(b)]	HR (mg/kg) [(c)]	Median CF [(d)]
			Enforcement (glyphosate)	Risk assessment (total [(e)])			
Maize	Import (USA)	outdoor	<0.02(24), 0.02, 0.03(3), 0.04, 0.04, <0.05(11), 0.05(4), 0.08, 0.08	<0.08(3), 0.08, 0.09(8), 0.1(7), 0.11(5), 0.12(3), 0.13, 0.13, 0.15, 0.17, 0.2(5), 0.21, 0.22, 0.22, 0.23, 0.23, 0.25, 0.3, 0.32, 0.36, 0.43, 0.6	0.11	0.6	3.0 (range 2.2 to 5.7) CF factor calculated from a dataset of 12 values where glyphosate residues were >LOQ.
Soya beans	Import (USA)	outdoor	<0.05(6), 0.05, 0.06, 0.06, 0.07, 0.08, 0.09(5), 0.1, 0.11, 0.12(3), 0.14, 0.14, 0.17, 0.19, 0.2, 0.21, 0.29, 0.32, 0.32, 0.45, 0.62, 0.86, 0.94, 1.7	<0.2, 0.33, 0.49, 0.64, 0.73, 0.82, 0.86, 1.04, 1.19, 1.19, 1.26, 1.3, 1.42, 1.52, 2.02, 2.04, 2.04, 2.18, 2.21, 2.36, 2.9, 3.01, 3.03, 3.08, 3.53, 3.59, 3.6, 5.52, 5.65, 5.66, 6.13, 6.61, 6.82, 6.87, 8.07, 8.64	2.2	8.64	17.7 (range 2.4 to 79) CF calculated from a dataset of 30 values where glyphosate residues were >LOQ.

(a): NEU, SEU, EU or Import (country code). In the case of indoor uses there is no necessity to differentiate between NEU and SEU.
(b): Median value of the individual trial results according to the risk assessment residue definition.
(c): Highest value of the individual trial results according to the risk assessment residue definition.
(d): The median conversion factor for enforcement to risk assessment is obtained by calculating the median of the individual conversion factors for each residues trial.
(e): sum of glyphosate, N-acetyl-glyphosate, AMPA and N-acetyl-AMPA, expressed as glyphosate

Option 2 - Residue definition for enforcement purposes: sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate

Table B.7.13-3: Residue in imported commodities based (Option 2)

Commo-dity	**Region** [(a)]	**Outdoor/ Indoor**	**Individual trial results (mg/kg)**		**STMR (mg/kg)** [(b)]	**HR (mg/kg)** [(c)]	**Median CF** [(d)]
			Enforcement (sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate)	Risk assessment (total [(e)])			
Maize	Import (USA)	outdoor	<0.04(3), 0.04, 0.05(8), 0.06(7), 0.07(5), 0.08(3), 0.09, 0.09, <0.1(4), 0.1, 0.11, 0.11, 0.12, 0.12, 0.13, 0.13, 0.15, 0.19, 0.2, 0.28, 0.32, 0.38, 0.54	<0.08(3), 0.08, 0.09(8), 0.1(7), 0.11(5), 0.12(3), 0.13, 0.13, 0.15, 0.17, 0.2(5), 0.21, 0.22, 0.22, 0.23, 0.23, 0.25, 0.3, 0.32, 0.36, 0.43, 0.6	0.11	0.6	1.7 (range 1.1 to 2) CF factor calculated from a dataset of 41 values where glyphosate residues were >LOQ.
Soya beans	Import (USA)	outdoor	<0.1, 0.23, 0.36, 0.43, 0.54, 0.71, 0.72, 0.81, 0.91, 0.96, 1.01, 1.11, 1.14, 1.31, 1.65, 1.75, 1.76, 1.82, 1.92, 1.99, 2.49, 2.49, 2.54, 2.87, 3.06, 3.26, 3.39, 4.37, 5.22, 5.45, 5.7, 5.89, 6.0, 6.14, 6.72, 8.02	<0.2, 0.33, 0.49, 0.64, 0.73, 0.82, 0.86, 1.04, 1.19, 1.19, 1.26, 1.3, 1.42, 1.52, 2.02, 2.04, 2.04, 2.18, 2.21, 2.36, 2.9, 3.01, 3.03, 3.08, 3.53, 3.59, 3.6, 5.52, 5.65, 5.66, 6.13, 6.61, 6.82, 6.87, 8.07, 8.64	2.2	8.64	1.2 (range 1 to 1.5) CF calculated from a dataset of 35 values where glyphosate residues were >LOQ.

(a): NEU, SEU, EU or Import (country code). In the case of indoor uses there is no necessity to differentiate between NEU and SEU.
(b): Median value of the individual trial results according to the risk assessment residue definition.
(c): Highest value of the individual trial results according to the risk assessment residue definition.
(d): The median conversion factor for enforcement to risk assessment is obtained by calculating the median of the individual conversion factors for each residues trial.
(e): sum of glyphosate, N-acetyl-glyphosate, AMPA and N-acetyl-AMPA, expressed as glyphosate

Option 3 - Residue definition for enforcement purposes: N-acetyl-glyphosate

Table B.7.13-4: Residue in imported commodities based (Option 3)

Commo-dity	**Region** [(a)]	**Outdoor/ Indoor**	**Individual trial results (mg/kg)**		**STMR (mg/kg)** [(b)]	**HR (mg/kg)** [(c)]	**Median CF** [(d)]
			Enforcement (N-acetyl-glyphosate)	Risk assessment (total [(e)])			
Maize	Import (USA)	outdoor	<0.02(4), 0.02, 0.02, 0.03(8), 0.04(11), <0.05(4), 0.05(3), 0.06(4), 0.07, 0.07, 0.08, 0.1, 0.1, 0.15, 0.17, 0.2, 0.24, 0.36, 0.52	<0.08(3), 0.08, 0.09(8), 0.1(7), 0.11(5), 0.12(3), 0.13, 0.13, 0.15, 0.17, 0.2(5), 0.21, 0.22, 0.22, 0.23, 0.23, 0.25, 0.3, 0.32, 0.36, 0.43, 0.6	0.11	0.6	2.5 (range 1.15 to 5.5), CF factor calculated from a dataset of 39 values where glyphosate residues were >LOQ.
Soya beans	Import (USA)	outdoor	<0.05, 0.18, 0.31, 0.38, 0.49, 0.61, 0.62, 0.63, 0.69, 0.86, 0.88, 0.9, 1.0, 1.0, 1.1, 1.3, 1.6, 1.6, 1.7, 1.7, 1.9, 2.4, 2.4, 2.7, 3.0, 3.2, 3.2, 4.0, 4.3, 4.3, 4.9, 5.0, 5.6, 5.9, 6.0, 6.6, 7.9	<0.2, 0.33, 0.49, 0.64, 0.73, 0.82, 0.86, 1.04, 1.19, 1.19, 1.26, 1.3, 1.42, 1.52, 2.02, 2.04, 2.04, 2.18, 2.21, 2.36, 2.9, 3.01, 3.03, 3.08, 3.53, 3.59, 3.6, 5.52, 5.65, 5.66, 6.13, 6.61, 6.82, 6.87, 8.07, 8.64	2.2	8.64	1.3 (range 1.1 to 2.3) CF calculated from a dataset of 35 values where glyphosate residues were >LOQ.

(a): NEU, SEU, EU or Import (country code). In the case of indoor uses there is no necessity to differentiate between NEU and SEU.
(b): Median value of the individual trial results according to the risk assessment residue definition.
(c): Highest value of the individual trial results according to the risk assessment residue definition.
(d): The median conversion factor for enforcement to risk assessment is obtained by calculating the median of the individual conversion factors for each residues trial.
(e): sum of glyphosate, N-acetyl-glyphosate, AMPA and N-acetyl-AMPA, expressed as glyphosate

B.7.14 Basis for differences, if any, in conclusion reached having regard to established or proposed Codex MRLs

On CODEX level glyphosate has been reviewed by the JMPR for residues in 2005 for non-tolerant, CP4-EPSPS- and GOX-modified crops (Pesticide residues in food – 2005, report 2005, FAO Plant Production and Protection Paper 183, Rome 2006). In 2011 residues in GAT modified crops were evaluated by the JMPR (Pesticide residues in food – 2011, report 2011, FAO Plant Production and Protection Paper 211, Rome 2012) in addition to the data already reviewed in 2005.

In 2005 the JMPR concluded that glyphosate and AMPA are the main residue in plant an animal commodities. Corresponding to the national systems, the definition of the residue for enforcement purposes was proposed as glyphosate for plant (non-tolerant and tolerant) and animal commodities. For dietary intake purposes the sum of glyphosate and AMPA, expressed as glyphosate, was proposed. Concerning the recommendation of maximum residue levels for none of the individual commodities tolerant crops posed a higher residue situation than non-tolerant crops. The established CXL were implemented into European legislation, if necessary.

Besides additional uses of glyphosate compared to 2005 the recent evaluation of glyphosate by the 2011 JMPR mainly dealt with the new mode of tolerance by the GAT modification and the impact on the residue definition and existing CXLs. The Meeting concluded that the GAT modification resulted in a strong change for the composition of the residue, leaving N-acetyl-glyphosate as major metabolite in soya beans and maize. Based on the toxicological properties of glyphosate and its metabolites it was concluded that N-acetyl-glyphosate, AMPA and N-acetyl-AMPA were not of higher toxicity than the parent. In summary the 2011 JMPR concluded on the following residue definitions for glyphosate:

Residue definition for enforcement purposes in plant commodities:

soya beans and maize:	sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate
other plant commodities:	glyphosate

Residue definition for enforcement purposes in animal commodities:
sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate

Residue definition for dietary intake purposes (plant and animal commodities):
sum of glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA, expressed as glyphosate

Since established MRLs in the EU based on glyphosate (parent only) also accommodate the CXLs for soya bean, maize and animal commodities established in 2012, no modification of the EU legislation became necessary.

B.7.15 Estimates of potential and actual dietary exposure through diet and other means (Annex IIA 6.9; Annex IIIA 8.10)

For glyphosate an acceptable daily intake (ADI) of 0.5 mg/kg bw as well as an acute reference dose (ARfD) of 0.5 mg/kg bw were ~~was~~ established. ~~Due to the low acute toxicity of the active substance the allocation of an ARfD was not necessary.~~ ~~t~~The main metabolites AMPA, N-acetyl-glyphosate and N-acetyl-AMPA were on no higher toxicity than glyphosate and can be evaluated based on the toxicological reference values for the parent.

B.7.15.1 Long-term dietary intake assessment

The long-term dietary intake assessment was based on the STMR values according to the representative uses and the corresponding residues in animal commodities:

Table B.7.15-1: Residue input values for the chronic intake assessment

Commodity	**Chronic risk assessment**	
	Input value (mg/kg)	Comment
Citrus fruits	0.05	Representative use for ground treatment does not result in detectable residues in orchards. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Tree nuts	0.05	Representative use for ground treatment does not result in detectable residues in orchards. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Pome fruit	0.05	Representative use for ground treatment does not result in detectable residues in orchards. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Stone fruit	0.05	Representative use for ground treatment does not result in detectable residues in orchards. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Grapes	0.05	STMR
Strawberries	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Table Olives	0.335~~19~~	STMR
~~Olive oil (olives for oil production)~~	~~0.017~~	~~STMR x PF 0.09~~
Root and tuber vegetables	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Bulb vegetables	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Fruiting vegetables	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Brassica vegetables	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Leaf vegetables & fresh herbs	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Legume vegetables	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Stem vegetables	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Pulses	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Oilseeds	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is

Commodity	**Chronic risk assessment**	
	Input value (mg/kg)	Comment
		irrelevant in non-tolerant crops.
Buckwheat, maize, millet, rice, sorghum	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Barley & oats, grain	5.85	STMR
Rye & wheat, grain	0.885	STMR
Rye, bran	1.3	STMR x PF 1.5
Rye, flour	0.39	STMR x PF 0.44
Rye, wholemeal flour	0.885	STMR x PF 1
Wheat, bran	1.6	STMR x PF 1.8
Wheat, flour	0.51	STMR x PF 0.57
Wheat, wholemeal flour	0.97	STMR x PF 1.1
Sugar plants	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Milk	0.05	STMR, LOQ based
Bovine muscle	0.125	STMR, LOQ based
Bovine, fat	0.131	STMR
Bovine, liver	0.11	STMR, LOQ based
Bovine, kidney	0.31	STMR
Sheep, goat, horses, asses, mules or hinnes and other farm animal matrices	variable	see bovine tissues
Eggs	0.04	STMR, LOQ based
Poultry, muscle, fat and liver	0.125	STMR, LOQ based
Poultry, kidney	0.155	STMR
Swine, muscle, fat and liver	0.125	STMR, LOQ based
Swine, kidney	0.059	STMR

The calculation was conducted with the EFSA PRIMo Rev. 2 and the German NVS II model. Based on the residue data listed above the EFSA PRIMo resulted in a maximum NEDI of 2.5% of the ADI (0.5 mg/kg bw) for children from Denmark. The German NVS II model resulted in a maximum utilisation of 1.5% of the ADI for the general population (14-80 years).

The chronic intake of glyphosate residues is unlikely to present a public health concern.

B.7.15.2 Short-term dietary intake assessment

~~For glyphosate and its metabolites AMPA, N-acetyl-glyphosate and N-acetyl-AMPA the allocation of an ARfD was not necessary. The estimation of the short-term dietary intake is not required.~~

The short-term dietary intake assessment was based on the HR and STMR values according to the representative uses and the corresponding residues in animal commodities:

Table B.7.15-2: Residue input values for the short-term intake assessment

Commodity	**Acute risk assessment**	
	Input value (mg/kg)	Comment
Citrus fruits	0.05	Representative use for ground treatment does not result in detectable residues in orchards. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Tree nuts	0.05	Representative use for ground treatment does not result in detectable residues in orchards. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Pome fruit	0.05	Representative use for ground treatment does not result in detectable residues in orchards. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Stone fruit	0.05	Representative use for ground treatment does not result in detectable residues in orchards. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Grapes	0.3	HR
Strawberries	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Table Olives	0.93	HR
Root and tuber vegetables	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Bulb vegetables	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Fruiting vegetables	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Brassica vegetables	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Leaf vegetables & fresh herbs	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Legume vegetables	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Stem vegetables	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Pulses	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Oilseeds	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Buckwheat, maize, millet,	0.05	Representative use before planting/sowing does not result in

Commodity	**Acute risk assessment**	
	Input value (mg/kg)	Comment
rice, sorghum		detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Barley & oats, grain	5.85	STMR, Case 3 commodity
Rye & wheat, grain	0.885	STMR Case 3 commodity
Rye, bran	1.3	STMR x PF 1.5, Case 3 commodity
Rye, flour	0.39	STMR x PF 0.44, Case 3 commodity
Rye, wholemeal flour	0.885	STMR x PF 1, Case 3 commodity
Wheat, bran	1.6	STMR x PF 1.8, Case 3 commodity
Wheat, flour	0.51	STMR x PF 0.57, Case 3 commodity
Wheat, wholemeal flour	0.97	STMR x PF 1.1, Case 3 commodity
Sugar plants	0.05	Representative use before planting/sowing does not result in detectable residues. LOQ for glyphosate used, since AMPA is irrelevant in non-tolerant crops.
Milk	0.05	STMR, LOQ based
Bovine muscle	0.125	HR, LOQ based
Bovine, fat	0.135	HR
Bovine, liver	0.2	HR
Bovine, kidney	1.82	HR
Sheep, goat, horses, asses, mules or hinnes and other farm animal matrices	variable	see bovine tissues
Eggs	0.04	HR, LOQ based
Poultry, muscle, fat and liver	0.125	HR, LOQ based
Poultry, kidney	0.155	HR
Swine, muscle, fat and liver	0.125	HR, LOQ based
Swine, kidney	0.059	HR

The calculation was conducted with the EFSA PRIMo Rev. 2 and the German NVS II model. Based on the residue data listed above the EFSA PRIMo as well as the German NVS II model resulted in a maximum IESTI of 4.7% of the ARfD (0.5 mg/kg bw) for the consumption of oats from children from Germany aged 2-4 years.
For adults the EFSA PRIMo resulted in a maximum utilisation of the ARfD of 8.5% for barley, based on the consumption data from the Netherlands. The German NVS II model indictated the highest intake for the General population aged 14-80 years with 5.7% of the ARfD, also based on barley.
All other commodities under consideration in this report gave lower utilisations of the ARfD.

The acute intake of glyphosate residues is unlikely to present a public health concern.

Glyphosate

Status of the active substance:	**Included**	Code no.	
LOQ (mg/kg bw):	0,05	proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0,5**	ARfD (mg/kg bw):	**0,5**
Source of ADI:	**PRAS**	Source of ARfD:	**PRAS**
Year of evaluation:	**2015**	Year of evaluation:	**2015**

Prepare workbook for refined calculations

Undo refined calculations

Explain choice of toxicological reference values.

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).
The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

	TMDI (range) in % of ADI minimum - maximum
	0 3
No of diets exceeding ADI:	---

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
2,6	IE adult	1,5	Barley	0,4	Wheat	0,2	Oats	0,2
2,5	DK child	1,0	Wheat	0,8	Rye	0,5	Oats	0,2
2,5	WHO Cluster diet B	1,5	Wheat	0,3	Barley	0,1	Table and wine grapes	0,3
2,2	WHO cluster diet E	0,9	Barley	0,7	Wheat	0,1	Oats	0,2
1,9	WHO Cluster diet F	0,7	Barley	0,6	Wheat	0,2	Oats	0,2
1,9	WHO cluster diet D	1,2	Wheat	0,3	Barley	0,1	Oats	0,2
1,7	NL child	0,8	Wheat	0,3	Milk and cream,	0,1	Oats	0,5
1,7	DE child	0,7	Wheat	0,2	Oats	0,1	Milk and cream,	0,4
1,4	UK Infant	0,5	Wheat	0,4	Milk and cream,	0,3	Oats	0,6
1,4	UK Toddler	0,7	Wheat	0,2	SUGAR PLANTS	0,2	Milk and cream,	0,6
1,3	WHO regional European diet	0,5	Wheat	0,4	Barley	0,0	Milk and cream,	0,2
1,3	IT kids/toddler	1,2	Wheat	0,0	Fruiting vegetables	0,0	Other cereal	0,1
1,2	ES adult	0,6	Barley	0,4	Wheat	0,0	Milk and cream,	0,1
1,2	FR toddler	0,5	Wheat	0,4	Milk and cream,	0,1	Root and tuber vegetables	0,6
1,2	ES child	0,8	Wheat	0,1	Milk and cream,	0,0	Bovine: Meat	0,2
1,1	NL general	0,4	Barley	0,4	Wheat	0,1	Milk and cream,	0,2
1,1	PT General population	0,7	Wheat	0,2	Table and wine grapes	0,0	Barley	0,1
1,0	FR all population	0,6	Wheat	0,2	Table and wine grapes	0,0	Milk and cream,	0,1
0,9	SE general population 90th percentile	0,6	Wheat	0,1	Milk and cream,	0,1	Root and tuber vegetables	0,3
0,8	IT adult	0,7	Wheat	0,0	Fruiting vegetables	0,0	Barley	0,1
0,8	DK adult	0,4	Wheat	0,1	Oats	0,1	Rye	0,1
0,7	LT adult	0,2	Rye	0,2	Wheat	0,1	Oats	0,1
0,6	UK vegetarian	0,4	Wheat	0,1	Oats	0,1	Table and wine grapes	0,1
0,6	FR infant	0,3	Milk and cream,	0,1	Wheat	0,1	Root and tuber vegetables	0,4
0,6	UK Adult	0,3	Wheat	0,1	Table and wine grapes	0,0	SUGAR PLANTS	0,1
0,5	FI adult	0,2	Wheat	0,1	Rye	0,1	Oats	0,1
0,1	PL general population	0,0	Root and tuber vegetables	0,0	Pome fruit	0,0	Table and wine grapes	0,1

Conclusion:
The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of Glyphosate is unlikely to present a public health concern.

Acute risk assessment /children

Acute risk assessment / adults / general population

The acute risk assessment is based on the ARfD.

For each commodity the calculation is based on the highest reported MS consumption per kg bw and the corresponding unit weight from the MS with the critical consumption. If no data on the unit weight was available from that MS an average European unit weight was used for the IESTI calculation.

In the **IESTI 1** calculation, the variability factors were 10, 7 or 5 (according to JMPR manual 2002), for lettuce a variability factor of 5 was used.
In the **IESTI 2** calculations, the variability factors of 10 and 7 were replaced by 5. For lettuce the calculation was performed with a variabilty factor of 3.

Threshold MRL is the calculated residue level which would leads to an exposure equivalent to 100 % of the ARfD.

Unprocessed commodities

No of commodities for which ARfD/ADI is exceeded (IESTI 1):		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 1):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**		---
IESTI 1	*)	**)	**IESTI 2**	*)	**)	**IESTI 1**	*)	**)	**IESTI 2**	*)	**)
Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)
4,7	Oats	5,85 / -	4,7	Oats	5,85 / -	8,5	Barley	5,85 / -	8,5	Barley	5,85 / -
3,9	Table grapes	0,3 / -	3,9	Table grapes	0,3 / -	1,9	Table grapes	0,3 / -	1,9	Table grapes	0,3 / -
2,6	Bovine: Edible offal	1,82 / -	2,6	Bovine: Edible	1,82 / -	1,7	Oats	5,85 / -	1,7	Oats	5,85 / -
2,6	Wheat	0,885 / -	2,6	Wheat	0,885 / -	1,4	Wine grapes	0,3 / -	1,4	Wine grapes	0,3 / -
2,1	Barley	5,85 / -	2,1	Barley	5,85 / -	1,4	Wheat	0,885 / -	1,4	Wheat	0,885 / -

Figure B.7.15-1: Screenshot of EFSA PRIMo Rev. 2 for glyphoste

Active Substance	Glyphosate
ADI (mg/kg bw)	0,5
ARfD for the general population (mg/kg bw)	0,5
ARfD for women in childbearing age (mg/kg bw)	n.n.

Reset

TMDI / NEDI	**Children 2-4 years (VELS-model)**	
	Average body weight	Individual consumption/body weight ratio
Chronic Intake	0,0067 mg/kg bw	0,0069 mg/kg bw
Utilisation ADI	1,3 %	1,4 %
Highest contributers (descending)	0,4 % ADI, Code: 0500090, Wheat, flour (and crushed wheat)	0,4 % ADI, Code: 0500090, Wheat, flour (and crushed wheat)
	0,2 % ADI, Code: 0500050, Oat, total	0,2 % ADI, Code: 0500050, Oat, total
	0,2 % ADI, Code: 1020000, Milk and cream, not concentrated, nor containing	0,2 % ADI, Code: 1020000, Milk and cream, not concentrated, nor containing
	0,1 % ADI, Code: 0130000, Pome fruit	0,1 % ADI, Code: 0130000, Pome fruit
	0,1 % ADI, Code: 0500070, Rye, flour	0,1 % ADI, Code: 0500070, Rye, flour

NESTI	**Children 2-4 years (VELS-model)**	
	Average body weight	Individual consumption/body weight ratio
Highest utilisations (descending)	4,7 % ARfD, Code: 0500050, Oat, bran	4,5 % ARfD, Code: 0500050, Oat, bran
	4,2 % ARfD, Code: 0500050, Oat flour (and crushed oat)	1,6 % ARfD, Code: 0500050, Oat flour (and crushed oat)
	2,5 % ARfD, Code: 0500050, Oat, raw	1,3 % ARfD, Code: 0500050, Oat, raw
	1,6 % ARfD, Code: 0233030, Watermelon, raw	1,3 % ARfD, Code: 0500010, Barley, bran (and hulled barley)
	1,4 % ARfD, Code: 0500010, Barley, bran (and hulled barley)	1,2 % ARfD, Code: 0500090, Wheat, flour (and crushed wheat)
	1,2 % ARfD, Code: 0500090, Wheat, flour (and crushed wheat)	1,2 % ARfD, Code: 0233030, Watermelon, raw
	1 % ARfD, Code: 0500070, Rye, raw	0,9 % ARfD, Code: 0130010, Apples, raw
	0,9 % ARfD, Code: 0130020, Pears, raw	0,9 % ARfD, Code: 0130020, Pears, raw
	0,9 % ARfD, Code: 0110010, Grapefruit, raw	0,9 % ARfD, Code: 0110020, Oranges, raw
	0,9 % ARfD, Code: , Sausages, unspecified	0,9 % ARfD, Code: , Sausages, unspecified

TMDI / NEDI	**General population 14-80 years (NVS-II-model)**	
	Average body weight	Individual consumption/body weight ratio
Chronic Intake	0,0073 mg/kg bw	0,0073 mg/kg bw
Utilisation ADI	1,5 %	1,5 %
Highest contributers (descending)	0,6 % ADI, Code: 0500010, Barley, total	0,6 % ADI, Code: 0500010, Barley, total
	0,3 % ADI, Code: 0500090, Wheat, processed	0,3 % ADI, Code: 0500090, Wheat, processed
	0,1 % ADI, Code: 1020000, Milk and cream, not concentrated, nor containing	0,1 % ADI, Code: 1020000, Milk and cream, not concentrated, nor containing
	0,1 % ADI, Code: 0500050, Oat, total	0,1 % ADI, Code: 0500050, Oat, total
	0,1 % ADI, Code: 0500070, Rye, processed	0,1 % ADI, Code: 0500070, Rye, processed

NESTI	**General population 14-80 years (NVS-II-model)**	
	Average body weight	Individual consumption/body weight ratio
Highest utilisations (descending)	6,9 % ARfD, Code: 0500010, Barley, processed	5,7 % ARfD, Code: 0500010, Barley, processed
	1,5 % ARfD, Code: 0500050, Oat, bran	1,8 % ARfD, Code: 0500050, Oat, bran
	1 % ARfD, Code: 0500070, Rye, destillate	1 % ARfD, Code: 0500090, Wheat, processed
	0,9 % ARfD, Code: 0500090, Wheat, processed	0,9 % ARfD, Code: 0500070, Rye, destillate
	0,7 % ARfD, Code: 0500050, Oat, processed	0,7 % ARfD, Code: 0500050, Oat, processed
	0,6 % ARfD, Code: 0500090, Wheat, wholemeal and bran	0,5 % ARfD, Code: 0500070, Rye, raw
	0,6 % ARfD, Code: 1012030, Bovine liver, processed	0,4 % ARfD, Code: 0500070, Rye, processed
	0,5 % ARfD, Code: 0270010, Asparagus, processed	0,4 % ARfD, Code: 0233010, Melons, raw
	0,4 % ARfD, Code: 0500070, Rye, processed	0,4 % ARfD, Code: 1012030, Bovine liver, processed
	0,4 % ARfD, Code: 0500070, Rye, raw	0,4 % ARfD, Code: 0500090, Wheat, wholemeal and bran

Figure B.7.15-2: Screenshot of German NVS II model for glyphosate

B.7.15.3 Public literature

The chronic dietary intake of numerous pesticides was investigated by Nougadère, A. et al (2011, ASB2012-11982) to introduce a ranking and scoring method for the active substances. The exposure of the Cammeroonian against glyphosate was investigated in a total diet study by Gimou et al (2008, ASB2012-12422). Harris C. et al published a case study to predict the chronic dietary intake of glyphosate based on several intake models in 2004 (2004, ASB2012-12428).

In view of the representative use based evaluation of glyphosate within this document, the provided literature was not applicable to support the dietary intake assessment for glyphosate.

B.7.16 Summary and evaluation of residue behaviour (Annex IIA 6.11.1; Annex IIIA 8.11)

The metabolism of glyphosate in non-tolerant plants was investigated in numerous crops, covering all crop groups. The active substance was applied via soil treatment, hydroponic application, stem or trunk treatment and foliar treatment. Following direct treatment via foliar, trunk, stem or hydroponic treatment unchanged glyphosate was the only significant residue. In presence of soil as a substrate the active substance is quickly degraded, leaving AMPA at

rates comparable or even higher than parent glyphosate. However, the uptake via the roots and the translocation in the plants was very low, not resulting in significant residue levels as confirmed by plant metabolism and confined rotational crop studies. A major part of the glyphosate was degraded into CO_2.

In glyphosate tolerant plants the metabolism may differ significantly. Depending on the kind of modification AMPA (GOX modification), N-acetyl-glyphosate (GAT modification) and N-acetyl-AMPA (GAT modification) become major metabolites in plant commodities, often being present at higher amounts than the unchanged parent. While CP4-EPSPS, an enzyme much lower susceptible to glyphosate, does not affect the metabolic pattern, the ratio between glyphosate and AMPA was approximately 1:1 in GOX modified plants. For GAT modified plants some commodities showed only little glyphosate remaining above the LOQ. N-acetyl-AMPA was the major residue in most commodities while parent glyphosate was only present at low level or even undetected.

The metabolism of glyphosate in rotational crops was investigated in several confined studies involving application rates to bare soil equivalent to 3.87 - 6.5 kg as/ha. The investigation of soil samples in these studies demonstrated the quick degradation of the parent substance, showing AMPA as major residue with levels up to 10 times higher than the glyphosate remaining. TRR levels in samples obtained from rotational crops contained substancial residues, equivalent to concentrations of up to 4.4 mg eq/kg. However, as demonstrated in soil treatment metabolism studies, most of the radioactivity remained unextracted due to incorperation of $^{14}CO_2$ from the degradation of glyphosate in the soil. In the extracts glyphosate levels depended on the interval between treatment and sampling. After short intervals up to 14 weeks glyphosate levels were higher than AMPA (glyphosate: 19.6 – 62 % of the TRR, AMPA: 2.3 - 15.6 % of the TRR). In samples collected after longer intervals glyphosate was only present in minor amounts of 10 % of the TRR or less (absolute levels <0.001 mg eq/kg to 0.026 mg eq/kg) while AMPA was the dominant residue with up to 20 % of the TRR (up to 0.05 mg eq/kg). Further metabolites were not identified.

In livestock animals the metabolism of glyphosate, AMPA and N-acetyl-glyphosate was investigated in lactating goats and laying hens. All three analytes are only slowly degraded. Most of the residues was recovered unchanged. The major part of the administered dose was excreted via the faeces. For bioavailable residues the excretion was observed mainly via urine, resulting in highest residue levels found in the kidney. Muscle, fat and milk gave very low residues, normally being present below the LOQ. In liver some metabolisation of glyphosate into AMPA was observed. However the levels of both analytes were much lower than in kidney. In eggs the residue increase during the whole dosing period of up to eight days. A plateau was observed in livestock feeding studies on laying hens after 14 days. Again, most of the residue was identified as unchanged substance administered.
No information on the metabolism of glyphosate or its metabolites in fish was provided.

Definition of the residue for plant commodities
Residue definition for enforcement purposes in sweet corn, oilseeds rape, soya beans and maize (non-tolerant and tolerant, all modifications):
sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate

Residue definition for enforcement purposes in other plant commodities:
glyphosate

Residue definition for dietary intake purposes in plant commodities:

sum of glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA, all expressed as glyphosate equivalents

(For the future generation of residue data N-acetly-glyphosate and N-acetyl-AMPA are only mandatory analytes in GAT-modified crops.)

Definition of the residue for animal commodities
Residue definition for enforcement purposes in animal commodities:
sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate

Residue definition for dietary intake purposes in animal commodities:
sum of glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA, all expressed as glyphosate equivalents

(For the calculation of the maximum dietary burden for the purpose of MRL setting, only glyphosate and N-acetyl-glyphosate need to considered, since the reformation of both analytes from AMPA or N-acetly-AMPA is unlikely).

The representative uses for glyphosate involve ground treatment for weed control in orchards and vineyards, the pre-emergence or pre-sowing application before replanting (all annual crops) and desiccation (cereal grain, linseed, mustard seed and rape seed). Except for the desiccation of linseed, mustard seed and rape seed all uses were provided with appropriate supervised field trial data corresponding to the representative GAP.

The confined rotational crops studies in combination with pre-emergence/pre-sowing supervised field trials indicate no glyphosate or AMPA residues above the LOQ in succeeding crops. Consideration of an uptake into rotational crops is not required. Based on these representative uses, the following MRLs were estimated using the OECD MRL-Calculator, if applicable:

Citrus fruits	0.05* mg/kg
Tree nuts	0.05* mg/kg
Pome fruit	0.05* mg/kg
Stone fruit	0.05* mg/kg
Grapes	0.5 mg/kg
Strawberries	0.05* mg/kg
Table olives	2 mg/kg
Root and tuber vegetables	0.05* mg/kg
Bulb vegetables	0.05* mg/kg
Fruiting vegetables, except sweet corn	0.05* mg/kg
Sweet corn	0.1* mg/kg (potential tolerant crop, higher LOQ)
Brassica vegetables	0.05* mg/kg
Leaf vegetables & fresh herbs	0.05* mg/kg
Legume vegetables	0.05* mg/kg
Stem vegetables	0.05* mg/kg
Pulses, except lentils	0.05* mg/kg
Oilseeds, except rape seed and soya beans	0.05* mg/kg
Maize, rape seed, lentils, soya beans	0.1* mg/kg (potential tolerant crop, higher LOQ)
~~Olives~~	~~2 mg/kg~~

Barley, oats	30 mg/kg
Rye, triticale, wheat	20 mg/kg
Buckwheat, millet, rice, sorghum, others	0.05* mg/kg
Herbal infusions	0.05* mg/kg
Sugar plants	0.05* mg/kg

For animal products MRLs were estimated on basis of potential glyphosate residues in feed commodities treated according to the representative uses:

Swine, muscle~~, fat and liver~~	0.05* mg/kg
Swine, fat and liver	0.1* mg/kg
Swine, kidney	0.2 mg/kg
Bovine, goat and sheep, muscle	0.05* mg/kg
Bovine, goat and sheep, fat	0.1 mg/kg
Bovine, goat and sheep, liver	0.1 mg/kg
Bovine, goat and sheep, kidney	2 mg/kg
Milk	0.05* mg/kg
Poultry, muscle~~, fat and liver~~	0.05* mg/kg
Poultry, fat and liver	0.1* mg/kg
Poultry, kidney	0.1 mg/kg
Eggs	0.05* mg/kg

Information on the freezer storage stability in plant and animal commodities was provided for glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA. The analytes were stable (>70% remaining) in the following matrices:

Table B.7.16-1: Summary of the storage stability data for glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl-AMPA

Matrix	Storage stability (months)			
	Glyphosate	AMPA	N-acetyl-glyphosate	N-acetyl-AMPA
High acid content				
Oranges	>24	>24	not investigated	not investigated
Tomato	>31	>31	not investigated	not investigated
High water content				
Clover	>31	6	not investigated	not investigated
Maize, forage	>9	>9	>9	>1
Maize, plant	>9	9	>9	>1
Maize stover	>12	>12	6	>12
Soya bean forage	31	24	>12	>1
Sorghum stover	31	9	not investigated	not investigated
Sugar beet roots	18	18	not investigated	not investigated
Sugar beet leaves	18	18	not investigated	not investigated
High oil content				
Linseed	>18	not investigated	not investigated	not investigated
Rape seed	>18	not investigated	not investigated	not investigated
Soya beans	>24	>24	>12	>1
High starch content				
Barley grain	18	18	not investigated	not investigated
Maize grain	31	>31	>12	>12
Rye grain	>45	10	not investigated	not investigated
Sorghum grain	>48	>48	not investigated	not investigated
Wheat grain	>45	>24	not investigated	not investigated
High protein content				
Beans, dry	>18	not investigated	not investigated	not investigated
Other				
Barley straw	18	18	not investigated	not investigated
Rye straw	>45	6	not investigated	not investigated
Soya bean hay	>12	>12	>12	>1
Soya bean straw	>24	>24	not investigated	not investigated
Wheat straw	>45	6	not investigated	not investigated
Animal commodities				
Swine, fat	>26	26	not investigated	not investigated
Swine, muscle	>26	>26	not investigated	not investigated
Swine, liver	>26	>26	not investigated	not investigated
Swine, kidney	>26	>26	not investigated	not investigated
Cattle, fat	>24	>24	not investigated	not investigated
Cattle, muscle	>24	>24	not investigated	not investigated
Cattle, liver	>24	>24	not investigated	not investigated
Cattle, kidney	>24	>24	not investigated	not investigated
Cattle, milk	>16	>16	not investigated	not investigated
Chicken, fat	>25	16	not investigated	not investigated
Chicken, muscle	>25	>25	not investigated	not investigated
Chicken, liver	>25	>25	not investigated	not investigated
Chicken, kidney	>13	>13	not investigated	not investigated
Chicken, eggs	14	14	not investigated	not investigated

During simulated processing glyphosate and N-acetyl-glyphosate were stable in sterilised buffer solutions. For the magnitude of residues in processed commodities the following processing factors were derived based on simulated processing studies and supervised field trials:

Table B.7.16-2: Summary of processing factors for glyphosate and AMPA

Commodity	Glyphosate		AMPA	
	Range of processing factors	**Median processing factor**	**Range of processing factors**	**Median processing factor**
Citrus, juice	0.45, <0.71, <0.83, <0.83, 0.83, <1	0.83	<LOQ	-
Citrus, peel	<0.83, 2.3, 2.8, 3.1, 3.1, 5.0	3	<LOQ	-
Citrus, feed meal	1.4, 1.8, 1.8, 3.3, 4.9, 5.3	2.6	<LOQ	-
Citrus, press liquor	<0.83, 1.7, 1.9, 2.1, 2.3, 2.7	2	<LOQ	-
Potato, chips	<LOQ	-	1.2, 1.3, 1.6	1.3
Potato, flakes	<LOQ	-	1.2, 1.5, 1.7	1.5
Potato, wet peel	<LOQ	-	0.26, <0.31, <0.71	0.31
Potato, dry peel	<LOQ	-	1.2, 1.5, 2.7	1.5
Potato, granules	<LOQ	-	1.4, 2.3, 2.4	2.3
Olive oil, crude (vergine)	<0.03, <0.03, <0.04, <0.05(4), <0.06, <0.06, <0.09, <0.13, <0.13, <0.17, <0.25, <0.35, <0.38, <0.42, <0.63, <0.63	0.09	<LOQ	-
Olive oil, refined	<0.05, <0.05, <0.09, <0.35, <0.38, <0.42	0.22	<LOQ	-
Linseed, oil	<0.1, <0.18, <0.31, <0.31	0.25	<LOQ	-
Linseed, press cake	1.1, <1.5, 1.6, 1.6	1.6	<LOQ	-
Rape, crude oil	<0.1, <0.13, <0.15, <0.27	0.14	<LOQ	-
Rape, refined oil	<0.05, <0.1, <0.13, <0.15, <0.27	0.13	<LOQ	-
Rape, press cake	1.2, <1.3, 1.4, 1.5, 2.2	1.4	<LOQ	-
Soya beans, fat free meal	0.95, 1.0	0.98	0.91, 0.99	0.95
Soya bean, hulls	4.4, 5.2	4.8	2.4, 2.5	2.45
Soya beans, crude oil	<0.01, 0.01	0.01	0.05, <0.06	0.055
Soya beans, soapstock	<0.04, <0.05	0.045	0.26, <0.32	0.29
Maize, fat free meal	1.0, 1.0, 1.2, 1.2	1.1	<0.4, 0.87	0.64
Maize, crude oil	<0.05, <0.08, <0.11, <0.14	0.1	<0.4, <0.6	0.5
Maize, refined oil	<0.05, <0.08, <0.11, <0.14	0.1	<0.4, <0.6	0.5
Maize, soapstock	<0.05, <0.08, <0.11, <0.14	0.1	not analysed	-
Maize, small grits	0.7, 1.1	0.9	not analysed	-
Maize, medium grits	0.6, 0.9	0.75	not analysed	-
Maize, large grits	0.6, 0.9	0.75	not analysed	-
Maize, flour	0.9, 0.9	0.9	<0.4, 0.79	0.59
Rye, bran	0.17, 1.3, 1.7, 4.8	1.5	0.5, 0.67, 0.84, 8	0.76
Rye, flour	0.11, 0.33, 0.55, 1.5	0.44	0.18, 1, 1.6, 4	1.3
Rye, wholemeal flour	0.01, 0.89, 1.1, 4.4	1	0.19, 0.22, 0.4, 13.8	0.31
Rye, wholemeal bread	0.07, 0.48, 0.78, 2.6	0.63	0.48, 0.59, 0.62, 6.8	0.61
Rye, middlings	0.07, 1.2, 1.5, 7.8	1.35	0.44, 0.74, 0.84, 13	0.79
Wheat, bran	0.96, 1.2, 1.3, 1.3, 1.6, 1.7, 1.8, 1.8, 1.8, 1.9, 2.0, 2.3, 2.8	1.8	1.2	1.2
Wheat, flour	0.08, 0.12, 0.17, 0.29, 0.52, 0.55, 0.57, 0.58,	0.57	0.81	0.81

Commodity	**Glyphosate**		**AMPA**	
	Range of processing factors	**Median processing factor**	**Range of processing factors**	**Median processing factor**
	0.63, 0.72, 0.72, 0.77, 0.92			
Wheat, wholemeal flour	0.54, 1.7	1.1	<LOQ	-
Wheat. wholemeal bread	0.34, 0.39	0.37	<LOQ	-
Wheat, middlings	0.32, 0.89	0.61	<LOQ	-
Wheat, semolina	0.14, 0.16	0.15	<LOQ	-
Wheat, semolina bran	1.4, 2.2	1.8	<LOQ	-

The chronic intake of glyphosate based on the representative uses resulted in a maximum utilisation of 2.5% of the ADI (0.5 mg/kg bw) for children from Denmark (EFSA PRIMo Rev. 2). The German NVS II model gave a utilisation of 1.5% of the ADI for the general population aged 14-80 years.

~~Due to the low acute toxicological properties of glyphosate and its metabolites the allocation of an ARfD was not necessary.~~

The acute intake based on the EFSA PRIMo as well as the German NVS II model resulted in a maximum IESTI of 4.7% of the ARfD (0.5 mg/kg bw) for the consumption of oats from children from Germany aged 2-4 years. For adults the EFSA PRIMo resulted in a maximum utilisation of the ARfD of 8.5% for barley, based on the consumption data from the Netherlands. The German NVS II model indictated the highest intake for the General population aged 14-80 years with 5.7% of the ARfD, also based on barley. All other commodities under consideration in this report gave lower utilisations of the ARfD.

In summary it can be concluded that the chronic and acute dietary intake is unlikely to present a public health concern.

B.7.17 References relied on

Studies marked in yellow were not part of the dossier for the renewal
Studies marked in grey are considered to be not acceptable

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
	EFSA	2009	Reasoned opinion: Modification of the residue definition of Glyphosate in genetically modified maize grain and soybeans, and in products of animal origin EFSA Journal 2009; 7(9):1310 ! EFSA-Q-2009-00372 ASB2012-3480	N	LIT
	EFSA	2012	Reasoned Opinion - Modification of the existing MRL for Glyphosate in lentils EFSA Journal 2012;10(1):2550 ! EFSA-Q-2011-00926 ASB2012-3184	N	LIT
OECD: KIIA 6.1.1	Hubbart, N. S.	1993	Determination of Glyphosate in soybean raw agricultural commodities (RAC) - Stability report Report No.: 91210 GLP: yes not published ASB2010-14765	Y	CHE
OECD: KIIA 6.1.1	Hubbart, N. S.	1993	Determination of Glyphosate in pasture grasses - Stability report Report No.: 91212 GLP: yes not published ASB2010-14766	Y	CHE
OECD: KIIA 6.1.1	Manning, M. J.; Mueth, M. G.	1988	Storage stability of Glyphosate and AMPA in swine tissues, dairy cow tissues and milk laying hen tissues and eggs Report No.: MSL-7515 GLP: yes not published RIP9501253	Y	MOD

[1] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
OECD: KIIA 6.1.1	McKay, J. C.	1989	Storage stability validation for ICIA0224 in raw agricultural commodities Report No.: WRC 89-22 GLP: yes not published RIP9500028	Y	SYN
OECD: KIIA 6.1.1	Morgenroth, U.	1995	Storage stability of Glyphosate and AMPA in wheat grain and straw and in rye grain and straw Report No.: 303614 GLP: yes not published ASB2010-14764	Y	CHE
OECD: KIIA 6.1.1	Mueth, M. G.	1991	Storage stability of Glyphosate residues in crop commodities Report No.: MSL-10843 GLP. yes not published RIP9501332	Y	MOD (owned by Glypho-sate Task Force)
OECD: KIIA 6.1.1	Mueth, M. G.; Allan, J. M.	2012	Storage stability of Glyphosate and AMPA on citrus fruit Report No.: MSL0023608 GLP: yes not published ASB2012-12452	Y	MOD
OECD: KIIA 6.1.1	Schulz, H.	1997	Determination of the storage stability of Glyphosate in beans, oilseed rape and linseed Report No.: IF-94/13882-00 GLP: yes not published ASB2010-14803	Y	CHE
OECD: KIIA 6.1.1	Schwartz, N. L.	2007	Stability of Glyphosate, N-Acetylglyphosate and Aminomethyl phosphonic acid in GAT corn forage, grain, and stover, stored frozen Report No.: DuPont-17379 GLP: yes not published ASB2008-2655	Y	DPB
OECD: KIIA 6.1.1	Schwartz, N. L.	2007	Stability of Glyphosate and metabolites in corn green plant, forage, grain, and stover contain-ing the GAT and ZM-HRA genes during fro-zen storage: Interim report Report No.: DuPont-20094 GLP: yes not published ASB2008-2656	Y	DPB

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
OECD: KIIA 6.1.1	Schwartz, N. L.	2007	Stability of Glyphosate, N-Acetylglyphosate, Aminomethyl phosphonic acid and N-Acetyl AMPA in GAT soybean forage, seed, and hay stored frozen: Second interim report Report No.: DuPont-17573 GLP: yes not published ASB2008-2654	Y	DPB
OECD: KIIA 6.1.1	Weber, H.	2010	Storage stability of residues of Glyphosate and AMPA in various plant materials Report No.: FSG-0707 GLP: yes not published ASB2012-12488	Y	ADM
OECD: KIIA 6.2.1	Anonymous	1976	Glyphosate residue and metabolism studies in sugarcane and soils Report No. RD93 GLP: no not published RIP9501198	Y	MOD
OECD: KIIA 6.2.1	Bleeke, M. S.	1997	Nature of Glyphosate residues in cotton plants tolerant to Roundup herbicide. Report MSL-14113 GLP: yes not published RIP9700619	Y	MOD
OECD: KIIA 6.2.1	Bohm, G. M. B.; Genovese, M. I.; Pigosso, G. et al.	2008	Residues of Glyphosate and Aminomethylphosphonic acid and levels of Isoflavones in BRS 244 RR and BRS 154 soybean GLP: no published: Ciéncia e Tecnologia de Alimentos, ISSN 0101-2061 ASB2012-12366	N	LIT
OECD: KIIA 6.2.1	Bresnahan, G. A.; Manthey, F. A.; Howatt, K. A. et al.	2003	Glyphosate applied preharvest induces shikimic acid accumulation in hard red spring wheat (Triticum aestivum) GLP: no published: Journal of Agricultural and Food Chemistry 2003, 51, 4004-4007 ASB2012-12365	N	LIT
OECD: KIIA 6.2.1	Cataneo, A. C.; Déstro, G. F. G.; Ferreira, L. C. et al.	2003	Glutathione S-transferase activity on the degradation of the herbicide Glyphosate in maize (Zea mays) plants GLP: no published: Planta Daninha, Vicosa-MG, v.21, n.2, p.307-312, 2003 ASB2012-12384	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
OECD: KIIA 6.2.1	Chapleo, S.; McLachlan, T.	2010	The metabolism of [14C]Glyphosate in 0827 canola DuPont-26109 GLP: yes not published ASB2011-13744	Y	DPB
OECD: KIIA 6.2.1	Duke, S. O.	2011	Glyphosate degradation in Glyphosate-resistant and -Susceptible crops and weeds GLP: no published: Journal of Agricultural and Food Chemistry 2011, 59, 5823-5841 ASB2012-12401	N	LIT
OECD: KIIA 6.2.1	Duke, S. O.; Rimando, A. M.; Pace, P. F. et al.	2003	Isoflavone, Glyphosate, and Ami-nomethylphosphonic acid levels in seeds of Glyphosate-treated, Glyphosate-resistant soy-bean GLP: no published: Journal of Agricultural and Food Chemistry 2003, 51, 340-344 ASB2012-12400	N	LIT
OECD: KIIA 6.2.1	George, Ch.	1995	Nature of Glyphosate residues in corn plants which are tolerant to Roundup herbicide Report MSL-14018 GLP: yes not published RIP9700618	Y	MOD
OECD: KIIA 6.2.1	Goure, W. F.	1994	Nature of Glyphosate residues in soybeans tolerant to Roundup herbicide MSL-13520 GLP: yes not published RIP9800117	Y	MOD
OECD: KIIA 6.2.1	Green, M. A.	2007	The metabolism of [14C]Glyphosate in Opti-mum GAT (Event DP-Ø9814Ø-6) field corn DuPont-19529 GLP: yes not published ASB2008-2657	Y	DPB
OECD: KIIA 6.2.1	Hasegawa, L. S.; Kumamoto, J.; Jordan, L. S.	1995	Degradation of Glyphosate in avocado fruit 10.04.1995, L365, ASB2011-13642	Y	LIT

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OECD: KIIA 6.2.1	MacDonald, A. M. G.	2007	The metabolism of [14C]Glyphosate in GAT/GM-HRA (DP-356Ø43-5, PHP20163a) soybeans DuPont-19530 GLP: yes not published ASB2008-2658	Y	DPB
OECD: KIIA 6.2.1	Malik, J. M.	1975	CP 67573 residue and metabolism Part 24: The metabolism of CP 67573 in coffee plants Report No. 344 GLP: no not published RIP9501192	Y	MOD
OECD: KIIA 6.2.1	Malik, J. M.; Brightwell, B. B.	1976	CP 67573 residue and metabolism Part 29: The metabolism of CP 67573 in sugar beets Report No. 394 GLP: no not published RIP9501195	Y	MOD
OECD: KIIA 6.2.1	Mehrsheikh, A.	1999	Protocol - Metabolism of Glyphosate in Roundup Ready(R) sugarbeet 99-63-M-7 GLP: yes not published RIP2003-1134	Y	MOD
OECD: KIIA 6.2.1	Mehrsheikh, A.	2000	Metabolism of Glyphosate in Roundup Ready Sugarbeet MSL-16247 GLP: yes not published RIP2001-906	Y	MOD
~~OECD: KIIA 6.2.1~~	~~Michaux, M.~~	~~1974~~	~~CP 67573: Determination of crop residues in winter wheat, spring wheat and spring barley~~ ~~Report A1~~ ~~GLP: no~~ ~~not published~~ ~~RIP9501209~~	~~Y~~	~~MOD~~
OECD: KIIA 6.2.1	Michaux, M.	1976	CP 67573: Determination of crop residues in kale, serradella, turnips Report A10 GLP: no not published RIP9501212	Y	MOD

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OECD: KIIA 6.2.1	Michaux, M.	1976	CP 67573: Determination of crop residues in grass Report A11 GLP: no not published RIP9501213	Y	MOD
OECD: KIIA 6.2.1	Nadeau, R. G.	1975	CP 67573 residue and metabolism Part 26: The metabolism of CP 67573 in potato plants Report No. 376 GLP. no not published RIP9501193	Y	MOD
OECD: KIIA 6.2.1	Nadeau, R. G.; Cozad, S. J.	1976	Absorption, translocation and metabolism of Roundup herbicide in walnut, almond and pecan trees Report No. 403 GLP: no not published RIP9501196	Y	MOD
OECD: KIIA 6.2.1	Parker, S.; Harris, M.	1991	Glyphosate-trimesium: Uptake and metabolism in USA grape vines RJ 1002B GLP: yes not published RIP9500012	Y	SYN
OECD: KIIA 6.2.1	Reddy, K. N.; Rimando, A. M.; Duke, S. O.; Nandula, V. K.	2008	Aminomethylphosphonic acid accumulation in plant species treated with glyphosate GLP: no published: Journal of Agricultural and Food Chemistry, 2008, 56, 2125-2130 ASB2012-12463	N	LIT
OECD: KIIA 6.2.1	Rogers, M. D.; Saeman Bleeke, M.; Goure, W. F.; George, C.; Nadeau, R. G.	1994	Nature of Glyphosate residues in Roundup herbicide tolerant canola MSL-13318 GLP: yes not published RIP9800118	Y	MOD
OECD: KIIA 6.2.1	Rojano-Delgado, A. M.; Cruz-Hipolito, H.; De Prado, R. et al.	2012	Limited uptake, translocation and enhanced metabolic degradation contribute to Glypho-sate tolerance in Mucuna pruriens var. utilis plants GLP: no published: Phytochemistry 73 (2012) 34-41 ASB2012-12462	N	LIT

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OECD: KIIA 6.2.1	Rueppel, M. L.; Moran, S. J.	1974	CP 67573 residue and metabolism Part 23: The metabolism of CP 67573 in apple trees Report No. 342 GLP: no not published RIP9501190	Y	MOD
OECD: KIIA 6.2.1	Rueppel, M. L.; Suba, L. A.	1973	CP 67573, Residue and Metabolism Part 10: The Metabolism of CP 67573 in soybeans, cotton, wheat, and corn Report No. 304 GLP: no not published RIP9600099	Y	MOD
OECD: KIIA 6.2.1	Rueppel, M. L.; Suba, L. A.; Moran, S. J.	1974	CP 67573 residue and metabolism Part 20: The metabolism of CP 67573 in grape plants Report No. 335 GLP: no not published RIP9501191	Y	MOD
OECD: KIIA 6.2.1	Stuart, C.; Parker, S.; Joseph, R. S. I.	1989	ICIA0224: Metabolism on wheat following a preharvest foliar spray RJ 0778B GLP: yes not published RIP9500014	Y	SYN
OECD: KIIA 6.2.1	Suba, L. A.; Georgieff, M. K.	1974	CP 67573 residue and metabolism Part 22: The metabolism of N-phosphonomethylglycine in barley, oats, rice and sorghum Report No. 341 GLP: no not published RIP9501189	Y	MOD
OECD: KIIA 6.2.1	Sutherland, M. L.	1975	The metabolism of CP 67573 by citrus - February 1973 - October 1974 Report No. 328 GLP: no not published RIP9501194	Y	MOD
OECD: KIIA 6.2.1	Sutherland, M. L.; Banduhn, M. C.	1976	The metabolism of Glyphosate in pasture crops Report No. 404 GLP: no not published RIP9501197	Y	MOD

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OECD: KIIA 6.2.1	Tambling, D. R.	1992	[^{14}C-Anion] ICIA0224: Nature of the residue: Soybeans RR 91-092B GLP: yes not published RIP9500015	Y	SYN
OECD: KIIA 6.2.1	Wagner, R.; Kogan, M.; Parada, A. M.	2003	Phytotoxic activity of root absorbed Glyphosate in corn seedlings (Zea mays L.) GLP: no published: Weed Biology and Management 3, 228-232 (2003) ASB2012-12484	N	LIT
OECD: KIIA 6.2.1	Wilkinson, M. J.; Joseph, R. S. I.	1990	ICIA0224: Uptake and metabolism in grapevines RJ 0815B GLP: yes not published RIP9500017	Y	SYN
OECD: KIIA 6.2.1	Bohn, T.; Cuhra, M.; Traavik, T.; Sanden, M.; Fagan, J.; Primicerio, R.;	2013	Compositional differences in soybeans on the market: Glyphosate accumulates in Roundup Ready GM soybean, Food Chemistry 153 (2014) 207–215, ASB2014-6353	N	LIT
OECD: KIIA 6.2.2	Bodden, R. M.	1988	Metabolism study of synthetic ^{13}C/^{14}C-labeled Glyphosate and Aminomethylphosphonic acid in laying hens. Part I Report No: MSL-7591 GLP: yes not published RIP9501205	Y	MOD
OECD: KIIA 6.2.2	Bowler, D. T.	1994	[^{14}C-PMG] Glyphosate-trimesium: Nature of the residue in tissues and eggs of laying hens Report No: RR-93-064B GLP: yes not published RIP9500020	Y	SYN
OECD: KIIA 6.2.2	Lowrie, Ch.	2007	The metabolism of [^{14}C]-N-Acetylglyphosate (IN-MCX20) in laying hens Report No.: DuPont-19795 GLP: yes not published ASB2008-2659	Y	DPB

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OECD: KIIA 6.2.2	Patanella, J. E.; Feng, P.	1988	Metabolism study of synthetic $^{13}C/^{14}C$-labeled Glyphosate and Aminomethylphosphonic acid in laying hens. Part II Report No: MSL-7420 GLP: yes not published RIP9501206	Y	MOD
OECD: KIIA 6.2.2	Powles, P.	1994	(^{14}C-Glyphosate): Absorption, distribution, metabolism and excretion following repeated oral administration to the laying hen Report No. 676/8-1011 GLP: yes not published RIP9501208	Y	MOD
OECD: KIIA 6.2.3	Bodden, R. M.	1988	Metabolism study of synthetic $^{13}C/^{14}C$-labeled Glyphosate and Aminomethylphosphonic acid in lactating goats. Part I Report: MSL 7586 GLP: yes not published RIP9501203	Y	MOD
OECD: KIIA 6.2.3	Ericson, J. L.	1994	The nature of residues of orally administered [Phosphonomethylene-^{14}C] glyphosate-trimesium in goat tissues and milk Report No: RR 93-062B GLP: yes not published RIP9500022	Y	SYN
OECD: KIIA 6.2.3	Lowrie, Ch.	2007	Metabolism of [^{14}C]-N-Acetylglyphosate (IN-MCX20) in the lactating goat Report No.: DuPont-19796 GLP: yes not published ASB2008-2660	Y	DPB
OECD: KIIA 6.2.3	Patanella, J. E.; Feng, P.	1988	Metabolism study of synthetic $^{13}C/^{14}C$-labeled Glyphosate and Aminomethylphosphonic acid in lactating goats. Part II Report: MSL-7458 GLP: yes not published RIP9501204	Y	MOD

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OECD: KIIA 6.2.3	Powles, P.	1994	(^{14}C-Glyphosate): Absorption, distribution, metabolism and excretion following repeated oral administration to the dairy goat Report No. 676/9-1011 GLP: yes not published RIP9501207	Y	MOD
OECD: KIIA 6.3	Anderson, L.; Butters, C.	2000	Glyphosate / Glyphosate-trimesium; Residue levels in barley from trials carried out in Nothern Europe during 1999 Report No.: RJ2907B GLP: yes not published RIP2000-2131	Y	SYN
OECD: KIIA 6.3	Anderson, L.; Butters, C.	2000	Glyphosate / Glyphosate-trimesium; Residue levels in wheat from trials carried out in Nothern Europe during 1999 Report No.: RJ2910B GLP: yes not published RIP2000-2130	Y	SYN
OECD: KIIA 6.3	Ando, C.; Se-gawa, R.; Gana, C. et al.	2003	Dissipation and offsite movement of forestry herbicides in plants of importance to native Americans in California National Forests GLP: no published: Bulletin of Environmental Contam-ination and Toxicology (2003) 71:354-361 ASB2012-12350	N	LIT
OECD: KIIA 6.3	Anon.	1975	Glyphosat - Pflanzenschutzmittel-Rückstände in Wein Report No.: BBA II-08 GLP: no not published ASB2009-5293	Y	MOD
OECD: KIIA 6.3	Anon.	1977	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln - Versuchsanstel-lung (Weintrauben) Report No.: 12703 GLP: no not published ASB2009-5277	Y	MOD

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OECD: KIIA 6.3	Anon.	1977	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln - Versuchsanstellung (Weintrauben) Report No.: 12703 GLP: no not published ASB2009-5279	Y	MOD
OECD: KIIA 6.3	Anon.	1977	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln - Versuchsanstellung (Weintrauben) Report No.: 12703 GLP: no not published ASB2009-5278	Y	MOD
OECD: KIIA 6.3	Anon.	1980	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenbehandlungsmitteln - Hafer Report No.: MOD 80-D/15 GLP: no not published ASB2009-5341	Y	MOD
OECD: KIIA 6.3	Anon.	1980	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenbehandlungsmitteln - Lagergetreide (Hafer) Report No.: CM 80-D-63 GLP: no not published ASB2009-5340	Y	MOD
OECD: KIIA 6.3	Anon.	1986	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenbehandlungsmitteln - Zuckerrüben Report No.: 002999 GLP: no not published ASB2009-2244	Y	MOD
OECD: KIIA 6.3	Anon.	1988	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenbehandlungsmitteln - Weinrebe Report No.:003118 GLP: yes not published ASB2009-2247	Y	MOD
OECD: KIIA 6.3	Anon.	1988	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenbehandlungsmitteln - Weinrebe Report No.:003119 GLP: yes not published ASB2009-2248	Y	MOD

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OECD: KIIA 6.3	Anon.	1988	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenbehandlungsmitteln - Weinrebe Report No.:005723 GLP: yes not published ASB2009-2255	Y	MOD
OECD: KIIA 6.3	Anon.	1988	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenbehandlungsmitteln - Weinrebe Report No.:005724 GLP: yes not published ASB2009-2258	Y	MOD
OECD: KIIA 6.3	Anon.	1988	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenbehandlungsmitteln - Weinrebe Report No.:01879 GLP: yes not published ASB2009-2249	Y	MOD
OECD: KIIA 6.3	Anon.	1988	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenbehandlungsmitteln - Weinrebe Report No.:005725 GLP: yes not published ASB2009-2265	Y	MOD
OECD: KIIA 6.3	Anon.	1991	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln - Wintergerste Report No.: 16227 GLP: no not published ASB2009-4415	Y	MOD
OECD: KIIA 6.3	Anon.	1991	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln - Wintergerste Report No.: 16229 GLP: no not published ASB2009-4420	Y	MOD
OECD: KIIA 6.3	Anon.	1991	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln - Wintergerste Report No.: 16246 GLP: no not published ASB2009-4421	Y	MOD

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OECD: KIIA 6.3	Anon.	1991	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln - Wintergerste Report No.: 11724 GLP: no not published ASB2009-4413	Y	MOD
OECD: KIIA 6.3	Anon.	1991	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln - Wintergerste Report No.: 16240 GLP: no not published ASB2009-4419	Y	MOD
OECD: KIIA 6.3	Anon.	1991	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln - Wintergerste Report No.: 16242 GLP: no not published ASB2009-4422	Y	MOD
OECD: KIIA 6.3	Anon.	1992	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln - Weizen Report No.: 12536 GLP: yes not published ASB2009-6583	Y	CHE
OECD: KIIA 6.3	Anon.	1992	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln - Weizen Report No.: 12492 GLP: yes not published ASB2009-6581	Y	CHE
OECD: KIIA 6.3	Anon.	1992	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln - Weizen Report No.: 12535 GLP: yes not published ASB2009-6582	Y	CHE
OECD: KIIA 6.3	Anon.	1992	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln - Weizen Report No.: 12491 GLP: yes not published ASB2009-6580	Y	CHE

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OECD: KIIA 6.3	Arregui, M. C.; Lenardon, A.; Sanchez, D. et al.	2004	Monitoring Glyphosate residues in transgenic Glyphosate-resistant soybean GLP: no published: Pest Management Science 60:163-166 (online 2003) ASB2012-12351	N	LIT
OECD: KIIA 6.3	Balluff, M.	1995	Determination of residues of Glistar in apples under field conditions at four locations in Germany Report No.: 94035 GLP: yes not published RIP9501344	Y	ALK
OECD: KIIA 6.3	Balluff, M.	1995	Determination of residues of Glistar in winter wheat under field conditions at four locations in Germany Report No.: 94035/01-FPWW GLP: yes not published RIP9501341	Y	ALK
OECD: KIIA 6.3	Block H.	2012	Determination of residues of Glyphosate and AMPA after one application of MON 52276 in sugar beet (outdoor) at 2 sites in Spain and Italy 2011 Report No.: S11-00266 GLP: yes not published ASB2012-12376	Y	MOD (owned by Glypho-sate Task Force)
OECD: KIIA 6.3	Block, H.	2012	Determination of residues of Glyphosate and AMPA after one application of MON 52276 in carrots (outdoor) at 4 sites in France, Spain and Poland 2011 Report No.: S11-00259 GLP: yes not published ASB2012-12369	Y	MOD (owned by Glypho-sate Task Force)
OECD: KIIA 6.3	Block, H.	2012	Determination of residues of Glyphosate and AMPA after one application of MON 52276 in potatoes (outdoor) at 4 sites in France, Germa-ny and Italy 2011 Report No.: S11-00258 GLP: yes not published ASB2012-12368	Y	MOD (owned by Glypho-sate Task Force)

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OECD: KIIA 6.3	Block, H.	2012	Determination of residues of Glyphosate and AMPA after one application of MON 52276 in bulb onions (outdoor) at 4 sites in France, Spain and Bulgaria 2011 Report No.: S11-00260 GLP: yes not published ASB2012-12370	Y	MOD (owned by Glypho-sate Task Force)
OECD: KIIA 6.3	Block, H.	2012	Determination of residues of Glyphosate and AMPA after one application of MON 52276 in tomato (outdoor) at 2 sites in Hungary and Germany 2011 Report No.: S11-00267 GLP: yes not published ASB2012-12377	Y	MOD (owned by Glypho-sate Task Force)
OECD: KIIA 6.3	Block, H.	2012	Determination of residues of Glyphosate and AMPA after one application of MON 52276 in cucumber and zucchini (outdoor) at 3 sites in Italy, France and Germany 2011 Report No.: S11-00261 GLP: yes not published ASB2012-12371	Y	MOD (owned by Glypho-sate Task Force)
OECD: KIIA 6.3	Block, H.	2012	Determination of residues of Glyphosate and AMPA after one application of MON 52276 in cauliflower (outdoor) at 4 sites in France, Hungary, Bulgaria and Italy 2011 Report No.: S11-00263 GLP: yes not published ASB2012-12373	Y	MOD (owned by Glypho-sate Task Force)
OECD: KIIA 6.3	Block, H.	2012	Determination of residues of Glyphosate and AMPA after one application of MON 52276 in head cabbage (outdoor) at 4 sites in Hungary, France (North), Spain and Bulgaria 2011 Report No.: S11-00262 GLP: yes not published ASB2012-12372	Y	MOD (owned by Glypho-sate Task Force)

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OECD: KIIA 6.3	Block, H.	2012	Determination of residues of Glyphosate and AMPA after one application of MON 52276 in leaf and head lettuce (outdoor) at 4 sites in France, Spain, UK and Germany 2011 Report No.: S11-00264 GLP: yes not published ASB2012-12374	Y	MOD (owned by Glypho-sate Task Force)
OECD: KIIA 6.3	Block, H.	2012	Determination of residues of Glyphosate and AMPA after one application of MON 52276 in leek (outdoor) at 4 sites in France, United Kingdom, Bulgaria and Italy 2011 Report No.: S11-00265 GLP: yes not published ASB2012-12375	Y	MOD (owned by Glypho-sate Task Force)
OECD: KIIA 6.3	Bonin, J.	1982	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenbehandlungsmitteln - Hafer Report No.: 003149 GLP: no not published ASB2009-5712	Y	MOD
OECD: KIIA 6.3	Bonin, J.	1982	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenbehandlungsmitteln - Hafer Report No.: 003150 GLP: no not published ASB2009-5711	Y	MOD
OECD: KIIA 6.3	C. Harrison	2007	Final Report on Project AF/10436/AV; to determine the magnitude of Glyphosate and Diquat residues at harvest in the agricultural oilseed rape resulting from one application of Glyphosate or Reglone, with or without the adjuvant Companion Gold, in the UK, 2006 Report No.: AF/10436/AV GLP: yes not published ASB2008-7571	Y	MOD
OECD: KIIA 6.3	Dittrich, R.; Thomas, U.	1992	Prüfung des Rückstandsverhaltens von CHE 3607 in Winterweizen unter Freilandbedingun-gen Report No.: RP-H 92 GLP: yes not published ASB2009-6573	Y	CHE

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OECD: KIIA 6.3	Euler	1986	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln - Winterraps (Jet Neuf) Report No.: 008369 GLP: no not published ASB2009-4823	Y	MOD
OECD: KIIA 6.3	Euler	1986	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln - Winterraps (Jet Neuf) Report No.: 008370 GLP: no not published ASB2009-4824	Y	MOD
OECD: KIIA 6.3	Euler	1986	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln - Winterraps (Jet Neuf) Report No.: 008371 GLP: no not published ASB2009-4821	Y	MOD
OECD: KIIA 6.3	Euler	1986	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln - Winterraps (Jet Neuf) Report No.: 008380 GLP: no not published ASB2009-4822	Y	MOD
OECD: KIIA 6.3	Euler	1986	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln - Winterraps (Jet Neuf) Report No.: 06851 GLP: no not published ASB2009-4831	Y	MOD
OECD: KIIA 6.3	Euler	1986	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenschutzmitteln - Winterraps (Jet Neuf) Report No.: 06852 GLP: no not published ASB2009-4832	Y	MOD

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OECD: KIIA 6.3	Granby, K.; Vahl, M.	2001	Investigation of the herbicide Glyphosate and the plant growth regulators Chlormequat and mepiquat in cereals produced in Denmark GLP: no published: Food Additives and Contaminants, 2001, Vol. 18, No. 10, 898-905 ASB2012-12423	N	LIT
OECD: KIIA 6.3	Grolleau, G.	2001	Magnitude of the Residue of Glyphosate in Cherry raw agricultural commodity; Germany - 2000; incl. Amendment Report No.: EA000181 GLP: yes not published RIP2001-558	Y	CHE MOD
OECD: KIIA 6.3	Grolleau, G.	2001	Magnitude of the Residue of Glyphosate in Peach raw agricultural commodity; Spain - 2000 Report No.: EA000182 GLP: yes not published RIP2001-557	Y	CHE MOD
OECD: KIIA 6.3	Gustin, C.	1999	Glyphosate and AMPA residues in wheat and barley treated pre-harvest with Roundup herbicide and MON 14420. 1998 Field trials in Belgium and France Report No.: MLL 30815 GLP: yes not published RIP2000-1243	Y	MOD
OECD: KIIA 6.3	Gustin, C.	2000	Glyphosate and AMPA residues in oil seed rape treated pre-harvest with Roundup (MON 2139) and MON 78294. 1999 Field trials in Belgium and France Report No.: MLL 31336 GLP: yes not published RIP2002-651	Y	MOD
OECD: KIIA 6.3	Hontis, A. M.	1989	Glyphosate and AMPA residues in oilseed rape following preharvest application of MON 14478 with Ammoniumsulfate and of Roundup herbicide. 1989 UK field trials Report No.: MLL 30235 GLP: no not published RIP9501286	Y	MOD

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OECD: KIIA 6.3	Hontis, A. M.	1991	Glyphosate and AMPA residues in wheat and barley following application of MON 52276, MON 44068 and Roundup herbicide, one week before crop harvest. French trials 1991 Report No.: MLL 30281 GLP: yes not published RIP9501287	Y	MOD
OECD: KIIA 6.3	Hontis, A. M.	1992	Residues of Glyphosate/AMPA in olives and olive oil following use of Sting SE - Spanish field trials 1990/1992 Report No.: MLL 30297 GLP: yes not published RIP9501289	Y	MOD
OECD: KIIA 6.3	Hontis, A. M.	1992	Glyphosate and AMPA residues in barley following preharvest application of MON 44068 and Roundup herbicide. German field trials 1991 Report No.: MLL 30286 GLP: yes not published RIP9500162	Y	MOD
OECD: KIIA 6.3	Hontis, A. M.	1992	Glyphosate and AMPA residues in barley following preharvest application of MON 44068 and Roundup herbicide. German field trials 1991 Report No.: MLL 30286 GLP: yes not published RIP9501288	Y	MOD
OECD: KIIA 6.3	Hontis, A. M.	1993	Residues of Glyphosate/AMPA in olives and olive oil following a soil treatment with MON 65040 herbicide. Italian field trials 1993 Report No.: MLL 30319 GLP: yes not published RIP9501290	Y	MOD
OECD: KIIA 6.3	Hontis, A. M.	1993	Residues of Glyphosate/AMPA in winter oilseed rape following an application of MON 52276, MON 44068 and Roundup herbicide, two weeks before harvest. UK field trials 1992 Report No.: MLL 30321 GLP: yes not published RIP9501292	Y	MOD

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OECD: KIIA 6.3	Hontis, A. M.	1993	Residues of Glyphosate/AMPA in winter wheat following an application of MON 52276, MON 44068 and Roundup herbicide, one week before harvest. - U.K. field trials 1992. Report No.: MLL 30320 GLP: yes not published RIP9501291	Y	MOD
OECD: KIIA 6.3	Hontis, A. M.	1996	esidues of Glyphosate and AMPA in peas treated pre-harvest with MON 52776 herbicide. U.K. and Belgian field trials, 1995 Report No.: MLL 30464 GLP: yes not published RIP9501283	Y	MOD
OECD: KIIA 6.3	Hontis, A. M.	1996	Residues of glyphosate and AMPA in olives and olive oil, following a soil treatment with Roundup herbicide. Spanish field trials, 1995 Report No.: MLL 30469 GLP: yes not published RIP9700184	Y	MOD
OECD: KIIA 6.3	Klimmek, S.; Weber, H.	2007	Decline and magnitude of residues of Glyphosate in lodged grain following application of Taifun Forte - Germany, season 2006 Report No.: FSG-0606 GLP: yes not published ASB2008-5610	Y	ADM
OECD: KIIA 6.3	Klimmek, S.; Weber, H.	2007	Decline and magnitude of residues of Glyphosate in sugar beet following application of Taifun Forte - Germany, season 2006 Report No.: FSG-0608 GLP: yes not published ASB2008-5609	Y	ADM
OECD: KIIA 6.3	Leak, S.	1994	Final report on project AS/2208/CN - Programme to generate crop samples for residue analysis following application of glyfos for pre-harvest desiccation of winter sown oilseed rape Report No.: AS/2208/CN GLP: yes not published ASB2010-14768	Y	CHE

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OECD: KIIA 6.3	Leak, S.; Tarpey, G.	1993	Report on the field phase of a study to generate crop samples for residue analysis following the application of CHE 3607 on linseed Report No.: AS/1902/CN GLP: yes not published RIP9501325	Y	CHE
OECD: KIIA 6.3	Leak, S.; Tarpey, G.	1993	Report on the field phase of a study to generate crop samples for residue analysis to monitor the dissipation of Glyphosate following the application of CHE 3607 on winter wheat Report No.: AS/1906/CN GLP: yes not published RIP9501303	Y	CHE
OECD: KIIA 6.3	Leak, S.; Webb R.	1993	Report on the field phase of the study to generate crop samples for residue analysis following the application of CHE 3607 on winter barley Report No.: AS/1896/CN GLP: yes not published RIP9501293	Y	CHE
OECD: KIIA 6.3	Leak, S.; Webb, R.	1993	Report on the field phase of a study to generate crop samples for residue analysis to monitor the dissipation of Glyphosate following the application of CHE 3607 on winter barley Report No.: AS/1905/CN GLP: yes not published RIP9501295	Y	CHE
OECD: KIIA 6.3	Leak, S.; Webb, R.	1993	Report on the field phase of a study to generate crop samples for residue analysis to monitor the dissipation of Glyphosate following the application of CHE 3607 on oats Report No.: AS/1907/CN GLP: yes not published RIP9501299	Y	CHE
OECD: KIIA 6.3	Leak, S.; Webb, R.	1993	Report on the field phase of a study to generate crop samples for residue analysis following the application of CHE 3607 with and without the adjuvant Frigate for preharvest desiccation of oats Report No.: AS/1897/CN GLP: yes not published RIP9501297	Y	CHE

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OECD: KIIA 6.3	Leak, S.; Webb, R.	1993	Report on the field phase of a study to generate crop samples for residue analysis following the application of CHE 3607 on winter wheat Report No.: AS/1898/CN GLP: yes not published RIP9501301	Y	CHE
OECD: KIIA 6.3	Lemaire, P.	1999	Glyphosate and AMPA residues in oilseed rape treated pre-harvest with Roundup herbicide and MON 14420. 1998 Field trials in Belgium and France Report No.: MLL 30817 GLP: yes not published RIP2000-1244	Y	MOD
OECD: KIIA 6.3	Lorenzatti, E.; Maitre, M.I.; Argelia, L. et al.	2004	Pesticide residues in immature soybeans of Argentina croplands GLP: no published: Fresenius Environmental Bulletin, Vol. 13 (7), 2004, 675-678 ASB2012-12448	N	LIT
OECD: KIIA 6.3	Losseau, F.	1988	Glyphosate residues in cereals following pre-harvest applications of MON 14478, with and without Ammoniumsulfate (AS), in comparison to Roundup herbicide - 1987 France - Field trials Report No.: MLL 30205 GLP: no not published RIP9501280	Y	MOD
OECD: KIIA 6.3	Losseau, F.	1988	Glyphosate residues in cereals following pre-harvest applications of MON 14474, MON 8791, MON 8755, MON 14456, in comparison to Roundup herbicide - 1987 Fed. Rep. Germany - Field trials. Report No.: MLL 30209 GLP: no not published RIP9501282	Y	MOD
OECD: KIIA 6.3	Losseau, F.	1989	Glyphosate and AMPA residues in grapes following MON 8755 (Arcade) herbicide applications in vineyards. German field trials 1988 Report No.: MLL 30227 GLP: yes not published RIP9501285	Y	MOD

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OECD: KIIA 6.3	Losseau, F.	1989	Glyphosate and AMPA residues in oilseedrape (OSR), peas and beans following preharvest applications of MON 14478 with Ammoni-umsulfate (AS) in comparison to Roundup or Roundup 480 herbicide applications. 1988 UK fieldtrials Report No.: MLL 30223 GLP: yes not published RIP9501284	Y	MOD
OECD: KIIA 6.3	Mastdagh, P.	1985	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenbehandlungsmitteln - Hafer Report No.: 003188 GLP: no not published ASB2009-5710	Y	MOD
OECD: KIIA 6.3	Mellet, M.; Wasser, Ch.	1994	Determination of the residues of Glyphosate and AMPA in wheat treated with Glifogarde in France in 1994 Report No.: RF 4061-2 GLP: yes not published ASB2009-3523	Y	INV
OECD: KIIA 6.3	Mestdagh, M.	1986	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenbehandlungsmitteln - Zuckerrüben Report No.: 008905 GLP: no not published ASB2009-2210	Y	MOD
OECD: KIIA 6.3	Mestdagh, M.	1986	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenbehandlungsmitteln - Zuckerrüben Report No.: 01811 GLP: no not published ASB2009-2242	Y	MOD
OECD: KIIA 6.3	Mestdagh, P.	1979	Glyphosate residues in cereals following pre-harvest application of Roundup in the United Kingdom Report No.: MLL 30037 GLP: no not published RIP9501230	Y	MOD

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OECD: KIIA 6.3	Mestdagh, P.	1980	Glyphosate residues in apples following Roundup application in Denmark Report No.: MLL 30053 GLP: no not published RIP9501235	Y	MOD
OECD: KIIA 6.3	Mestdagh, P.	1980	Glyphosate residues in cereals following pre-harvest application of Roundup in France Report No.: MLL 30046 GLP: no not published RIP9501231	Y	MOD
OECD: KIIA 6.3	Mestdagh, P.	1982	Glyphosate residues in cereal grain and straw following preharvest treatment with Roundup herbicide in the United Kingdom - 1982 trials - Part I. Report No.: MLL 30087 GLP: no not published RIP9501249	Y	MOD
OECD: KIIA 6.3	Mestdagh, P.	1983	Residue analysis for Glyphosate and AMPA in flax and processed fractions following preharvest Roundup herbicide treatments. UK and Ireland 1982 trials Report No.: MLL 30106 GLP: no not published RIP9501266	Y	MOD
OECD: KIIA 6.3	Mestdagh, P.	1983	Residue analysis for Glyphosate and AMPA in brassica seedcrops and processed fractions following preharvest Roundup herbicide treatments. UK and Scandinavian trials 1980-1982 Report No.: MLL 30104 GLP: no not published RIP9501265	Y	MOD
OECD: KIIA 6.3	Mestdagh, P.	1983	Glyphosate residues in cereals following Roundup herbicide preharvest applications using low spray water volumes and/or additional surfactant/active ingredient ratios. UK 1982 trails Report No.: ML 30112 GLP: no not published RIP9501269	Y	MOD

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OECD: KIIA 6.3	Mestdagh, P.	1985	Glyphosate residues in rye and oat following Roundup herbicide preharvest applications. Denmark 1984 trials Report No.: MLL 30150 GLP: no not published RIP9501275	Y	MOD
OECD: KIIA 6.3	Mestdagh, P.	1988	Glyphosate and AMPA residues in oilseed rape and peas following preharvest Roundup herbicide applications. 1986-1987 field trials Fed. Rep. of Germany Report No.: MLL 30204 GLP: no not published RIP9501279	Y	MOD
OECD: KIIA 6.3	Mestdagh, P.	1988	Glyphosate residues in cereals following preharvest applications of MON 14478, with and without Ammoniumsulfate (AS), in comparison to Roundup (MON 2139) and/or Roundup 480 (MON 8762) herbicide - 1987 UK field trials Report No.: MLL 30200 GLP: no not published RIP9501278	Y	MOD
OECD: KIIA 6.3	Michaux, M.	1975	CP 67573: Determination of crop residues in grapes - Final Report Report No.: A2 GLP: no not published ASB2009-5294	Y	MOD
OECD: KIIA 6.3	Michaux, M.	1975	CP 67573 : Determination of crop residues in sugar beets tops and roots Report No.: A3 GLP:no not published RIP9501210	Y	MOD
OECD: KIIA 6.3	Michaux, M.	1976	CP 67573 : Determination of crop residues in apples and pears - Final report Report No.: A9 GLP: no not published RIP9501211	Y	MOD

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OECD: KIIA 6.3	Michaux, M.	1977	CP 67573: Determination of crop residues in grapes and apples Report No.: MON 2139 GLP: no not published ASB2009-5276	Y	MOD
OECD: KIIA 6.3	Michaux, M.	1977	CP 67573 : Determination of crop residues in salads, onions, carrots, peas and beans Report No.: A16 GLP: no not published RIP9501216	Y	MOD
OECD: KIIA 6.3	Nassoy, G.	1994	Gilfogarde (Glyphosat): Residus de glyphosate sur cultures de ble tendre d`hiver traitees avec le Glifogarde Report No.: 94 H CP HX GLP: yes not published RIP2002-1448	Y	INV
OECD: KIIA 6.3	Nassoy, G.	2000	Determination des residus a la recolte sur ble tendre d' hiver apres application avant recolte de l'herbicide Madrigal (Glyphosate) Dans le nord et le sud de la France Report No.: 97 H CP HX P/A GLP: yes not published ASB2009-3524	Y	INV
OECD: KIIA 6.3	Nassoy, G.	2000	Determination of Glyphosate and its main metabolite AMPA residues in wheat (grains and straw) after applications of the formulation HERBEX Report No.: 15920 ADR GLP: yes not published ASB2009-3522	Y	INV
OECD: KIIA 6.3	Perny, A.	2002	Glyphosate and AMPA residues in wheat and barley treated pre-harvest with Roundup(r) (MON 2139), MON 78273 and MON 78568. 2001 Field Trials in France, Germany and Italy. Report No.: RA1157 GLP: yes not published RIP2005-200	Y	MOD

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OECD: KIIA 6.3	Puy, E.	1993	Détermination des résidus de glyphosate et de son métabolite l'AMPA dans des échantillons de pailles et de grains de céréales traitées avec Glistar en France en 1992 Report No.: RF 2052 GLP: yes not published RIP9501345	Y	ALK
OECD: KIIA 6.3	Reding, M. A.	1978	Determination of crop residues in apples Report No.: A22 GLP: no not published RIP9501218	Y	MOD
OECD: KIIA 6.3	Reding, M. A.	1978	Final report on CP 67573: Determination of crop residues in olives and olive oil Report No.: A20-II GLP: no not published RIP9501217	Y	MOD
OECD: KIIA 6.3	Reding, M. A.	1986	Glyphosate and Aminomethylphosphonic acid residues in cereal grain and straw following preharvest treatment with Roundup herbicide in Europe Report No.: MLL 30177 GLP: no not published RIP9501276	Y	MOD
OECD: KIIA 6.3	Reding, M. A.	1987	Residual Glyphosate and AMPA in oilseed rape, beans and peas following application of MON 8762 - MON 8795 and Roundup herbi-cide. UK 1986 field trials Report No.: MLL 30180 GLP: no not published RIP9501277	Y	MOD
OECD: KIIA 6.3	Reding, M. A.	1988	Residue determination of Glyphosate and Aminomethylphosphonic acid in various crops following different Roundup, or Glyphosate based formulation, applications. 1978-1987 trial period Report No.: MLL 30206 GLP: no not published RIP9501281	Y	MOD

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OECD: KIIA 6.3	Riffart, K.	1981	Berichtsbogen für Rückstandsuntersuchungen mit Pflanzenbehandlungsmitteln - Hafer Report No.: 001639 GLP: no not published ASB2009-5713	Y	MOD
OECD: KIIA 6.3	Schulz, H.	1992	Determination of the residues of Glyphosate and AMPA in cereals (SAG 539 00) Report No.: 275837 GLP: yes not published RIP9501328	Y	CHE
OECD: KIIA 6.3	Schulz, H.	1994	Determination of the dissipation of Glyphosate in apples following treatment with Glifos under field conditions in Portugal 1993 Report No.: IF-93/04768-01 GLP: yes not published RIP9501330	Y	CHE
OECD: KIIA 6.3	Schulz, H.	1997	Glyphosate residues in barley and wheat following preharvest application of Roundup and Glyfos in France 1996 Report No.: IF-96/07067-00 GLP: yes not published ASB2010-14804	Y	CHE
OECD: KIIA 6.3	Schulz, H.; Mirbach, M. J.	1994	Determination of residues of Glyphosate in linseed - Treatment with CHE 3607 - UK, season 1992, Agrisearch UK Ltd., Study Plan AS/1902/CN Report No.: IF-93/13836-01 GLP: yes not published RIP9501326	Y	CHE
OECD: KIIA 6.3	Schulz, H.; Mirbach, M. J.	1994	Determination of residues of Glyphosate in winter sown oilseed rape (seed and pods) - Treatment with CHE 3607 - UK Season 1992 - Agrisearch UK Ltd. Study Plan AS/1908/CN Report No.: IF-93/13839-01 GLP: yes not published RIP9501324	Y	CHE

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OECD: KIIA 6.3	Schulz, H.; Mirbach, M. J.	1994	Determination of residues of Glyphosate in winter sown oilseed rape - Treatment with CHE 3607 - UK Season 1992, Agrisearch UK Ltd. Study Plan AS/1899/CN Report No.: IF-93/13831-01 GLP: yes not published RIP9501322	Y	CHE
OECD: KIIA 6.3	Schulz, H.; Mirbach, M. J.	1994	Determination of residues of Glyphosate in winter barley (whole plant, grains and straws - Treatment with CHE 3607/Frigate - UK, Season 1992, Agrisearch UK Ltd, Study Plan AS/1905/CN Report No.: IF-93/04573-01 GLP: yes not published RIP9501296	Y	CHE
OECD: KIIA 6.3	Schulz, H.; Mirbach, M. J.	1994	Determination of residues of Glyphosate in winter barley (grains and straws) - Treatment with CHE 3607/Frigate - UK, Season 1992, Agrisearch UK Ltd, Study Plan AS/1896/CN Report No.: IF-93/04568-01 GLP: yes not published RIP9501294	Y	CHE
OECD: KIIA 6.3	Schulz, H.; Mirbach, M. J.	1994	Determination of residues of Glyphosate in winter oats (whole plant, grains and straw - Treatment with CHE 3607/Frigate - UK, Season 1992, Agrisearch UK Ltd, Study Plan AS/1907/CN Report No.: IF-93/04575-01 GLP: yes not published RIP9501300	Y	CHE
OECD: KIIA 6.3	Schulz, H.; Mirbach, M. J.	1994	Determination of residues of Glyphosate in winter oats (grains and straw) - Treatment with CHE 3607/Frigate - UK, Season 1992, Agrisearch UK Ltd, Study Plan AS/1897/CN Report No.: IF-93/04569-01 GLP: yes not published RIP9501298	Y	CHE

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OECD: KIIA 6.3	Schulz, H.; Mirbach, M. J.	1994	Determination of residues of Glyphosate in winter wheat (grain and straw) - Treatment with CHE 3607/Frigate - UK, Season 1992, Agrisearch UK Ltd, Study Plan AS/1898/CN Report No.: IF-93/04570-01 GLP: yes not published RIP9501302	Y	CHE
OECD: KIIA 6.3	Schulz, H.; Mirbach, M. J.	1994	Determination of residues of Glyphosate in winter wheat (whole plant, grains and straw - Treatment with CHE 3607/Frigate - UK, Season 1992, Agrisearch UK Ltd, Study Plan AS/1906/CN Report No.: IF-93/04574-01 GLP: yes not published RIP9501304	Y	CHE
OECD: KIIA 6.3	Vanbellinghen, C.	2000	Glyphosate and AMPA residues in wheat and barley treated pre-harvest with Roundup herbicide (MON 2139) and MON 78294. 1999 Field trials in Belgium and France Report No.: MLL 31337 GLP: yes not published RIP2002-650	Y	MOD
OECD: KIIA 6.3	Zietz, E.	1993	Determination of residues of Glyphosate in cereals and processing products - Treatment with GLYFOS - Germany season 1992 Report No.: IF-92/11567-01 GLP: yes not published RIP9501329, RIP9500134 & ASB2011-9181	Y	CHE
OECD: KIIA 6.3 & 6.5.3	Mestdagh, P.	1981	Residual Glyphosate in processed barley grains following a preharvest application of Roundup herbicide in the United Kingdom Report No: MLL.30.070 GLP: no not published RIP9501238	Y	MOD
OECD: KIIA 6.3 & 6.5.3	Mestdagh, P.	1981	Residual Glyphosate in processed oat grains following a preharvest application of Roundup herbicide in the United Kingdom Report No.: MLL 30.071 GLP: no not published RIP9501239	Y	MOD

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OECD: KIIA 6.3 & 6.5.3	Mestdagh, P.	1982	Residual Glyphosate in beer, wort and malt obtained from barley grains following a pre-harvest application of Roundup herbicide in France Report No.: MLL 30.084 GLP: no not published RIP9501248	Y	MOD
OECD: KIIA 6.3 & 6.5.3	Mestdagh, P.	1981	Residual Glyphosate in processed wheat grains following a preharvest application of Roundup herbicide in the United Kingdom Report No.: MLL 30.069 GLP: no not published RIP9501237		MOD
OECD: KIIA 6.3 & 6.5.3	Mestdagh, P.; Dirks, R. C.	1987	Glyphosate residues in processed fractions obtained from Roundup herbicide treated wheat, barley, and oat. European field trials. Report No.: MLL 30.179 GLP: no not published RIP9501256		MOD
OECD: KIIA 6.3 & 6.5.3	Mestdagh, P.	1982	Analytical residues methods - Glyphosate residues in cereal grain and straw following preharvest treatment with Roundup herbicide in the United Kingdom Report No.: MLL 30.087 GLP: no not published ASB2009-5491		
OECD: KIIA 6.3 & 6.5.3	Schulz, H.	1992	Determination of residues of Glyphosate and AMPA in cereals - (CHE 03690H) Report No.: 275848 GLP: yes not published RIP9501327	Y	CHE
OECD: KIIA 6.3 & 6.5.3	Schulz, H.	1992	Determination of residues of Glyphosate and AMPA in cereals - (SAG 539 00) Report No.: 275837 GLP: yes not published RIP9501328	Y	CHE

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OECD: KIIA 6.3 & 6.5.3	Steinmetz, J. R.	1984	Glyphosate residues in wheat and wheat grain milling/fractionation products following pre-harvest applications with Roundup herbicide Report No.: MSL-3677 GLP: no not published RIP9501254	Y	MOD
OECD: KIIA 6.3 & 6.5.3	Steinmetz, J. R.; Cowell, J. E.	1984	Addendum to MSL-3612: Glyphosate residues in wheat grain milling/fractionation products. Report No.: MSL-4005 GLP: no not published RIP9501255	Y	MOD
OECD: KIIA 6.4.1	Dibb-Fuller, M.; Bramble, F. Q.	2007	Magnitude of residues of N-Acetylglyphosate and degradates in laying hen tissues and eggs Report No.: DuPont-20088 GLP: yes not published ASB2008-2652	Y	DPB
OECD: KIIA 6.4.1	Graham, D. G.	1987	Magnitude of SC-0224 residues in eggs and poultry Report No.: RRC 87-43 GLP: yes not published RIP9500025	Y	SYN
OECD: KIIA 6.4.1	Manning, M. J.; Wilson, G. R.	1987	Residue determination of Glyphosate and AMPA in laying hen tissues and eggs following a 28 day feeding study Report No.: MSL-6676 GLP: yes not published RIP9501252	Y	MOD
OECD: KIIA 6.4.2	Cowell, J. E.	1987	Residue determination of Glyphosate and AMPA in dairy cow tissues and milk following a 28 day feeding study Report No.: MSL-6729 GLP: yes not published RIP9501250	Y	MOD
OECD: KIIA 6.4.2	Graham, D. G.	1987	Magnitude of SC-0224 residues in meat and milk Report No.: RRC 87-44 GLP: yes not published RIP9500024	Y	SYN

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OECD: KIIA 6.4.2	McLellan, G.; Bramble, F. Q.	2007	Magnitude of residues of N-Acetylglyphosate and degradates in dairy cow tissues and milk Report No.: DuPont-20087 GLP: yes not published ASB2008-2653	Y	DPB
OECD: KIIA 6.4.2	Krüger, M. et al	2014	Detection of Glyphosate Residues in Animals and Humans J. Environ. Anal. Toxicol. 4:210 doi: 10.4172/2161-0525.1000210 ISSN: 2161-0525 GLP: no Published ASB2014-5024	N	LIT
OECD: KIIA 6.4.3	Manning, M. J.; Wilson, G. R.	1987	Residue determination of Glyphosate and AMPA in swine tissues following a 28-day feeding study Report-No.: MSL-6627 GLP: yes not published RIP9501251	Y	MOD
OECD: KIIA 6.5.1	Hiler, T.	2010	Nature of [^{14}C]Glyphosate residues in processed commodities - High temperature hydrolysis Report No.: MSL0023072 GLP: yes not published ASB2012-12432	Y	MOD (owned by Glyphosate Task Force)
OECD: KIIA 6.5.1	Umstätter, S.; Peterson, B.	2006	High temperature hydrolysis of [^{14}C]IN-MCX20 in buffered aqueous solution at pH 4, 5, and 6 Report No.: DuPont-19797 GLP: yes not published ASB2008-2675	Y	DPB
OECD: KIIA 6.5.3	Beasley, R. K.	1975	CP 57573, Residue and metabolism part 27: Determination of CP 67573 and CP 50435 residues in citrus process fractions Report No.: 377 GLP: no not published RIP9501260	Y	MOD

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OECD: KIIA 6.5.3	Caierao, E.; Acosta, A. D. S.	2007	Industrial suitability for malting of grains from desiccated pre-harvest barley GLP: no published: Pesq. agropec. bras., Brasilia, v.42, p.1277-1282, set. 2007 ASB2012-12382	N	LIT
OECD: KIIA 6.5.3	Cowell, J. E.	1986	Determination of Glyphosate and Ami-nomethylphosphonic acid residues in citrus fruit and process fractions following post-directed treatment with Roundup herbicide Report No.: MSL-6194 GLP: no not published RIP9501261	Y	MOD
OECD: KIIA 6.5.3	Kunda, U. S.	1990	Glyphosate residues in or on corn grits and flour following preharvest applications of Roundup herbicide to corn Report No.: MSL-9797 GLP: yes not published RIP9501258	Y	MOD
OECD: KIIA 6.5.3	Kunstman, J. L.	1987	Glyphosate residues in corn grain fractions following preharvest applications to corn with Roundup herbicide Report No.: MSL-6917 GLP: yes not published RIP9501257	Y	MOD
OECD: KIIA 6.5.3	Kunstman, J. L.; Steinmetz, J. R.; Farmer, P. S.; Blount, L. M.	1983	Glyphosate residues in soybeans and soybean fractions following recirculating sprayer and preharvest topical treatment with Roundup herbicide Report No.: MSL-3259 GLP: no not published RIP9501259	Y	MOD
OECD: KIIA 6.5.3	Low, F.; Shaw, I.; Gerrard, J.	2005	The effect of Saccharomyces cerevisiae on the stability of the herbicide Glyphosate during bread leavening GLP: no published: Letters in Applied Microbiology 2005, 40, 133-137 ASB2012-12449	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
OECD: KIIA 6.5.3	Mueth, M. G.	1988	Glyphosate residues in potatoes and processed fractions of potatoes after treatment with Roundup herbicide Report No.: MSL-7877 GLP: yes not published RIP9501263	Y	MOD
OECD: KIIA 6.5.3	Oppenhuizen, M. E.	1995	Magnitude of Glyphosate residues in corn processed commodities following preharvest application of Roundup herbicide Report No.: MSL-13655 GLP: yes not published RIP9800120	Y	MOD
OECD: KIIA 6.6.2	Brightwell, B.; Cooper, B. J.	1978	Uptake and metabolism of Glyphosate in root, leaf and cereal type rotation crops Report No.: MSL 0882 GLP: no not published RIP9501200	Y	MOD
OECD: KIIA 6.6.2	Hatterman, D. R.	1998	LX1146-02 (Glyphosate technical) confined rotational crop study on lettuce, radish, and wheat in California Report No.: 1651-91-146-01-09B-17 GLP: yes not published RIP2003-1112	Y	MOD
OECD: KIIA 6.6.2	McMullan, P. C.; Honegger, J. L.; Logusch, E. W.	1990	Confined rotational crops study of Glyphosate - Part II: Quantitation, characterization and identification of Glyphosate and its metabolites in rotational crops Report No.: MSL 9811 GLP: yes not published RIP9501202	Y	MOD
OECD: KIIA 6.6.2	Nicholls, R. G.	1990	Confined rotational crops study of Glyphosate - Part I: In-field portion Report No.: MSL 9810 GLP: yes not published RIP9501201	Y	MOD
OECD: KIIA 6.6.2	Spillner, C. J.; Bowler, D. T.	1993	[^{14}C-Anion] Glyphosate-trimesium: Confined accumulation studies on rotational crops Report No.:RR92-096B GLP: yes not published RIP9500018	Y	SYN

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
OECD: KIIA 6.6.2	Suba, L. A.	1976	Metabolism of CP 67573 in representative vegetables and rotation crops Report No.: 406 GLP: no not published RIP9501199	Y	MOD
OECD: KIIA 6.9	Gimou, M. M.; Charrondiere, U. R.; Leblanc, J. C. et al.	2008	Dietary exposure to pesticide residues in Yaounde: the Cameroonian total diet study GLP: no published: Food Additives and Contaminants, April 2008, 25(4): 458-471 ASB2012-12422	N	LIT
OECD: KIIA 6.9	Harris, C. A.; Gaston, C. P.	2004	Effects of refining predicted chronic dietary intakes of pesticide residues: A case study using Glyphosate GLP: no published: Food Additives and Contaminants, 2004, Vol. 21, No. 9, 857-864 ASB2012-12428	N	LIT
OECD: KIIA 6.9	Nougadère, A., Reninger, J.-C., Volatier, J.-L., Leblanc, J.-C.	2011	Chronic dietary risk characterization for pesticide residues: A ranking and scoring method integrating agricultural uses and food contamination data GLP: no published: Food and Chemical Toxicology 49 (2011) 1484-1510 ASB2012-11982	N	LIT

Codes of owner

ALK	Alkaloida Europe
CHE	Cheminova A/S
DPB	DuPont de Nemours
ADM	ADAMA Agan Ltd
INV	INVISTA Textiles (U.K.) Ltd
LIT	Published literature
MOD	Monsanto Europe S.A./N.V.
SYN	Syntana Handelsgesellschaft

Studies marked in yellow were not part of the dossier for the renewal
Studies marked in grey are considered to be not acceptable

B.7.18 Appendix – Supervised field trials

B.7.18.1 Tree nuts

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360~~355.7~~ g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : European Hazel

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.219 , MLL 30219, 89, 1A Italy Nola 1988-11-01	San Giovanni	1) 1976 (planting) 2) 3)	0.72	200	0.36	1988-08-02[4)]	BBCH 75-76	nut	<0.05 <0.05 <0.05	<0.05 <0.05 <0.05	20 20 20	4) spraying analytical method: XA 001 (HPLC with OPA), LOQ 0.05 mg/kg, 3 replicate field samples, max. sample storage: 2 months RIP9501283
MLL 30.219 , MLL 30219, 89, 1B Italy Nola 1988-11-01	San Giovanni	1) 1976 (planting) 2) 3)	1.4	200	0.72	1988-08-02[4)]	BBCH 75-76	nut	<0.05 <0.05 <0.05	<0.05 <0.05 <0.05	20 20 20	4) spraying analytical method: XA 001 (HPLC with OPA), LOQ 0.05 mg/kg, 3 replicate field samples, max. sample storage: 2 months RIP9501283

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.219 , MLL 30219, 89, 2A Italy Nola 1988-11-01	San Giovanni	1) 1976 (planting) 2) 3)	4.3	200	2.2	1988-06-16[4)]	BBCH 75-76	nut	<0.05 <0.05 <0.05	<0.05 <0.05 <0.05	67 67 67	4) spraying analytical method: XA 001 (HPLC with OPA), LOQ 0.05 mg/kg, 3 replicate field samples, max. sample storage: 2 months RIP9501283
MLL 30.219 , MLL 30219, 89, 2B Italy Nola 1988-11-01	San Giovanni	1) 1976 (planting) 2) 3)	8.6	200	4.3	1988-06-16[4)]	BBCH 75-76	nut	<0.05 <0.05 <0.05	<0.05 <0.05 <0.05	67 67 67	4) spraying analytical method: XA 001 (HPLC with OPA), LOQ 0.05 mg/kg, 3 replicate field samples, max. sample storage: 2 months RIP9501283
MLL 30.219 , MLL 30219, 89, 2A Italy Vetralla 1988-11-01	Tanda Romana	1) 1976 (planting) 2) 3)	0.72	200	0.36	1988-08-26	BBCH 75-76	nut	<0.05 <0.05 <0.05	<0.05 <0.05 <0.05	44 44 44	4) spraying analytical method: XA 001 (HPLC with OPA), LOQ 0.05 mg/kg, 3 replicate field samples, max. sample storage: 1 month, RIP9501283
MLL 30.219 , MLL 30219, 89, 2B Italy Vetralla 1988-11-01	Tanda Romana	1) 1976 (planting) 2) 3)	1.4	200	0.72	1988-08-26[4)]	BBCH 75-76	nut	<0.05 <0.05 <0.05	<0.05 <0.05 <0.05	20 20 20	4) spraying analytical method: XA 001 (HPLC with OPA), LOQ 0.05 mg/kg, 3 replicate field samples, max. sample storage 1 month, RIP9501283

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.219 , MLL 30219, 89, 2C Italy Vetralla 1988-11-01	Tanda Romana	1) 1976 (planting) 2) 3)	4.3	200	2.2	1988-06-17[4)]	BBCH 75-76	nut	<0.05 <0.05 <0.05	<0.05 <0.05 <0.05	90 90 90	4) spraying analytical method: XA 001 (HPLC with OPA), LOQ 0.05 mg/kg, 3 replicate field samples, max. sample storage: 1 month, RIP9501283
MLL 30.219 , MLL 30219, 89, 2D Italy Vetralla 1988-11-01	Tanda Romana	1) 1976 (planting) 2) 3)	8.6	200	4.3	1988-06-17[4)]	BBCH 75-76	nut	<0.05 <0.05 <0.05	<0.05 <0.05 <0.05	90 90 90	4) spraying analytical method: XA 001 (HPLC with OPA), LOQ 0.05 mg/kg, 3 replicate field samples, max. sample storage: 1 month, RIP9501283

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.2 Pome fruit

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Apple

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA
(Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report MLL 30,053, A 42, 1 Denmark Lille Salby 1980-07-28	Johnathan	1) (planting) 2) 3)	1.8	no data		1979-08-05[4)]	BBCH 61	fruit	<0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05	8 30 47 75	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501235
report MLL 30,053, A 42, 2 Denmark Western Part of Zealand 1980-07-28	Cortland	1) (planting) 2) 3)	2.9	no data		1979-09-20[4)]	BBCH 7	fruit	<0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05	0 4 6 9	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501235

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360~~355.7~~ g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Apple

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 22, addendum to report A9, 526/75-1,4,5, plot 1 Denmark Tystofte 1978-04-26	Lobo	1) (planting) 2) 3)	1.5	no data		1974-11-18[4)]	no data	fruit	<0.05	<0.05	1387	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501218
report A 22, addendum to report A9, 526/75-1,4,5, plot 2 Denmark Tystofte 1978-04-26	Lobo	1) (planting) 2) 3)	3.0	no data		1974-11-18[4)]	no data	fruit	<0.05	<0.05	1387	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501218
report A 22, addendum to report A9, 526/75-76-1,4,5, plot 1 Denmark Tystofte 1978-04-26	Lobo	1) (planting) 2) 3)	1.5	no data		1975-11-06[4)]	no data	fruit	<0.05	<0.05	314	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501218

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 22, addendum to report A9, 526/75-76-1,4,5, plot 2 Denmark Tystofte 1978-04-26	Lobo	1) (planting) 2) 3)	3.0	no data		1975-11-06[4)]	no data	fruit	<0.05	<0.05	314	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501218
report A 22, addendum to report A9, 526/75-76-8,9, plot 1 Denmark Tystofte 1978-04-26	Lobo	1) (planting) 2) 3)	1.5	no data		1976-03-29[4)]	no data	fruit	<0.05	<0.05	170	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501218
report A 22, addendum to report A9, 526/75-76-8,9, plot 2 Denmark Tystofte 1978-04-26	Lobo	1) (planting) 2) 3)	3.0	no data		1976-03-29[4)]	no data	fruit	<0.05	<0.05	170	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501218
report A 22, addendum to report A9, 526/75-8,9, plot 1 Denmark Tystofte 1978-04-26	Lobo	1) (planting) 2) 3)	1.5	no data		1975-05-05[4)]	no data	fruit	<0.05	<0.05	133	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501218

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 22, addendum to report A9, 526/75-8,9, plot 2 Denmark Tystofte 1978-04-26	Lobo	1) (planting) 2) 3)	3.0	no data		1975-05-05[4)]	no data	fruit	<0.05	<0.05	133	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501218
report A 22, addendum to report A9, 528/72-74-1,6,7, plot 1 Denmark Guldhojgärd, Tornemark 1978-04-26	Grästen	1) (planting) 2) 3)	1.5	no data		1974-03-14[4)]	no data	fruit	<0.05	<0.05	172	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501218
report A 22, addendum to report A9, 528/72-74-1,6,7, plot 2 Denmark Guldhojgärd, Tornemark 1978-04-26	Grästen	1) (planting) 2) 3)	2.0	no data		1974-03-14[4)]	no data	fruit	<0.05	<0.05	172	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501218
report A 22, addendum to report A9, 528/72-75-1,6,7 plot 1 Denmark Guldhojgärd, Tornemark 1978-04-26	Grästen	1) (planting) 2) 3)	1.5	no data		1975-03-18[4)]	no data	fruit	<0.05	<0.05	170	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501218

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 22, addendum to report A9, 528/72-75-1,6,7 plot 2 Denmark Guldhojgärd, Tornemark 1978-04-26	Grästen	1) (planting) 2) 3)	2.0	no data		1975-03-18[4)]	no data	fruit	<0.05	<0.05	170	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501218
report A 9, 1, plot 1 Germany 49328 Bergsieker, Meesdorf 1976-09-15	Cox Orange	1) (planting) 2) 3)	1.8	no data		1976-05-20[4)]	BBCH 65	fruit	<0.05	<0.05	56	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 1, plot2 Germany 49328 Bergsieker, Meesdorf 1976-09-15	Cox Orange	1) (planting) 2) 3)	3.6	no data		1976-05-20[4)]	BBCH 65	fruit	<0.05	<0.05	56	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 10, plot 1 United Kingdom Wisbech, Norfolk 1976-09-15	Grenadier	1) (planting) 2) 3)	1.8	no data		1976-04-29[4)]	BBCH 69	fruit	<0.05	<0.05	88	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 9, 10, plot 2 United Kingdom Wisbech, Norfolk 1976-09-15	Grenadier	1) (planting) 2) 3)	3.6	no data		1976-04-29[4)]	BBCH 69	fruit	<0.05	<0.05	88	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 11, plot1 Netherlands Ophemert, de Betuwe 1976-09-15	Cox	1) (planting) 2) 3)	1.4	no data		1976-06-11[4)]	no data	fruit	<0.05	<0.05	88	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 11, plot 2 Netherlands Ophemert, de Betuwe 1976-09-15	Cox	1) (planting) 2) 3)	2.2	no data		1976-06-11[4)]	no data	fruit	<0.05	<0.05	88	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 12 Netherlands Heesselt, de Betuwe 1976-09-15	Cox	1) (planting) 2) 3)	1.4	no data		1975-10-27[4)]	no data	fruit	<0.05	<0.05	316	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 9, 13 Netherlands Varik, de Betuwe 1976-09-15	Laxton	1) (planting) 2) 3)	1.4 2.2	no data		1975-10-27 1976-07-29[4)]	no data	fruit	<0.05	<0.05	40	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 2, plot 1 Germany 49328 Bergsieker, Meesdorf 1976-09-15	Goldparmäne	1) (planting) 2) 3)	1.8	no data		1976-05-20[4)]	BBCH 65	fruit	<0.05	<0.05	56	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 2, plot 2 Germany 49328 Bergsieker, Meesdorf 1976-09-15	Goldparmäne	1) (planting) 2) 3)	3.6	no data		1976-05-20[4)]	BBCH 65	fruit	<0.05	<0.05	56	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 28 France 49330 Etriche 1976-09-15	Golden Delicious	1) (planting) 2) 3)	3.6	no data		1976-07-01[4)]	BBCH 75	fruit	<0.05	<0.05	26	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 9, 29, plot 1 France 67700 Grands Fruits 1976-09-15	Golden Delicious	1) (planting) 2) 3)	4.5	no data		1976-06-22[4)]	BBCH 73	fruit	<0.05	<0.05	36	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 29, plot 2 France 67700 Grands Fruits 1976-09-15	Golden Delicious	1) (planting) 2) 3)	9.0	no data		1976-06-22[4)]	BBCH 73	fruit	<0.05	<0.05	36	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 3, plot 1 Germany Lauffach 1976-09-15	Cox Orange	1) (planting) 2) 3)	1.8	no data		1976-05-25[4)]	BBCH 69	fruit	<0.05	<0.05	50	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 3, plot 2 Germany Lauffach 1976-09-15	Cox Orange	1) (planting) 2) 3)	3.6	no data		1976-05-25[4)]	BBCH 69	fruit	<0.05	<0.05	50	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 9, 30 France 21690 St.Germain 1976-09-15	Golden Delicious	1) (planting) 2) 3)	4.3	no data		1976-05-07[4)]	BBCH 69	fruit	<0.05	<0.05	81	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 31, plot 1 France 45370 Mareau aux Pres 1976-09-15	Reinette du Canada	1) (planting) 2) 3)	4.5	no data		1976-05-20[4)]	BBCH 69	fruit	<0.05	<0.05	69	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 31, plot 2 France 45370 Mareau aux Pres 1976-09-15	Reinette du Canada	1) (planting) 2) 3)	9.0	no data		1976-05-20[4)]	BBCH 69	fruit	<0.05	<0.05	69	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 32 Belgium Zepperen 1976-09-15	Golden Delicious	1) (planting) 2) 3)	1.8	no data		1975-10-28[4)]	no data	fruit	<0.05	<0.05	323	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 9, 33 Belgium Vilvoorde 1976-09-15	Golden Delicious	1) (planting) 2) 3)	1.4	no data		1976-04-09[4)]	no data	fruit	<0.05	<0.05	159	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 34 Belgium Schepdaal 1976-09-15	Golden Delicious	1) (planting) 2) 3)	1.4	no data		1976-03-28[4)]	no data	fruit	<0.05	<0.05	171	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 35 Belgium Stabroek 1976-09-15	Golden Delicious	1) (planting) 2) 3)	1.8	no data		1975-09-26[4)]	no data	fruit	<0.05	<0.05	355	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 4, plot1 Germany Langendiebach, Hanau 1976-09-15	Cox Orange	1) (planting) 2) 3)	1.8	no data		1976-05-25[4)]	BBCH 69	fruit	<0.05	<0.05	50	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 9, 4, plot 2 Germany Langendiebach, Hanau 1976-09-15	Cox Orange	1) (planting) 2) 3)	3.6	no data		1976-05-25[4)]	BBCH 69	fruit	<0.05	<0.05	50	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 5, plot 1 Germany Lauffach 1976-09-15	Goldparmäne	1) (planting) 2) 3)	1.8	no data		1976-05-25[4)]	BBCH 69	fruit	<0.05	<0.05	50	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 5, plot 2 Germany Lauffach 1976-09-15	Goldparmäne	1) (planting) 2) 3)	3.6	no data		1976-05-25[4)]	BBCH 69	fruit	<0.05	<0.05	50	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 6, plot 1 Germany Langendiebach, Hanau 1976-09-15	Goldparmäne	1) (planting) 2) 3)	1.8	no data		1976-05-25[4)]	BBCH 69	fruit	<0.05	<0.05	50	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 9, 6, plot 2 Germany Langendiebach, Hanau 1976-09-15	Goldparmäne	1) (planting) 2) 3)	3.6	no data		1976-05-25[4)]	BBCH 69	fruit	<0.05	<0.05	50	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 7, plot 1 United Kingdom Wisbech, Norfolk 1976-09-15	Bramley's Seedling	1) (planting) 2) 3)	1.8	no data		1976-04-29[4)]	BBCH 69	fruit	<0.05	<0.05	88	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 7, plot 2 United Kingdom Wisbech, Norfolk 1976-09-15	Bramley's Seedling	1) (planting) 2) 3)	3.6	no data		1976-04-29[4)]	BBCH 69	fruit	<0.05	<0.05	88	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 8, plot 1 United Kingdom Wisbech, Norfolk 1976-09-15	Discovery	1) (planting) 2) 3)	1.8	no data		1976-04-29[4)]	BBCH 69	fruit	<0.05	<0.05	88	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 9, 8, plot 2 United Kingdom Wisbech, Norfolk 1976-09-15	Discovery	1) (planting) 2) 3)	3.6	no data		1976-04-29[4)]	BBCH 69	fruit	<0.05	<0.05	88	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 9, plot 1 United Kingdom Wisbech, Norfolk 1976-09-15	Laxton Superb	1) (planting) 2) 3)	1.8	no data		1976-04-29[4)]	BBCH 69	fruit	<0.05	<0.05	88	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 9, plot 2 United Kingdom Wisbech, Norfolk 1976-09-15	Laxton Superb	1) (planting) 2) 3)	3.6	no data		1976-04-29[4)]	BBCH 69	fruit	<0.05	<0.05	88	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360~~355.7~~ g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Alkaloida

Active ingredient : Glyphosate
Crop / crop group : Apple

Submission date : 2012-09-25

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
94035/01-FPAP, 1 B Germany 75245 Neulingen-Bauschlott 1995-04-12	Golden Delicious	1) 1960 (sowing) 2) 3) 1994-10-13	2.5	285	0.88	1994-06-24[4)]	BBCH 74	fruit	<0.02	<0.02	88	4) spraying with protective screen 2 replicate field samples, analytical method: DFG method 405 (HPLC-FD), LOQ 0.02 mg/kg, max. sample storage: 4 months, RIP9501344
94035/01-FPAP, 2 B Germany 88069 Tettnang 1995-04-12	Cox Orange	1) 1989 (sowing) 2) 3) 1994-09	2.0	230	0.88	1994-06-28[4)]	BBCH 74-75	fruit	<0.02	<0.02	76	4) spraying with protective screen, 2 replicate field samples, analytical method: DFG method 405 (HPLC-FD), LOQ 0.02 mg/kg, max. sample storage: 4 months, RIP9501344

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
94035/01-FPAP, 3 B Germany 74613 Öhringen-Untermaßholder-bach 1995-04-12	Boskop	1) 1976 (planting) 2) 3) 1994-09-20	1.5	190	0.81	1994-06-284)	BBCH 74	fruit	<0.02	<0.02	83	4) spraying with protective screen, 2 replicate field samples, analytical method: DFG method 405 (HPLC-FD), LOQ 0.02 mg/kg, max. sample storage: 4 months, RIP9501344
94035/01-FPAP, 4 B Germany 53340 Meckenheim 1995-04-12	Elstar	1) 1985 (planting) 2) 3) 1994-09-15	1.9	219	0.89	1994-07-074)	BBCH 74	fruit	<0.02	<0.02	67	4) spraying with protective screen, 2 replicate field samples, analytical method: DFG method 405 (HPLC-FD), LOQ 0.02 mg/kg, max. sample storage: 4 months, RIP9501344

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 694 g/kg
Formulation (e.g. WP) : WG
Commercial product (name) : Glistar
Applicant : Alkaloida

Active ingredient : Glyphosate
Crop / crop group : Apple

Submission date : 2012-09-25

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
94035/01-FPAP, 1 A Germany 75245 Neulingen-Bauschlott 1995-04-12	Golden Delicious	1) 1960 (sowing) 2) 3) 1994-10-13	2.0	250	0.82	1994-06-24[4)]	BBCH 74	fruit	<0.02 <0.02	<0.02 <0.02	88 88	4) spraying with protective screen, 2 replicate field samples, analytical method: DFG method 405 (HPLC-FD), LOQ 0.02 mg/kg, max. sample storage: 4 months, RIP9501344
94035/01-FPAP, 2 A Germany 88069 Tettnang 1995-04-12	Cox Orange	1) 1989 (sowing) 2) 3) 1994-09	1.9	219	0.87	1994-06-28[4)]	BBCH 74-75	fruit	<0.02 <0.02	<0.02 <0.02	76 76	4) spraying with protective screen, 2 replicate field samples, analytical method: DFG method 405 (HPLC-FD), LOQ 0.02 mg/kg, max. sample storage: 4 months, RIP9501344

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
94035/01-FPAP, 3 A Germany 74613 Öhringen-Untermaßholder-bach 1995-04-12	Boskop	1) 1976 (planting) 2) 3) 1994-09-20	1.9	218	0.87	1994-06-28[4)]	BBCH 74	fruit	<0.02 <0.02	<0.02 <0.02	83 83	4) spraying with protective screen, at 2012-09-25 2 replicate field samples, analytical method: DFG method 405 (HPLC-FD), LOQ 0.02 mg/kg, max. sample storage: 4 months, RIP9501344
94035/01-FPAP, 4 A Germany 53340 Meckenheim 1995-04-12	Elstar	1) 1985 (planting) 2) 3) 1994-09-15	1.8	218	0.83	1994-07-07[4)]	BBCH 74	fruit	<0.02 <0.02	<0.02 <0.02	67 67	4) spraying with protective screen, at 2012-09-25 2 replicate field samples, analytical method: DFG method 405 (HPLC-FD), LOQ 0.02 mg/kg, max. sample storage: 4 months, RIP9501344

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360~~355.7~~ g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate-Dimethylammonium
Crop / crop group : Apple

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate-dimethylammonium
8.2 AMPA (Aminomethyl phosphonic acid), calculated as Glyphosate-dimethylammonium

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 9, 14 France 73150 Valdition 1976-09-15	Starkrinson	1) (planting) 2) 3)	4.3	no data		1976-06-18[4)]	no data	fruit	<0.05	<0.05	40	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 15 France 17190 Les Vignieres 1976-09-15	Starkrinson	1) (planting) 2) 3)	4.5 3.6	no data		1973-07-061976-07-02[4)]	BBCH 74	fruit	<0.05	<0.05	26	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 9, 16 France 84300 Cavallion 1976-09-15	Golden Delicious	1) (planting) 2) 3)	4.3	no data		1976-06-07[4)]	BBCH 72	fruit	<0.05	<0.05	51	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 17 France 13610 Le Puy Ste, Reparade 1976-09-15	Granny Smith	1) (planting) 2) 3)	4.5	no data		1976-04-29[4)]	BBCH 69	fruit	<0.05	<0.05	90	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 20 France 84270 Vedene 1976-09-15	Granny Smith	1) (planting) 2) 3)	4.3	no data		1976-06-02[4)]	BBCH 72	fruit	<0.05	<0.05	61	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 21 France 84250 Le Thor 1976-09-15	Golden Delicious	1) (planting) 2) 3)	4.3	no data		1976-06-18[4)]	BBCH 74	fruit	<0.05	<0.05	45	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 9, 22 France 82600 Aucamville 1976-09-15	Granny Smith	1) (planting) 2) 3)	4.3	no data		1976-06-11[4)]	BBCH 72	fruit	<0.05	<0.05	40	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 23 France 81310 Peyrole 1976-09-15	Golden Delicious	1) (planting) 2) 3)	4.3	no data		1976-06-21[4)]	BBCH 75	fruit	<0.05	<0.05	30	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 24, plot1 France 82200 Moissac 1976-09-15	Golden Delicious	1) (planting) 2) 3)	4.5	no data		1976-06-10[4)]	BBCH 72	fruit	<0.05	<0.05	42	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 24, plot2 France 82200 Moissac 1976-09-15	Golden Delicious	1) (planting) 2) 3)	9.0	no data		1976-06-10[4)]	BBCH 72	fruit	<0.05	<0.05	42	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 9, 25 France 81800 Rabastens 1976-09-15	Golden Delicious	1) (planting) 2) 3)	4.5	no data		1976-06-14[4)]	BBCH 71	fruit	<0.05	<0.05	38	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 26 France 65230 Villemur 1976-09-15	Richared	1) (planting) 2) 3)	4.3	no data		1976-06-04[4)]	BBCH 71	fruit	<0.05	<0.05	47	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 27, plot1 France 81500 Lavaur 1976-09-15	Reine des reinettes	1) (planting) 2) 3)	4.5	no data		1976-06-17[4)]	BBCH 76	fruit	<0.05	<0.05	35	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 27, plot2 France 81500 Lavaur 1976-09-15	Reine des reinettes	1) (planting) 2) 3)	9.0	no data		1976-06-17[4)]	BBCH 76	fruit	<0.05	<0.05	35	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : GLYFOS
Applicant : Cheminova A/S

Active ingredient : Glyphosate-Dimethylammonium
Crop / crop group : Apple

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate-dimethylammonium
8.2 AMPA (Aminomethyl phosphonic acid), calculated as Glyphosate-dimethylammonium

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
IF-93/04768-01, A plot2 Portugal 4520 Souto Ereira 1994-07-05	Golden Delicious	1) (planting) 2) 3) 1993-09-06	3.5	600	0.58	1993-06-08[4)]	no data	fruit	<0.03 <0.03 <0.03 <0.03		28 45 65 90	4) spraying height of tree 2.50m analytical method: DFG-Methode Nr.405, (HPLC/fluorescence detector), LOQ: 0.03 mg/kg, max. sample storage: 2 months RIP9501330
IF-93/04768-01, A plot4 Portugal 4520 Souto Ereira 1994-07-05	Golden Delicious	1) (planting) 2) 3) 1993-09-06	3.7	600	0.61	1993-06-08[4)]	no data	fruit	<0.03 <0.03 <0.03 <0.03		28 45 65 90	4) spraying height of tree 2.50m analytical method: DFG-Methode Nr.405, (HPLC/fluorescence detector), LOQ: 0.03 mg/kg, max. sample storage: 2 months RIP9501330

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
IF-93/04768-01, B plot2 Portugal Costa 1994-07-05	Golden Delicious	1) (planting) 2) 3) 1993-09-06	3.4	600	0.56	1993-06-08[4)]	no data	fruit	<0.03 <0.03 <0.03 <0.03		28 45 65 90	4) spraying height of tree 2.50m analytical method: DFG-Methode Nr.405, (HPLC/fluorescence detector), LOQ: 0.03 mg/kg, max. sample storage: 2 months RIP9501330
IF-93/04768-01, B plot4 Portugal Costa 1994-07-05	Golden Delicious	1) (planting) 2) 3) 1993-09-06	3.5	600	0.58	1993-06-08[4)]	no data	fruit	<0.03 <0.03 <0.03 <0.03		28 45 65 90	4) spraying height of tree 2.50m analytical method: DFG-Methode Nr.405, (HPLC/fluorescence detector), LOQ: 0.03 mg/kg, max. sample storage: 2 months RIP9501330

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360~~355.7~~ g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Pear

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 9, 18, plot 1 France 84460 Cheval-Blanc 1976-09-15	Dr.Guyot/Cognassier de Provence	1) (planting) 2) 3)	4.3	no data		1976-06-24[4)]	BBCH 74	fruit	<0.05	<0.05	40	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 18, plot 2 France 84460 Cheval-Blanc 1976-09-15	Dr.Guyot/Cognassier de Provence	1) (planting) 2) 3)	8.6	no data		1976-06-24[4)]	BBCH 74	fruit	<0.05	<0.05	40	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211
report A 9, 19 France 17190 Les Vignieres 1976-09-15	Dr.Guyot/Cognassier de Provence	1) (planting) 2) 3)	4.5 3.6 3.6	no data		1973-06-281974-07-111976-07-02[4)]	BBCH 74	fruit	<0.05	<0.05	32	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 9, 36 Italy Salerano 1976-09-15	Passacrassana	1) (planting) 2) 3)	2.7	no data		1975-07-16[4)]	BBCH 74	fruit	<0.05	<0.05	84	4) spraying analytical method: (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: no data RIP9501211

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.3 Stone fruit

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Active ingredient : Glyphosat (Isopropyl-salt)
Crop / crop group : Cherries

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Submission date : 2005-04-25

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup Ultra
Applicant : MON Monsanto Europe S.A.

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1Glyphosat
8.2 AMPA

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)		PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Study No. EA 000181, GE01 DE-47918 Tönisvorst 2001-04-03	Morellenfeuer	1) 1980 2) 2000-04-08 -04-24 3) 2000-07-12	4.2	486	0.86	2000-06-28[4)]	BBCH 85	whole fruits	<0.05	<0.05	13	4) spraying onto the soil under the canopy of the trees analytical method: GC/MS-MS LOQ: 0.05 mg/kg, height of trees: 3 m, max sample storage: 5 months RIP2001-558
Study No. EA 000181, GE02 DE-53501 Echendorf 2001-04-03	Schatten-morelle	1) 1980 2) 2000-04-10 -04-29 3) 2000-07-14	4.4	512	0.86	2000-06-28[4)]	BBCH 85	whole Fruits	<0.05	<0.05	14	4) spraying onto the soil under the canopy of the trees analytical method: GC/MS-MS LOQ: 0.05 mg/kg, height of trees: 3 m, max sample storage: 5 months RIP2001-558

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup Ultra
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Peach

Submission date : 2005-04-25

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
EA000182, 633 GLY, SP01 Spain 46170 Villar del Arzobispo, Valencia 2001-03-21	Sudanell	1) 1997 (planting) 2) 2000-04-15 3) 2000-07-24	4.3	500	0.86	2000-07-17[4)]	BBCH 78	fruit, without stone fruit, whole	<0.05 <0.05	<0.05 <0.05	7 7	4) spraying analytical method: GC-MS/MS, LOQ 0.05 mg/kg, max. sample storage: 5 months, RIP2001-557
EA000182, 633 GLY, SP02 Spain 46011 Chulilla, Valencia 2001-03-21	Sudanell	1) 1985 (planting) 2) 2000-04-04 3) 2000-07-21	4.3	465	0.93	2000-07-14[4)]	BBCH 78	fruit, without stone fruit, whole	<0.05 <0.05	<0.05 <0.05	7 7	4) spraying analytical method: GC-MS/MS, LOQ 0.05 mg/kg, max. sample storage: 5 months, RIP2001-557

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.4 Grapes

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 180 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : SWING
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Grape Vine

Submission date : 1989-09-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
003118 Germany 6730 Neustadt 1988-12-27	Riesling (white variety)	1) 1977 (sowing) 2) 1988-06-14 - 1988-06-19 3) 1988-09-01	0.72 0.72	400 400	0.18 0.18	1988-05-181988-09-01[4)]	BBCH 85	grapes (normal, low, ground), all of them	<0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05	0 4 8 14 21	4) spraying analytical method: XA001 (HPLC) for both substances, LOQ 0.05 mg/kg, max. sample storage: 7 months, sampling according height of vine RIP9501285 ASB2009-2247

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
003119 Germany 6741 Roschbach 1988-12-27	Müller-Thurgau (white variety)	1) 1976 (sowing) 2) 1988-06-14 - 1988-06-18 3) 1988-08-25	0.72 0.72	400 400	0.18 0.18	1988-05-191988-08-25[4)]	BBCH 85	grapes (normal, low), all of them	<0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05	0 4 8 14 21	4) spraying analytical method: XA001 (HPLC) for both substances, LOQ 0.05 mg/kg, max. sample storage: 7 months, sampling according height of vine RIP9501285 ASB2009-2248
005723 Germany 8702 Thüngersheim 1989-04-10	Müller-Thurgau (white variety)	1) 1983 (sowing) 2) 1988-06-17 - 1988-06-25 3) 1988-10-05	0.72 0.72	400 400	0.18 0.18	1988-06-141988-08-01[4)]	BBCH 77-79	grapes, normal low ground normal low ground all of them	 <0.05 0.20 <0.05 <0.05 <0.05 0.07 <0.05 <0.05 <0.05 <0.05 <0.05	 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05	 0 0 0 4 4 4 9 15 21 65	4) spraying analytical method: XA001 (HPLC) for both substances, LOQ 0.05 mg/kg, max. sample storage: 8 months, sampling according height of vine RIP9501285 ASB2009-2255

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
005724 Germany 7102 Weinsberg 1989-04-27	Riesling (white variety)	1) 1976 (sowing) 2) 1988-06-14 - 1988-06-23 3) 1988-10-20	0.72	400	0.18	1988-09-27[4)]	BBCH 85	grapes, normal low ground normal low ground normal low ground normal low ground normal low ground	 <0.05 0.30 0.50 <0.05 <0.05 0.07 0.20 <0.05 <0.05 <0.05 <0.05 0.30 <0.05 <0.05 0.10	 <0.05 <0.05 <0.05 <0.05 <0.05 	 0 0 0 5 5 5 9 9 9 14 14 14 21 21 21	4) spraying analytical method: XA001 (HPLC) for both substances, LOQ 0.05 mg/kg, max. sample storage: 5 months, sampling according height of vine RIP9501285 ASB2009-2258
01879 Germany 7800 Freiburg 1989-04-10	Blauer Spät-burgunder (red variety)	1) 1981 (sowing) 2) 1988-06-15 - 1988-06-20 3) 1988-10-18	0.72 0.72	400 400	0.18 0.18	1988-04-261988-07-29[4)]	BBCH 77-79	grapes (normal, low), all of them	<0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05	0 3 10 14 21	4) spraying analytical method: XA001 (HPLC) for both substances, LOQ 0.05 mg/kg, max. sample storage: 7 months, sampling according height of vine RIP9501285 ASB2009-2249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
005725 Germany 6551 Siefersheim 1989-04-27	Morio Muskat (white variety)	1) 1971 (sowing) 2) 1988-06-21 - 1988-06-28 3) 1988-09-29	0.72 0.72	400 400	0.18 0.18	1988-05-251988-08-16[4)]	BBCH 79-81	grapes	<0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05	0 3 6 13 20	4) spraying analytical method: XA001 (HPLC) for both substances, LOQ 0.05 mg/kg, max. sample storage: 8 months RIP9501285 ASB2009-2265

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Grape Vine

Submission date : 1977-12-16

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 17, addendum to report No.2+9, 1 Germany 6551 Neu Bamberg 1977-01-01	Ortega (white variety)	1) 1962 (planting) 2) 1977-06 - 1977-07 3) 1977-10-05	3.6	600	0.60	1977-08-15[4)]	BBCH 75	grape segments	<0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05	10 21 31 41 51	4) spraying analytical method: AG1-X8+AG 50-X8 (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: 3 months ASB2009-5277 (report form) ASB2009-5276
report A 17, addendum to report No.2+9, 2 Germany 6551 Wonsheim 1977-01-01	Müller Thurgau (white variety)	1) 1965 (planting) 2) 1977-07 3) 1977-10-05	3.6	600	0.60	1977-08-15[4)]	BBCH 75	grape segments	<0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05	10 21 31 41 51	4) spraying analytical method: AG1-X8+AG 50-X8 (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: 3 months ASB2009-5279 (report form) ASB2009-5276

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 17, addendum to report No.2+9, 3 Germany 6521 Dalsheim 1977-01-01	Ortega (white variety)	1) 1966 (planting) 2) 1977-06 - 1977-07 3) 1977-10-05	3.6	600	0.60	1977-08-15[4)]	BBCH 75	grape segments	<0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05	10 21 31 41 51	4) spraying analytical method: AG1-X8+AG 50-X8 (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: 3 months ASB2009-5278 (report form) ASB2009-5276

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Grape Vine

Submission date : 1975-03-04

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 2, G-R-V-23-T Germany 65345 Rauenthal, Reinhessen 1975-01-17	Riesling (white variety)	1) (planting) 2) 3)	3.6	600	0.60	1974-07-08[4)]	BBCH 71	grape seg-ments	<0.05	<0.05	78	4) spraying analytical method: AG1-X8+AG 50-X8 (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: 3 months ASB2009-5293 (report form) ASB2009-5294
report A 2, G-R-V-24-T Germany 67246 Dirmstein Pfalz 1975-01-17	Müller-Thurgau (white variety)	1) (planting) 2) 3)	3.6	600	0.60	1974-08-14[4)]	BBCH 71	grape seg-ments	<0.05	<0.05	41	4) spraying analytical method: AG1-X8+AG 50-X8 (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: 3 months ASB2009-5293 (report form) ASB2009-5294

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
report A 2, G-R-V-25-T Germany 97332 Astheim, Franken 1975-01-17	Müller-Thurgau (white variety)	1) (planting) 2) 3)	3.6	600	0.60	1974-07-02[4)]	BBCH 71	grape seg-ments	<0.05	<0.05	85	4) spraying analytical method: AG1-X8+AG 50-X8 (GLC-FPD), LOQ: 0.05 mg/kg, max. sample storage: 3 months ASB2009-5293 (report form) ASB2009-5294

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.5 Olives

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : MON 65040
Applicant : Monsanto SA (MOD)

Active ingredient : Glyphosate
Crop/crop group : Olive

Submission date : 1995-09-01

Indoors/outdoors : Outdoors (European South)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)		DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL30.319 IT-Francavilla, Puglia 1995-04-27	Nardo	1) – 2) – 3) 1993-01-27	1.44	200	0.72	1993-01-13	no data	olives, taken from tree olives, ground fallen olive oil	<0.05 [1] 0.4 [1] <0.05 [2]	<0.05 [3] <0.05 [3] <0.05 [4]	6 6 6	Results are corrected for method recovery [1] : (80%) [2] : (92%) [3] : (59%) [4] : (78%) analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg, max sample storage: 4 months RIP9501290

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)		DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL30.319 IT-Francavilla, Puglia 1995-04-27	Nardo	1) – 2) – 3) 1993-01-27	1.44	200	0.72	1993-01-13	no data	olives, taken from tree olives, ground fallen olive oil	0.08 [1] 0.4 [1] <0.05 [2]	<0.05 [3] <0.05 [3] <0.05 [4]	13 13 13	Results are corrected for method recovery [1] : (80%) [2] : (92%) [3] : (59%) [4] : (78%) analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg, max sample storage: 4 months RIP9501290
MLL30.319 IT-San Casciano, Toscana 1995-04-27	Leccino	1) – 2) – 3) 1992-12-18	1.44	300	0.48	1992-12-04	no data	olives, taken from tree olives, ground fallen olive oil	<0.05 [1] <0.05 [1] <0.05 [2]	<0.05 [3] <0.05 [3] <0.05 [4]	7 7 7	Results are corrected for method recovery [1] : (80%) [2] : (92%) [3] : (59%) [4] : (78%) analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg, max sample storage: 4 months RIP9501290
MLL30.319 IT-San Casciano, Toscana 1995-04-27	Leccino	1) – 2) – 3) 1992-12-18	1.44	300	0.48	1992-12-04	no data	olives, taken from tree olives, ground fallen olive oil	<0.05 [1] <0.05 [1] <0.05 [2]	<0.05 [3] <0.05 [3] <0.05 [4]	14 14 14	Results are corrected for method recovery [1] : (80%) [2] : (92%) [3] : (59%) [4] : (78%) analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg, max sample storage: 4 months RIP9501290

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)		DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
A 20-II ES-Santaella, Cordoba 1995-04-27	Nevadillo	1) – 2) – 3) 1977-11-14	2.16	–	no data	1977-10-25	no data	olives	<0.05	<0.05	20	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217
A 20-II ES-Santaella, Cordoba 1995-04-27	Nevadillo	1) – 2) – 3) 1977-11-14	4.32	–	no data	1977-10-25	no data	olives	<0.05	<0.05	20	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217
A 20-II ES-Santaella, Cordoba 1995-04-27	Lechin	1) – 2) – 3) 1977-11-14	2.16	–	no data	1977-10-25	no data	olives	<0.05	<0.05	20	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217
A 20-II ES-Santaella, Cordoba 1995-04-27	Lechin	1) – 2) – 3) 1977-11-14	4.32	–	no data	1977-10-25	no data	olives	<0.05	<0.05	20	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)		DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
A 20-II ES-Santaella, Cordoba 1995-04-27	Hojiblanco	1) – 2) – 3) 1977-11-14	2.16	–	no data	1977-10-25	no data	olives	<0.05	<0.05	20	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217
A 20-II ES-Santaella, Cordoba 1995-04-27	Hojiblanco	1) – 2) – 3) 1977-11-14	4.32	–	no data	1977-10-25	no data	olives	<0.05	<0.05	20	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217
A 20-II ES-Santaella, Cordoba 1995-04-27	Ocal	1) – 2) – 3) 1977-11-14	2.16	–	no data	1977-10-25	no data	olives	<0.05	<0.05	20	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217
A 20-II ES-Santaella, Cordoba 1995-04-27	Ocal	1) – 2) – 3) 1977-11-14	4.32	–	no data	1977-10-25	no data	olives	<0.05	<0.05	20	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217
A 20-II ES-Savilla 1995-04-27	Manzanilla	1) – 2) – 3) 1977-11-09	2.16	–	no data	1977-10-13	no data	olives	<0.05	<0.05	27	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)		DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
A 20-II ES-Savilla 1995-04-27	Manzanilla	1) – 2) – 3) 1977-11-09	4.32	–	no data	1977-10-13	no data	olives	<0.05	<0.05	27	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217
A 20-II ES-Savilla 1995-04-27	Gordal	1) – 2) – 3) 1977-11-09	2.16	–	no data	1977-10-13	no data	olives	<0.05	<0.05	27	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217
A 20-II ES-Savilla 1995-04-27	Gordal	1) – 2) – 3) 1977-11-09	4.32	–	no data	1977-10-13	no data	olives	<0.05	<0.05	27	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217
A 20-II ES-La Canaleja-Montilla 1995-04-27	Hojiblanco	1) – 2) – 3) 1977-01-15	2.16	–	no data	1976-11-22	no data	olive oil	<0.05 <0.05	<0.05 <0.05	38 54	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217
A 20-II ES-La Canaleja-Montilla 1995-04-27	Hojiblanco	1) – 2) – 3) 1977-01-15	4.32	–	no data	1976-11-22	no data	olive oil	<0.05 <0.05	<0.05 <0.05	38 54	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)		DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
A 20-II ES-Aguadulce, Sevilla 1995-04-27	Hojiblanco	1) – 2) – 3) 1976-12-18	2.16	–	no data	1976-11-16	no data	olive oil	<0.05 <0.05	<0.05 <0.05	30 32	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217
A 20-II ES-La Canaleja-Montilla 1995-04-27	Hojiblanco	1) – 2) – 3) 1976-12-18	4.32	–	no data	1976-11-16	no data	olive oil	<0.05 <0.05	<0.05 <0.05	30 32	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217
A 20-II ES-LA Canaleja-Montilla 1995-04-27	Lechin	1) – 2) – 3) 1976-12-30	2.16	–	no data	1976-11-22	no data	olive oil	<0.05	<0.05	38	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217
A 20-II ES-LA Canaleja-Montilla 1995-04-27	Lechin	1) – 2) – 3) 1976-12-30	4.32	–	no data	1976-11-22	no data	olive oil	<0.05	<0.05	38	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)		DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
A 20-II ES-Utera- Servilla 1995-04-27	Lechin	1) – 2) – 3) 1976-12-09	2.16	–	no data	1976-11-09	no data	olive oil	<0.05	<0.05	30	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217
A 20-II ES-Utera- Servilla 1995-04-27	Lechin	1) – 2) – 3) 1976-12-09	4.32	–	no data	1976-11-09	no data	olive oil	<0.05	<0.05	30	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217
A 20-II ES-EL Frontanal, Montilla 1995-04-27	Nevadillo	1) – 2) – 3) 1977-01-15	2.16	–	no data	1976-11-22	no data	olive oil	<0.05	<0.05	54	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217
A 20-II ES-EL Frontanal, Montilla 1995-04-27	Nevadillo	1) – 2) – 3) 1977-01-15	4.32	–	no data	1976-11-22	no data	olive oil	<0.05	<0.05	54	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217
A 20-II ES-Aguilarde, La Frontera 1995-04-27	Picudo	1) – 2) – 3) –	2.16	–	no data	1976-11-22	no data	olive oil	<0.05	<0.05	30	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)		DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
A 20-II ES-Aguilarde, La Frontera 1995-04-27	Picudo	1) – 2) – 3) –	4.32	–	no data	1976-11-22	no data	olive oil	<0.05	<0.05	30	analytical method: GC-FPD LOQ: 0.05 mg/kg, recoveries Glyphosate: 69%, AMPA 54%, max sample storage: 12 months RIP9501217

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Active ingredient : Glyphosate
Crop/crop group : Olive

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Submission date : May 1997

Content of a.i. (g/kg or g/l) : 120 g/l
Formulation (e.g. WP) : SC
Commercial product (name) : Star
Applicant : Industrias Afrasa S.A.

Indoors/outdoors : Outdoors (European South)
Other a. i. in formulation (common name and content) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
ES-Rosell Castel-lón	Picual	1) – 2) – 3) 1992-01-21	0.36	30	1.2	1992-01-14	no data	fruit, without stone	1 0.88 0.76 0.63	0 1 3 7	Spraying on soil; fruit from soil.

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 120 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Sting* SE
Applicant : Monsanto

Active ingredient : Glyphosate
Crop/crop group : Olive

Submission date : May 1997

Indoors/outdoors : Outdoors (European South)
Other a. i. in formulation (common name and content) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
90-Gly-02 ES- Pedrera, (Servilla)	Hojiblanca	1) – 2) – 3) 1990-02-08	0.36	200	0.18	1990-02-01	no data	fruit olive oil	0.9 1.1 <0.05 <0.05	1 7 1 7	ground spraying; ground laying fruit analytical method: XA001 (HPLC with OPA) LOQ: mg/kg, recoveries for AMPA in fruit 53, 58 and 70%, next validated level 0.1 mg/kg, max. sample storage: 16 months RIP9501289
90-Gly-02 ES- Puebla de Cazalla, (Servilla)	Lechin	1) – 2) – 3) 1990-02-08	0.36	200	0.18	1990-02-01	no data	fruit olive oil	1.1 1.2 <0.05 <0.05	1 7 1 7	ground spraying; ground laying fruit analytical method: XA001 (HPLC with OPA) LOQ: mg/kg, recoveries for AMPA in fruit 53, 58 and 70%, next validated level 0.1 mg/kg, max. sample storage: 16 months RIP9501289

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30297 ES-Torreblasco-pedro, (Jaén)	Picual	1) nodata 2) nodata 3) 1992-01-15	0.36	200	0.18	1991-12-13	no data	fruit olive oil	1.8 0.8 <0.05 <0.05	0 32 0 32	ground spraying; ground laying fruit analytical method: XA001 (HPLC with OPA) LOQ: mg/kg, recoveries for AMPA in fruit 53, 58 and 70%, next validated level 0.1 mg/kg, max. sample storage: 16 months RIP9501289
MLL 30297 ES-Sierra Yegua, (Málaga)	Hojiblanca	1) nodata 2) nodata 3) 1992-02-14	0.36	200	0.18	1992-01-20	no data	fruit olive oil	0.3 0.4 <0.05 <0.05	0 24 0 24	ground spraying; ground laying fruit analytical method: XA001 (HPLC with OPA) LOQ: mg/kg, recoveries for AMPA in fruit 53, 58 and 70%, next validated level 0.1 mg/kg, max. sample storage: 16 months RIP9501289
MLL 30297 ES-Linares, (Jaén)	Picual	1) nodata 2) nodata 3) 1992-02-14	0.36	200	0.18	1992-01-15	no data	fruit olive oil	0.2 2 <0.05 <0.05	0 30 0 30	ground spraying; ground laying fruit analytical method: XA001 (HPLC with OPA) LOQ: mg/kg, recoveries for AMPA in fruit 53, 58 and 70%, next validated level 0.1 mg/kg, max. sample storage: 16 months RIP9501289

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30297 ES-Córdoba, (Córdoba)	Hojiblanca	1) nodata 2) nodata 3) 1992-02-25	0.36	200	0.18	1992-01-15	no data	fruit olive oil	0.08 0.4 <0.05 <0.05	0 41 0 41	ground spraying; ground laying fruit analytical method: XA001 (HPLC with OPA) LOQ: mg/kg, recoveries for AMPA in fruit 53, 58 and 70%, next validated level 0.1 mg/kg, max. sample storage: 16 months RIP9501289

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360~~355.7~~ g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Olive

Submission date : 1997-05-13

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30469 , 95-GLY-20 SP, AP/3065/ME/1 A Spain 29250 Chartaojal, Provincia de Malaga 1996-05-01	Hojiblanca	1) 1965 (planting) 2) 3) 1995-12-05	2.2	400	0.54	1995-11-07[4)]	BBCH 79-81	fruit, from ground fruit, picked crude oil refined oil	9.8 <0.05 <0.05 <0.05 ~~<0.05~~ <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 ~~<0.05~~ <0.05 <0.05	0 28 0 28 28 28	4) spraying analytical method: XA 001 (HPLC with OPA), LOQ 0.05 mg/kg, max. sample storage: 7 months, RIP9700184
MLL 30469 , 95-GLY-20 SP, AP/3065/ME/1 B Spain 29250 Chartaojal, Provincia de Malaga 1996-05-01	Hojiblanca	1) 1965 (planting) 2) 3) 1995-12-05	2.2	400	0.54	1995-11-21[4)]	BBCH 79-81	fruit, from ground fruit, picked crude oil refined oil	12.6 <0.05 <0.05 <0.05 ~~<0.05~~ <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 ~~<0.05~~ <0.05 <0.05	0 14 0 14 14 14	4) spraying analytical method: XA 001 (HPLC with OPA), LOQ 0.05 mg/kg, max. sample storage: 7 months, RIP9700184

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30469 , 95-GLY-20 SP, AP/3065/ME/1 C Spain 29250 Chartaojal, Provincia de Malaga 1996-05-01	Hojiblanca	1) 1965 (planting) 2) 3) 1995-12-05	2.2	400	0.54	1995-11-28[4)]	BBCH 79-81	fruit, from ground fruit, picked crude oil refined oil	9.2 0.14 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05	0 7 0 7 7 7	4) spraying analytical method: XA 001 (HPLC with OPA), LOQ 0.05 mg/kg, max. sample storage: 7 months, RIP9700184
MLL 30469 , 95-GLY-20 SP, AP/3065/ME/2 A Spain 14100 La Carlota, Provincia de Cordoba 1996-05-01	Picual	1) 1988 (planting) 2) 3) 1995-12-05	2.2	400	0.54	1995-11-09[4)]	BBCH 79-81	fruit, from ground fruit, picked crude oil	12.6 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05	0 28 0 28 28	4) spraying analytical method: XA 001 (HPLC with OPA), LOQ 0.05 mg/kg, max. sample storage: 6 months, RIP9700184
MLL 30469 , 95-GLY-20 SP, AP/3065/ME/2 B Spain 14100 La Carlota, Provincia de Cordoba 1996-05-01	Picual	1) 1988 (planting) 2) 3) 1995-12-07	2.2	400	0.54	1995-11-23[4)]	BBCH 79-81	fruit, from ground fruit, picked crude oil refined oil	5.8 0.11 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05	0 14 0 14 14 14	4) spraying analytical method: XA 001 (HPLC with OPA), LOQ 0.05 mg/kg, max. sample storage: 6 months, RIP9700184

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30469 , 95-GLY-20 SP, AP/3065/ME/2 C Spain 14100 La Carlota, Provincia de Cordoba 1996-05-01	Picual	1) 1988 (planting) 2) 3) 1995-12-08	2.2	400	0.54	1995-12-01[4)]	BBCH 79-81	fruit, from ground fruit, picked crude oil refined oil	4.3 0.11 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05	0 6 0 6 6 6	4) spraying analytical method: XA001 (HPLC with OPA), LOQ 0.05 mg/kg, max. sample storage: 5 months, RIP9700184
MLL 30469 , 95-GLY-20 SP, AP/3065/ME/3 A Spain 14640 Villa del Rio, Provincia del Cordoba 1996-05-01	Picual	1) (planting) 2) 3) 1995-12-04	2.2	400	0.54	1995-11-06[4)]	BBCH 79-81	fruit, from ground fruit, picked crude oil refined oil	9.3 <0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05	0 28 0 28 28 28	4) spraying analytical method: XA001 (HPLC with OPA), LOQ 0.05 mg/kg, max. sample storage: 6 months, RIP9700184
MLL 30469 , 95-GLY-20 SP, AP/3065/ME/3 B Spain 14640 Villa del Rio, Provincia del Cordoba 1996-05-01	Picual	1) (planting) 2) 3) 1995-12-04	2.2	400	0.54	1995-11-20[4)]	BBCH 79-81	fruit, from ground fruit, picked crude oil refined oil	11.8 0.13 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05	0 14 0 14 14 14	4) spraying analytical method: XA001 (HPLC with OPA), LOQ 0.05 mg/kg, max. sample storage: 6 months, RIP9700184

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30469 , 95-GLY-20 SP, AP/3065/ME/3 C Spain 14640 Villa del Rio, Provincia del Cordoba 1996-05-01	Picual	1) (planting) 2) 3) 1995-12-04	2.2	400	0.54	1995-11-27[4)]	BBCH 79-81	fruit, from ground fruit, picked crude oil refined oil	5.9 0.53 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05	0 7 0 7 7 7	4) spraying analytical method: XA001 (HPLC with OPA), LOQ 0.05 mg/kg, max. sample storage: 6 months, RIP9700184
MLL 30469 , 95-GLY-20 SP, AP/3065/ME/4 A Spain 23400 Ubeda, Provincia de Jaen 1996-05-01	Picual	1) 1971 (planting) 2) 3) 1995-12-06	2.2	400	0.54	1995-11-08[4)]	BBCH 79-81	fruit, from ground fruit, picked crude oil refined oil	6.4 <0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05	0 28 0 28 28 28	4) spraying analytical method: XA001 (HPLC with OPA), LOQ 0.05 mg/kg, max. sample storage: 6 months, RIP9700184
MLL 30469 , 95-GLY-20 SP, AP/3065/ME/4 B Spain 23400 Ubeda, Provincia de Jaen 1996-05-01	Picual	1) 1971 (planting) 2) 3) 1995-12-06	2.2	400	0.54	1995-11-22[4)]	BBCH 79-81	fruit, from ground fruit, picked crude oil refined oil	6.7 0.93 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05	0 14 0 14 14 14	4) spraying analytical method: XA001 (HPLC with OPA), LOQ 0.05 mg/kg, max. sample storage: 6 months, RIP9700184

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30469 , 95-GLY-20 SP, AP/3065/ME/4 C Spain 23400 Ubeda, Provincia de Jaen 1996-05-01	Picual	1) 1971 (planting) 2) 3) 1995-12-06	2.2	400	0.54	1995-11-29[4)]	BBCH 79-81	fruit, from ground fruit, picked crude oil refined oil	6.0 0.93 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05	0 7 0 7 7 7	4) spraying analytical method: XA001 (HPLC with OPA), LOQ 0.05 mg/kg, max. sample storage: 6 months, RIP9700184

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.6 Carrots

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360~~355.7~~ g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop/crop group : Carrot

Submission date : 1995-09-01

Indoors/outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A 16, CP 67573, 16 A France 44 La Planche 1977-10-31	no data	1) (sowing) 2) 3)	1.4			1977-03-18[4)]	BBCH 00	root body	<0.05		101	4) spraying, pre-seeding (10 days), analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery Glyphosat: 62%, AMPA: 48%, max. sample storage: 4 months RIP9501216
REPORT A 16, CP 67573, 16 B France 44 La Planche 1977-10-31	no data	1) (sowing) 2) 3)	4.3			1977-03-18[4)]	BBCH 00	root body	<0.05	<0.05	101	4) spraying, pre-seeding (10 days), analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery Glyphosat: 62%, AMPA: 48%, max. sample storage: 4 months RIP9501216

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A 16, CP 67573, 16 C France 44 La Planche 1977-10-31	no data	1) (sowing) 2) 3)	8.6			1977-03-18[4)]	BBCH 00	root body	<0.05	<0.05	101	4) spraying, pre-seeding (10 days), analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery Glyphosat: 62%, AMPA 48%, max. sample storage: 4 months RIP9501216

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : MON 52276, SL~~C~~ (360 g/l Glyphosate)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Carrot

Submission date : 2012-08-16

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./ hl							
	(a)	(b)				(c)		(a)			(d)	(e)
S11-00259-01 France 49650 Allonnes, Maine et Loire 2012-03-08	Montdibell	1) 2011-06-09 (planting) 2) 3) 2011-09-15	2.3	187	1.2	2011-06-14[4)]	BBCH 00	root body	<0.05	<0.05	93	4) spraying analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 4 months, ASB2012-12369
S11-00259-02 Poland 64-600 Uscikowo, Wielkopolska 2012-03-08	Laguna	1) 2011-04-18 (planting) 2) 3) 2011-09-15	2.3	193	1.2	2011-04-19[4)]	BBCH 00	root body	<0.05	<0.05	176	4) spraying analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 3 months, ASB2012-12369

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360~~355.7~~ g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Carrot

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A 16, CP 67573, 13 A France 73544 Gould 1977-10-31	no data	1) (sowing) 2) 3)	1.4			1977-03-15[4)]	BBCH 00	root body	<0.05	<0.05	119	4) spraying, pre-seeding (same day), analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery Glyphosat: 62%, AMPA 48%, max. sample storage: 4 months RIP9501216
REPORT A 16, CP 67573, 13 B France 73544 Gould 1977-10-31	no data	1) (sowing) 2) 3)	4.3			1977-03-15[4)]	BBCH 00	root body	0.070	<0.05	119	4) spraying, pre-seeding (same day), analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery Glyphosat: 62%, AMPA 48%, max. sample storage: 4 months RIP9501216

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A 16, CP 67573, 13 C France 73544 Gould 1977-10-31	no data	1) (sowing) 2) 3)	8.6			1977-03-15[4)]	BBCH 00	root body	0.080	<0.05	119	4) spraying, pre-seeding (same day), analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery Glyphosat: 62%, AMPA 48%, max. sample storage: 4 months RIP9501216
REPORT A 16, CP 67573, 14 A France 31 Toulouse 1977-10-31	no data	1) (sowing) 2) 3)	1.4			1977-05-31[4)]	BBCH 00	root body	<0.05	<0.05	104	4) spraying, pre-seeding (one day), analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery Glyphosat: 62%, AMPA 48%, max. sample storage: 1 months RIP9501216
REPORT A 16, CP 67573, 14 B France 31 Toulouse 1977-10-31	no data	1) (sowing) 2) 3)	4.3			1977-05-31[4)]	BBCH 00	root body	<0.05	<0.05	104	4) spraying, pre-seeding (one day), analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery Glyphosat: 62%, AMPA 48%, max. sample storage: 1 months RIP9501216

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A 16, CP 67573, 14 C France 31 Toulouse 1977-10-31	no data	1) (sowing) 2) 3)	8.6			1977-05-31[4)]	BBCH 00	root body	<0.05	<0.05	104	4) spraying, pre-seeding (one day), analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery Glyphosat: 62%, AMPA 48%, max. sample storage: 1 months RIP9501216
REPORT A 16, CP 67573, 15 A France 84210 Althen les Paluds 1977-10-31	no data	1) (sowing) 2) 3)	1.4			1977-03-18[4)]	BBCH 00	root body	<0.05	<0.05	140	4) spraying, pre-seeding (one month), analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery Glyphosat: 62%, AMPA 48%, max. sample storage: 2 months RIP9501216
REPORT A 16, CP 67573, 15 B France 84210 Althen les Paluds 1977-10-31	no data	1) (sowing) 2) 3)	4.3			1977-03-18[4)]	BBCH 00	root body	<0.05	<0.05	140	4) spraying, pre-seeding (one month), analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery Glyphosat: 62%, AMPA 48%, max. sample storage: 2 months RIP9501216

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A 16, CP 67573, 15 C France 84210 Althen les Paluds 1977-10-31	no data	1) (sowing) 2) 3)	8.6			1977-03-18[4)]	BBCH 00	root body	<0.05		140	4) spraying, pre-seeding (one month), analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery Glyphosat: 62%, AMPA 48%, max. sample storage: 2 months RIP9501216

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : MON 52276, SL~~C~~ (360 g/l Glyphosate)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Carrot

Submission date : 2012-08-16

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
S11-00259-04 France 31700 Balgnac, Haute-Garonne 2012-03-08	Maestro	1) 2011-05-25 (planting) 2) 3) 2011-10-14	2.5	210	1.2	2011-05-30[4)]	BBCH 00-03	root body	<0.05	<0.05	137	4) spraying analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 3 months, ASB2012-12369
S11-00259-05 Spain 03400 Villena, Alicante 2012-03-08	Maestro	1) 2011-04-13 (planting) 2) 3) 2011-09-21	2.1	169	1.2	2011-04-20[4)]	BBCH 00-03	root body	<0.05	<0.05	154	4) spraying analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 4 months, ASB2012-12369

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.7 Potato

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : MON 52276, SL~~C~~ (360 g/l Glyphosate)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Potato

Submission date : 2012-08-16

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
S11-00258-01 France 67870 Griesheim, Bas-Rhin 2012-03-08	Charlotte	1) 2011-03-12 (planting) 2) 2011-05-30 - 2011-06-20 3) 2011-07-26	2.2	175	1.2	2011-03-29[4)]	BBCH 00	tuber	<0.05	<0.05	119	4) spraying analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 7 months, ASB2012-12368
S11-00258-02 Germany 21739 Dollern, Lower Saxony 2012-03-08	Milva	1) 2011-04-17 (planting) 2) 2011-06-20 - 2011-07-03 3) 2011-09-05	2.3	184	1.2	2011-04-20[4)]	BBCH 00	tuber	<0.05	<0.05	138	4) spraying analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 5 months, ASB2012-12368

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : MON 52276, SL~~C~~ (360 g/l Glyphosate),
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Potato

Submission date : 2012-08-16

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation
(content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
S11-00258-03 Italy 82290 Meauzac, Midi Pyrenées 2012-03-08	Noisette	1) 2011-07-12 (planting) 2) 2011-08 - 2011-08 3) 2011-11-03	2.2	187	1.2	2011-07-12[4)]	BBCH 00	tuber	<0.05	<0.05	114	4) spraying analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 3 months, ASB2012-12368
S11-00258-04 Italy 40024 Gaiana, Bologna 2012-03-08	Primura	1) 2011-03-15 (planting) 2) 2011-05 - 2011-05 3) 2011-06-24	2.4	200	1.2	2011-03-18[4)]	BBCH 00	tuber	<0.05	<0.05	98	4) spraying analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 8 months, ASB2012-12368

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.8 Bulb onions

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360~~355.7~~ g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop/crop group : Onion

Submission date : 1995-09-01

Indoors/outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Report No. A 16, CP 67573, 09 A United Kingdom Drayton 1977-10-31	no data	1) (sowing) 2) 3)	1.8			1977-04-06[4)]	BBCH 00	bulb	<0.05	<0.05	89	4) spraying, post-drilling, pre-emergence analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 47%, for AMPA: 37%, max. sample storage: 3 months RIP9501216

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Report No. A 16, CP 67573, 09 B United Kingdom Drayton 1977-10-31	no data	1) (sowing) 2) 3)	3.6			1977-04-06[4)]	BBCH 00	bulb	<0.05	<0.05	89	4) spraying, post-drilling, pre-emergence analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 47%, for AMPA: 37%, max. sample storage: 3 months RIP9501216
Report No. A 16, CP 67573, 10 A United Kingdom Marlow 1977-10-31	no data	1) (sowing) 2) 3)	1.8			1977-04-15[4)]	BBCH 00	bulb	<0.05	<0.05	80	4) spraying, post-drilling, pre-emergence analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 47%, for AMPA: 37%, max. sample storage: 3 months RIP9501216
Report No. A 16, CP 67573, 10 B United Kingdom Marlow 1977-10-31	no data	1) (sowing) 2) 3)	3.6			1977-04-15[4)]	BBCH 00	bulb	<0.05	<0.05	80	4) spraying, post-drilling, pre-emergence analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 47%, for AMPA: 37%, max. sample storage: 3 months RIP9501216

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Report No. A 16, CP 67573, 11 A United Kingdom Fosdyke I 1977-10-31	no data	1) (sowing) 2) 3)	1.8			1977-04-22[4)]	BBCH 00	bulb	<0.05	<0.05	94	4) spraying, pre-seed and incorporation analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 47%, for AMPA: 37%, max. sample storage: 3 months RIP9501216
Report No. A 16, CP 67573, 11 B United Kingdom Fosdyke I 1977-10-31	no data	1) (sowing) 2) 3)	3.6			1977-04-22[4)]	BBCH 00	bulb	<0.05	<0.05	94	4) spraying, pre-seed and incorporation analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 47%, for AMPA: 37%, max. sample storage: 3 months RIP9501216
Report No. A 16, CP 67573, 12 A United Kingdom Fosdyke II 1977-10-31	no data	1) (sowing) 2) 3)	1.8			1977-04-22[4)]	BBCH 00	bulb	<0.05	<0.05	94	4) spraying, pre-seed and incorporation analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 47%, for AMPA: 37%, max. sample storage: 3 months RIP9501216

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Report No. A 16, CP 67573, 12 B United Kingdom Fosdyke II 1977-10-31	no data	1) (sowing) 2) 3)	3.6			1977-04-22[4)]	BBCH 00	bulb	<0.05	<0.05	94	4) spraying, pre-seed and incorporation analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 47%, for AMPA: 37%, max. sample storage: 3 months RIP9501216

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : MON 52276, SL~~C~~ (360 g/l Glyphosate)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Onion

Submission date : 2012-08-16

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
S11-00260-01 France 67370 Woellenheim, Bas-Rhin 2012-02-28	Takmark F1	1) 2011-03-22 (sowing) 2) 3) 2011-08-11	2.3	187	1.2	2011-04-04[4)]	BBCH 01	bulb	<0.05	<0.05	129	4) spraying analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 5 months, ASB2012-12370
S11-00260-02 Poland 64-600 Uscikowo, Wielkopolska 2012-02-28	Kristine	1) 2011-04-08 (sowing) 2) 3) 2011-09-01	2.4	203	1.2	2011-04-11[4)]	BBCH 03	bulb	<0.05	<0.05	143	4) spraying analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 5 months, ASB2012-12370

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360~~355.7~~ g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop/crop group : Onion

Submission date : 1995-09-01

Indoors/outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Report No. A 16, CP 67573, 08 A France 84440 Robion 1977-10-31	no data	1) (sowing) 2) 3)	1.4			1977-03-14[4)]	BBCH 00	bulb	<0.05	<0.05	120	4) spraying, pre-seeding (11 days) analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 47%, for AMPA: 37%, max. sample storage: 3 months RIP9501216

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Report No. A 16, CP 67573, 08 B France 84440 Robion 1977-10-31	no data	1) (sowing) 2) 3)	4.3			1977-03-14[4)]	BBCH 00	bulb	<0.05	<0.05	120	4) spraying, pre-seeding (11 days) analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 47%, for AMPA: 37%, max. sample storage: 3 months RIP9501216
Report No. A 16, CP 67573, 08 C France 84440 Robion 1977-10-31	no data	1) (sowing) 2) 3)	8.6			1977-03-14[4)]	BBCH 00	bulb	0.050	<0.05	120	4) spraying, pre-seeding (11 days) analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 47%, for AMPA: 37%, max. sample storage: 3 months RIP9501216

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : MON 52276, SL~~C~~ (360 g/l Glyphosate)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Onion

Submission date : 2012-08-16

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.3 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
S11-00260-04 Bulgaria 5570 Letnitsa, Letnitsa 2012-02-28	Stutgarten Rijsen	1) 2011-03-27 (sowing) 2) 3) 2011-08-27	2.4	193	1.2	2011-03-31[4)]	BBCH 00	bulb	<0.05	<0.05	149	4) spraying analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 5 months, ASB2012-12370
S11-00260-03 Spain 02140 El, Salobral, Albacete 2012-02-28	Eso	1) 2011-03-10 (sowing) 2) 3) 2011-09-01	2.4	205	1.2	2011-03-25[4)]	BBCH 03	bulb	<0.05	<0.05	154	4) spraying analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 5 months, ASB2012-12370

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.9 Tomato

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : MON 52276, SL~~C~~ (360 g/l Glyphosate)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Tomato

Submission date : 2012-08-16

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
S11-00267-01 Germany 69124 Kirchheim, Baden-Württemberg 2012-02-28	Vanessa	1) 2011-05-06 (sowing) 2) 2011-05-10 - 2011-07-31 3) 2011-08-04	2.3	187	1.2	2011-05-03[4)]	BBCH 01	fruit	<0.05	<0.05	93	4) spraying analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 5 months, ASB2012-12377
S11-00267-02 Hungary 2454 Ivánsca, Fejér 2012-02-28	Claudius F1	1) 2011-07 (sowing) 2) 2011-07 - 2011-08 3) 2011-10-14	2.3	185	1.2	2011-07-12[4)]	BBCH 01	fruit	<0.05	<0.05	94	4) spraying analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 5 months, ASB2012-12377

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.10 Cucumber

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : MON 52276, SL~~C~~ (360 g/l Glyphosate)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Cucumber

Submission date : 2012-08-16

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
S11-00261-04 Italy 04022 Fondi, Latina 2012-02-28	Ekron	1) 2011-05-15 (planting) 2) 2011-05 - 2011-06 3) 2011-06-23	2.2	181	1.2	2011-05-12[4)]	no data	fruit	<0.05	<0.05	42	4) spraying 3 days before planting the seedlings analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 5 months, ASB2012-12371

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.11 Courgette (Zucchini)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : MON 52276, SL~~C~~ (360 g/l Glyphosate)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Zucchini

Submission date : 2012-08-16

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
S11-00261-01 Germany 69124 Neurott, Baden-Württemberg 2012-02-28	Monitor	1) 2011-06-24 (planting) 2) 2011-08 - 2011-08 3) 2011-08-15	2.6	207	1.2	2011-07-21[4)]	no data	fruit	<0.05	<0.05	25	4) spraying 3 days before planting the seedlings, analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 5 months, ASB2012-12371

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : MON 52276, SL~~C~~ (360 g/l Glyphosate)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Zucchini

Submission date : 2012-08-16

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
S11-00261-03 France 66540 Baho, Pyrénées-Orientales 2012-02-28	Cigal F1	1) 2011-05-12 (planting) 2) 2011-07 3) 2011-06-30	2.2	180	1.2	2011-05-09[4)]	no data	fruit	<0.05	<0.05	52	4) spraying 3 days before planting the seedlings, analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 5 months, ASB2012-12371

Remarks: (a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.12 Cauliflower

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : MON 52276, SL~~C~~ (360 g/l Glyphosate)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Cauliflower

Submission date : 2012-08-16

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
S11-00263-01 France 91140 La Pointevine, Essonne 2012-02-28	Aviso	1) 2011-06-27 (planting) 2) 3)	2.3	182	1.2	2011-06-24[4)]	no data	flower	<0.05	<0.05	75	4) spraying 3 days before planting the seedlings analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 4 months, ASB2012-12373
S11-00263-02 Hungary 2454 Ivánsca, Fejér 2012-02-28	Cortes	1) 2011-07-15 (planting) 2) 3)	2.2	176	1.2	2011-07-12[4)]	no data	flower	<0.05	<0.05	125	4) spraying 3 days before planting the seedlings analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 4 months, ASB2012-12373

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : MON 52276, SL~~C~~ (360 g/l Glyphosate)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Cauliflower

Submission date : 2012-08-16

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
S11-00263-03 Italy 40057 Granarolo, Emilia Romagna 2012-02-28	Castellum	1) 2011-03-28 (planting) 2) 3)	2.4	203	1.2	2011-03-25[4)]	no data	flower	<0.05	<0.05	80	4) spraying 3 days before planting the seedlings analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 7 months, ASB2012-12373
S11-00263-04 Bulgaria 40024 Letnitsa, Letnitsa 2012-02-28	Snowball	1) 2011-07-16 (planting) 2) 3)	2.3	189	1.2	2011-07-13[4)]	no data	flower	<0.05	<0.05	120	4) spraying 3 days before planting the seedlings analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 2 months, ASB2012-12373

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.13 Head cabbage

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : MON 52276, SL~~C~~ (360 g/l Glyphosate)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : White Cabbage

Submission date : 2012-08-16

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
S11-00262-01 France 21130 Auxonne, Côte d'Or 2012-03-08	Padoc	1) 2011-07-11 (planting) 2) 3)	2.5	207	1.2	2011-07-08[4)]		head	<0.05	<0.05	67	4) spraying 3 days before planting the seedlings, analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 5 months, ASB2012-12372
S11-00262-02 Hungary 6635 Szegvár, Csongrád 2012-03-08	Pandion	1) 2011-09-10 (planting) 2) 3) 2011-12-13	2.1	172	1.2	2011-09-07[4)]		head	<0.05	<0.05	97	4) spraying 3 days before planting the seedlings, analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 2 months, ASB2012-12372

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : MON 52276, SL~~C~~ (360 g/l Glyphosate)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : White Cabbage

Submission date : 2012-08-16

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
S11-00262-03 Spain 50180 Utebo, Zaragoza 2012-03-08	Melissa	1) 2011-08-11 (planting) 2) 3) 2011-11-14	2.1	173	1.2	2011-08-08[4)]		head	<0.05	<0.05	98	4) spraying 3 days before planting the seedlings, analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 3 months, ASB2012-12372
S11-00262-04 Bulgaria 5570 Letnitsa, Letnitsa 2012-03-08	Kyose	1) 2011-08-20 (planting) 2) 3) 2011-11-24	2.3	190	1.2	2011-08-17[4)]		head	<0.05	<0.05	99	4) spraying 3 days before planting the seedlings, analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 3 months, ASB2012-12372

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.14 Lettuce

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360~~355.7~~ g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Head Lettuce

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Report No. A 16, CP 67573, 02 A France 44470 Thouaré 1977-10-31	no data (salat)	1) (planting) 2) 3)	1.4			1977-03-15	no data	plant	<0.05	<0.05	82	before planting (3 weeks), analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 59%, for AMPA: 42%, max. sample storage: 5 months RIP9501216

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Report No. A 16, CP 67573, 02 B France 44470 Thouaré 1977-10-31	no data (salat)	1) (planting) 2) 3)	4.3			1977-03-15	no data	plant	<0.05	<0.05	82	before planting (3 weeks), analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 59%, for AMPA: 42%, max. sample storage: 5 months RIP9501216
Report No. A 16, CP 67573, 02 C France 44470 Thouaré 1977-10-31	no data (salat)	1) (planting) 2) 3)	8.6			1977-03-15	no data	plant	<0.05	<0.05	82	before planting (3 weeks), analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 59%, for AMPA: 42%, max. sample storage: 5 months RIP9501216
Report No. A 16, CP 67573, 03 A France 44470 Carquefou 1977-10-31	no data (salat)	1) (planting) 2) 3)	1.4			1977-04-13[4)]	no data	plant	<0.05	<0.05	54	4) spraying before planting (8 days), analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 59%, for AMPA: 42%, max. sample storage: 4 months RIP9501216

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Report No. A 16, CP 67573, 03 B France 44470 Carquefou 1977-10-31	no data (salat)	1) (planting) 2) 3)	4.3			1977-04-13[4)]	no data	plant	<0.05	<0.05	54	4) spraying before planting (8 days), analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 59%, for AMPA: 42%, max. sample storage: 4 months RIP9501216
Report No. A 16, CP 67573, 03 C France 44470 Carquefou 1977-10-31	no data (salat)	1) (planting) 2) 3)	8.6			1977-04-13[4)]	no data	plant	<0.05	<0.05	54	4) spraying before planting (8 days), analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 59%, for AMPA: 42%, max. sample storage: 4 months RIP9501216
Report No. A 16, CP 67573, 04 A France 45 St. Benoit S/Loire 1977-10-31	no data (salat)	1) (planting) 2) 3)	1.4			1977-03-25[4)]	no data	plant	<0.05	<0.05	74	4) spraying, before planting analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 59%, for AMPA: 42%, max. sample storage: 4 months RIP9501216

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Report No. A 16, CP 67573, 04 B France 45 St. Benoit S/Loire 1977-10-31	no data (salat)	1) (planting) 2) 3)	4.3			1977-03-25[4)]	no data	plant	0.060	<0.05	74	4) spraying, before planting analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 59%, for AMPA: 42%, max. sample storage: 4 months RIP9501216
Report No. A 16, CP 67573, 04 C France 45 St. Benoit S/Loire 1977-10-31	no data (salat)	1) (planting) 2) 3)	8.6			1977-03-25[4)]	no data	plant	<0.05	<0.05	74	4) spraying, before planting analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 59%, for AMPA: 42%, max. sample storage: 4 months RIP9501216

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360~~355.7~~ g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Head Lettuce

Submission date : 1995-09-01

Indoors / outdoors : ?
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Report No. A 16, CP 67573, 05 A France Merville 1977-10-31	no data (salat)	1) (sowing) 2) 3)	1.4			1977-06-15[4)]	no data	plant	<0.05	<0.05	89	4) spraying pre-seeding (37 days before) analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 59%, for AMPA: 42%, postcode: no data, could be European North or South, max. sample storage: 1 months RIP9501216

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Report No. A 16, CP 67573, 05 B France Merville 1977-10-31	no data (salat)	1) (sowing) 2) 3)	4.3			1977-06-15[4)]	no data	plant	<0.05	<0.05	89	4) spraying pre-seeding (37 days before) analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 59%, for AMPA: 42%, postcode: no data, could be European North or South, max. sample storage: 1 months RIP9501216
Report No. A 16, CP 67573, 05 C France Merville 1977-10-31	no data (salat)	1) (sowing) 2) 3)	8.6			1977-06-15[4)]	no data	plant	<0.05	<0.05	89	4) spraying pre-seeding (37 days before) analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 59%, for AMPA: 42%, postcode: no data, could be European North or South, max. sample storage: 1 months RIP9501216

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : MON 52276, SL~~C~~ (360 g/l Glyphosate)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Head Lettuce

Submission date : 2012-08-16

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
S11-00264-03 France 66600 Rivesaltes, Pyrénées 2012-03-08	Sucrine	1) 2011-05-26 (planting) 2) 3)	2.3	197	1.2	2011-05-23[4)]	no data	head	<0.05	<0.05	38	4) spraying 3 days before planting the seedlings, analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 8 months, ASB2012-12374
S11-00264-04 Spain 46820 Anna, Valencia 2012-03-08	Cervantes	1) 2011-06-10 (planting) 2) 3)	2.4	203	1.2	2011-06-07[4)]	no data	head	<0.05	<0.05	48	4) spraying 3 days before planting the seedlings, analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 7 months, ASB2012-12374

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360~~355.7~~ g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Head Lettuce

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Report No. A 16, CP 67573, 01 A France 31 Toulouse 1977-10-31	no data (salat)	1) (sowing) 2) 3)	1.4			1977-03-04[4)]	no data	plant	0.10	<0.05	63	4) spraying, before planting (1 week) analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 59%, for AMPA: 42%, max. sample storage: 5 months RIP9501216

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Report No. A 16, CP 67573, 01 B France 31 Toulouse 1977-10-31	no data (salat)	1) (planting) 2) 3)	4.3			1977-03-04[4)]	no data	plant	<0.05	<0.05	63	4) spraying, before planting (1 week) analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 59%, for AMPA: 42%, max. sample storage: 5 months RIP9501216
Report No. A 16, CP 67573, 01 C France 31 Toulouse 1977-10-31	no data (salat)	1) (planting) 2) 3)	4.3			1977-03-04[4)]	no data					4) spraying, before planting (1 week) analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 59%, for AMPA: 42%, max. sample storage: 5 months RIP9501216
Report No. A 16, CP 67573, 06 A France 84300 Cavaillon 1977-10-31	no data (salat)	1) (planting) 2) 3)	1.4			1977-05-18[4)]	no data	plant	<0.05	<0.05	51	4) spraying, before planting (20 days) analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 59%, for AMPA: 42%, max. sample storage: 2 months RIP9501216

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Report No. A 16, CP 67573, 06 B France 84300 Cavaillon 1977-10-31	no data (salat)	1) (planting) 2) 3)	4.3			1977-05-18[4)]	no data	plant	<0.05	<0.05	51	4) spraying, before planting (20 days) analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 59%, for AMPA: 42%, max. sample storage: 2 months RIP9501216
Report No. A 16, CP 67573, 06 C France 84300 Cavaillon 1977-10-31	no data (salat)	1) (planting) 2) 3)	8.6			1977-05-18[4)]	no data	plant	<0.05	<0.05	51	4) spraying, before planting (20 days) analytical method: GLC/FPD, LOQ 0.05 mg/kg, recovery for Glyphosat: 59%, for AMPA: 42%, max. sample storage: 2 months RIP9501216

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : MON 52276, SL~~C~~ (360 g/l Glyphosate)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Cutting Lettuce

Submission date : 2012-08-16

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
S11-00264-01 Germany 69124 Neurott, Baden-Württemberg 2012-03-08	Kirinia	1) 2011-04-21 (planting) 2) 3)	2.5	200	1.2	2011-04-18[4)]	no data	leaf	<0.05	<0.05	42	4) spraying 3 days before planting the seedlings, analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 9 months, ASB2012-12374
S11-00264-02 United Kingdom (UK) C07 7KU Ardleigh, Essex 2012-03-08	Oak Leaf - Red	1) 2011-05-23 (planting) 2) 3)	2.3	190	1.2	2011-05-20[4)]	no data	leaf	<0.05	<0.05	56	4) spraying 3 days before planting the seedlings, analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 7 months, ASB2012-12374

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.15 Leek

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : MON 52276, SL~~C~~ (360 g/l Glyphosate)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Leek

Submission date : 2012-08-16

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
S11-00265-02 United Kingdom L40 0RF Burscough, Lancashire 2012-02-28	Parvella	1) 2011-03-25 (planting) 2) 3)	2.4	203	1.2	2011-03-23[4)]	no data	whole plant without root	<0.05	<0.05	183	4) spraying 3 days before planting the seedlings, analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 5 months, ASB2012-12375
S11-00265-01 France 69124 Auxone, Cote-d'Or 2012-02-28	Kenton	1) 2011-07-01 (planting) 2) 3)	2.5	213	1.2	2011-06-28[4)]	no data	whole plant, without root	<0.05	<0.05	77	4) spraying 3 days before planting the seedlings, analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 5 months, ASB2012-12375

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : MON 52276, SL~~C~~ (360 g/l Glyphosate)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Leek

Submission date : 2012-08-16

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
S11-00265-04 Bulgaria 5570 Letnitsa, Letnitsa 2012-02-28	Starozagorski 72	1) 2011-06-19 (planting) 2) 3)	2.1	173	1.2	2011-06-16[4)]	no data	whole plant without root	<0.05	<0.05	125	4) spraying 3 days before planting the seedlings, analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 3 months, ASB2012-12375
S11-00265-03 Italy 45020 Luisa, Rovigo 2012-02-28	Maxim	1) 2011-06-04 (planting) 2) 3)	2.3	190	1.2	2011-06-01[4)]	no data	whole plant, without root	<0.05	<0.05	65	4) spraying 3 days before planting the seedlings, analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 5 months, ASB2012-12375

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.16 Linseeds

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup Ultra
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Linseed

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.106, 52, 1 A Ireland Lambeg, Lisburn 1983-05-25	Reina	1) 1982-05-02 (sowing) 2) 3)	1.4	250	0.58	1982-08-06[4)]	BBCH 79-81	bolls fibre seed(s)	44.5 6.2 128	0.83 <0.2 1.3	46 46 46	4) spraying analytical method: HPLC with OPA/Ninhydrin, LOQ Glyphosat / AMPA: capsules: 8 mg/kg/0.4 mg/kg, seeds: 1 mg/kg / 0.2 mg/kg, plant: 1 mg/kg, 0.2 mg/kg, no control value for seed or capsules reported, max. sample storage: 18 months RIP9501266

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.106, 52, 1 B Ireland Lambeg, Lisburn 1983-05-25	Reina	1) 1982-05-02 (sowing) 2) 3)	2.9	250	1.2	1982-08-06[4)]	BBCH 79-81	bolls fibre seed(s)	45 5.9 189	0.90 0.21 1.9	46 46 46	4) spraying analytical method: HPLC with OPA/Ninhydrin, LOQ Glyphosat / AMPA: capsules: 8 mg/kg/0.4 mg/kg, seeds: 1 mg/kg / 0.2 mg/kg, plant: 1 mg/kg, 0.2 mg/kg, no control value for seed or capsules reported, max. sample storage: 18 months, RIP9501266
MLL 30.106, 52, 1 C Ireland Lambeg, Lisburn 1983-05-25	Reina	1) 1982-05-02 (sowing) 2) 3)	1.4	250	0.58	1982-08-17[4)]	BBCH 79-81	bolls fibre seed(s)	22.0 6.6 24.0	0.82 0.23 0.31	35 35 35	4) spraying analytical method: HPLC with OPA/Ninhydrin, LOQ Glyphosat / AMPA: capsules: 8 mg/kg/0.4 mg/kg, seeds: 1 mg/kg / 0.2 mg/kg, plant: 1 mg/kg, 0.2 mg/kg, no control value for seed or capsules reported, max. sample storage: 18 months, RIP9501266
MLL 30.106, 52, 1 D Ireland Lambeg, Lisburn 1983-05-25	Reina	1) 1982-05-02 (sowing) 2) 3)	2.9	250	1.2	1982-08-17[4)]	BBCH 79-81	bolls fibre seed(s)	22.5 6.3 23.7	0.40 <0.2 0.26	35 35 35	4) spraying analytical method: HPLC with OPA/Ninhydrin, LOQ Glyphosat / AMPA: capsules: 8 mg/kg/0.4 mg/kg, seeds: 1 mg/kg/0.2 mg/kg, plant: 1 mg/kg/0.2 mg/kg, no control value for seed or capsules reported, max. sample storage: 18 months, RIP9501266

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.106, 52, 2 A United Kingdom Arbroath, Scotland 1983-05-25	Regina	1) 1982-05-02 (sowing) 2) 3)	1.4	250	0.58	1982-07-27[4)]	BBCH 79-81	plant	1.0	<0.2	82	4) spraying analytical method: HPLC with OPA/ Ninhydrin, LOQ: Glyphosat, AMPA: plant: 1 mg/kg, 0.2 mg/kg, max. sample storage: 18 months RIP9501266
MLL 30.106, 52, 2 B United Kingdom Arbroath, Scotland 1983-05-25	Regina	1) 1982-05-02 (sowing) 2) 3)	2.9	250	1.2	1982-07-27[4)]	BBCH 79-81	plant	1.5	<0.2	82	4) spraying analytical method: HPLC with OPA/ Ninhydrin, LOQ Glyphosat, AMPA : plant: 1 mg/kg, 0.2 mg/kg, max. sample storage: 18 months RIP9501266
MLL 30.106, 52, 2 C United Kingdom Arbroath, Scotland 1983-05-25	Regina	1) 1982-05-02 (sowing) 2) 3)	1.4	250	0.58	1982-08-28[4)]	BBCH 79-81	bolls plant seed (RAC) seed oil cake, press	250 <1.0 1.3 1.3 <0.4 2.1	1.2 <0.2 <0.2 <0.2 <0.08 <0.2	5 50 5 5 5 5	4) spraying analytical method: HPLC with OPA/ Ninhydrin, LOQ Glyphosat / AMPA: capsules: 8 mg/kg/0.4 mg/kg, seeds: 1 mg/kg / 0.2 mg/kg, plant: 1 mg/kg / 0.2 mg/kg, control value for seeds 1.1 mg/kg, no control value for capsules, replicate field samples for seed, max. sample storage: 18 months RIP9501266

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.106, 52, 2 D United Kingdom Arbroath, Scotland 1983-05-25	Regina	1) 1982-05-02 (sowing) 2) 3)	2.9	250	1.2	1982-08-28[4)]	BBCH 79-81	bolls oil cake, press plant seed (RAC) seed	230 <0.4 2.4 1.9 2.2 2.3	1.2 <0.08 <0.2 <0.2 <0.2 <0.2	5 5 5 50 5 5	4) spraying analytical method: HPLC with OPA/ Ninhydrin, LOQ Glyphosat / AMPA: capsules: 8 mg/kg/0.4 mg/kg, seeds: 1 mg/kg / 0.2 mg/kg, plant: 1 mg/kg / 0.2 mg/kg, oil: 0.4 mg/kg/ 0.08 mg/kg, cake: 2 mg/kg/ 0.2 mg/kg, replicate field samples for seed, max. sample storage: 18 months, RIP9501266
MLL 30.106, 52, 3 A United Kingdom Backboath, Scotland 1983-05-25	Hera	1) 1982-05-02 (sowing) 2) 3)	1.4	250	0.58	1982-07-27[4)]	BBCH 79-81	plant	<1.0	<0.2	72	4) spraying analytical method: HPLC with OPA/ Ninhydrin, LOQ Glyphosat / AMPA: plant: 1 mg/kg/ 0.2 mg/kg, max. sample storage: 18 months, RIP9501266
MLL 30.106, 52, 3 B United Kingdom Backboath, Scotland 1983-05-25	Hera	1) 1982-05-02 (sowing) 2) 3)	2.9	250	1.2	1982-07-27[4)]	BBCH 79-81	plant	1.2	<0.2	72	4) spraying analytical method: HPLC with OPA/ Ninhydrin, LOQ Glyphosat / AMPA: plant: 1 mg/kg, 0.2 mg/kg, max. sample storage: 18 months, RIP9501266

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.106, 52, 3 C United Kingdom Backboath, Scotland 1983-05-25	Hera	1) 1982-05-02 (sowing) 2) 3)	1.4	250	0.58	1982-08-28[4)]	BBCH 79-81	bolls oil cake, press plant seed (RAC) seed	103 <0.4 <2.0 <1.0 1.3 1.0	0.99 <0.08 <0.2 <0.2 <0.2 <0.2	5 5 5 5 5 5	4) spraying analytical method: HPLC with OPA/Ninhydrin, LOQ Glyphosat / AMPA: capsules: 8 mg/kg/0.4 mg/kg, seeds: 1 mg/kg/0.2 mg/kg, plant: 1 mg/kg/0.2 mg/kg, oil: 0.4 mg/kg/0.08 mg/kg, cake: 2 mg/kg/0.2 mg/kg, replicate field samples for seed, control value for seed 2.3 g/kg, max. sample storage: 18 months, RIP9501266
MLL 30.106, 52, 3 D United Kingdom Backboath, Scotland 1983-05-25	Hera	1) 1982-05-02 (sowing) 2) 3)	2.9	250	1.2	1982-08-28[4)]	BBCH 79-81	bolls oil cake, press plant seed (RAC) seed	85.0 <0.4 6.3 <1.0 4.0 2.9	3.2 <0.08 <0.2 <0.2 <0.2 <0.2	5 5 5 5 5 5	4) spraying analytical method: HPLC with OPA/ Ninhydrin, LOQ Glyphosat / AMPA: capsules: 8 mg/kg / 0.4 mg/kg, seeds: 1 mg/kg / 0.2 mg/kg, plant: 1 mg/kg, 0.2 mg/kg, oil: 0.4 mg/kg/0.08 mg/kg, cake: 2 mg/kg/ 0.2 mg/kg, replicate field samples for seed, control value for seed 2.4 mg/kg, max. sample storage: 18 months, RIP9501266

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : CHE 3607
Applicant : Cheminova A/S

Active ingredient : Glyphosate
Crop / crop group : Linseed

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
IF-93/13836-01, A United Kingdom Melbourne, Derbyshire 1994-07-14	Flanders	1) 1992-04-19 (sowing) 2) 3)	1.4	200	0.72	1992-07-10[4)]	12 Turner	seed(s)	5.7 4.5 3.6	7 7 7	4) spraying treated with, analytical method: DFG No. 405 (HPLC/FPD), LOQ: 0.06 mg/kg, 3 replicate field samples, max. sample storage: 18 months, RIP9501325 (field) RIP9501326
IF-93/13836-01, B United Kingdom Melbourne, Derbyshire 1994-07-14	Flanders	1) 1992-04-19 (sowing) 2) 3)	0.72	200	0.36	1992-07-10[4)]	12 Turner	seed(s)	2.0 1.8 1.9	7 7 7	4) spraying treated with CHE 3607 SL (360 g a.i. /kg), analytical method: DFG No. 405 (HPLC/FPD), LOQ: 0.06 mg/kg 3 replicate field samples, max. sample storage: 18 months, RIP9501325 (field) RIP9501326

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.17 Oilseed rape

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : (MON 2139) Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Oilseed rape

Submission date : 2001-11-28

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 31336 99-GLY-08-B/F Trt 2 BE-5380 Franc Waret 15.07.2001	Olara	1) 01.09.1998 (planting) 2) – 3)	1.4	200	0.72	14.07.1999	BBCH 88	seed	5.4 4.6	<0.05 <0.05	0 12	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 12 months RIP2002-651
MLL 31336 9076 CT1 Plot C FR-36110 Levroux 15.07.2001	Pollen	1) 01.09.1998 (sowing) 2) – 3)	1.5	183	0.82	22.06.1999	BBCH 80	seed	2.9 0.86	<0.05 <0.05	0 17	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 12 months RIP2002-651
MLL 31336 9076 AL1 Plot C FR-68740	Coctail	1) 20.09.1998 (sowing) 2) – 3)	1.4	175	0.82	30.06.1999	BBCH 87	seed	6.6 0.87	<0.05 <0.05	0 10	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg,

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Fessenheim 15.07.2001												max. sample storage: 12 months RIP2002-651
MLL 31336 9076 AN1 Plot C FR-67160 Seebach 15.07.2001	Capitol	1) 28.08.1998 (sowing) 2) – 3)	1.4	169	0.82	28.06.1999	BBCH 87	seed	6.5 0.32	<0.05 <0.05	0 11	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 12 months RIP2002-651

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 450 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : MON 78294
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Oilseed rape

Submission date : 2001-11-28

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 31336 99-GLY-08-B/F Trt 2 BE-5380 Franc Waret 15.07.2001	Olara	1) 01.09.1998 (planting) 2) – 3)	1.4	200	0.72	14.07.1999	BBCH 88	seed	2.8 0.40	<0.05 <0.05	0 12	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 12 months RIP2002-651
MLL 31336 9076 CT1 Plot D FR-36110 Levroux 15.07.2001	Pollen	1) 01.09.1998 (sowing) 2) – 3)	1.4	170	0.82	22.06.1999	BBCH 80	seed	2.3 0.96	<0.05 <0.05	0 17	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 12 months RIP2002-651

MLL 31336 9076 AL1 Plot D FR-68740 Fessenheim 15.07.2001	Coctail	1) 20.09.1998 (sowing) 2) – 3)	1.5	182	0.82	30.06.1999	BBCH 87	seed	5.8 0.54	<0.05 <0.05	0 10	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 12 months RIP2002-651
MLL 31336 9076 AN1 Plot D FR-67160 Seebach 15.07.2001	Capitol	1) 28.08.1998 (sowing) 2) – 3)	1.4	172	0.82	28.06.1999	BBCH 87	seed	2.0 0.35	<0.05 <0.05	0 11	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 12 months RIP2002-651

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : (MON 2139) Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Oilseed rape

Submission date : 2000-05-30

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation
(common name and content) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30817 R 8145 CT1 FR- 36110 Levroux 15.03.2000	Contact	1) 05.09.1997 (sowing) 2) – 3) 09.07.1998	1.5	202	0.72	29.06.1998	BBCH 85	seed	0.80 0.32	0 10	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 9 months RIP2000-1244
9MLL 30817 R 8145 AL1 FR- 68740 Fassenheim 15.03.2000	Lisabeth	1) 12.09.1997 (sowing) 2) – 3) 03.07.1998	1.4	192	0.72	23.06.1998	BBCH 89	seed	2.0 1.9	0 10	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 9 months RIP2000-1244

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30817 R 8145 R 8145 AN1 FR- 54330 Vaudemont 15.03.2000	Colombus	1) 22.08.1997 (sowing) 2) – 3) 25.07.1998	1.4	195	0.72	11.07.1998	BBCH 87	seed	0.98 0.06	0 14	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 8 months RIP2000-1244
MLL 30817 98-GLY-07 B/F BE- 5380 Franc- Waret 15.03.2000	Synergie	1) 29.08.1997 (sowing) 2) – 3) 24.07.1998	1.4	200	0.72	10.07.1998	BBCH 92	seed	1.7 0.23	0 14	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 8 months RIP2000-1244 Blank value: PHI 0: 0.182 mg/kg

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Active ingredient : Glyphosate
Crop / crop group : Oilseed rape

Submission date : 2000-05-30

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : (MON 2139) Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : AMPA

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30817 R 8145 CT1 FR- 36110 Levroux 15.03.2000	Contact	1) 05.09.1997 (sowing) 2) – 3) 09.07.1998	1.5	202	0.72	29.06.1998	BBCH 85	seed	<0.05 <0.05	0 10	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 9 months RIP2000-1244
9MLL 30817 R 8145 AL1 FR- 68740 Fassenheim 15.03.2000	Lisabeth	1) 12.09.1997 (sowing) 2) – 3) 03.07.1998	1.4	192	0.72	23.06.1998	BBCH 89	seed	<0.05 <0.05	0 10	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 9 months RIP2000-1244
MLL 30817 R 8145 R 8145 AN1 FR- 54330 Vaudemont 15.03.2000	Colombus	1) 22.08.1997 (sowing) 2) – 3) 25.07.1998	1.4	195	0.72	11.07.1998	BBCH 87	seed	<0.05 <0.05	0 14	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 8 months

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl						
	(a)	(b)				(c)		(a)		(d)	(e)
											RIP2000-1244
MLL 30817 98-GLY-07 B/F BE- 5380 Franc- Waret 15.03.2000	Synergie	1) 29.08.1997 (sowing) 2) – 3) 24.07.1998	1.4	200	0.72	10.07.1998	BBCH 92	seed	<0.05 <0.05	0 14	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 8 months RIP2000-1244

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 680 g/kg
Formulation (e.g. WP) : WG
Commercial product (name) : MON 14420
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Oilseed rape

Submission date : 2000-05-30

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30817 R 8145 CT1 FR- 36110 Levroux 15.03.2000	Contact	1) 05.09.1997 (sowing) 2) – 3) 09.07.1998	1.5	203	0.715	29.06.1998	BBCH 85	seed	1.4 0.50	0 10	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 9 months RIP2000-1244
9MLL 30817 R 8145 AL1 FR- 68740 Fassenheim 15.03.2000	Lisabeth	1) 12.09.1997 (sowing) 2) – 3) 03.07.1998	1.5	204	0.716	23.06.1998	BBCH 89	seed	4.6 1.5	0 10	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 9 months RIP2000-1244
MLL 30817 R 8145 R 8145 AN1 FR- 54330 Vaudemont 15.03.2000	Colombus	1) 22.08.1997 (sowing) 2) – 3) 25.07.1998	1.4	197	0.716	11.07.1998	BBCH 87	seed	2.1 0.21	0 14	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 8 months

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl						
	(a)	(b)				(c)		(a)		(d)	(e)
											RIP2000-1244
MLL 30817 98-GLY-07 B/F BE- 5380 Franc- Waret 15.03.2000	Synergie	1) 29.08.97 (sowing) 2) – 3) 24.07.1998	1.4	200	0.714	10.07.1998	BBCH 92	seed	2.7 0.12	0 14	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 8 months RIP2000-1244 Blank value: PHI 0: 0.182 mg/kg

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Active ingredient : Glyphosate
Crop / crop group : Oilseed rape

Submission date : 2000-05-30

Content of a.i. (g/kg or g/l) : 680 g/kg
Formulation (e.g. WP) : WG
Commercial product (name) : MON 14420
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : AMPA

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30817 R 8145 CT1 FR- 36110 Levroux 15.03.2000	Contact	1) 05.09.1997 (sowing) 2) – 3) 09.07.1998	1.5	203	0.715	29.06.1998	BBCH 85	seed	<0.05 <0.05	0 10	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 9 months RIP2000-1244
9MLL 30817 R 8145 AL1 FR- 68740 Fassenheim 15.03.2000	Lisabeth	1) 12.09.1997 (sowing) 2) – 3) 03.07.1998	1.5	204	0.716	23.06.1998	BBCH 89	seed	<0.05 <0.05	0 10	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 9 months RIP2000-1244
MLL 30817 R 8145 R 8145 AN1 FR- 54330 Vaudemont 15.03.2000	Colombus	1) 22.08.1997 (sowing) 2) – 3) 25.07.1998	1.4	197	0.716	11.07.1998	BBCH 87	seed	<0.05 <0.05	0 14	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 8 months

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl						
	(a)	(b)				(c)		(a)		(d)	(e)
											RIP2000-1244
MLL 30817 98-GLY-07 B/F BE- 5380 Franc- Waret 15.03.2000	Synergie	1) 29.08.97 (sowing) 2) – 3) 24.07.1998	1.4	200	0.714	10.07.1998	BBCH 92	seed	<0.05 <0.05	0 14	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 8 months RIP2000-1244

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Clinic (= Durano)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Rape

Submission date : 2008-08-29

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
AF/10436/AV/1 (plot 3), with adjuvant United Kingdom DE74 2QQ Diseworth, Derbyshire 2007-02-13	Apex	1) (sowing) 2) 3) 2006-07-26	1.1	244	0.43	2006-07-10[4)]	BBCH 80-81	seed(s)	0.96	16	4) spraying analytical method: Agrisearch Glypho-sate/Crops/GW/06/2 (HPLC-MS-MS), LOQ 0.05 mg/kg, max. sample storage: 3 months adjuvant "Companion Gold" = "Aquasol" TGSII-007B (1.25 l/ha) ASB2008-7571
AF/10436/AV/1 (plot 2) United Kingdom DE74 2QQ Diseworth, Derbyshire 2007-02-13	Apex	1) (sowing) 2) 3) 2006-07-26	1.0	240	0.43	2006-07-10[4)]	BBCH 80-81	seed(s)	0.84	16	4) spraying analytical method: Agrisearch Glypho-sate/Crops/GW/06/2 (HPLC-MS-MS) ASB2008-7571

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
AF/10436/AV/2 (plot 3), with adjuvant United Kingdom DE11 7BA Boundary, Swadlincote 2007-02-13	Lioness	1) 2005-09-03 (sowing) 2) 3) 2006-07-26	1.1	243	0.43	2006-07-10[4)]	BBCH 81-82	seed(s)	1.4	16	4) spraying analytical method: Agrisearch Glypho-sate/Crops/GW/06/2 (HPLC-MS-MS), LOQ 0.05 mg/kg, max. sample storage: 3 months adjuvant "Companion Gold" = "Aquasol" TGSII-007B (1.25 l/ha) ASB2008-7571
AF/10436/AV/2 (plot 2) United Kingdom DE11 7BA Boundary, Swadlincote 2007-02-13	Lioness	1) 2005-09-03 (sowing) 2) 3) 2006-07-26	1.1	243	0.43	2006-07-10[4)]	BBCH 81-82	seed(s)	1.3	16	4) spraying analytical method: Agrisearch Glypho-sate/Crops/GW/06/2 (HPLC-MS-MS) ASB2008-7571

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
AF/10436/AV/3 (plot 3), with adjuvant United Kingdom NG10 5NP Sandiacre, Nottingham 2007-02-13	Apex	1) 2005-08-28 (sowing) 2) 3) 2006-07-26	1.1	255	0.43	2006-07-11[4)]	BBCH 81-82	seed(s)	3.6	15	4) spraying analytical method: Agrisearch Glypho-sate/Crops/GW/06/2 (HPLC-MS-MS), LOQ 0.05 mg/kg, max. sample storage: 3 months adjuvant "Companion Gold" = "Aquasol" TGSII-007B (1.25 l/ha) ASB2008-7571
AF/10436/AV/3 (plot 2) United Kingdom NG10 5NP Sandiacre, Nottingham 2007-02-13	Apex	1) 2005-08-28 (sowing) 2) 3) 2006-07-26	1.1	255	0.43	2006-07-11[4)]	BBCH 81-82	seed(s)	2.4	15	4) spraying analytical method: Agrisearch Glypho-sate/Crops/GW/06/2 (HPLC-MS-MS) ASB2008-7571

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
AF/10436/AV/4 (plot 3), with adjuvant United Kingdom Draycott, Derbyshire 2007-02-13	Apex	1) 2005-09-02 (sowing) 2) 3) 2006-07-26	1.0	237	0.43	2006-07-11[4)]	BBCH 81-83	seed(s)	1.9	15	4) spraying analytical method: Agrisearch Glypho-sate/Crops/GW/06/2 (HPLC-MS-MS), LOQ 0.05 mg/kg, max. sample storage: 3 months adjuvant "Companion Gold" = "Aquasol" TGSII-007B (1.25 l/ha) ASB2008-7571
AF/10436/AV/4 (plot 2) United Kingdom Draycott, Derbyshire 2007-02-13	Apex	1) 2005-09-02 (sowing) 2) 3) 2006-07-26	1.0	242	0.43	2006-07-11[4)]	BBCH 81-83	seed(s)	2.6	15	4) spraying analytical method: Agrisearch Glypho-sate/Crops/GW/06/2 (HPLC-MS-MS) ASB2008-7571

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Glyfos
Applicant : Cheminova A/S

Active ingredient : Glyphosate
Crop / crop group : Winter Rape

Submission date : 2010-10-20

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
AS/2208/CN, trial AS/2208/CN/2A United Kingdom East Rounton, North Yorkshire 1995-03-01	Falcon	1) 1992-08-28 (sowing) 2) 3) 1993-08-07	0.72	200	0.36	1993-07-24[4)]	BBCH 89	seed(s)	0.28	14	4) spraying analytical method: DFG method No. 405 (HPLC-FD), LOQ: 0.06 mg/kg, max. sample storage: 11 months ASB2010-14768
AS/2208/CN, trial AS/2208/CN/2B United Kingdom East Rounton, North Yorkshire 1995-03-01	Falcon	1) 1992-08-28 (sowing) 2) 3) 1993-08-07	1.4	200	0.72	1993-07-24[4)]	BBCH 89	seed(s)	0.48	14	4) spraying analytical method: DFG method No. 405 (HPLC-FD), LOQ: 0.06 mg/kg, max. sample storage: 11 months ASB2010-14768

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
AS/2208/CN, trial AS/2208/CN/3A United Kingdom Tetsworth, Oxfordshire 1995-03-01	Capricorn	1) 1992-09-17 (sowing) 2) 3) 1993-08-06	0.72	200	0.36	1993-07-23[4)]	BBCH 89	seed(s)	0.37	14	4) spraying analytical method: DFG method No. 405 (HPLC-FD), LOQ: 0.06 mg/kg, max. sample storage: 11 months ASB2010-14768
AS/2208/CN, trial AS/2208/CN/3B United Kingdom Tetsworth, Oxfordshire 1995-03-01	Capricorn	1) 1992-09-17 (sowing) 2) 3) 1993-08-06	1.4	200	0.72	1993-07-23[4)]	BBCH 89	seed(s)	0.57	14	4) spraying analytical method: DFG method No. 405 (HPLC-FD), LOQ: 0.06 mg/kg, max. sample storage: 11 months ASB2010-14768

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Rape

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Report MLL 30.204, trial no. 008369 Germany 8913 Schondorf 1988-03-07	Jet Neuf	1) 1985-08-30 (sowing) 2) 3) 1986-07-25	1.1	200	0.54	1986-07-16[4)]	BBCH 93-99	seed(s) straw	4.0 0.60 0.60 0.30 11.0		0 4 7 12 12	4) spraying analytical method: XA001 (HPLC), LOQ: 0.05 mg/kg, max. sample storage: not reported report form: ASB2009-4823 RIP9501279
Report MLL 30.204, trial no. 008370 Germany 8084 Inning 1988-03-07	Jet Neuf	1) 1985-08-28 (sowing) 2) 3) 1986-07-28	1.1	200	0.54	1986-07-17[4)]	BBCH 93-99	seed(s) straw	6.5 0.80 0.20 0.70 28.0		0 5 8 12 12	4) spraying analytical method: XA001 (HPLC), LOQ: 0.05 mg/kg, max. sample storage: not reported report form: ASB2009-4824 RIP9501279

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Report MLL 30.204, trial no. 008371 Germany 8081 Malching 1988-03-07	Jet Neuf	1) 1985-08-29 (sowing) 2) 3) 1986-07-28	1.1	200	0.54	1986-07-16[4)]	BBCH 93-99	seed(s) straw	3.0 0.40 0.70 0.70 35.0		0 4 7 12 12	4) spraying analytical method: XA001 (HPLC), LOQ: 0.05 mg/kg, max. sample storage: not reported report form: ASB2009-4821 RIP9501279
Report MLL 30.204, trial no. 008380 Germany 3006 Burgwedel 1988-03-07	Lirabon	1) 1985-09-05 (sowing) 2) 3) 1986-08-18	1.1	200	0.54	1986-07-28[4)]	BBCH 93-99	seed(s) straw	3.6 1.5 0.90 0.40 0.60 19.0		0 3 7 10 21 21	4) spraying analytical method: XA001 (HPLC), LOQ: 0.05 mg/kg, max. sample storage: not reported report form: ASB2009-4822 RIP9501279
Report MLL 30.204, trial no. 06851 Germany 3582 Felsberg 1988-03-07	Belinda	1) 1986-08 (sowing) 2) 3) 1987-08-17	1.1	200	0.54	1986-07-22[4)]	BBCH 93-99	plant pod seed(s)	56.0 32.0 46.0 29.0 38.0 19.0 0.30 6.8 6.8 6.8	0.30 0.30 0.30 0.20 0.40 0.40 <0.05 <0.05 0.10 0.10	0 5 8 13 16 26 8 13 16 26	4) spraying analytical method: XA001 (HPLC), LOQ: 0.05 mg/kg, recovery AMPA: 51 %, max. sample storage: not reported report form: ASB2009-4831 RIP9501279

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Report MLL 30.204, trial no. 06852 Germany 3578 Schwalmstadt 1988-03-07	Jet Neuf	1) 1986-08 (sowing) 2) 3) 1987-08-12	1.1	200	0.54	1986-07-22[4)]	BBCH 93-99	plant pod seed(s)	11.0 10.0 1.4 8.4 8.8 0.40 0.30 <0.05		5 8 13 17 21 13 17 21	4) spraying analytical method: XA001 (HPLC), LOQ: 0.05 mg/kg, max. sample storage: not reported report form: ASB2009-4832 RIP9501279

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : CHE 3607
Applicant : Cheminova A/S

Active ingredient : Glyphosate
Crop / crop group : Winter Rape

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
IF-93/13839-01, trial AS/1908/CN1, plot 2 United Kingdom SK 414 239 Wilson, Derby-shire 1994-07-18	Lictor	1) 1991-09-18 (sowing) 2) 3) 1992-07-30	1.4	200	0.72	1992-07-16[4)]	no data	seed(s) pod, with seed	0.40 50.4 13.1 0.50 0.60	14 0 3 7 10	4) spraying analytical method: DFG method No. 405 (HPLC), LOQ: 0.06 mg/kg, max. sample storage: 21 months RIP9501324
IF-93/13839-01, trial AS/1908/CN1, plot 3 United Kingdom SK 414 239 Wilson, Derby-shire 1994-07-18	Lictor	1) 1991-09-18 (sowing) 2) 3) 1992-07-30	0.72	200	0.36	1992-07-16[4)]	no data	seed(s) pod, with seed	0.20 31.4 6.1 0.10 0.20	14 0 3 7 10	4) spraying analytical method: DFG method No. 405 (HPLC), LOQ: 0.06 mg/kg, max. sample storage: 21 months RIP9501324

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : CHE 3607
Applicant : Cheminova A/S

Active ingredient : Glyphosate
Crop / crop group : Winter Rape

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
IF-93/13831-01, trial AS/1899/CN1, plot 2 United Kingdom SK 416 238 Wilson, Derby-shire 1994-07-01	Lictor	1) 1991-09-18 (sowing) 2) 3) 1992-08-07	1.4	200	0.72	1992-07-24[4)]	no data	seed(s)	0.30	14	4) spraying analytical method: DFG method No. 405 (HPLC), LOQ: 0.06 mg/kg, max. sample storage: 20 months RIP9501322
IF-93/13831-01, trial AS/1899/CN1, plot 3 United Kingdom SK 416 238 Wilson, Derby-shire 1994-07-01	Lictor	1) 1991-09-18 (sowing) 2) 3) 1992-08-07	0.72	200	0.36	1992-07-24[4)]	no data	seed(s)	0.10	14	4) spraying analytical method: DFG method No. 405 (HPLC), LOQ: 0.06 mg/kg, max. sample storage: 20 months RIP9501322

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
IF-93/13831-01, trial AS/1899/CN2, plot 2 United Kingdom SE 922 720 Wintringham, Yorkshire 1994-07-01	Samurai	1) 1991-09-06 (sowing) 2) 3) 1992-08-07	1.4	200	0.72	1992-07-24[4)]	no data	seed(s)	0.060	14	4) spraying analytical method: DFG method No. 405 (HPLC), LOQ: 0.06 mg/kg, max. sample storage: 20 months RIP9501322
IF-93/13831-01, trial AS/1899/CN2, plot 3 United Kingdom SE 922 720 Wintringham, Yorkshire 1994-07-01	Samurai	1) 1991-09-06 (sowing) 2) 3) 1992-08-07	0.72	200	0.36	1992-07-24[4)]	no data	seed(s)	0.20	14	4) spraying analytical method: DFG method No. 405 (HPLC), LOQ: 0.06 mg/kg, max. sample storage: 20 months RIP9501322
IF-93/13831-01, trial AS/1899/CN3, plot 2 United Kingdom TL 792 144 NR Chelmsford, Essex 1994-07-01	Falcon	1) 1991-09-18 (sowing) 2) 3) 1992-07-24	1.4	200	0.72	1992-07-10[4)]	no data	seed(s)	0.40	14	4) spraying analytical method: DFG method No. 405 (HPLC), LOQ: 0.06 mg/kg, max. sample storage: 20 months RIP9501322

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
IF-93/13831-01, trial AS/1899/CN3, plot 3 United Kingdom TL 792 144 NR Chelmsford, Essex 1994-07-01	Falcon	1) 1991-09-18 (sowing) 2) 3) 1992-07-24	0.72	200	0.36	1992-07-10[4)]	no data	seed(s)	0.20	14	4) spraying analytical method: DFG method No. 405 (HPLC), LOQ: 0.06 mg/kg, max. sample storage: 20 months RIP9501322

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 480 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup 480
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop/crop group : Winter Rape

Submission date : 1995-09-01

Indoors/outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.223 A 3 United Kingdom, Bentley, Warwick 1995-09-01	Bienvenue	1) 1987 (sowing) 2) 3)	1.1	200	0.54	1988-07-18[4)]	BBCH 89	seed(s)	9.3 8.0 7.0 7.0	not analysed	6 6 6 6	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg (recovery 60%), next validated level: 5 mg/kg, replicate field samples, values corrected for method recovery, max. sample storage: 4 months RIP9501284

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.223 B 3 United Kingdom, Kingscote 1995-09-01	Bienvenue	1) 1987 (sowing) 2) 3)	1.1	200	0.54	1988-07-19[4)]	BBCH 89	seed(s)	4.9 5.6 5.0 5.0	not analysed	16 16 16 16	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg (recovery 65%), next validated level 5 mg/kg, replicate field samples, values corrected for method recovery, max. sample storage: 4 months RIP9501284
MLL 30.223 C 3 United Kingdom, Fordham Essex 1995-09-01	Ariana	1) 1987 (sowing) 2) 3)	1.1	200	0.54	1988-07-18[4)]	BBCH 89	seed(s) straw	0.70 3.9 4.6 1.8 38.0	<0.05 <0.05 <0.05 <0.05 0.40	14 14 14 14 14	4) spraying treated with tankmix Spin-tone SL (MON 14478) + A-Sulfate (2-5%), analytical method: XA001 (HPLC with OPA), LOQ: 0.05 mg/kg, Glyphosat: recovery for plant at 20 mg/kg: 115%, AMPA: recovery at 0.05 mg/kg: 41-74%, replicate field samples, values are corrected for method recovery, max. sample storage: 4 months RIP9501284

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop/crop group : Winter Rape

Submission date : 1995-09-01

Indoors/outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/1, 2 A United Kingdom, Wilson, Derby-shire 1993-06-01	Lictor	1) (sowing) 2) 3)	1.4	200	0.072	1992-07-16[4)]	BBCH 81-87	seed(s)	6.6 1.3	<0.05 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: 0.05 mg/kg (recoveries Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level: Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), control for Glyphosate. 0.2 mg/kg, max. sample storage: 9 months RIP9501292

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/1, 2 B United Kingdom, Wilson, Derby-shire 1993-06-01	Lictor	1) (sowing) 2) 3)	2.9	200	0.14	1992-07-16[4)]	BBCH 81-87	seed(s)	17.0 3.4	0.050 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: Glyphosat: 0.05 mg/kg (recoveries Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), control for Glyphosate. 0.2 mg/kg, max. sample storage: 9 months RIP9501292

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/2, 2 A United Kingdom, Burnham, Essex 1993-06-01	Samurai	1) (sowing) 2) 3)	1.4	200	0.072	1992-07-24[4)]	BBCH 83-88	seed(s)	6.8 0.60	<0.05 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: Glyphosat: 0.05 mg/kg (recoveries Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level: Glyphosate: 0.1 mg/kg, AMPA 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), control value for Glyphosate 0.1 mg/kg, max. sample storage: 9 months RIP9501292

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/2, 2 B United Kingdom, Burnham, Essex 1993-06-01	Samurai	1) (sowing) 2) 3)	2.9	200	0.14	1992-07-24[4)]	BBCH 83-88	seed(s)	38.0 1.8	0.10 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg (recover-ies Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), control value for Glyphosate 0.1 mg/kg, max. sample storage: 9 months RIP9501292

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/3, 2 A United Kingdom, Terling, Essex 1993-06-01	Falcon	1) (sowing) 2) 3)	1.4	200	0.072	1992-07-10[4)]	BBCH 81-87	seed(s)	6.4 0.70	<0.05 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg (recover-ies Glyphosate: 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), max. sample storage: 9 months RIP9501292

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/3, 2 B United Kingdom, Terling, Essex 1993-06-01	Falcon	1) (sowing) 2) 3)	2.9	200	0.14	1992-07-10[4)]	BBCH 81-87	seed(s)	12.0 1.3	<0.05 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: Glyphosat: 0.05 mg/kg (recoveries Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), max. sample storage: 9 months RIP9501292

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/4, 2 A United Kingdom, Burnham, Essex 1993-06-01	Lictor	1) (sowing) 2) 3)	1.4	200	0.072	1992-07-10[4)]	BBCH 87	seed(s)	4.2 0.40	<0.05 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg (recoveries Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), max. sample storage: 9 months RIP9501292

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/4, 2 B United Kingdom, Burnham, Essex 1993-06-01	Lictor	1) (sowing) 2) 3)	2.9	200	0.14	1992-07-10[4)]	BBCH 87	seed(s)	7.9 1.6	<0.05 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: 0.05 mg/kg (recoveries Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), max. sample storage: 9 months RIP9501292

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 400 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Spintone SL
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop/crop group : Winter Rape

Submission date : 1995-09-01

Indoors/outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.223 A 1 United Kingdom, Bentley, Warwick 1995-09-01	Bienvenue	1) 1987 (sowing) 2) 3)	1.0	200	0.50	1988-07-18[4)]	BBCH 89	seed(s)	6.7 7.4 8.7 10.4	not analysed	6 6 6 6	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: 0.05 mg/kg (recovery 60%), next validated level 5 mg/kg, replicate field samples, values corrected for method recovery, max. sample storage: 4 months RIP9501284
MLL 30.223 A 2 United Kingdom, Bentley, Warwick 1995-09-01	Bienvenue	1) 1987 (sowing) 2) 3)	2.0	200	1.0	1988-07-18[4)]	BBCH 89	seed(s)	9.8 11.0 13.0 14.0	not analysed	6 6 6 6	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: 0.05 mg/kg (recovery 60%), next validated level 5 mg/kg, replicate field samples, values corrected for method recovery, max. sample storage: 4 months RIP9501284

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.223 B 1 United Kingdom, Kingscote 1995-09-01	Bienvenue	1) 1987 (sowing) 2) 3)	1.0	200	0.50	1988-07-19[4)]	BBCH 89	seed(s)	3.9 4.4 4.4 3.2		16 16 16 16	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg (recovery 65%), next validated level 5 mg/kg, replicate field samples, values corrected for method recovery, max. sample storage: 4 months RIP9501284
MLL 30.223 B 2 United Kingdom, Kingscote 1995-09-01	Bienvenue	1) 1987 (sowing) 2) 3)	2.0	200	1.0	1988-07-19[4)]	BBCH 89	seed(s)	7.5 7.6 8.6 9.0		16 16 16 16	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg (recovery 65%), next validated level 5 mg/kg, replicate field samples, values corrected for method recovery, max. sample storage: 4 months RIP9501284
MLL 30.223 C 1 United Kingdom, Fordham Essex 1995-09-01	Ariana	1) 1987 (sowing) 2) 3)	1.0	200	0.50	1988-07-18[4)]	BBCH 89	seed(s) straw	2.7 3.1 0.30 2.0 24.0	<0.05 <0.05 <0.05 <0.05 1.0	14 14 14 14 14	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: 0.05 mg/kg, recovery Glyphosat: for plant at 20 mg/kg 115%, AMPA: 41-74% at 0.05 mg/kg, replicate field samples, values are corrected for method recovery, max. sample storage: 4 months RIP9501284

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.223 C 2 United Kingdom, Fordham Essex 1995-09-01	Ariana	1) 1987 (sowing) 2) 3)	2.0	200	1.0	1988-07-18[4)]	BBCH 89	seed(s) straw	6.4 3.1 2.7 2.8 46.0	<0.05 <0.05 <0.05 <0.05 0.90	14 14 14 14 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg, recovery Glyphosat: for plant at 20 mg/kg 115%, AMPA: 41-74% at 0.05 mg/kg, replicate field samples, values are corrected for method recovery, max. sample storage: 4 months RIP9501284

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : MON 52276
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop/crop group : Winter Rape

Submission date : 1995-09-01

Indoors/outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/1, 1 A United Kingdom, Wilson, Derby-shire 1993-06-01	Lictor	1) (sowing) 2) 3)	1.4	200	0.072	1992-07-16[4)]	BBCH 81-87	seed(s)	6.1 <0.05	<0.05 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: Glyphosat: 0.05 mg/kg (recoveries Glyphosate: 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level: Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), control for Glyphosate. 0.2 mg/kg, max. sample storage: 9 months RIP9501292

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/1, 1 B United Kingdom, Wilson, Derby-shire 1993-06-01	Lictor	1) (sowing) 2) 3)	2.9	200	0.14	1992-07-16[4)]	BBCH 81-87	seed(s)	6.7 2.4	<0.05 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: Glyphosat: 0.05 mg/kg (recoveries Glypho-sate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, compo-site of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), control for Glyphosate. 0.2 mg/kg, max. sample storage: 9 months RIP9501292

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/2, 1 A United Kingdom, Burnham, Essex 1993-06-01	Samurai	1) (sowing) 2) 3)	1.4	200	0.072	1992-07-24[4)]	BBCH 83-88	seed(s)	4.9 0.60	<0.05 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg (recoveries Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), control value for glyphosate 0.1 mg/kg, max. sample storage: 9 months RIP9501292

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/2, 1 B United Kingdom, Burnham, Essex 1993-06-01	Samurai	1) (sowing) 2) 3)	2.9	200	0.14	1992-07-24[4)]	BBCH 83-88	seed(s)	31.0 1.5	0.10 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg (recover-ies Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), control value for glyphosate 0.1 mg/kg, max. sample storage: 9 months RIP9501292

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/3, 1 A United Kingdom, Terling, Essex 1993-06-01	Falcon	1) (sowing) 2) 3)	1.4	200	0.072	1992-07-10[4)]	BBCH 81-87	seed(s)	5.7 0.90	<0.05 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: Glyphosat: 0.05 mg/kg (recoveries Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), max. sample storage: 9 months RIP9501292

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/3, 1 B United Kingdom, Terling, Essex 1993-06-01	Falcon	1) (sowing) 2) 3)	2.9	200	0.14	1992-07-10[4)]	BBCH 81-87	seed(s)	8.0 1.3	<0.05 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: Glyphosat: 0.05 mg/kg (recoveries Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), max. sample storage: 9 months RIP9501292
MLL 30321, 92-GLY-02, AS/1933/MO/4, 1 A United Kingdom, Burnham, Essex 1993-06-01	Lictor	1) (sowing) 2) 3)	1.4	200	0.072	1992-07-10[4)]	BBCH 87	seed(s)	9.5 0.60	<0.05 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg (recoveries Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), max. sample storage: 9 months RIP9501292

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/4, 1 B United Kingdom, Burnham, Essex 1993-06-01	Lictor	1) (sowing) 2) 3)	2.9	200	0.14	1992-07-10[4)]	BBCH 87	seed(s)	10.0 0.40	0.050 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg (recover-ies Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), max. sample storage: 9 months RIP9501292

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 420 g/kg
Formulation (e.g. WP) : WG
Commercial product (name) : MON 44068 WG
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop/crop group : Winter Rape

Submission date : 1995-09-01

Indoors/outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/1, 3 A United Kingdom, Wilson, Derby-shire 1993-06-01	Lictor	1) (sowing) 2) 3)	1.5	200	0.074	1992-07-16[4)]	BBCH 81-87	seed(s)	10.0 1.2	<0.05 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg (recover-ies Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), control for Glyphosate. 0.2 mg/kg, max. sample storage: 9 months RIP9501292

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/1, 3 B United Kingdom, Wilson, Derby-shire 1993-06-01	Lictor	1) (sowing) 2) 3)	2.9	200	0.15	1992-07-16[4)]	BBCH 81-87	seed(s)	18.0 3.5	0.080 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg (recover-ies Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), control for Glyphosate. 0.2 mg/kg, max. sample storage: 9 months RIP9501292

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/2, 3 A United Kingdom, Burnham, Essex 1993-06-01	Samurai	1) (sowing) 2) 3)	1.5	200	0.074	1992-07-24[4)]	BBCH 83-88	seed(s)	11.0 1.0	<0.05 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg (recover-ies Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78 next validat-ed level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), control value for glyphosate 0.1 mg/kg, max. sample storage: 9 months RIP9501292

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/2, 3 B United Kingdom, Burnham, Essex 1993-06-01	Samurai	1) (sowing) 2) 3)	2.9	200	0.15	1992-07-24[4)]	BBCH 83-88	seed(s)	61.0 1.4	0.30 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg (recover-ies Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), control value for glyphosate 0.1 mg/kg, max. sample storage: 9 months RIP9501292

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/3, 3 A United Kingdom, Terling, Essex 1993-06-01	Falcon	1) (sowing) 2) 3)	1.5	200	0.074	1992-07-10[4)]	BBCH 81-87	seed(s)	4.2 0.80	<0.05 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg (recoveries Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), max. sample storage: 9 months RIP9501292
MLL 30321, 92-GLY-02, AS/1933/MO/3, 3 B United Kingdom, Terling, Essex 1993-06-01	Falcon	1) (sowing) 2) 3)	2.9	200	0.15	1992-07-10[4)]	BBCH 81-87	seed(s)	24.0 0.90	<0.05 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg (recoveries Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), max. sample storage: 9 months RIP9501292

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30321, 92-GLY-02, AS/1933/MO/4, 3 A United Kingdom, Burnham, Essex 1993-06-01	Lictor	1) (sowing) 2) 3)	1.5	200	0.074	1992-07-10[4)]	BBCH 87	seed(s)	9.0 0.60	<0.05 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg (recoveries Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), max. sample storage: 9 months RIP9501292
MLL 30321, 92-GLY-02, AS/1933/MO/4, 3 B United Kingdom, Burnham, Essex 1993-06-01	Lictor	1) (sowing) 2) 3)	2.9	200	0.15	1992-07-10[4)]	BBCH 87	seed(s)	14.0 1.4	<0.05 <0.05	0 14	4) spraying analytical method: XA001 (HPLC with OPA) LOQ: 0.05 mg/kg (recoveries Glyphosate 84%, 60%, AMPA: 70%, 87%, 78%, 55%, 57%, 78%), next validated level Glyphosate: 0.1 mg/kg, AMPA: 0.2 mg/kg, composite of 3 replicate field samples, values are corrected for average recovery of all levels (Glyphosate: 73%, AMPA 79%), max. sample storage: 9 months RIP9501292

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 240 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : MON 8795
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop/crop group : Winter Rape

Submission date : 1995-09-01

Indoors/outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30.180, MLL 30180, 73, 1 A United Kingdom, Doggington 1987-01-01	Calypso	1) (sowing) 2) 3)	2.9	80	3.6	1986-08-17[4)]	BBCH 81-88	seed(s)	3.7	12	4) spraying analytical method: XA0100 (HPLC with OPA), LOQ: 0.05 mg/kg, composite sample of 4 replicate field samples, values are corrected for recovery (85 %), max. sample storage: 5 months RIP9501277
MLL 30.180, MLL 30180, 73, 1 B United Kingdom, Doggington 1987-01-01	Calypso	1) (sowing) 2) 3)	2.9	250	1.2	1986-08-17[4)]	BBCH 81-88	seed(s)	2.8	12	4) spraying analytical method: HPLC with OPA, LOQ: 0.05 mg/kg, composite sample of 4 replicate field samples, values are corrected for recovery (85 %), max. sample storage: 5 months RIP9501277

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30.180, MLL 30180, 73, 2 A United Kingdom, Tadcaster 1987-01-01	Rafal	1) (sowing) 2) 3)	2.9	80	3.6	1986-08-01[4)]	BBCH 81-88	seed(s)	0.50	13	4) spraying analytical method: HPLC with OPA, LOQ: 0.05 mg/kg, composite sample of 4 replicate field samples, values are corrected for recovery (85 %), max. sample storage: 5 months RIP9501277
MLL 30.180, MLL 30180, 73, 2 B United Kingdom, Tadcaster 1987-01-01	Rafal	1) (sowing) 2) 3)	2.9	250	1.2	1986-08-01[4)]	BBCH 81-88	seed(s)	0.60	13	4) spraying analytical method: HPLC with OPA, LOQ: 0.05 mg/kg, composite sample of 4 replicate field samples, values are corrected for recovery (85 %), max. sample storage: 5 months RIP9501277
MLL 30.180, MLL 30180, 73, 3 A United Kingdom, Sto Longa, Cambridgeshire 1987-01-01	Bienvenue	1) 1985-09-07 (sowing) 2) 3)	2.9	80	3.6	1986-07-29[4)]	BBCH 81-88	seed(s)	0.40	14	4) spraying analytical method: HPLC with OPA, LOQ: 0.05 mg/kg, composite sample of 4 replicate field samples, values are corrected for recovery (85 %), max. sample storage: 5 months RIP9501277

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30.180, MLL 30180, 73, 3 B United Kingdom, Sto Longa, Cambridgeshire 1987-01-01	Bienvenue	1) 1985-09-07 (sowing) 2) 3)	5.8	250	2.3	1986-07-29[4)]	BBCH 81-88	seed(s)	0.60	14	4) spraying analytical method: XA0100 (Glyphosat: HPLC with OPA, LOQ: 0.05 mg/kg, composite sample of 4 replicate field samples, values are corrected for recovery (85 %), max. sample storage: 5 months RIP9501277

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Active ingredient : Glyphosate
Crop/crop group : Winter Rape

Submission date : 1995-09-01

Content of a.i. (g/kg or g/l) : 480 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup Four 80
Applicant : Monsanto Agrar Deutschland GmbH

Indoors/outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30.180, MLL 30180, 73, 1 E United Kingdom, Doggington 1987-01-01	Calypso	1) (sowing) 2) 3)	2.9	250	1.2	1986-08-17[4)]	BBCH 81-88	seed(s)	3.5	12	4) spraying analytical method: HPLC with OPA, LOQ: 0.05 mg/kg, composite sample of 4 replicate field samples, values are corrected for recovery (85 %), max. sample storage: 5 months RIP9501277
MLL 30.180, MLL 30180, 73, 2 E United Kingdom, Tadcaster 1987-01-01	Rafal	1) (sowing) 2) 3)	2.9	250	1.2	1986-08-01[4)]	BBCH 81-88	seed(s)	0.60	13	4) spraying analytical method: HPLC with OPA, LOQ: 0.05 mg/kg, composite sample of 4 replicate field samples, values are corrected for recovery (85 %), max. sample storage: 5 months RIP9501277

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30.180, MLL 30180, 73, 3 E United Kingdom, Stow Longa Cambridgeshire 1987-01-01	Bienvenue	1) 1985-09-07 (sowing) 2) 3)	2.9	250	1.2	1986-08-01[4)]	BBCH 81-88		0.70		4) spraying analytical method: XA0100 (HPLC with OPA), LOQ: 0.05 mg/kg, composite sample of 4 replicate field samples, values are corrected for recovery (85 %), max. sample storage: 5 months RIP9501277

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Active ingredient : Glyphosate
Crop/crop group : Winter Rape

Submission date : 1995-09-01

Content of a.i. (g/kg or g/l) : 480 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup Four 80
Applicant : Monsanto Agrar Deutschland GmbH

Indoors/outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30.180, MLL 30180, 73, 1 C United Kingdom, Doggington 1987-01-01	Calypso	1) (sowing) 2) 3)	2.9	250	1.2	1986-08-17[4)]	BBCH 81-88	seed(s)	6.3	12	4) spraying analytical method: HPLC with OPA, LOQ: 0.05 mg/kg, composite sample of 4 replicate field samples, values are corrected for recovery (85 %), max. sample storage: 5 months RIP9501277
MLL 30.180, MLL 30180, 73, 2 C United Kingdom, Tadcaster 1987-01-01	Rafal	1) (sowing) 2) 3)	2.9	250	1.2	1986-08-01[4)]	BBCH 81-88	seed(s)	0.50	13	4) spraying analytical method: HPLC with OPA, LOQ: 0.05 mg/kg, composite sample of 4 replicate field samples, values are corrected for recovery (85 %), max. sample storage: 5 months RIP9501277

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30.180, MLL 30180, 73, 3 C United Kingdom, Sto Longa, Cambridgeshire 1987-01-01	Bienvenue	1) 1985-09-07 (sowing) 2) 3)	2.9	250	1.2	1986-07-29[4)]	BBCH 81-88	seed(s)	0.40	14	4) spraying analytical method: HPLC with OPA, LOQ: 0.05 mg/kg, composite sample of 4 replicate field samples, values are corrected for recovery (85 %), max. sample storage: 5 months RIP9501277

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360~~355.7~~ g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop/crop group : Winter Rape

Submission date : 1995-09-01

Indoors/outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.180, MLL 30180, 73, 1 D United Kingdom, Doggington 1987-01-01	Calypso	1) (sowing) 2) 3)	2.8	250	1.1	1986-08-17[4)]	BBCH 81-88	seed(s)	3.6	not analysed	12	4) spraying treated with Roundup, analytical method: HPLC with OPA, LOQ: 0.05 mg/kg, composite sample of 4 replicate field samples, values are corrected for recovery (85 %), max. sample storage: 5 months RIP9501277

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.180, MLL 30180, 73, 2 D United Kingdom, Tadcaster 1987-01-01	Rafal	1) (sowing) 2) 3)	2.8	250	1.1	1986-08-01[4)]	BBCH 81-88	seed(s)	0.40	not analysed	13	4) spraying analytical method: HPLC with OPA, LOQ: 0.05 mg/kg, composite sample of 4 replicate field samples, values are corrected for recovery (85 %), max. sample storage: 5 months RIP9501277
MLL 30.180, MLL 30180, 73, 3 D United Kingdom, Stow Longa, Cambridgeshire 1987-01-01	Bienvenue	1) (sowing) 2) 3)	2.8	250	1.1	1986-07-29[4)]	BBCH 81-88	seed(s)	0.80	not analysed	14	4) spraying analytical method: HPLC with OPA, LOQ: 0.05 mg/kg, composite sample of 4 replicate field samples, values are corrected for recovery (85 %), max. sample storage: 5 months RIP9501277

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Glyfos
Applicant : Cheminova A/S

Active ingredient : Glyphosate
Crop / crop group : Spring Rape

Submission date : 2010-10-20

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
AS/2208/CN, trial AS/2208/CN/1A United Kingdom Caunton, Notting-hamshire 1995-03-01	Bingo	1) 1993-03-06 (sowing) 2) 3) 1993-09-09	0.72	200	0.36	1993-08-26[4)]	BBCH 89	seed(s)	0.14	14	4) spraying analytical method: DFG method No. 405 (HPLC-FD), LOQ: 0.06 mg/kg, max. sample storage: 10 months ASB2010-14768
AS/2208/CN, trial AS/2208/CN/1B United Kingdom Caunton, Notting-hamshire 1995-03-01	Bingo	1) 1993-03-06 (sowing) 2) 3) 1993-09-09	1.4	200	0.72	1993-08-26[4)]	BBCH 89	seed(s)	0.28	14	4) spraying analytical method: DFG method No. 405 (HPLC-FD), LOQ: 0.06 mg/kg, max. sample storage: 10 months ASB2010-14768

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360~~355.7~~ g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop/crop group : Summer Rape

Submission date : 1995-09-01

Indoors/outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.235, MLL 30235, 93, 1 C United Kingdom, Witchford, Cambs 1989-11-07	Libravo	1) 1988-09-10 (sowing) 2) 3)	1.1	200	0.54	1989-07-10[4)]	BBCH 82-83	seed(s)	0.90 0.80 0.70 0.70	<0.05 <0.05 <0.05 <0.05	7 7 7 7	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: 0.05 mg/kg, recoveries for AMPA: 66%, 98%, 89%, 4 replicate field samples, max. sample storage: 3 months RIP9501286
MLL 30.235, MLL 30235, 93, 2 C United Kingdom, Graveley 1989-11-07	Libravo	1) 1988-09-10 (sowing) 2) 3)	1.1	200	0.54	1989-07-12[4)]	BBCH 82-83	seed(s)	1.7 2.0 1.8 1.6	<0.05 <0.05 <0.05 <0.05	7 7 7 7	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: 0.05 mg/kg, recoveries for AMPA: 66%, 98%, 89%, 4 replicate field samples, max. sample storage: 3 months RIP9501286

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.235, MLL 30235, 93, 3 C United Kingdom, Cirencester 1989-11-07	Cobra	1)1989-08-26 (sowing) 2) 3)	1.1	200	0.54	1989-07-124)	BBCH 82-83	seed(s)	0.20 0.40 0.50 0.70	<0.05 <0.05 <0.05 <0.05	8 8 8 8	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: 0.05 mg/kg, 4 replicate field samples, max. sample storage: 3 months RIP9501286
MLL 30.235, MLL 30235, 93, 4 C United Kingdom, Rumbelow 1989-11-07	Ariana	1) (sowing) 2) 3)	1.1	200	0.54	1989-07-14[4)]	BBCH 82-83	seed(s)	2.3 4.3 4.4 3.0	<0.05 <0.05 <0.05 <0.05	7 7 7 7	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: Glyphosat: 0.1 mg/kg, AM-PA: 0.05 mg/kg, 4 replicate field samples, max. sample storage: 3 months RIP9501286
MLL 30.235, MLL 30235, 93, 5 C United Kingdom, Hunter Scotland 1989-11-07	Cobra	1) (sowing) 2) 3)	1.1	200	0.54	1989-08-02[4)]	BBCH 82-83	seed(s)	1.7 1.1 0.70 0.70	<0.05 <0.05 <0.05 <0.05	7 7 7 7	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: Glyphosat: 0.1 mg/kg, AM-PA: 0.05 mg/kg (recoveries. 89%, 65%), 4 replicate field samples, max. sample storage: 2 months RIP9501286

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 400 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : MON 14478
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop/crop group : Summer Rape

Submission date : 1995-09-01

Indoors/outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.235, MLL 30235, 93, 1 A United Kingdom, Witchford, Cambs 1989-11-07	Libravo	1) 1988-09-10 (sowing) 2) 3)	1.0	200	0.50	1989-07-10[4)]	BBCH 82-83	seed(s)	0.60 0.60 0.40 0.40	<0.05 <0.05 <0.05 <0.05	7 7 7 7	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: 0.05 mg/kg, recoveries for AMPA: 66%, 98%, 98%, 4 replicate field samples, max. sample storage: 3 months RIP9501286
MLL 30.235, MLL 30235, 93, 1 B United Kingdom, Witchford, Cambs 1989-11-07	Libravo	1) 1988-09-10 (sowing) 2) 3)	2.0	200	1.0	1989-07-10[4)]	BBCH 82-83	seed(s)	1.4 1.1 0.60 1.7	<0.05 <0.05 <0.05 <0.05	7 7 7 7	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: 0.05 mg/kg, recoveries for AMPA: 66%, 98%, 89%, 4 replicate field samples, max. sample storage: 3 months RIP9501286

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.235, MLL 30235, 93, 2 A United Kingdom, Graveley 1989-11-07	Libravo	1) (sowing) 2) 3)	1.0	200	0.50	1989-07-12[4)]	BBCH 82-83	seed(s)	1.0 1.1 1.0 0.80	<0.05 <0.05 <0.05 <0.05	7 7 7 7	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: 0.05 mg/kg, recoveries for AMPA: 66%, 98%, 89%, 4 replicate field samples, max. sample storage: 3 months RIP9501286
MLL 30.235, MLL 30235, 93, 2 B United Kingdom, Graveley 1989-11-07	Libravo	1) (sowing) 2) 3)	2.0	200	1.0	1989-07-12[4)]	BBCH 82-83	seed(s)	1.0 1.3 0.90 0.70	<0.05 <0.05 <0.05 <0.05	7 7 7 7	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: 0.05 mg/kg, recoveries for AMPA: 66%, 98%, 89%, 4 replicate field samples, max. sample storage: 3 months RIP9501286
MLL 30.235, MLL 30235, 93, 3 A United Kingdom, Cirencester 1989-11-07	Cobra	1) (sowing) 2) 3)	1.0	200	0.50	1989-07-12[4)]	BBCH 82-83	seed(s)	0.20 0.50 0.60 0.40	<0.05 <0.05 <0.05 <0.05	8 8 8 8	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: 0.05 mg/kg, recoveries for AMPA: 66%, 98%, 89%, 4 replicate field samples, max. sample storage: 3 months RIP9501286

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.235, MLL 30235, 93, 3 B United Kingdom, Cirencester 1989-11-07	Cobra	1) (sowing) 2) 3)	2.0	200	1.0	1989-07-12[4)]	BBCH 82-83	seed(s)	0.50 0.80 0.30 0.60	<0.05 <0.05 <0.05 <0.05	8 8 8 8	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: 0.05 mg/kg, recoveries for AMPA: 66%, 98%, 89%, 4 replicate field samples, max. sample storage: 3 months RIP9501286
MLL 30.235, MLL 30235, 93, 4 A United Kingdom, Rumbelow 1989-11-07	Ariana	1) (sowing) 2) 3)	1.0	200	0.50	1989-07-14[4)]	BBCH 82-83	seed(s)	0.50 0.60 0.90 0.90	<0.05 <0.05 <0.05 <0.05	7 7 7 7	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: Glyphosat: 0.1 mg/kg, AMPA: 0.05 mg/kg, 4 replicate field samples, max. sample storage: 3 months RIP9501286
MLL 30.235, MLL 30235, 93, 4 B United Kingdom, Rumbelow 1989-11-07	Ariana	1) (sowing) 2) 3)	2.0	200	1.0	1989-07-14[4)]	BBCH 82-83	seed(s)	1.5 1.3 0.90 1.1	<0.05 <0.05 <0.05 <0.05	7 7 7 7	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: Glyphosat: 0.1 mg/kg, AMPA: 0.05 mg/kg, 4 replicate field samples, max. sample storage: 3 months RIP9501286

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.235, MLL 30235, 93, 5 A United Kingdom, Hunter Scotland 1989-11-07	Cobra	1) (sowing) 2) 3)	1.0	200	0.50	1989-08-02[4)]	BBCH 82-83	seed(s)	1.1 1.4 1.5 0.80	<0.05 <0.05 <0.05 <0.05	7 7 7 7	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: Glyphosat: 0.1 mg/kg, AMPA: 0.05 mg/kg (recov-eries. 89%, 65%), 4 replicate field samples, max. sample storage: 2 months RIP9501286
MLL 30.235, MLL 30235, 93, 5 B United Kingdom, Hunter Scotland 1989-11-07	Cobra	1) (sowing) 2) 3)	2.0	200	1.0	1989-08-02[4)]	BBCH 82-83	seed(s)	0.80 1.0 1.8 0.70	<0.05 <0.05 <0.05 <0.05	7 7 7 7	4) spraying analytical method: XA001 (HPLC with OPA), LOQ: Glyphosat: 0.1 mg/kg, AMPA: 0.05 mg/kg (recov-eries. 89%, 65%), 4 replicate field samples, max. sample storage: 2 months RIP9501286

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360~~355.7~~ g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Summer Rape

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.104, MLL 30104, 51, p 84 Denmark Slagelse 1983-04-18	Karat	1) (sowing) 2) 3)	1.5			1982-08-06[4)]	no data	seed(s)	4.1	<0.2	20	4) spraying analytical method: XA001 (Glyphosat: HPLC with OPA, AMPA: HPLC with Ninhydrin), LOQ: Glyphosat: 0.5 mg/kg, AMPA: 0.2 mg/kg, max. sample storage: no data RIP9501265
MLL 30.104, MLL 30104, 51, p 85 Denmark Horsens 1983-04-18	Brutor	1) (sowing) 2) 3)	1.5			1982-08-09[4)]	no data	seed(s)	6.7	<0.2	21	4) spraying analytical method: XA001 (Glyphosat: HPLC with OPA, AMPA: HPLC with Ninhydrin), LOQ: Glyphosat: 0.5 mg/kg, AMPA: 0.2 mg/kg, max. sample storage: no data RIP9501265

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.104, MLL 30104, 51, p 86 Denmark Hasley 1983-04-18	Niklon	1) (sowing) 2) 3)	1.5			1982-08-12[4)]	no data	seed(s), RAC Cake, press Cake, roasted refined oil	8.6 11.8 11.4 <0.4	<0.2 <0.2 <0.2 <0.2	21 21 21 21	4) spraying analytical method: XA001 (Glyphosat: HPLC with OPA, AMPA: HPLC with Ninhydrin), LOQ: 1.0 mg/kg in seeds, 2.0 mg/kg in press cake, 0.4 mg/kg in oil, AMPA: 0.2 mg/kg, max. sample storage: no data RIP9501265
MLL 30.104, MLL 30104, 51, p 99 A United Kingdom (UK) Bottisham 1983-04-18	Brutor	1) 1982-03-31 (sowing) 2) 3)	1.4			1982-08-19[4)]	BBCH 75-79	seed(s)	<1.0 <1.0 <1.0 <1.0	<0.2 <0.2 <0.2 <0.2	14 14 14 14	4) spraying analytical method: XA001 (Glyphosat: HPLC with OPA, AMPA: HPLC with Ninhydrin), LOQ: Glyphosat: 1.0 mg/kg, AMPA: 0.2 mg/kg, 4 replicate field samples, max. sample storage: no data RIP9501265
MLL 30.104, MLL 30104, 51, p 99 B United Kingdom (UK) Bottisham 1983-04-18	Brutor	1) 1982-03-31 (sowing) 2) 3)	2.8			1982-08-19[4)]	BBCH 75-79	seed(s)	<1.0 <1.0 <1.0 <1.0	<0.2 <0.2 <0.2 <0.2	14 14 14 14	4) spraying analytical method: XA001 (Glyphosat: HPLC with OPA, AMPA: HPLC with Ninhydrin), LOQ: Glyphosat: 1.0 mg/kg, AMPA: 0.2 mg/kg, 4 replicate field samples, max. sample storage: no data RIP9501265

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360~~355.7~~ g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Rape

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.104, MLL 30104, 51, p 100 A United Kingdom Easton 1983-04-18	Jet Neuf	1) (sowing) 2) 3)	1.4			1982-07-12[4)]	no data	seed(s) seed(s), RAC Cake, press crude oil refined oil	1.0 1.5 1.4 2.0 1.5 <2.0 <0.4 <0.4	<0.2 <0.2 <0.2 <0.2 <0.2 <0.2 <0.2 <0.2	14 14 14 14 14 14 14 14	4) spraying analytical method: XA001 (Glyphosat: HPLC with OPA, AMPA: HPLC with Ninhydrin), LOQ: Glyphosat: 1.0 mg/kg in seeds, 2.0 mg/kg in press cake, 0.4 mg/kg in oil AMPA: 0.2 mg/kg, 4 replicate field samples, max. sample storage: no data RIP9501265

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.104, MLL 30104, 51, p 100 B United Kingdom Easton 1983-04-18	Jet Neuf	1) (sowing) 2) 3)	2.8			1982-07-12[4)]	no data	seed(s) seed(s), RAC Cake, press crude oil refined oil	3.3 3.0 2.6 3.9 3.2 4.9 <0.4 <0.4	<0.2 <0.2 <0.2 <0.2 <0.2 <0.2 <0.2 <0.2	14 14 14 14 14 14 14 14	4) spraying analytical method: XA001 (Glyphosat: HPLC with OPA, AMPA: HPLC with Ninhydrin), LOQ: 1.0 mg/kg in seeds, 2.0 mg/kg in press cake, 0.4 mg/kg in oil, AMPA: 0.2 mg/kg, 4 replicate field samples, max. sample storage: no data RIP9501265
MLL 30.104, MLL 30104, 51, p 101 A United Kingdom Easton 1983-04-18	Jet Neuf	1) (sowing) 2) 3)	1.4			1982-07-12[4)]	no data	seed(s) seed(s), RAC Cake, press crude oil refined oil	1.7 4.1 2.1 3.0 2.7 3.1 <0.4 <0.4	<0.2 <0.2 <0.2 <0.2 <0.2 <0.2 <0.2 <0.2	14 14 14 14 14 14 14 14	4) spraying analytical method: XA001 (Glyphosat: HPLC with OPA, AMPA: HPLC with Ninhydrin), LOQ: 1.0 mg/kg in seeds, 2.0 mg/kg in press cake, 0.4 mg/kg in oil, AMPA: 0.2 mg/kg, 4 replicate field samples, max. sample storage: no data RIP9501265

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.104, MLL 30104, 51, p 101 B United Kingdom Easton 1983-04-18	Jet Neuf	1) (sowing) 2) 3)	2.8			1982-07-12[4)]	no data	seed(s) seed(s), RAC Cake, press crude oil refined oil	3.2 3.9 5.2 3.6 4.0 8.9 <0.4 <0.4	<0.2 <0.2 <0.2 <0.2 <0.2 <0.2 <0.2 <0.2	14 14 14 14 14 14 14 14	4) spraying analytical method: XA001 (Glyphosat: HPLC with OPA, AMPA: HPLC with Ninhydrin), LOQ: 1.0 mg/kg in seeds, 2.0 mg/kg in press cake, 0.4 mg/kg in oil, AMPA: 0.2 mg/kg, 4 replicate field samples, max. sample storage: no data RIP9501265
MLL 30.104, MLL 30104, 51, p 89 Sweden 1983-04-18	no data	1) (sowing) 2) 3)	1.4			1980-08-23[4)]		seed(s) straw	2.0 29.9	<0.2 0.30	18 18	4) spraying analytical method: GC-FDP, LOQ: Glyphosat: 0.5 mg/kg, AMPA: 0.2 mg/kg, max. sample storage: no data RIP9501265
MLL 30.104, MLL 30104, 51, p 89 B Sweden 1983-04-18	no data	1) (sowing) 2) 3)	2.2			1980-08-23[4)]	no data	seed(s) straw	1.4 16.1	<0.2 <0.2	18 18	4) spraying analytical method: GC-FDP, LOQ: Glyphosat: 0.5 mg/kg, AMPA: 0.2 mg/kg, max. sample storage: no data RIP9501265

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.104, MLL 30104, 51, p 90 A United Kingdom Cottenham 1983-04-18	Jet Neuf	1) (sowing) 2) 3)	0.72			1980-07-22[4)]	no data	seed(s)	<0.5 <0.5 <0.5 <0.5	<0.2 <0.2 <0.2 <0.2	7 7 7 7	4) spraying analytical method: GC-FDP, LOQ: Glyphosat: 0.5 mg/kg, AMPA: 0.2 mg/kg, 4 replicate field samples, max. sample storage: no data RIP9501265
MLL 30.104, MLL 30104, 51, p 90 B United Kingdom Cottenham 1983-04-18	Jet Neuf	1) (sowing) 2) 3)	1.4			1980-07-22[4)]		seed(s)	0.70 0.50 0.60 0.60	<0.2 <0.2 <0.2 <0.2	7 7 7 7	4) spraying analytical method: GC-FDP, LOQ: Glyphosat: 0.5 mg/kg, AMPA: 0.2 mg/kg, 4 replicate field samples, max. sample storage: no data RIP9501265
MLL 30.104, MLL 30104, 51, p 90 C United Kingdom Cottenham 1983-04-18	Jet Neuf	1) (sowing) 2) 3)	2.9			1980-07-22[4)]		seed(s)	1.3 2.5 1.9 1.7	<0.2 <0.2 <0.2 <0.2	7 7 7 7	4) spraying analytical method: GC-FDP, LOQ: Glyphosat: 0.5 mg/kg, AMPA: 0.2 mg/kg, 4 replicate field samples, max. sample storage: no data RIP9501265

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.104, MLL 30104, 51, p 91 A United Kingdom Sheardown 1983-04-18	Brutor	1) (sowing) 2) 3)	1.4	200	0.72	1980-09-08[4)]	no data	seed(s)	<0.5 <0.5 <0.5 <0.5	<0.2 <0.2 <0.2 <0.2	11 11 11 11	4) spraying analytical method: GC-FDP, LOQ: Glyphosat: 0.5 mg/kg, AMPA: 0.2 mg/kg, 4 replicate field samples, max. sample storage: no data RIP9501265
MLL 30.104, MLL 30104, 51, p 91 B United Kingdom Sheardown 1983-04-18	Brutor	1) (sowing) 2) 3)	2.9	200	1.4	1980-09-08[4)]	no data	seed(s)	0.80 0.80 1.2 1.0	<0.2 <0.2 <0.2 <0.2	11 11 11 11	4) spraying analytical method: GC-FDP, LOQ: Glyphosat: 0.5 mg/kg, AMPA: 0.2 mg/kg, 4 replicate field samples, max. sample storage: no data RIP9501265
MLL 30.104, MLL 30104, 51, p 92 United Kingdom Sheardown 1983-04-18	Brutor	1) (sowing) 2) 3)	1.4			1980-09-08[4)]		seed(s)	<0.5	<0.2	11	4) spraying analytical method: GC-FDP, LOQ: Glyphosat: 0.5 mg/kg, AMPA: 0.2 mg/kg, max. sample storage: no data RIP9501265

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.104, MLL 30104, 51, p 93 Denmark 1983-04-18	Line	1) (sowing) 2) 3)	1.4			1981-10-08[4)]	no data	seed(s)	<0.5	<0.2	17	4) spraying analytical method: GC-FDP, LOQ: Glyphosat: 0.5 mg/kg, AMPA: 0.2 mg/kg, no control value, max. sample storage: no data RIP9501265
MLL 30.104, MLL 30104, 51, p 94 Denmark 1983-04-18	Gulliver	1) (sowing) 2) 3)	1.4			1981-10-08[4)]		seed(s)	11.6	<0.2	17	4) spraying analytical method: GC-FDP, LOQ: Glyphosat: 0.5 mg/kg, AMPA: 0.2 mg/kg, no control value, max. sample storage: no data RIP9501265
MLL 30.104, MLL 30104, 51, p 95 Finland 1983-04-18	Torch	1) (sowing) 2) 3)	1.5			1981-09-07[4)]	no data	seed(s)	6.3 1.5	<0.2 <0.2	4 15	4) spraying analytical method: GC-FDP, LOQ: Glyphosat: 0.5 mg/kg, AMPA: 0.2 mg/kg, no controls, max. sample storage: no data RIP9501265
MLL 30.104, MLL 30104, 51, p 96 Finland 1983-04-18	Oro	1) (sowing) 2) 3)	1.5			1981-09-09[4)]	no data	seed(s)	4.3 0.80	<0.2 <0.2	2 20	4) spraying analytical method: GC-FDP, LOQ: Glyphosat: 0.5 mg/kg, AMPA: 0.2 mg/kg, max. sample storage: no data RIP9501265

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.104, MLL 30104, 51, p 97 Sweden 1983-04-18	Svalof	1) (sowing) 2) 3)	1.5			1981-08-24[4)]	no data	plant, dry seed(s)	30.0 <0.5	0.40 <0.2	10 10	4) spraying analytical method: GC-FDP, LOQ: Glyphosat: 0.5 mg/kg, AMPA: 0.2 mg/kg, no recovery for plant, max. sample storage: no data RIP9501265
MLL 30.104, MLL 30104, 51, p 98 A Sweden Skaberjo 1983-04-18	no data	1) (sowing) 2) 3)	0.72			1981-08-10[4)]	no data	seed(s)	1.1	<0.2	16	4) spraying analytical method: GC-FDP, LOQ: Glyphosat: 0.5 mg/kg, AMPA: 0.2 mg/kg, max. sample storage: no data RIP9501265
MLL 30.104, MLL 30104, 51, p 98 B Sweden Skaberjo 1983-04-18	no data	1) (sowing) 2) 3)	1.1			1981-08-10[4)]	no data	seed(s)	1.8	<0.2	16	4) spraying analytical method: GC-FDP, LOQ: Glyphosat: 0.5 mg/kg, AMPA: 0.2 mg/kg, max. sample storage: no data RIP9501265
MLL 30.104, MLL 30104, 51, p 98 C Sweden Skaberjo 1983-04-18	no data	1) (sowing) 2) 3)	1.5			1981-08-10[4)]	no data	seed(s)	2.8	<0.2	16	4) spraying analytical method: GC-FDP, LOQ: Glyphosat: 0.5 mg/kg, AMPA: 0.2 mg/kg, max. sample storage: no data RIP9501265

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : (MON 2139) Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Oilseed rape

Submission date : 2001-11-28

Indoors / outdoors : Outdoors (European South)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 31336 9076 DR1 Plot C FR-47120 Duras 15.07.2001	Corrida	1) 29.08.1998 (sowing) 2) – 3)	1.4	172	0.82	18.06.1999	BBCH 85	seed	2.4 1.2	<0.05 <0.05	0 10	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 12 months RIP2002-651
MLL 31336 9076 BD1 Plot C FR-26750 St. Paul les Romans 15.07.2001	Bristol	1) 09.09.1998 (sowing) 2) -- 3)	1.5	184	0.82	18.06.1999	BBCH 89	seed	1.4 0.23	<0.05 <0.05	0 13	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 12 months RIP2002-651

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 450 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : (MON 2139) Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Oilseed rape

Submission date : 2001-11-28

Indoors / outdoors : Outdoors (European South)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 31336 9076 DR1 Plot D FR-47120 Duras 15.07.2001	Corrida	1) 29.08.1998 (sowing) 2) – 3)	1.4	171	0.82	18.06.1999	BBCH 85	seed	1.3 1.4	<0.05 <0.05	0 10	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 12 months RIP2002-651
MLL 31336 9076 BD1 Plot D FR-26750 St. Paul les Romans 15.07.2001	Bristol	1) 09.09.1998 (sowing) 2) -- 3)	1.5	183	0.82	18.06.1999	BBCH 89	seed	2.3 0.08	<0.05 <0.05	0 13	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 12 months RIP2002-651

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : (MON 2139) Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Oilseed rape

Submission date : 2000-05-30

Indoors / outdoors : Outdoors (European South)
Other a. i. in formulation
(common name and content) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30817 R 8145 DR1 FR- 47120 Loubes- Bernac 15.03.2000	Mavaja	1) 05.09.1997 (sowing) 2) – 3) 04.07.1998	1.5	212	0.719	22.06.1998	BBCH 87	seed	3.4 0.40	0 12	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 9 months RIP2000-1244
MLL 30817 R 8145 BD1 FR- 26100 Romans 15.03.2000	Cocktail	1) 04.09.1997 (sowing) 2) – 3) 29.06.1998	1.5	212	0.719	19.06.1998	BBCH 89	seed	22 1.8	0 10	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 9 months RIP2000-1244

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Active ingredient : Glyphosate
Crop / crop group : Oilseed rape

Submission date : 2000-05-30

Content of a.i. (g/kg or g/l) : 680 g/kg
Formulation (e.g. WP) : WG
Commercial product (name) : (MON 14420) Roundup TURBO
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Indoors / outdoors : Outdoors (European South)
Other a. i. in formulation (common name and content) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30817 R 8145 DR1 FR- 47120 Loubes- Bernac 15.03.2000	Mavaja	1) 05.09.1997 (sowing) 2) – 3) 04.07.1998	1.5	214	0.716	22.06.1998	BBCH 87	seed	3.1 0.93	0 12	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 9 months RIP2000-1244
MLL 30817 R 8145 BD1 FR- 26100 Romans 15.03.2000	Cocktail	1) 04.09.1997 (sowing) 2) – 3) 29.06.1998	1.5	204	0.714	19.06.1998	BBCH 89	seed	19 5.6	0 10	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 9 months RIP2000-1244

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Active ingredient : Glyphosate
Crop / crop group : Oilseed rape

Submission date : 2000-05-30

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : (MON 2139) Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Indoors / outdoors : Outdoors (European South)
Other a. i. in formulation (common name and content) :
Residues calculated as : AMPA

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30817 R 8145 DR1 FR- 47120 Loubes- Bernac (S-FR) 15.03.2000	Mavaja	1) 05.09.1997 (sowing) 2) – 3) 04.07.1998	1.53	212	0.719	22.06.1998	BBCH 87	seed	<0.05 <0.05	0 12	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 9 months RIP2000-1244
MLL 30817 R 8145 BD1 FR- 26100 Romans (S-FR) 15.03.2000	Cocktail	1) 04.09.1997 (sowing) 2) – 3) 29.06.1998	1.53	212	0.719	19.06.1998	BBCH 89	seed	0.07 <0.05	0 10	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 9 months RIP2000-1244

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Active ingredient : Glyphosate
Crop / crop group : Oilseed rape

Submission date : 2000-05-30

Content of a.i. (g/kg or g/l) : 680 g/kg
Formulation (e.g. WP) : WG
Commercial product (name) : (MON 14420) Roundup TURBO
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Indoors / outdoors : Outdoors (European South)
Other a. i. in formulation
(common name and content) :
Residues calculated as : AMPA

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30817 R 8145 DR1 FR- 47120 Loubes- Bernac (S-FR) 15.03.2000	Mavaja	1) 05.09.1997 (sowing) 2) – 3) 04.07.1998	1.53	214	0.716	22.06.1998	BBCH 87	seed	<0.05 <0.05	0 12	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 9 months RIP2000-1244
MLL 30817 R 8145 BD1 FR- 26100 Romans (S-FR) 15.03.2000	Cocktail	1) 04.09.1997 (sowing) 2) – 3) 29.06.1998	1.46	204	0.714	19.06.1998	BBCH 89	seed	0.07 0.07	0 10	analytical method: LLN-ES-ASOP-051-8 (HPLC-FD), LOQ 0.05 mg/kg, max. sample storage: 9 months RIP2000-1244

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.18 Barley (Northern Europe)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate (Isopropylamin-salt)
Crop / crop group : Spring Barley

Submission date : 01.09.1995

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)			(d)	(e)
report MLL 30037, A 36, trial 16524	Lofa Abed	1) – (sowing)	1.4	200	0.70	30.08.1979	BBCH 91	grain	3.6	<0.05	7	
		2) --										analytical method: GC-FPD), LOQ's: 0.05 mg/kg
GB-		3) --						straw	48.0	0.62	7	
Lecklade, Gloucester			2.9	200	1.4	30.08.1979	BBCH 91	grain	7.9	<0.05	7	RIP9501230
23.10.1979								straw	119	1.2	7	
report MLL 30046, A 36, trial 41 Viev I	Aramin	1) – (sowing)	4.3	500	0.86	06.08.1979	BBCH 93	grain	5.2	<0.05	1	
		2) --							2.7	<0.05	7	
FR-41		3) --							1.4	<0.05	14	
Vievry le Rayé									1.2	<0.05	21	
								straw	232	0.80	1	
25.01.1980									45.5	0.15	7	analytical method: GC-FPD), LOQ's: 0.05 mg/kg
									39.2	0.18	14	
									34.6	0.13	21	
			8.6	500	1.7	06.08.1979	BBCH 93	grain	18.0	0.09	1	RIP9501231
									4.7	<0.05	7	
									4.8	<0.05	14	
									2.8	<0.05	21	

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)			(d)	(e)
								straw	857 88.7 65.0 67.9	0.80 0.15 0.18 0.13	1 7 14 21	
report MLL 30046, A 36, trial 41 Viev II FR-41 Vievry le Rayé 25.01.1980	Aramin	1) – (sowing) 2) -- 3) --	4.3	500	0.86	06.08.1979	BBCH 93	grain straw grain straw	12.2 7.8 5.4 4.2 548 90.0 46.0 39.0 42.0 9.7 11.4 9.3 985 147 86.0 67.0	<0.05 <0.05 <0.05 <0.05 1.6 0.59 0.40 0.26 <0.05 <0.05 <0.05 <0.05 1.95 0.71 0.67 0.66	0 7 14 21 0 7 14 21 0 7 14 21 0 7 14 21	analytical method: GC-FPD), LOQ's: 0.05 mg/kg RIP9501231
			8.6	500	1.7	06.08.1979	BBCH 93					
report MLL 30070, A 36, trial RS 5E/AN, processing GB- Debden, Essex 28.10.1981	Keg	1) – (sowing) 2) -- 3) --	1.4	200	0.70	10.08.1980	BBCH 91	grain grain, RAC malt	2.7 3.2 2.0 2.6 0.10	<0.05 <0.05 <0.05 n.a. n.a.	6 6 6 6 6	analytical method: GC-FPD), LOQ's: 0.05 mg/kg, replicate field samples
			2.9	200	1.4	10.08.1980	BBCH 91	grain grain, RAC malt	5.0 6.4 5.9 6.0 0.19	<0.05 <0.05 <0.05 n.a. n.a.	6 6 6 6 6	RIP9501238, RIP9501256
			2.9	200	1.4	13.08.1980	BBCH 91	grain	5.7 6.3 6.9	<0.05 <0.05 <0.05	3 3 3	

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)			(d)	(e)
								grain, RAC malt	6.3 0.15	n.a. n.a.	3 3	
report MLL 30070, A 36, trial RS 6E/AN, processing GB- Cottenham, Cambridgeshire 28.10.1981	Sundance	1) – (sowing) 2) -- 3) --	1.4	200	0.70	01.09.1980	BBCH 91	grain grain, RAC malt	5.6 6.1 6.8 6.2 0.06	<0.05 <0.05 <0.05 n.a. n.a.	7 7 7 7 7	analytical method: GC-FPD), LOQ's: 0.05 mg/kg, replicate field samples
			2.9	200	1.4	01.09.1980	BBCH 91	grain grain, RAC malt	9.5 9.8 14.2 11.2 0.20	0.05 0.05 <0.05 n.a. n.a.	7 7 7 7 7	RIP9501238, RIP9501256
			2.9	200	1.4	05.09.1980	BBCH 91	grain grain, RAC malt	11.9 9.3 11.0 10.7 0.17	<0.05 <0.05 <0.05 n.a. n.a.	3 3 3 3 3	
report MLL 30070, A 36, trial RS 7E/AN, processing GB- Balsham, Cambridgeshire 28.10.1981	Arc Royal	1) – (sowing) 2) -- 3) --	1.4	200	0.70	01.09.1980	BBCH 91	grain grain, RAC malt	14.8 13.2 10.8 12.8 0.10	0.08 0.13 0.12 n.a. n.a.	8 8 8 8 8	analytical method: GC-FPD), LOQ's: 0.05 mg/kg, replicate field samples
			2.9	200	1.4	01.09.1980	BBCH 91	grain grain, RAC malt	14.4 14.5 12.2 13.7 0.15	<0.05 <0.05 <0.05 n.a. n.a.	8 8 8 8 8	RIP9501238, RIP9501256
			2.9	200	1.4	05.09.1980	BBCH 91	grain grain, RAC malt	15.1 16.2 15.0 15.4 0.19	0.05 0.09 0.05 n.a. n.a.	4 4 4 4 4	

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)			(d)	(e)
report MLL 30070, A 36, trial CPH 80/2, processing GB- Kelso-Borders- Scotland 28.10.1981	Golden Promise	1) – (sowing) 2) -- 3) --	1.4	200	0.70	02.09.1980	BBCH 93	grain grain, RAC malt	12.5 12.6 12.4 12.5 0.22	<0.05 <0.05 <0.05 n.a. n.a.	7 7 7 7 7	analytical method: GC-FPD), LOQ's: 0.05 mg/kg, replicate field samples
			2.9	200	1.4	02.09.1980	BBCH 93	grain grain, RAC malt	27.6 36.0 34.8 32.8 0.40	0.05 0.1 0.1 n.a. n.a.	7 7 7 7 7	RIP9501238, RIP9501256
			2.9	200	1.4	05.09.1980	BBCH 93	grain grain, RAC malt	27.7 24.1 25.5 25.7 0.28	0.05 0.05 0.05 n.a. n.a.	4 4 4 4 4	
report MLL 30070, A 36, processing GB- Fulbourn Cambridge 28.10.1981	Maris Otter	1) – (sowing) 2) -- 3) --	1.4	200	0.70	21.08.1980	BBCH 93	grain grain, RAC malt	3.2 5.0 5.4 4.5 0.07	<0.05 <0.05 <0.05 n.a. n.a.	7 7 7 7 7	analytical method: GC-FPD), LOQ's: 0.05 mg/kg, replicate field samples
			2.9	200	1.4	21.08.1980	BBCH 93	grain grain, RAC malt	8.8 8.7 11.0 9.5 0.18	<0.05 0.05 0.1 n.a. n.a.	7 7 7 7 7	RIP9501238, RIP9501256
			2.9	200	1.4	24.08.1980	BBCH 93	grain grain, RAC malt	7.6 9.9 7.2 8.2 0.21	<0.05 <0.05 <0.05 n.a. n.a.	4 4 4 4 4	

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)			(d)	(e)
report MLL 30070, A 36, processing GB- Fulbourn Cambridge 28.10.1981	Maris Otter	1) – (sowing) 2) -- 3) --	1.4	200	0.70	21.08.1980	BBCH 93	grain grain, RAC malt for ale production malt for lager production	2.8 2.7 0.05 0.11	<0.05 n.a n.a. n.a.	7 7 7 7	analytical method: GC-FPD), LOQ's: 0.05 mg/kg, replicate field samples RIP9501238, RIP9501256
			2.9	200	1.4	21.08.1980	BBCH 93	grain grain, RAC malt for ale production malt for lager production beer (ale, yeast 240) beer (ale, yeast 1026) beer (lager, yeast 1342)	6.6 6.2 0.10 0.13 <0.05 <0.05 <0.05	<0.05 n.a n.a. n.a. <0.05 <0.05 <0.05	4 4 4 4 4 4 4	

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : August 2000

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : (MON 2139) Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30815, 98-GLY-06-B/F, R 8144 CT2/C FR- 36110 Levroux 05/1999	Nevada	1) 13.02.1998 (sowing) 2) – 3) 22.07.1998	2.2	204	1.1	13.07.1998	BBCH 87	grain straw	17 5.8 4.2 250 11 12	0.05 0.05 0.05 0.97 0.26 0.40	0 9 14 0 9 14	analytical method: (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Replicate Plot to 98-GLY-06-B/F, R 8144 CT2/D RIP2000-1243
MLL 30815,98-GLY-06-B/F,R 8144 AN1/C FR- 68740 Favieres 05/1999	Scarlet	1) 20.02.1998 (sowing) 2) – 3) 18.07.1998	2.3	200	1.1	11.07.1998	BBCH 87	grain straw	22 1.8 1.4 225 4.4 6.0	0.06 <0.05 <0.05 0.74 <0.05 0.06	0 7 14 0 7 14	analytical method: (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Replicate Plot to 98-GLY-06-B/F,R 8144 AN1/D RIP2000-1243

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 680 g/kg
Formulation (e.g. WP) : WG
Commercial product (name) : MON 14420
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : August 2000

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30815, 98-GLY-06-B/F, R 8144 CT2/D FR- 36110 Levroux 05/1999	Nevada	1) 13.02.1998 (sowing) 2) – 3) 22.07.1998	2.2	204	1.1	13.07.1998	BBCH 87	grain straw	15 5.9 4.0 206 11 9.2	<0.05 0.05 <0.05 0.78 0.35 0.30	0 9 14 0 9 14	analytical method: (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Replicate Plot to 98-GLY-06-B/F, R 8144 CT2/C RIP2000-1243
MLL 30815, 98-GLY-06-B/F, R 8144 AN1/D FR- 68740 Favieres 05/1999	Scarlet	1) 20.02.1998 (sowing) 2) – 3) 18.07.1998	2.3	200	1.1	11.07.1998	BBCH 87	grain straw	17 2.0 2.2 208 <0.05 3.2	0.06 <0.05 <0.05 0.79 <0.05 <0.05	0 7 14 0 7 14	analytical method: (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Replicate Plot to 98-GLY-06-B/F,R 8144 AN1/C RIP2000-1243

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Taifun forte
Applicant : ADAMA Agan Ltd

Active ingredient : Glyphosate (Isopropylamin-salt)
Crop / crop group : Spring Barley

Submission date : 2008-07-25

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AM-PA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
FSG-0606, trial 3 Germany 47574 Goch-Asperden 2007-09-26	Estana (lodged grain)	1) 2006-03-30 (sowing) 2) 2006-05-24 - 2006-06-07 3) 2006-07-18	1.86	310	0.6	2006-07-05[4)]	BBCH 77-83	grain straw	8.0 54	0.17 4.5	13 13	4) spraying analytical method: DFG method 405 (HPLC-FLD) for both substances, LOQ's: 0.05 mg/kg, max. sample storage: 11 months ASB2008-5610

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 650 g/l
Formulation (e.g. WP) : SG
Commercial product (name) : MON 78568
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2005-04-21

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 BP2, plot D France 45170 Frapuy-Attray 2002-06-13	Scarlett	1) 2001-02-19 (sowing) 2) 3)	2.2	305	0.72	2001-07-16[4)]	BBCH 87	grain straw	4.1 41.2	0.16 0.97	7 7	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 5 months for grain, 7 months for straw Replicate to R A1157, trial A1157 BP2, plot C RIP2005-200
R A1157,	Scarlett	1) 2001-02-20	2.1	288	0.72	2001-07-09[4)]	BBCH 87	grain	2.4	<0.05	7	4) spraying

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
trial A1157 CT2, plot D France 36110 Bois Clair-Levroux 2002-06-13		(sowing) 2) 3)						straw	13.3	0.26	7	analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 5 months for grain, 7 months for straw Replicate to R A1157, trial A1157 CT2, plot C RIP2005-200
R A1157, trial A1157 HA1, plot D Germany 31515 Wunstorf 2002-06-13	Theresa	1) 2000-09-26 (sowing) 2) 3)	2.1	297	0.72	2001-07-06[4)]	BBCH 85	grain straw	3.5 18.0	0.070 0.19	7 7	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 5 months for grain, 7 months for straw Replicate to R A1157, trial A1157 HA1, plot C RIP2005-200
R A1157,	Barke	1) 2001-02-21	2.0	283	0.72	2001-07-26[4)]	BBCH 87-89	grain	6.6	0.11	7	4) spraying

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
trial A1157 OB1, plot D France 29830 Kerloroc-Ploudalmézeau 2002-06-13		(sowing) 2) 3)						straw	82.9	1.2	7	analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 4 months for grain, 6 months for straw Replicate to R A1157, trial A1157 OB1, plot C RIP2005-200

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 540 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : MON 78273
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2005-04-21

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 BP2, plot C France 45170 Frapuy-Attray 2002-06-13	Scarlett	1) 2001-02-19 (sowing) 2) 3)	2.1	297	0.72	2001-07-16[4)]	BBCH 87	grain straw	3.8 41.7	0.17 0.98	7 7	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 5 months for grain, 7 months for straw Replicate to R A1157, trial A1157 BP2, plot D RIP2005-200
R A1157,	Scarlett	1) 2001-02-20	2.1	296	0.72	2001-07-09[4)]	BBCH 87	grain	2.8	0.050	7	4) spraying

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
trial A1157 CT2, plot C France 36110 Bois Clair-Levroux 2002-06-13		(sowing) 2) 3)						straw	12.3	0.23	7	analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 5 months for grain, 7 months for straw Replicate to R A1157, trial A1157 CT2, plot D RIP2005-200
R A1157, trial A1157 HA1, plot C Germany 31515 Wunstorf 2002-06-13	Theresa	1) 2000-09-26 (sowing) 2) 3)	2.3	313	0.72	2001-07-06[4)]	BBCH 85	grain straw	5.1 34.5	0.060 0.37	7 7	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 5 months for grain, 7 months for straw Replicate to R A1157, trial A1157 HA1, plot D RIP2005-200
R A1157,	Barke	1) 2001-02-21	2.0	283	0.72	2001-07-26[4)]	BBCH 87-89	grain	8.8	0.13	7	4) spraying

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
trial A1157 OB1, plot C France 29830 Kerloroc-Ploudalmézeau 2002-06-13		(sowing) 2) 3)						straw	97.5	1.2	7	analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 4 months for grain, 6 months for straw Replicate to R A1157, trial A1157 OB1, plot D RIP2005-200

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2005-04-21

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 BP2, plot B France 45170 Frapuy-Attray 2002-06-13	Scarlett	1) 2001-02-19 (sowing) 2) 3)	2.2	308	0.72	2001-07-16[4)]	BBCH 87	grain straw	5.9 36.9	0.17 0.76	7 7	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 5 months for grain, 7 months for straw RIP2005-200
R A1157, trial A1157 CT2, plot B France 36110 Bois Clair-Levroux 2002-06-13	Scarlett	1) 2001-02-20 (sowing) 2) 3)	2.2	299	0.72	2001-07-09[4)]	BBCH 87	grain straw	2.8 12.8	0.050 0.19	7 7	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 5 months for grain, 7 monthsfor straw RIP2005-200

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 HA1, plot B Germany 31515 Wunstorf 2002-06-13	Theresa	1) 2000-09-26 (sowing) 2) 3)	2.2	311	0.72	2001-07-06[4)]	BBCH 85	grain straw	5.2 32.2	0.080 0.33	7 7	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 5 months for grain, 7 months for straw RIP2005-200
R A1157, trial A1157 OB1, plot B France 29830 Kerloroc-Ploudalmézeau 2002-06-13	Barke	1) 2001-02-21 (sowing) 2) 3)	2.1	288	0.72	2001-07-26[4)]	BBCH 87-89	grain straw	6.7 69.6	0.10 0.70	7 7	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 4 months for grain, 6 monthsfor straw RIP2005-200

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2010-10-20

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
IF-96/07067-00, trial F-96-3, plot 3 France 67170 Berstheim 1997-06-23	Nevacla	1) 1996-03-25 (sowing) 2) 1996-06-03 - 1996-06-20 3) 1996-07-21	2.1	198	1.0	1996-07-17[4)]	BBCH 92	grain straw	11.1 11.6 11.5 127 133 119	7 7 7 7 7 7	4) spraying analytical method: DFG-method No.405, (HPLC/FLD), LOQ: 0.05 mg/kg straw, LOQ: 0.03 mg/kg grain, replicate field samples, max. sample storage: 4 months for grain, 5 months for straw Replicate Plot to IF-96/07067-00, trial F-96-3, plot 2 ASB2010-14804

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : GLYFOS
Applicant : Cheminova A/S

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2010-10-20

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
IF-96/07067-00, trial F-96-3, plot 2 France 67170 Berstheim 1997-06-23	Nevacla	1) 1996-03-25 (sowing) 2) 1996-06-03 - 1996-06-20 3) 1996-07-21	2.1	198	1.0	1996-07-17[4)]	BBCH 92	grain straw	12.7 16.0 11.4 123 76.7 78.1	7 7 7 7 7 7	4) spraying analytical method: DFG-method No.405, (HPLC/FLD), LOQ: 0.05 mg/kg straw, LOQ: 0.03 mg/kg grain, replicate field samples, max. sample storage: 4 months for grain, 5 months for straw Replicate Plot to IF-96/07067-00, trial F-96-3, plot 3 ASB2010-14804
IF-96/07067-00, trial F-96-3, plot 4 France 67170 Berstheim 1997-06-23	Nevacla	1) 1996-03-25 (sowing) 2) 1996-06-03 - 1996-06-20 3) 1996-07-21	1.7	210	0.79	1996-07-17[4)]	BBCH 92	grain straw	5.2 5.6 7.9 131 109 106	7 7 7 7 7 7	4) spraying analytical method: DFG-method No.405, (HPLC/FLD), LOQ: 0.05 mg/kg straw, LOQ: 0.03 mg/kg grain, replicate field samples, max. sample storage: 4 months for grain, 7 months for straw ASB2010-14804

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 41 VIEV I, 1 WBH, plot 1 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-07	2.1	500	0.43	1979-08-06[4)]	BBCH 87	grain straw	3.9 136	<0.05 0.32	1 1	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Reverse decline study RIP9501231
MLL 30046, A 36, trial 41 VIEV I, 1 WBH, plot 1, 1 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-13	2.1	500	0.43	1979-08-06[4)]	BBCH 87	grain straw	1.2 28.4	<0.05 0.11	7 7	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Reverse decline study RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 41 VIEV I, 1 WBH, plot 1, 2 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-20	2.1	500	0.43	1979-08-06[4)]	BBCH 87	grain straw	0.74 17.0	<0.05 0.090	14 14	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Reverse decline study RIP9501231
MLL 30046, A 36, trial 41 VIEV I, 1 WBH, plot 1, 3 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-27	2.1	500	0.43	1979-08-06[4)]	BBCH 87	grain straw	0.89 16.2	<0.05 0.080	21 21	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months, blind value grain 0.52 mg/kg Reverse decline study RIP9501231
MLL 30046, A 36, trial 41 VIEV I, 1 WBH, plot 2 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-07	4.3	500	0.86	1979-08-06[4)]	BBCH 87	grain straw	5.2 232	<0.05 0.80	1 1	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Reverse decline study RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 41 VIEV I, 1 WBH, plot 2, 1 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-13	4.3	500	0.86	1979-08-06[4)]	BBCH 87	grain straw	2.7 45.5	<0.05 0.15	7 7	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Reverse decline study RIP9501231
MLL 30046, A 36, trial 41 VIEV I, 1 WBH, plot 2, 2 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-20	4.3	500	0.86	1979-08-06[4)]	BBCH 87	grain straw	1.4 39.2	<0.05 0.18	14 14	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Reverse decline study RIP9501231
MLL 30046, A 36, trial 41 VIEV I, 1 WBH, plot 2, 3 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-27	4.3	500	0.86	1979-08-06[4)]	BBCH 87	grain straw	1.2 34.6	<0.05 0.13	21 21	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months, blind value grain 0.52 mg/kg Reverse decline study RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 41 VIEV I, 1 WBH, plot 3 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-07	8.6	500	1.7	1979-08-06[4)]	BBCH 87	grain straw	18.0 857	0.090 3.2	1 1	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Reverse decline study RIP9501231
MLL 30046, A 36, trial 41 VIEV I, 1 WBH, plot 3, 1 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-13	8.6	500	1.7	1979-08-06[4)]	BBCH 87	grain straw	4.7 88.7	<0.05 0.41	7 7	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Reverse decline study RIP9501231
MLL 30046, A 36, trial 41 VIEV I, 1 WBH, plot 3, 2 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-20	8.6	500	1.7	1979-08-06[4)]	BBCH 87	grain straw	4.8 65.0	<0.05 0.38	14 14	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Reverse decline study RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 41 VIEV I, 1 WBH, plot 3, 3 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-27	8.6	500	1.7	1979-08-06[4)]	BBCH 87	grain straw	2.8 67.9	<0.05 0.36	21 21	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months, blind value grain 0.52 mg/kg Reverse decline study RIP9501231
MLL 30046, A 36, trial 41 VIEV II, 1 WBH, plot 1 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-06	2.1	500	0.43	1979-08-06[4)]	BBCH 87	grain straw	10.1 417	<0.05 0.90	0 0	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Reverse decline study RIP9501231
MLL 30046, A 36, trial 41 VIEV II, 1 WBH, plot 1, 1 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-13	2.1	500	0.43	1979-08-06[4)]	BBCH 87	grain straw	2.4 44.0	<0.05 0.24	7 7	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Reverse decline study RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 41 VIEV II, 1 WBH, plot 1, 2 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-20	2.1	500	0.43	1979-08-06[4)]	BBCH 87	grain straw	2.3 30.0	<0.05 0.15	14 14	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months, blind value grain 1.4 mg/kg, straw 9.6 Reverse decline study RIP9501231
MLL 30046, A 36, trial 41 VIEV II, 1 WBH, plot 1, 3 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-27	2.1	500	0.43	1979-08-06[4)]	BBCH 87	grain straw	2.7 24.0	<0.05 0.20	21 21	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Reverse decline study RIP9501231
MLL 30046, A 36, trial 41 VIEV II, 1 WBH, plot 2 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-06	4.3	500	0.86	1979-08-06[4)]	BBCH 87	grain straw	12.2 548	<0.05 1.6	0 0	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Reverse decline study RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 41 VIEV II, 1 WBH, plot 2, 1 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-13	4.3	500	0.86	1979-08-06[4)]	BBCH 87	grain straw	7.8 90.0	<0.05 0.59	7 7	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Reverse decline study RIP9501231
MLL 30046, A 36, trial 41 VIEV II, 1 WBH, plot 2, 2 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-20	4.3	500	0.86	1979-08-06[4)]	BBCH 87	grain straw	5.5 46.0	<0.05 0.40	14 14	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months, blind value grain 1.4 mg/kg, straw 9.6 Reverse decline study RIP9501231
MLL 30046, A 36, trial 41 VIEV II, 1 WBH, plot 2, 3 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-27	4.3	500	0.86	1979-08-06[4)]	BBCH 87	grain straw	4.2 39.0	<0.05 0.26	21 21	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Reverse decline study RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 41 VIEV II, 1 WBH, plot 3 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-06	8.6	500	1.7	1979-08-06[4)]	BBCH 87	grain straw	42.0 985	<0.05 2.0	0 0	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Reverse decline study RIP9501231
MLL 30046, A 36, trial 41 VIEV II, 1 WBH, plot 3, 1 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-13	8.6	500	1.7	1979-08-06[4)]	BBCH 87	grain straw	9.7 147	<0.05 0.71	7 7	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Reverse decline study RIP9501231
MLL 30046, A 36, trial 41 VIEV II, 1 WBH, plot 3, 2 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-20	8.6	500	1.7	1979-08-06[4)]	BBCH 87	grain straw	11.4 86.0	<0.05 0.67	14 14	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months, blind value grain 1.4 mg/kg, straw 9.6 Reverse decline study RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 41 VIEV II, 1 WBH, plot 3, 3 France 41 Vievry le Raye, Oncques 1980-01-25	Aramin	1) 1979 (sowing) 2) 3) 1979-08-27	8.6	500	1.7	1979-08-06[4)]	BBCH 87	grain straw	9.3 67.0	<0.05 0.66	21 21	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Reverse decline study RIP9501231

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : MON 52276
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06F06; T1 France 91 Champotteux / Maisse 1991-12-01	Natacha	1) 1991 (planting) 2) 3)	1.1	200	0.54	1991-07-12[4)]	BBCH 87	grain straw	1.2 7.9	<0.05 0.10	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON52276, SL~~C~~ (360g/l Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months, RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06F06; T2 France 91 Champotteux / Maisse 1991-12-01	Natacha	1) 1991 (planting) 2) 3)	2.2	200	1.1	1991-07-12[4)]	BBCH 87	grain straw	1.6 17.0	<0.05 <0.05	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON52276, SL~~C~~ (360g/l Glypho-sate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months Replicate Plot to 06F06; T5 RIP9501287
MLL 30281; 91-GLY-01; 06F06; T5 France 91 Champotteux / Maisse 1991-12-01	Natacha	1) 1991 (planting) 2) 3)	2.2	200	1.1	1991-07-12[4)]	BBCH 87	grain straw	3.3 12.0	<0.05 0.10	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~C~~ (360g/l Glypho-sate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months Replicate Plot to 06F06; T2 RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06F08; T1 France 22 Verneuil sur Serre / Laon 1991-12-01	Natacha	1) 1991 (planting) 2) 3)	1.1	200	0.54	1991-07-30[4)]	BBCH 87	grain straw	4.4 70.0	0.060 0.70	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON52276, SL~~C~~ (360g/l Glypho-sate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months, RIP9501287
MLL 30281; 91-GLY-01; 06F08; T2 France 22 Verneuil sur Serre / Laon 1991-12-01	Natacha	1) 1991 (planting) 2) 3)	2.2	200	1.1	1991-07-30	BBCH 87	grain straw	5.9 160	0.090 1.6	25 25	submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON52276, SL~~C~~ (360g/l Glypho-sate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months Replicate to 91-GLY-01; 06F08; T5 & 91-GLY-01; 06F08; T4 RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06F08; T5 France 22 Verneuil sur Serre / Laon 1991-12-01	Natacha	1) 1991 (planting) 2) 3)	2.2	200	1.1	1991-07-30[4)]	BBCH 87	grain straw	9.6 120	0.090 1.2	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~C~~ (360g/l Glypho-sate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months Replicate to 91-GLY-01; 06F08; T2 & 91-GLY-01; 06F08; T4 RIP9501287

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 420 g/kg
Formulation (e.g. WP) : WG
Commercial product (name) : MON 44068
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06F06; T3 France 91 Champotteux / Maisse 1991-12-01	Natacha	1) 1991 (planting) 2) 3)	1.1	200	0.53	1991-07-12[4)]	BBCH 87	grain straw	1.1 4.2	<0.05 <0.05	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON44068, WG (420g/kg Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months, RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06F06; T4 France 91 Champotteux / Maisse 1991-12-01	Natacha	1) 1991 (planting) 2) 3)	2.1	200	1.1	1991-07-12[4)]	BBCH 87	grain straw	2.2 16.0	<0.05 0.20	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON44068, WG (420g/kg Glypho-sate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months, RIP9501287
MLL 30281; 91-GLY-01; 06F08; T3 France 22 Verneuil sur Serre / Laon 1991-12-01	Natacha	1) 1991 (planting) 2) 3)	1.1	200	0.53	1991-07-30[4)]	BBCH 87	grain straw	2.8 30.0	<0.05 0.30	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON44068, WG (420g/kg Glypho-sate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months, RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06F08; T4 France 22 Verneuil sur Serre / Laon 1991-12-01	Natacha	1) 1991 (planting) 2) 3)	2.1	200	1.1	1991-07-30[4)]	BBCH 87	grain straw	4.0 140	0.060 1.8	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON44068, WG (420g/kg Glypho-sate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months Replicate to 91-GLY-01; 06F08; T2 & 91-GLY-01; 06F08; T5 RIP9501287

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i.	(g/kg or g/l)	:	360 g/l
Formulation	(e.g. WP)	:	SL
Commercial product	(name)	:	Roundup
Applicant		:	Monsanto Agrar Deutschland GmbH

Active ingredient	:	Glyphosate
Crop / crop group	:	Spring Barley
Submission date	:	2012-11-01
Indoors / outdoors	:	Outdoors (European North)
Other a.i. in formulation (content and common name)	:	
Residues calculated as	:	8.1 Glyphosate 8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A1; CP 67573; UK/73/1, part 1 United Kingdom (UK) Mollington 1974-01-07	Julia	1) (planting) 2) - 3) 1973-08-19	1.1			1973-03-10[4]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	162 162	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 16 months, RIP9501209

1	2	3	4			5	6	7	8.1	8.2	9	10
Report No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A1; CP 67573; UK/73/1, part 2 United Kingdom (UK) Mollington 1974-01-07	Julia	1) (planting) 2) 3) 1973-08-19	2.2			1973-03-10[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	162 162	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 16 months, RIP9501209
REPORT A1; CP 67573; UK/73/14, part 1 United Kingdom (UK) Yelden 1974-01-07	Vada	1) (planting) 2) 3) 1973-08-20	2.2			1973-03-08[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	165 165	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 16 months, RIP9501209

1	2	3	4			5	6	7	8.1	8.2	9	10
Report No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A1; CP 67573; UK/73/14, part 2 United Kingdom (UK) Yelden 1974-01-07	Vada	1) (planting) 2) - 3) 1973-08-20	4.5			1973-03-08[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	165 165	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 16 months, RIP9501209
REPORT A1; CP 67573; UK/73/15, part 1 United Kingdom (UK) Huish 1974-01-07	Lofa Abed	1) (planting) 2) - 3) 1973-09-14	2.2			1973-03-15[4)]	BBCH 00	grain	<0.05	<0.05	183	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 16 months, RIP9501209

1	2	3	4			5	6	7	8.1	8.2	9	10
Report No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A1; CP 67573; UK/73/8, part 1 United Kingdom (UK) Swineshead 1974-01-07	Julia	1) (planting) 2) 3) 1973-09-08	2.2			1973-03-21[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	171 171	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 16 months, RIP9501209
REPORT A1; CP 67573; UK/73/8, part 2 United Kingdom (UK) Swineshead 1974-01-07	Julia	1) (planting) 2) 3) 1973-09-08	4.5			1973-03-21[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	171 171	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 16 months, RIP9501209

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~C~~
Commercial product (name) : ROUNDUP
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, SU 82/10 RP, 09 United Kingdom (UK) Cottenham 1991-12-01	Triumph	1) 1982 (planting) 2) 3)	1.4	200	0.72	1982-08-03[4)]		grain straw	1.2 1.5 1.5 8.5 17.4 13.0	<0.2 <0.2 <0.2 0.70 0.80 <0.4	7 7 7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : ROUNDUP
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 11 United Kingdom (UK) Dundee-Tayside 1983-09-11	Golden Promise	1) (planting) 2) 3)	0.36	20	1.8	1982-08-27[4)]		grain straw	1.7 1.2 9.9	<0.2 <0.2 <0.4	4 4 4	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 12 United Kingdom (UK) Dundee-Tayside 1983-09-11	Golden Prom-ise	1) (planting) 2) 3)	0.36	200	0.18	1982-08-27[4)]		grain straw	1.1 1.2 18.9	<0.2 <0.2 <0.4	4 4 4	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glypho-sate),analytical method: (HPLC/OPA),LOQ Glypho-sate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 13 United Kingdom (UK) Dundee-Tayside 1983-09-11	Golden Prom-ise	1) (planting) 2) 3)	0.36	20	1.8	1982-08-27[4)]		grain straw	1.6 1.3 13.3	<0.2 <0.2 <0.4	4 4 4	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (+ Frigate) (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sam-ple storage: no da-ta,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 14 United Kingdom (UK) Dundee-Tayside 1983-09-11	Golden Prom-ise	1) (planting) 2) 3)	0.36	200	0.18	1982-08-27[4)]		grain straw	1.3 0.90 16.9	<0.2 <0.2 0.42	4 4 4	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (+ Frigate) (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sam-ple storage: no da-ta,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 15 United Kingdom (UK) Dundee-Tayside 1983-09-11	Golden Prom-ise	1) (planting) 2) 3)	0.54	20	2.7	1982-08-27[4)]		grain straw	3.1 2.2 27.4	<0.2 <0.2 0.62	4 4 4	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glypho-sate),analytical method: (HPLC/OPA),LOQ Glypho-sate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 16 United Kingdom (UK) Dundee-Tayside 1983-09-11	Golden Promise	1) (planting) 2) 3)	0.54	20	2.7	1982-08-27[4)]		grain straw	2.3 1.3 1.0 1.4 14.4	<0.2 <0.2 <0.2 <0.2 <0.4	4 4 4 4 4	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (+ Frigate) (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 17 United Kingdom (UK) Dundee-Tayside 1983-09-11	Golden Promise	1) (planting) 2) 3)	0.72	20	3.6	1982-08-27[4)]		grain straw	2.2 3.0 28.0	<0.2 0.50 0.49	4 4 4	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 18 United Kingdom (UK) Dundee-Tayside 1983-09-11	Golden Prom-ise	1) (planting) 2) 3)	0.72	200	0.36	1982-08-27[4)]		grain straw	1.5 2.0 27.7	<0.2 <0.2 0.68	4 4 4	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glypho-sate),analytical method: (HPLC/OPA),LOQ Glypho-sate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 19 United Kingdom (UK) Dundee-Tayside 1983-09-11	Golden Prom-ise	1) (planting) 2) 3)	0.72	20	3.6	1982-08-27[4)]		grain straw	6.0 4.3 38.4	<0.2 0.26 0.61	4 4 4	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (+ Frigate) (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sam-ple storage: no da-ta,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 20 United Kingdom (UK) Dundee-Tayside 1983-09-11	Golden Prom-ise	1) (planting) 2) 3)	0.72	200	0.36	1982-08-27[4)]		grain straw	2.0 1.9 31.3	<0.2 0.63 0.68	4 4 4	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (+ Frigate) (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 485 g/l
Formulation (e.g. WP) : SC
Commercial product (name) : RUP Four80
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87/283/12; 09 United Kingdom (UK) Arncroach 1988-02-05	Golden Promise	1) (planting) 2) 3)	1.6	200	0.80	1987-08-31[4)]		grain straw	4.6 29.0	<0.4 <0.4	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with RUP Four80, SC (480g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data Replicate to MLL 30.200; 87/283/12; 07 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87/283/12; 10 United Kingdom (UK) Arncroach 1988-02-05	Golden Prom-ise	1) (planting) 2) 3)	2.0	200	1.0	1987-08-31[4)]		grain straw	6.2 35.0	<0.4 <0.4	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with RUP Four80, SC (480g/l Glypho-sate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data Replicate to MLL 30.200; 87/283/12; 04 & MLL 30.200; 87/283/12; 08 RIP9501278

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 400 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : MON14478
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87/283/12; 01 United Kingdom (UK) Arncroach 1988-02-05	Golden Promise	1) (planting) 2) 3)	0.60	200	0.30	1987-08-31[4)]		grain straw	1.6 7.1	<0.4 <0.4	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87/283/12; 02 United Kingdom (UK) Arncroach 1988-02-05	Golden Prom-ise	1) (planting) 2) 3)	1.0	200	0.50	1987-08-31[4)]		grain straw	3.0 9.9	<0.4 <0.4	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glypho-sate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, RIP9501278
MLL 30.200; 87/283/12; 04 United Kingdom (UK) Arncroach 1988-02-05	Golden Prom-ise	1) (planting) 2) 3)	2.0	200	1.0	1987-08-31[4)]		grain straw	4.7 47.0	<0.4 <0.4	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glypho-sate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data Replicate to MLL 30.200; 87/283/12; 10 & MLL 30.200; 87/283/12; 08 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87/283/12; 05 United Kingdom (UK) Arncroach 1988-02-05	Golden Prom-ise	1) (planting) 2) 3)	0.60	200	0.30	1987-08-31[4)]		grain straw	1.6 8.1	<0.4 <0.4	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, RIP9501278
MLL 30.200; 87/283/12; 06 United Kingdom (UK) Arncroach 1988-02-05	Golden Prom-ise	1) (planting) 2) 3)	1.0	200	0.50	1987-08-31[4)]		grain straw	4.2 16.0	<0.4 <0.4	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87/283/12; 07 United Kingdom (UK) Arncroach 1988-02-05	Golden Prom-ise	1) (planting) 2) 3)	1.6	200	0.80	1987-08-31[4)]		grain straw	4.6 24.0	<0.4 <0.4	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data Replicate to MLL 30.200; 87/283/12; 09 RIP9501278
MLL 30.200;	Golden Prom-	1) (planting)	2.0	200	1.0	1987-08-31[4)]		grain	4.8	<0.4	7	4) spraying

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
								straw	18.0	<0.4	7	

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : ROUNDUP
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87/283/12; 11 United Kingdom (UK) Arncroach 1988-02-05	Golden Promise	1) (planting) 2) 3)	0.72	200	0.36	1987-08-31[4)]		grain straw	1.5 9.6	<0.4 <0.4	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87/283/12; 12 United Kingdom (UK) Arncroach 1988-02-05	Golden Prom-ise	1) (planting) 2) 3)	1.4	200	0.72	1987-08-31[4)]		grain straw	4.6 26.0	<0.4 <0.4	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glypho-sate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, RIP9501278

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 400 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : MON14478
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2012-10-30

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-310-013, 01 France 02 Cugny 1988-02-05	Triumph	1) 1987-03-12 (planting) 2) 3)	1.1	200	0.55	1987-08-07[4)]		grain straw	3.4 2.9 25.0 <4.0	<0.08 <0.08 0.76 <0.4	12 12 12 12	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples, RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-310-013, 02 France 02 Cugny 1988-02-05	Triumph	1) 1987-03-12 (planting) 2) 3)	2.2	200	1.1	1987-08-07[4)]		grain straw	7.2 3.6 38.0 <4.0	<0.08 <0.08 1.1 <0.4	12 12 12 12	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples Replicate to MLL 30.205; 80, 87-310-013, 06 & MLL 30.205; 80, 87-310-013, 09 Straw value <4.0 mg/kg not considered valid. RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-310-013, 03 France 02 Cugny 1988-02-05	Triumph	1) 1987-03-12 (planting) 2) 3)	4.4	200	2.2	1987-08-07[4)]		grain straw	8.0 7.2 102 <4.0	<0.08 <0.08 1.9 <0.4	12 12 12 12	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples, RIP9501280
MLL 30.205; 80, 87-310-013, 04 France 02 Cugny 1988-02-05	Triumph	1) 1987-03-12 (planting) 2) 3)	8.8	200	4.4	1987-08-07[4)]		grain straw	11.0 11.4 197 322	<0.08 <0.08 3.7 <0.4	12 12 12 12	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples, RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-310-013, 05 France 02 Cugny 1988-02-05	Triumph	1) 1987-03-12 (planting) 2) 3)	1.1	200	0.55	1987-08-07[4)]		grain straw	4.1 2.3 13.0 <4.0	<0.08 <0.08 0.58 <0.4	12 12 12 12	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples, RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-310-013, 06 France 02 Cugny 1988-02-05	Triumph	1) 1987-03-12 (planting) 2) 3)	2.2	200	1.1	1987-08-07[4)]		grain straw	3.0 5.2 41.0 <4.0	<0.08 <0.08 0.76 <0.4	12 12 12 12	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples Replicate to MLL 30.205; 80, 87-310-013, 06 & MLL 30.205; 80, 87-310-013, 09 Straw value <4.0 mg/kg not considered valid. RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-310-013, 07 France 02 Cugny 1988-02-05	Triumph	1) 1987-03-12 (planting) 2) 3)	4.4	200	2.2	1987-08-07[4)]		grain straw	6.2 5.2 74.0 <4.0	<0.08 <0.08 1.2 <0.4	12 12 12 12	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples, RIP9501280
MLL 30.205; 80, 87-310-013, 08 France 02 Cugny 1988-02-05	Triumph	1) 1987-03-12 (planting) 2) 3)	8.8	200	4.4	1987-08-07[4)]		grain straw	11.0 9.7 140 221	<0.08 <0.08 2.5 <0.4	12 12 12 12	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples, RIP9501280

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2012-10-30

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-310-013, 09 France 02 Cugny 1988-02-05	Triumph	1) 1987-03-12 (planting) 2) 3)	2.2	200	1.1	1987-08-07[4)]		grain straw	3.7 5.8 70.0 <4.0	<0.08 <0.08 1.5 <0.4	12 12 12 12	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with Roundup, SL~~SC~~ (360g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples Replicate to MLL 30.205; 80, 87-310-013, 06 & MLL 30.205; 80, 87-310-013, 06 Straw value <4.0 mg/kg not considered valid. RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-310-013, 11 France 02 Cugny 1988-02-05	Triumph	1) 1987-03-12 (planting) 2) 3)	8.6	200	4.3	1987-08-07[4)]		grain straw	9.8 12.8 209 146	<0.08 <0.08 2.4 <0.4	12 12 12 12	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with Roundup, SL~~SC~~ (360g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data, replicate field samples, RIP9501280
MLL 30.205; 80, 87-310-013,10 France 02 Cugny 1988-02-05	Triumph	1) 1987-03-12 (planting) 2) 3)	4.3	200	2.2	1987-08-07[4)]		grain straw	9.5 6.4 90.0 <4.0	<0.08 <0.08 1.7 <0.4	12 12 12 12	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with Roundup, SL~~SC~~ (360g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field sample, RIP9501280

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 400 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : MON14474
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 16 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.1			1987-08-25[4)]		grain	6.6 11.0 7.2 2.8	<0.03 0.060 0.080 0.050	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14474, SL~~SC~~ (400g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data, RIP9501282

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 17 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.2			1987-08-25[4)]		grain	7.0 9.0 12.0 2.7	<0.03 0.060 0.10 0.050	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14474, SL~~SC~~ (400g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data, RIP9501282
MLL 30.209; 81, 18 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.8			1987-08-25[4)]		grain	9.2 8.0 12.0 4.4	<0.03 0.060 0.12 0.080	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14474, SL~~SC~~ (400g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data Replicate to MLL 30.209; 81, 02, MLL 30.209; 81, 04, MLL 30.209; 81, 06 & MLL 30.209; 81, 15 RIP9501282

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 22 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.1			1987-08-25[4)]		grain	9.9 7.3 7.8 2.5	<0.03 <0.03 <0.03 <0.03	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14474, SL~~SC~~ (400g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data, RIP9501282
MLL 30.209; 81, 23 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.2			1987-08-25[4)]		grain	<0.03 <0.03 <0.03 <0.03	<0.03 <0.03 <0.03 <0.03	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14474, SL~~SC~~ (400g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data Trial not considered valid, since no residue for desiccation is unlikely. RIP9501282

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 24 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.8			1987-08-25[4)]		grain	<0.03 <0.03 <0.03 <0.03	<0.03 <0.03 <0.03 <0.03	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14474, SL~~SC~~ (400g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data Trial not considered valid, since no residue for desiccation is unlikely. RIP9501282
MLL 30.209; 81, 34 Germany Garbsen 1988-06-20	no data	1) (planting) 2) 3)	1.1			1987-08-06[4)]		grain	9.4 4.1 2.3 1.3	<0.03 0.040 0.030 0.030	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14474, SL~~SC~~ (400g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data, RIP9501282

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 35 Germany Garbsen 1988-06-20	no data	1) (planting) 2) 3)	1.2			1987-08-06[4)]		grain	9.1 9.9 4.0 0.60	<0.03 0.040 0.040 <0.03	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14474, SL~~SC~~ (400g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data, RIP9501282
MLL 30.209; 81, 36 Germany Garbsen 1988-06-20	no data	1) (planting) 2) 3)	1.8			1987-08-06[4)]		grain	17.4 14.0 7.4 6.4	<0.03 0.090 0.080 0.050	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14474, SL~~SC~~ (400g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data Replicate to MLL 30.209; 81, 26, MLL 30.209; 81, 28 & MLL 30.209; 81, 30 RIP9501282

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : ROUNDUP
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 01 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.1			1987-08-25[4)]		grain	4.7 3.4 3.3 1.2	<0.03 0.030 0.040 <0.03	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data, RIP9501282

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 02 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.8			1987-08-25[4)]		grain	7.8 8.5 8.9 3.2	<0.03 0.050 0.090 0.050	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data Replicate to MLL 30.209; 81, 18, MLL 30.209; 81, 04, MLL 30.209; 81, 06 & MLL 30.209; 81, 15 RIP9501282
MLL 30.209; 81, 07 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.1			1987-08-25[4)]		grain	3.3 <0.03 <0.03 <0.03	<0.03 <0.03 <0.03 <0.03	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data Trial not considered valid, since no residue for desiccation is unlikely. RIP9501282

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 08 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.8			1987-08-25[4)]		grain	<0.03 <0.03 <0.03 <0.03	<0.03 <0.03 <0.03 <0.03	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data Trial not considered valid, since no residue for desiccation is unlikely. RIP9501282
MLL 30.209; 81, 25 Germany Garbsen 1988-06-20	no data	1) (planting) 2) 3)	1.1			1987-08-06[4)]		grain	4.9 5.1 2.9 2.0	<0.03 0.050 0.050 0.050	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data, RIP9501282

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 26 Germany Garbsen 1988-06-20	no data	1) (planting) 2) 3)	1.8			1987-08-06[4)]		grain	14.0 14.0 6.7 7.0	<0.03 0.080 0.070 0.10	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data Replicate to MLL 30.209; 81, 36, MLL 30.209; 81, 28 & MLL 30.209; 81, 30 RIP9501282

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 270 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : MON8791
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 03 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.1			1987-08-25[4)]		grain	6.1 3.6 3.5 1.0	<0.03 0.040 0.030 <0.03	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON8791, SL~~SC~~ (270g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data, RIP9501282

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 04 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.8			1987-08-25[4)]		grain	8.2 8.0 7.6 3.6	<0.03 0.030 0.050 0.040	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON8791, SL~~SC~~ (270g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data Replicate to MLL 30.209; 81, 02, MLL 30.209; 81, 18, MLL 30.209; 81, 06 & MLL 30.209; 81, 15 RIP9501282
MLL 30.209; 81, 09 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.1			1987-08-25[4)]		grain	4.3 <0.03 <0.03 <0.03	<0.03 <0.03 <0.03 <0.03	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON8791, SL~~SC~~ (270g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data Trial not considered valid, since no residue for desiccation is unlikely. RIP9501282

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 10 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.8			1987-08-25[4)]		grain	<0.03 <0.03 <0.03 <0.03	<0.03 <0.03 <0.03 <0.03	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON8791, SL~~SC~~ (270g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data Trial not considered valid, since no residue for desiccation is unlikely. RIP9501282
MLL 30.209; 81, 27 Germany Garbsen 1988-06-20	no data	1) (planting) 2) 3)	1.1			1987-08-06[4)]		grain	6.5 4.1 1.9 2.0	<0.03 <0.03 <0.03 <0.03	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON8791, SL~~SC~~ (270g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data, RIP9501282

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 28 Germany Garbsen 1988-06-20	no data	1) (planting) 2) 3)	1.8			1987-08-06[4)]		grain	19.0 21.0 11.0 4.4	<0.03 0.10 0.10 0.70	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON8791, SL~~SC~~ (270g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data Replicate to MLL 30.209; 81, 26, MLL 30.209; 81, 36 & MLL 30.209; 81, 30 RIP9501282

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 180 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : LLN8306
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 05 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.1			1987-08-25[4)]		grain	2.9 4.5 2.8 1.2	<0.03 0.030 0.030 <0.03	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with LLN8306, SL~~SC~~ (180g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data, RIP9501282

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 06 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.8			1987-08-25[4)]		grain	8.1 9.6 11.0 4.2	<0.03 0.040 0.080 0.030	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with LLN8306, SL~~SC~~ (180g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data Replicate to MLL 30.209; 81, 02, MLL 30.209; 81, 04, MLL 30.209; 81, 18 & MLL 30.209; 81, 15 RIP9501282
MLL 30.209; 81, 11 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.1			1987-08-25[4)]		grain	5.3 <0.03 <0.03 <0.03	<0.03 <0.03 <0.03 <0.03	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with LLN8306, SL~~SC~~ (180g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data Trial not considered valid, since no residue for desiccation is unlikely. RIP9501282

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 12 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.8			1987-08-25[4)]		grain	<0.03 <0.03 <0.03 <0.03	<0.03 <0.03 <0.03 <0.03	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with LLN8306, SL~~SC~~ (180g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data Trial not considered valid, since no residue for desiccation is unlikely. RIP9501282
MLL 30.209; 81, 29 Germany Garbsen 1988-06-20	no data	1) (planting) 2) 3)	1.1			1987-08-06[4)]		grain	8.5 8.9 4.7 4.0	<0.03 <0.03 0.050 0.050	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with LLN8306, SL~~SC~~ (180g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data, RIP9501282

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 30 Germany Garbsen 1988-06-20	no data	1) (planting) 2) 3)	1.8			1987-08-06[4)]		grain	14.0 12.0 6.8 6.0	<0.03 0.15 0.16 0.13	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with LLN8306, SL~~SC~~ (180g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data Replicate to MLL 30.209; 81, 26, MLL 30.209; 81, 28 & MLL 30.209; 81, 36 RIP9501282

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 120 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : MON14456
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 13 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	0.72			1987-08-25[4)]		grain	3.5 3.9 4.0 1.7		0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14456, SL~~SC~~ (120g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data, RIP9501282

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 14 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.1			1987-08-25[4)]		grain	4.3 4.4 6.0 1.6	<0.03 0.030 0.060 0.030	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14456, SL~~SC~~ (120g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data, RIP9501282
MLL 30.209; 81, 15 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.8			1987-08-25[4)]		grain	6.8 7.6 9.6 2.7	<0.03 0.050 0.10 0.050	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14456, SL~~SC~~ (120g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data Replicate to MLL 30.209; 81, 02, MLL 30.209; 81, 04, MLL 30.209; 81, 06 & MLL 30.209; 81, 18 RIP9501282

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 19 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	0.72			1987-08-25[4)]		grain	<0.03 <0.03 <0.03 <0.03	<0.03 <0.03 <0.03 <0.03	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14456, SL~~SC~~ (120g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data Trial not considered valid, since no residue for desiccation is unlikely. RIP9501282
MLL 30.209; 81, 20 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.1			1987-08-25[4)]		grain	5.6 9.0 3.4 1.6	<0.03 <0.03 <0.03 <0.03	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14456, SL~~SC~~ (120g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data, RIP9501282

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 21 Germany Walsrode 1988-06-20	no data	1) (planting) 2) 3)	1.8			1987-08-25[4)]		grain	<0.03 <0.03 <0.03 <0.03	<0.03 <0.03 <0.03 <0.03	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14456, SL~~SC~~ (120g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data Trial not considered valid, since no residue for desiccation is unlikely. RIP9501282
MLL 30.209; 81, 31 Germany Garbsen 1988-06-20	no data	1) (planting) 2) 3)	0.18			1987-08-06[4)]		grain	2.6 1.4 0.80 0.80	<0.03 <0.03 <0.03 <0.03	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14456, SL~~SC~~ (120g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data, RIP9501282

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.209; 81, 32 Germany Garbsen 1988-06-20	no data	1) (planting) 2) 3)	0.72			1987-08-06[4)]		grain	2.2 3.5 1.2 1.2	0.070 0.030 <0.03 0.030	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14456, SL~~SC~~ (120g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data, RIP9501282
MLL 30.209; 81, 33 Germany Garbsen 1988-06-20	no data	1) (planting) 2) 3)	1.1			1987-08-06[4)]		grain	5.7 5.2 2.0 1.3	<0.03 0.040 <0.03 0.030	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14456, SL~~SC~~ (120g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate and AMPA: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: no data, RIP9501282

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : (MON 2139) Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : 30.05.2000

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30815, 98-GLY-06 B/F, L 06, Trt 2 BE- 5380 Franc- Waret 05/1999	Marilor	1) 22.09.1997 (sowing) 2) – 3) 09.07.1998	2.2	200	1.1	02.07.1998	BBCH 94	grain straw	16 20 7.8 117 86 25	<0.05 0.12 <0.05 0.83 1.88 0.92	0 7 14 0 7 14	analytical method: LLN-E/S-ASOP-051 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 10 months Replicate to MLL 30815, 98-GLY-06 B/F, L 06, Trt 3 RIP2000-1243
MLL 30815, 98-GLY-06 B/F, R 8144 OB2/C FR- 29830 Ploudalmezau 05/1999	Intro	1) 06.12.1997 (sowing) 2) – 3) 12.08.1998	2.1	193	1.1	05.08.1998	BBCH 89-92	grain straw	9.9 12 19 174 124 84	<0.05 0.11 0.16 1.01 2.4 2.4	0 7 14 0 7 14	analytical method: LLN-E/S-ASOP-051 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 10 months Replicate to MLL 30815, 98-GLY-06 B/F, R 8144 OB2/D RIP2000-1243

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 680 g/kg
Formulation (e.g. WP) : WG
Commercial product (name) : MON 14420
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : 30.05.2000

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30815, 98-GLY-06 B/F, L 06, Trt 3 BE- 5380 Franc- Waret 05/1999	Marilor	1) 22.09.1997 (sowing) 2) – 3) 09.07.1998	2.2	200	1.1	02.07.1998	BBCH 94	grain straw	12 14 7.5 145 75 30	<0.05 0.12 <0.05 0.79 2.2 1.1	0 7 14 0 7 14	analytical method: LLN-E/S-ASOP-051 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 10 months Replicate to MLL 30815, 98-GLY-06 B/F, L 06, Trt 2 RIP2000-1243
MLL 30815, 98-GLY-06 B/F, R 8144 OB2/D FR- 29830 Ploudalmezau 05/1999	Intro	1) 06.12.1997 (sowing) 2) – 3) 12.08.1998	2.1	190	1.1	05.08.1998	BBCH 89-92	grain straw	9.3 9.9 0.93 164 147 56	<0.05 0.13 <0.05 1.5 3.4 1.8	0 7 14 0 7 14	analytical method: LLN-E/S-ASOP-051 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 10 months Replicate to MLL 30815, 98-GLY-06 B/F, R 8144 OB2/C RIP2000-1243

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : 29.11.2000

Content of a.i. (g/kg or g/l) : 347 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : YF11357
Applicant : Syngenta Agro GmbH

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl						
	(a)	(b)				(c)		(a)		(d)	(e)
RJ2907B, AF/4678/ZE/1 GB- Denton, Lincolnshire 31.01.2000	Gleam	1) 22.09.1998 (sowing) 2) – 3) 20.07.1999	2.1	301	0.70	13.07.1999	BBCH 87	grain straw	5.1 56	7 7	analytical method: report RR92-042B RES2 (GC-MSD), LOQ's: 0.05 mg/kg, max. sample storage: 5 months RIP2000-2131
RJ2907B, AF/4678/ZE/2 GB- Elford, Staffordshire 31.01.2000	Regina	1) 20.09.1998 (sowing) 2) – 3) 26.07.1999	2.1	300	0.70	19.07.1999	BBCH 87	grain straw	8.4 22	7 7	analytical method: report RR92-042B RES2 (GC-MSD), LOQ's: 0.05 mg/kg, max. sample storage: 5 months RIP2000-2131
RJ2907B, AF/4678/ZE/3 FR- 45300 Ansonville, Loiret 31.01.2000	Esterel	1) 05.11.1998 (sowing) 2) – 3) 02.07.1999	2.1	306	0.70	25.06.1999	BBCH 89	grain straw	5.5 18	7 7	analytical method: report RR92-042B RES2 (GC-MSD), LOQ's: 0.05 mg/kg, max. sample storage: 5 months RIP2000-2131

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl						
	(a)	(b)				(c)		(a)		(d)	(e)
RJ2907B, AF/4678/ZE/4 FR- 77320 Coupigny, Seine-et-Marne 31.01.2000	Esterel	1) 07.10.1998 (sowing) 2) – 3) 07.07.1999	2.1	300	0.70	30.06.1999	BBCH 87	grain straw	1.5 6.9	7 7	analytical method: report RR92-042B RES2 (GC-MSD), LOQ's: 0.05 mg/kg, max. sample storage: 5 months **Blank value:** *straw 0.06 mg/kg* RIP2000-2131

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : (MON 2139) Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : July 2002

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (kg/ha)	Residues (kg/ha)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 31337 99-GLY-07-B/F, IF-99/35656-00, Trt 2 BE-5380 Franc-Waret 05/2000	Marilor	1) 25.09.1998 (planting) 2) -- 3) 09.07.1999	2.0	189	1.1	02.07.1999	BBCH 90	grain straw	29 11 10 80 25 22	0.05 0.13 0.15 0.66 0.40 0.42	0 7 14 0 7 14	analytical method: DFG method 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Replicate to MLL 31337 99-GLY-07-B/F, IF-99/35656-00, Trt 3 RIP2002-650
MLL 31337, 99-GLY-07-B/F, IF-99/35656-00, 9075 CT1, Plot C FR-36110 Levroux 15.07.2001	Scarlett	1) 11/1998 (sowing) 2) -- 3) 23.07.1999	2.1	170	1.2	16.07.1999	BBCH 89	grain straw	12 4.4 4.8 189 41 24	<0.05 <0.05 0.08 1.4 0.69 0.78	0 7 14 0 7 14	analytical method: DFG method 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Replicate to MLL 31337, 99-GLY-07-B/F, IF-99/35656-00, 9075 CT1, Plot D RIP2002-650

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (kg/ha)	Residues (kg/ha)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 31337, 99-GLY-07-B/F, IF-99/35656-00, 9075 AN2, Plot C FR-54115 Favières 05/2000	Scarlett	1) 23.10.1998 (sowing) 2) -- 3) 22.07.1999	2.1	168	1.2	15.07.1999	BBCH 87	grain straw	12 12 14 110 31 29	<0.05 0.13 0.20 0.83 0.49 0.84	0 7 14 0 7 14	analytical method: DFG method 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Replicate to MLL 31337, 99-GLY-07-B/F, IF-99/35656-00, 9075 AN2, Plot D RIP2002-650
MLL 31337, 99-GLY-07-B/F, IF-99/35656-00, 9075 OB2, Plot C FR-29830 Ploudalmézeau 05/2000	Emeraude	1) 23.11.1998 (sowing) 2) -- 3) 02.08.1999	2.2	178	1.2	26.08.1999	BBCH 87	grain straw	15 16 1.8 184 62 9.1	0.08 0.22 0.10 2.0 2.1 0.95	0 7 14 0 7 14	analytical method: DFG method 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 7 months Replicate to MLL 31337, 99-GLY-07-B/F, IF-99/35656-00, 9075 OB2, Plot D RIP2002-650

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 450 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : MON 78294
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : July 2002

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (kg/ha)	Residues (kg/ha)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 31337, 99-GLY-07-B/F, IF-99/35656-00, Trt 3 BE-5380 Franc-Waret 05/2000	Marilor	1) 25.09.1998 (planting) 2) -- 3) 09.07.1999	2.1	195	1.1	02.07.1999	BBCH 90	grain straw	20 9.5 7.6 90 29 18	<0.05 0.11 0.12 0.82 0.44 0.34	0 7 14 0 7 14	analytical method: DFG method 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Replicate to MLL 31337 99-GLY-07-B/F, IF-99/35656-00, Trt 2 RIP2002-650
MLL 31337, 99-GLY-07-B/F, IF-99/35656-00, 9075 CT1 Plot D FR-36110 Levroux 05/2000	Scarlett	1) 11/98 (sowing) 2) -- 3) 23.07.1999	2.2	180	1.2	16.07.1999	BBCH 89	grain straw	16 3.8 5.5 180 80 31	0.06 <0.05 0.08 2.1 1.2 0.90	0 7 14 0 7 14	analytical method: DFG method 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Replicate to MLL 31337, 99-GLY-07-B/F, IF-99/35656-00, 9075 CT1, Plot C RIP2002-650

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (kg/ha)	Residues (kg/ha)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 31337, 99-GLY-07-B/F, IF-99/35656-00, 9075 AN2, Plot D FR-54115 Favières 05/2000	Scarlett	1) 23.10.1998 (sowing) 2) -- 3) 22.07.1999	2.1	172	1.2	15.07.1999	BBCH 87	grain straw	19 11 10 110 43 38	0.06 0.14 0.15 0.87 0.57 0.66	0 7 14 0 7 14	analytical method: DFG method 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Replicate to MLL 31337, 99-GLY-07-B/F, IF-99/35656-00, 9075 AN2, Plot C RIP2002-650
MLL 31337, 99-GLY-07-B/F, IF-99/35656-00, 9075 OB2, Plot D FR-29830 Ploudalmézeau 05/2000	Emeraude	1) 23.11.1998 (sowing) 2) -- 3) 02.08.1999	2.1	174	1.2	26.07.1999	BBCH 89	grain straw	16 19 4.8 145 110 15	0.08 0.21 0.16 1.4 3.0 1.7	0 7 14 0 7 14	analytical method: DFG method 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 7 months Replicate to MLL 31337, 99-GLY-07-B/F, IF-99/35656-00, 9075 OB2, Plot C RIP2002-650

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Active ingredient : Glyphosate (Isopropylamin-salt)
Crop / crop group : Winter Barley

Submission date : 01.09.1995

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)			(d)	(e)
report MLL 30037, A 36, trial RWB/1 GB- York, Yorkshire 23.10.1979	Sonja	1) – (sowing) 2) -- 3) --	1.4	200	0.70	21.07.1979	BBCH 88	grain straw	6.6 78	<0.05 0.27	6 6	analytical method: GC-FPD), LOQ's: 0.05 mg/kg RIP9501230
			2.9	200	1.4	21.07.1979	BBCH 88	grain straw	9.8 118	<0.05 0.31	6 6	
report MLL 30070, A 36, trial RS 1E/AN GB- Fulbourn, Cambridge 28.10.1981	Maris Otter	1) – (sowing) 2) -- 3) --	1.4	200	0.70	21.07.1980	BBCH 91	grain	2.8	<0.05	7	analytical method: GC-FPD), LOQ's: 0.05 mg/kg RIP9501238
			2.9	200	1.4	24.07.1980	BBCH 91	grain	6.6	<0.05	4	
report MLL 30070, A 36, trial RS 2E/AN GB- Fulbourn, Cambridge 28.10.1981	Maris Otter	1) – (sowing) 2) -- 3) --	1.4	200	0.70	21.07.1980	BBCH 91	grain	3.2 5.0 5.4	<0.05 <0.05 <0.05	7 7 7	analytical method: GC-FPD), LOQ's: 0.05 mg/kg, replicate field samples RIP9501238
			2.9	200	1.4	21.07.1980	BBCH 91	grain	8.8 8.7 11.0	<0.05 <0.05 0.1	7 7 7	
			2.9	200	1.4	24.08.1980	BBCH 91	grain	7.6 9.9 7.2	<0.05 <0.05 <0.05	4 4 4	

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Taifun forte
Applicant : ADAMA Agan L

Active ingredient : Glyphosate (Isopropylamin-salt)
Crop / crop group : Winter Barley

Submission date : 2008-07-25

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AM-PA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
FSG-0606, trial 1	Lomerid (lodged grain)	1) 2005-09-20 (sowing)	1.85	308	0.60	2006-07-10[4)]	BBCH 87	rest of plant	34	0.12	0	4) spraying
									43	0.33	3	
Germany		2) 2006-05-29							22	0.28	7	analytical method: DFG
04668		- 2006-06-08							29	0.39	9	method 405 (HPLC-FLD)
Motterwitz		3) 2006-07-24						ears of grain	46	0.23	0	for both substances, LOQ's:
									65	0.59	3	0.05 mg/kg, max. sample
									33	0.47	7	storage: 11 months
2007-09-26									31	0.55	9	
								grain	10	0.17	14	ASB2008-5610
								straw	26	2.2	14	

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : CHE 3607
Applicant : Cheminova A/S

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate

1	2	3	4			5	6	7	8.1	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
IF-93/04573-01; AS/1905/CN; 01 United Kingdom (UK) SK412237 Wilson, Derbyshire 1992-02-10	Magie	1) 1991-10-05 (planting) 2) 3)	1.4	200	0.72	1992-07-16[4)]	BBCH 89-92	plant grain straw	77.9 20.0 20.6 3.3 8.4	0 1 3 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with CHE 3607, SL (360g/l Glyphosate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: grain 0.03mg/kg, whole plant 0.04mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501295, RIP9501296
IF-93/04573-01; AS/1905/CN; 02 United Kingdom (UK) SK412237 Wilson, Derbyshire 1992-02-10	Magie	1) 1991-10-05 (planting) 2) 3)	0.72	200	0.36	1992-07-16[4)]	BBCH 89-92	plant grain straw	58.6 6.8 8.9 2.2 2.5	0 1 3 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with CHE 3607, SL (360g/l Glyphosate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: grain 0.03mg/kg, whole plant 0.04mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501295, RIP9501296

1	2	3	4			5	6	7	8.1	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
IF-93/04573-01; AS/1905/CN; 03 United Kingdom (UK) SK412237 Wilson, Derbyshire 1992-02-10	Magie	1) 1991-10-05 (planting) 2) 3)	0.72	200	0.36	1992-07-16[4)]	BBCH 89-92	plant grain straw	24.9 10.8 8.3 2.1 1.5	0 1 3 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with tankmix CHE 3607, SL /Frigate, ADJ (360g/l Glyphosate, 800g/l Tallow amine ethoxylate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: grain 0.03mg/kg, whole plant 0.04mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501295, RIP9501296
IF-93/04573-01; AS/1905/CN; 04 United Kingdom (UK) SK412237 Wilson, Derbyshire 1992-02-10	Magie	1) 1991-10-05 (planting) 2) 3)	0.36	200	0.18	1992-07-16[4)]	BBCH 89-92	plant grain straw	8.0 4.8 4.3 0.70 2.2	0 1 3 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with tankmix CHE 3607, SL /Frigate, ADJ (360g/l Glyphosate, 800g/l Tallow amine ethoxylate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: grain 0.03mg/kg, whole plant 0.04mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501295, RIP9501296

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 356 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : CHE 3607
Applicant : Cheminova A/S

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30286; 104/91-GLY-03; BBA 16227 Germany 6209 Aarbergen-Panrod 1992-02-10	Pastoral	1) 1990-09-18 (planting) 2) 3) 1991-08-14	1.8	200	0.89	1991-07-29[4)]	BBCH 89-92	grain	8.9 7.4 4.3 3.6	<0.05 0.060 <0.05 <0.05	0 5 10 16	4) spraying submitted to Glyphosat 004282-00/00 at 1993-06-26,treated with formulation Roundup, SL~~SC~~ 042389-00 (356 g/l Glyphosate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: 0,05 mg/kg,max. sample storage: 6 months Replicate to MLL 30286; 104/91-GLY-03; BBA 11724 ASB2009-4415, RIP9500162, RIP9501288

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30286; 104/91-GLY-03; BBA 16229 Germany 3006 Burgwedel-Thönse 1992-02-10	Marinka	1) 1990-09-26 (planting) 2) 3) 1991-08-11	1.8	200	0.89	1991-07-27[4)]	BBCH 89-92	grain	18.0 16.0 18.0 4.6	0.10 0.20 0.30 0.20	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1993-06-26,treated with formulation Roundup, SL~~SC~~ 042389-00 (356 g/l Glypho-sate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: 0,05 mg/kg,max. sample storage: 6 months Replicate to MLL 30286; 104/91-GLY-03; BBA 16240 ASB2009-4420, RIP9500162, RIP9501288
MLL 30286; 104/91-GLY-03; BBA 16246 Germany 3050 Wunstorf-Liete 1992-02-10	Catania	1) 1990-09-26 (planting) 2) 3) 1991-08-11	1.8	200	0.89	1991-07-27[4)]	BBCH 89-92	grain	16.0 11.0 8.6 2.4	0.080 0.10 0.090 <0.05	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1993-06-26,treated with formulation Roundup, SL~~SC~~ 042389-00 (356 g/l Glypho-sate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: 0,05 mg/kg,max. sample storage: 6 months Replicate to MLL 30286; 104/91-GLY-03; BBA 16242 ASB2009-4421, RIP9500162, RIP9501288

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 428 g/kg
Formulation (e.g. WP) : WG
Commercial product (name) : MON44068
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30286; 104/91-GLY-03; BBA 11724 Germany 6209 Aarbergen-Panrod 1992-02-10	Pastoral	1) 1990-09-18 (planting) 2) 3) 1991-08-14	2.1	200	1.1	1991-07-29[4)]	BBCH 89-92	grain	12.0 7.3 4.7 3.2	0.060 0.060 <0.05 <0.05	0 5 10 16	4) spraying submitted to Glyphosat 004282-00/00 at 1993-06-26,treated with formulation MON44068, WG (428 g/kg Glyphosate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: 0,05 mg/kg,max. sample storage: 6 months Replicate to MLL 30286; 104/91-GLY-03; BBA 16227 ASB2009-4413, RIP9500162, RIP9501288

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30286; 104/91-GLY-03; BBA 16240 Germany 3006 Burgwedel-Thönse 1992-02-10	Marinka	1) 1990-09-26 (planting) 2) 3) 1991-08-11	2.1	200	1.1	1991-07-27[4)]	BBCH 89-92	grain	12.0 13.0 13.0 3.0	0.080 0.20 0.30 0.20	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1993-06-26,treated with formulation MON44068, WG (428 g/kg Glyphosate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: 0,05 mg/kg,max. sample storage: 6 months Replicate to MLL 30286; 104/91-GLY-03; BBA 16229 ASB2009-4419, RIP9500162, RIP9501288
MLL 30286; 104/91-GLY-03; BBA 16242 Germany 3050 Wunstorf-Liete 1992-02-10	Catania	1) 1990-09-26 (planting) 2) 3) 1991-08-11	2.1	200	1.1	1991-07-27[4)]	BBCH 89-92	grain	14.0 12.0 6.2 1.9	0.090 0.10 0.070 <0.05	0 5 10 15	4) spraying submitted to Glyphosat 004282-00/00 at 1993-06-26,treated with formulation MON44068, WG (428 g/kg Glyphosate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: 0,05 mg/kg,max. sample storage: 6 months Replicate to MLL 30286; 104/91-GLY-03; BBA 16246 ASB2009-4422, RIP9500162, RIP9501288

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i.	(g/kg or g/l)	:	360 g/l	Active ingredient	: Glyphosate
Formulation	(e.g. WP)	:	SL	Crop / crop group	: Winter Barley
Commercial product	(name)	:	Roundup	Submission date	: 2012-11-01
Applicant		:	Monsanto Agrar Deutschland GmbH	Indoors / outdoors	: Outdoors (European North)
				Other a.i. in formulation (content and common name)	:
				Residues calculated as	: 8.1 Glyphosate 8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A1; CP 67573; UK/73/13, part 1 United Kingdom (UK) Mollington 1974-01-07	Julia	1) (planting) 2) - 3) 1973-08-19	2.2			1972-10-02[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 0.050	321 321	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 16 months, RIP9501209

1	2	3	4			5	6	7	8.1	8.2	9	10
Report No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A1; CP 67573; UK/73/13, part 2 United Kingdom (UK) Mollington 1974-01-07	Julia	1) (planting) 2) 3) 1973-08-19	4.5			1972-10-02[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	321 321	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 16 months, RIP9501209

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : ROUNDUP
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, SU 82/10 RP, 06 United Kingdom (UK) Normanton 1991-12-01	Igri	1) 1982 (planting) 2) 3)	1.4	200	0.72	1982-07-18[4)]		grain straw	3.6 4.7 1.6 50.4 57.0 34.0	<0.2 <0.2 <0.2 1.0 0.89 0.57	9 9 9 9 9 9	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, SU 82/10 RP, 07 United Kingdom (UK) Lechlade 1991-12-01	Igri	1) 1982 (planting) 2) 3)	1.4	200	0.72	1982-07-09[4)]		grain straw	1.7 1.5 1.7 10.6 11.9 18.0	<0.2 <0.2 <0.2 <0.4 <0.4 0.44	12 12 12 12 12 12	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249
MLL 30.087; MLL 30087; A 36; P III, SU 82/10 RP, 08 United Kingdom (UK) Bossal 1991-12-01	Igri	1) 1982 (planting) 2) 3)	1.4	200	0.72	1982-07-26[4)]		grain straw	10.7 9.2 12.8 29.5 84.6 71.8	<0.2 <0.2 <0.2 <0.4 1.6 2.1	8 8 8 8 8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, SU 82/10 RP, 10 United Kingdom (UK) East Lothians 1991-12-01	Igris	1) 1982 (planting) 2) 3)	1.4	200	0.72	1982-07-28[4)]		grain straw	4.3 4.2 4.7 35.1 39.4 48.1	<0.2 <0.2 <0.2 0.64 0.57 0.97	6 6 6 6 6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : ROUNDUP
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; SU 82/11,12/RP; 01 United Kingdom (UK) Rise-Berverley 1983-09-11	Igri	1) (planting) 2) 3)	0.72	20	3.6	1982-07-20[4)]		grain straw	3.9 6.9 44.8 34.0	<0.2 <0.2 0.63 0.46	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; SU 82/11,12/RP; 02 United Kingdom (UK) Rise-Berverley 1983-09-11	Igri	1) (planting) 2) 3)	0.72	40	1.8	1982-07-20[4)]		grain straw	5.7 7.9 54.3 30.0	<0.2 <0.2 0.71 <0.4	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; SU 82/11,12/RP; 03 United Kingdom (UK) Rise-Berverley 1983-09-11	Igri	1) (planting) 2) 3)	0.72	100	0.72	1982-07-20[4)]		grain straw	5.2 4.6 48.7 65.7	<0.2 <0.2 0.50 0.54	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; SU 82/11,12/RP; 04 United Kingdom (UK) Rise-Berverley 1983-09-11	Igri	1) (planting) 2) 3)	0.72	200	0.36	1982-07-20[4)]		grain straw	5.7 4.7 45.6 43.0	<0.2 <0.2 0.50 0.47	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; SU 82/11,12/RP; 05 United Kingdom (UK) Rise-Berverley 1983-09-11	Igri	1) (planting) 2) 3)	1.4	20	7.2	1982-07-20[4)]		grain straw	10.8 11.6 63.0 74.5	0.21 <0.2 0.78 0.85	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; SU 82/11,12/RP; 06 United Kingdom (UK) Rise-Berverley 1983-09-11	Igri	1) (planting) 2) 3)	1.4	40	3.6	1982-07-20[4)]		grain straw	3.7 8.1 51.4 61.4	<0.2 <0.2 0.64 0.76	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; SU 82/11,12/RP; 07 United Kingdom (UK) Rise-Berverley 1983-09-11	Igri	1) (planting) 2) 3)	1.4	100	1.4	1982-07-20[4)]		grain straw	<0.4 11.2 2.0[5)] 76.0	<0.2 <0.2 <0.4 0.62	7 7 7 7	4) spraying 5) LOQ: 4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; SU 82/11,12/RP; 08 United Kingdom (UK) Rise-Berverley 1983-09-11	Igri	1) (planting) 2) 3)	1.4	200	0.72	1982-07-20[4)]		grain straw	19.6 9.8 236 86.8	<0.2 <0.2 1.4 0.81	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; SU 82/11,12/RP; 09 United Kingdom (UK) Rise-Berverley 1983-09-11	Igri	1) (planting) 2) 3)	2.9	20	14	1982-07-20[4)]		grain straw	10.8 14.5 64.8 46.4	<0.2 <0.2 1.1 0.59	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; SU 82/11,12/RP; 10 United Kingdom (UK) Rise-Berverley 1983-09-11	Igri	1) (planting) 2) 3)	2.9	40	7.2	1982-07-20[4)]		grain straw	20.2 28.0 62.1 123	<0.2 0.21 0.82 1.6	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; SU 82/11,12/RP; 11 United Kingdom (UK) Rise-Berverley 1983-09-11	Igri	1) (planting) 2) 3)	2.9	100	2.9	1982-07-20[4)]		grain straw	27.0 19.8 137 123	<0.2 0.20 1.2 1.3	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; SU 82/11,12/RP; 12 United Kingdom (UK) Rise-Berverley 1983-09-11	Igri	1) (planting) 2) 3)	2.9	200	1.4	1982-07-20[4)]		grain straw	19.8 12.4 150 89.5	<0.2 <0.2 0.98 1.7	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 485 g/l
Formulation (e.g. WP) : SC
Commercial product (name) : RUP Four80
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; UK/CL01/P; 11 United Kingdom (UK) Neston, Wilts. 1988-02-05	Igri	1) 1986-09-22 (planting) 2) 3)	1.6	200	0.80	1987-07-28[4)]		grain straw	2.2 11.0	<0.4 0.40	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with RUP Four80, SC (480g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data Replicate to MLL 30.200; UK/CL01/P; 04 & MLL 30.200; UK/CL01/P; 09 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; UK/CL01/P; 12 United Kingdom (UK) Neston, Wilts. 1988-02-05	Igri	1) 1986-09-22 (planting) 2) 3)	2.0	200	1.0	1987-07-28[4)]		grain straw	5.0 36.0	<0.4 1.1	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with RUP Four80, SC (480g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data Replicate to MLL 30.200; UK/CL01/P; 05 & MLL 30.200; UK/CL01/P; 10 RIP9501278

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 400 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : MON14478
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; UK/CL01/P; 01 United Kingdom (UK) Neston, Wilts. 1988-02-05	Igri	1) 1986-09-22 (planting) 2) 3)	0.60	200	0.30	1987-07-28[4)]		grain straw	1.1 7.7	<0.4 <0.4	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; UK/CL01/P; 02 United Kingdom (UK) Neston, Wilts. 1988-02-05	Igri	1) 1986-09-22 (planting) 2) 3)	0.80	200	0.40	1987-07-28[4)]		grain straw	1.2 12.0	<0.4 <0.4	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, RIP9501278
MLL 30.200; UK/CL01/P; 03 United Kingdom (UK) Neston, Wilts. 1988-02-05	Igri	1) 1986-09-22 (planting) 2) 3)	1.0	200	0.50	1987-07-28[4)]		grain straw	2.2 13.0	<0.4 <0.4	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; UK/CL01/P; 04 United Kingdom (UK) Neston, Wilts. 1988-02-05	Igri	1) 1986-09-22 (planting) 2) 3)	1.6	200	0.80	1987-07-28[4)]		grain straw	3.2 20.0	<0.4 0.48	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data Replicate to MLL 30.200; UK/CL01/P; 11 & MLL 30.200; UK/CL01/P; 09 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; UK/CL01/P; 05 United Kingdom (UK) Neston, Wilts. 1988-02-05	Igri	1) 1986-09-22 (planting) 2) 3)	2.0	200	1.0	1987-07-28[4)]		grain straw	4.6 24.0	<0.4 0.75	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data Replicate to MLL 30.200; UK/CL01/P; 12 & MLL 30.200; UK/CL01/P; 10 RIP9501278
MLL 30.200; UK/CL01/P; 06 United Kingdom (UK) Neston, Wilts. 1988-02-05	Igri	1) 1986-09-22 (planting) 2) 3)	0.60	200	0.30	1987-07-28[4)]		grain straw	1.4 7.6	<0.4 <0.4	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; UK/CL01/P; 07 United Kingdom (UK) Neston, Wilts. 1988-02-05	Igri	1) 1986-09-22 (planting) 2) 3)	0.80	200	0.40	1987-07-28[4)]		grain straw	1.3 9.7	<0.4 <0.4	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, RIP9501278
MLL 30.200; UK/CL01/P; 08 United Kingdom (UK) Neston, Wilts. 1988-02-05	Igri	1) 1986-09-22 (planting) 2) 3)	1.0	200	0.50	1987-07-28[4)]		grain straw	1.8 12.0	<0.4 <0.4	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; UK/CL01/P; 09 United Kingdom (UK) Neston, Wilts. 1988-02-05	Igri	1) 1986-09-22 (planting) 2) 3)	1.6	200	0.80	1987-07-28[4)]		grain straw	2.5 20.0	<0.4 0.57	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data Replicate to MLL 30.200; UK/CL01/P; 04 & MLL 30.200; UK/CL01/P; 11 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; UK/CL01/P; 10 United Kingdom (UK) Neston, Wilts. 1988-02-05	Igri	1) 1986-09-22 (planting) 2) 3)	2.0	200	1.0	1987-07-28[4)]		grain straw	3.5 22.0	<0.4 0.88	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data Replicate to MLL 30.200; UK/CL01/P; 05 & MLL 30.200; UK/CL01/P; 12 RIP9501278

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 400 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : MON14478
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : 2012-10-30

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-289-017, 01 France 62130 Saint Paul 1988-02-05	Gerbel	1) 1986-09 (planting) 2) 3)	1.1	200	0.55	1987-07-24[4)]		grain straw	0.80 1.4 5.5 8.1	<0.08 <0.08 <0.4 <0.4	20 20 20 20	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-289-017, 02 France 62130 Saint Paul 1988-02-05	Gerbel	1) 1986-09 (planting) 2) 3)	2.2	200	1.1	1987-07-24[4)]		grain straw	1.4 1.0 15.0 9.3	<0.08 <0.08 <0.4 <0.4	20 20 20 20	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples RIP9501280
MLL 30.205; 80, 87-289-017, 03 France 62130 Saint Paul 1988-02-05	Gerbel	1) 1986-09 (planting) 2) 3)	4.4	200	2.2	1987-07-24[4)]		grain straw	2.0 1.8 21.0 20.0	0.24 <0.08 <0.4 <0.4	20 20 20 20	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples, RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-289-017, 04 France 62130 Saint Paul 1988-02-05	Gerbel	1) 1986-09 (planting) 2) 3)	8.8	200	4.4	1987-07-24[4)]		grain straw	5.7 3.8 38.0 48.0	0.29 <0.08 <0.4 <0.4	20 20 20 20	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples, RIP9501280
MLL 30.205; 80, 87-289-017, 05 France 62130 Saint Paul 1988-02-05	Gerbel	1) 1986-09 (planting) 2) 3)	1.1	200	0.55	1987-07-24[4)]		grain straw	1.0 2.1 11.0 7.9	0.17 <0.08 <0.4 <0.4	20 20 20 20	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples, RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-289-017, 06 France 62130 Saint Paul 1988-02-05	Gerbel	1) 1986-09 (planting) 2) 3)	2.2	200	1.1	1987-07-24[4)]		grain straw	1.4 1.6 12.0 12.0	0.14 <0.08 <0.4 <0.4	20 20 20 20	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples, RIP9501280
MLL 30.205; 80, 87-289-017, 07 France 62130 Saint Paul 1988-02-05	Gerbel	1) 1986-09 (planting) 2) 3)	4.4	200	2.2	1987-07-24[4)]		grain straw	3.0 2.9 18.0 15.0	0.10 <0.08 <0.4 <0.4	20 20 20 20	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples, RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-289-017, 08 France 62130 Saint Paul 1988-02-05	Gerbel	1) 1986-09 (planting) 2) 3)	8.8	200	4.4	1987-07-24[4)]		grain straw	5.6 5.1 40.0 23.0	0.37 <0.08 <0.4 <0.4	20 20 20 20	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples, RIP9501280

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : 2012-10-30

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-289-017, 09 France 62130 Saint Paul 1988-02-05	Gerbel	1) 1986-09 (planting) 2) 3)	2.2	200	1.1	1987-07-24[4)]		grain straw	3.0 2.8 14.0 16.0	0.20 <0.08 <0.4 <0.4	20 20 20 20	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with Roundup, SL~~SC~~ (360g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples, RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-289-017, 10 France 62130 Saint Paul 1988-02-05	Gerbel	1) 1986-09 (planting) 2) 3)	4.3	200	2.2	1987-07-24[4)]		grain straw	4.0 3.0 32.0 26.0	0.30 <0.08 0.40 <0.4	20 20 20 20	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with Roundup, SL~~SC~~ (360g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples, RIP9501280
MLL 30.205; 80, 87-289-017, 11 France 62130 Saint Paul 1988-02-05	Gerbel	1) 1986-09 (planting) 2) 3)	8.6	200	4.3	1987-07-24[4)]		grain straw	2.1 2.5 16.0 23.0	0.30 <0.08 <0.4 <0.4	20 20 20 20	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with Roundup, SL~~SC~~ (360g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data,replicate field samples, RIP9501280

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : MON52276
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06F05; T1 France 91 Vayres sur Essonne 1991-12-01	Volga	1) 1991 (planting) 2) 3)	1.1	200	0.54	1991-07-22[4)]	BBCH 87	grain straw	1.0 9.0	<0.05 0.070	8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON52276, SL~~SC~~ (360g/l Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months, RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06F05; T2 France 91 Vayres sur Essonne 1991-12-01	Volga	1) 1991 (planting) 2) 3)	2.2	200	1.1	1991-07-22[4)]	BBCH 87	grain straw	1.8 15.0	<0.05 0.10	8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON52276, SL~~SC~~ (360g/l Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months Replicate to MLL 30281; 91-GLY-01; 06F05; T5 & MLL 30281; 91-GLY-01; 06F05; T4 RIP9501287
MLL 30281; 91-GLY-01; 06F05; T5 France 91 Vayres sur Essonne 1991-12-01	Volga	1) 1991 (planting) 2) 3)	2.2	200	1.1	1991-07-22[4)]	BBCH 87	grain straw	1.6 9.0	<0.05 0.060	8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months Replicate to MLL 30281; 91-GLY-01; 06F05; T2 & MLL 30281; 91-GLY-01; 06F05; T4 RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06F07; T1 France 51 Sept Sauly / Reims 1991-12-01	Volga	1) 1991 (planting) 2) 3)	1.1	200	0.54	1991-07-29[4)]	BBCH 87	grain straw	4.3 61.0	0.060 0.50	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON52276, SL~~SC~~ (360g/l Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months, RIP9501287
MLL 30281; 91-GLY-01; 06F07; T2 France 51 Sept Sauly / Reims 1991-12-01	Volga	1) 1991 (planting) 2) 3)	2.2	200	1.1	1991-07-29[4)]	BBCH 87	grain straw	5.4 140	0.080 1.3	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON52276, SL~~SC~~ (360g/l Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months Replicate to MLL 30281; 91-GLY-01; 06F07; T4 & MLL 30281; 91-GLY-01; 06F07; T5 RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06F07; T5 France 51 Sept Sauly / Reims 1991-12-01	Volga	1) 1991 (planting) 2) 3)	2.2	200	1.1	1991-07-29[4)]	BBCH 87	grain straw	7.2 140	0.070 1.0	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months Replicate to MLL 30281; 91-GLY-01; 06F07; T4 & MLL 30281; 91-GLY-01; 06F07; T2 RIP9501287

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 420 g/kg
Formulation (e.g. WP) : WG
Commercial product (name) : MON44068
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06F05; T3 France 91 Vayres sur Essonne 1991-12-01	Volga	1) 1991 (planting) 2) 3)	1.1	200	0.53	1991-07-22[4)]	BBCH 87	grain straw	0.070 0.30	<0.05 <0.05	8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON44068, WG (420g/kg Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months, RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06F05; T4 France 91 Vayres sur Essonne 1991-12-01	Volga	1) 1991 (planting) 2) 3)	2.1	200	1.1	1991-07-22[4)]	BBCH 87	grain straw	2.8 7.5	<0.05 0.060	8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON44068, WG (420g/kg Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months Replicate to MLL 30281; 91-GLY-01; 06F05; T5 & MLL 30281; 91-GLY-01; 06F05; T2 RIP9501287
MLL 30281; 91-GLY-01; 06F07; T3 France 51 Sept Sauly / Reims 1991-12-01	Volga	1) 1991 (planting) 2) 3)	1.1	200	0.53	1991-07-29[4)]	BBCH 87	grain straw	3.2 49.0	<0.05 0.40	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON44068, WG (420g/kg Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months, RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06F07; T4 France 51 Sept Sauly / Reims 1991-12-01	Volga	1) 1991 (planting) 2) 3)	2.1	200	1.1	1991-07-29[4)]	BBCH 87	grain straw	4.4 72.0	0.060 0.50	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON44068, WG (420g/kg Glypho-sate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months Replicate to MLL 30281; 91-GLY-01; 06F07; T2 & MLL 30281; 91-GLY-01; 06F07; T5 RIP9501287

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : CHE 3607
Applicant : Cheminova A/S

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
IF-93/04568-01; AS/1896/CN; trial 1; A United Kingdom (UK) SE890713 Wintringham / Yorkshire 1994-03-22	Halcyon	1) 1991-10-02 (planting) 2) 3)	1.4	200	0.72	1992-07-14[4)]	BBCH 85-87	grain straw	3.3 13.5		7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation CHE 3607, SL (360 g/l Glyphosate,analytical method: DFG No. 405 (HPLC/FPD),LOQ grain: 0.03 mg/kg, LOQ straw: 0,05 mg/kg,max. sample storage: 15 months, RIP9501293, RIP9501294

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
IF-93/04568-01; AS/1896/CN; trial 1; B United Kingdom (UK) SE890713 Wintringham / Yorkshire 1994-03-22	Halcyon	1) 1991-10-02 (planting) 2) 3)	0.72	200	0.36	1992-07-14[4)]	BBCH 85-87	grain straw	1.5 8.9		7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation CHE 3607, SL (360 g/l Glyphosate,analytical method: DFG No. 405 (HPLC/FPD),LOQ grain: 0.03 mg/kg, LOQ straw: 0,05 mg/kg,max. sample storage: 15 months RIP9501293, RIP9501294
IF-93/04568-01; AS/1896/CN; trial 1; C United Kingdom (UK) SE890713 Wintringham / Yorkshire 1994-03-22	Halcyon	1) 1991-10-02 (planting) 2) 3)	0.72	200	0.36	1992-07-14[4)]	BBCH 85-87	grain straw	1.6 6.6		7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation CHE 3607, SL (360 g/l Glyphosate) + Frigate, LI 004698-00 (800 g/l Tallow amine ethoxylate),analytical method: DFG No. 405 (HPLC/FPD),LOQ grain: 0.03 mg/kg, LOQ straw: 0,05 mg/kg,max. sample storage: 15 months, RIP9501293, RIP9501294

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
IF-93/04568-01; AS/1896/CN; trial 1; D United Kingdom (UK) SE890713 Wintringham / Yorkshire 1994-03-22	Halcyon	1) 1991-10-02 (planting) 2) 3)	0.36	200	0.18	1992-07-14[4)]	BBCH 85-87	grain straw	0.60 2.7		7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation CHE 3607, SL (360 g/l Glyphosate) + Frigate, LI 004698-00 (800 g/l Tallow amine ethoxylate),analytical method: DFG No. 405 (HPLC/FPD),LOQ grain: 0.03 mg/kg, LOQ straw: 0,05 mg/kg,max. sample storage: 15 months, RIP9501293, RIP9501294
IF-93/04568-01; AS/1896/CN; trial 2; A United Kingdom (UK) SE419242 Derbyshire 1994-03-22	Halcyon	1) 1991-10-04 (planting) 2) 3)	1.4	200	0.72	1992-07-15[4)]	BBCH 89-92	grain straw	3.1 17.1		7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation CHE 3607, SL (360 g/l Glyphosate,analytical method: DFG No. 405 (HPLC/FPD),LOQ grain: 0.03 mg/kg, LOQ straw: 0,05 mg/kg,max. sample storage: 15 months, RIP9501293, RIP9501294

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
IF-93/04568-01; AS/1896/CN; trial 2; B United Kingdom (UK) SE419242 Derbyshire 1994-03-22	Halcyon	1) 1991-10-04 (planting) 2) 3)	0.72	200	0.36	1992-07-15[4)]	BBCH 89-92	grain straw	1.3 6.8		7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation CHE 3607, SL (360 g/l Glyphosate,analytical method: DFG No. 405 (HPLC/FPD),LOQ grain: 0.03 mg/kg, LOQ straw: 0,05 mg/kg,max. sample storage: 15 months, RIP9501293, RIP9501294
IF-93/04568-01; AS/1896/CN; trial 2; C United Kingdom (UK) SE419242 Derbyshire 1994-03-22	Halcyon	1) 1991-10-04 (planting) 2) 3)	0.72	200	0.36	1992-07-15[4)]	BBCH 89-92	grain straw	0.90 10.8		7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation CHE 3607, SL (360 g/l Glyphosate) + Frigate, LI 004698-00 (800 g/l Tallow amine ethoxylate),analytical method: DFG No. 405 (HPLC/FPD),LOQ grain: 0.03 mg/kg, LOQ straw: 0,05 mg/kg,max. sample storage: 15 months, RIP9501293, RIP9501294

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
IF-93/04568-01; AS/1896/CN; trial 2; D United Kingdom (UK) SE419242 Derbyshire 1994-03-22	Halcyon	1) 1991-10-04 (planting) 2) 3)	0.36	200	0.18	1992-07-15[4)]	BBCH 89-92	grain straw	0.60 4.5		7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation CHE 3607, SL (360 g/l Glyphosate) + Frigate, LI 004698-00 (800 g/l Tallow amine ethoxylate),analytical method: DFG No. 405 (HPLC/FPD),LOQ grain: 0.03 mg/kg, LOQ straw: 0,05 mg/kg,max. sample storage: 15 months, RIP9501293, RIP9501294
IF-93/04568-01; AS/1896/CN; trial 3; A United Kingdom (UK) SO634338 Hereford 1994-03-22	Marinka	1) 1991-10-26 (planting) 2) 3)	1.4	200	0.72	1992-07-16[4)]	BBCH 87-92	grain straw	3.4 21.9		7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation CHE 3607, SL (360 g/l Glyphosate,analytical method: DFG No. 405 (HPLC/FPD),LOQ grain: 0.03 mg/kg, LOQ straw: 0,05 mg/kg,max. sample storage: 15 months, RIP9501293, RIP9501294

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
IF-93/04568-01; AS/1896/CN; trial 3; B United Kingdom (UK) SO634338 Hereford 1994-03-22	Marinka	1) 1991-10-26 (planting) 2) 3)	0.72	200	0.36	1992-07-16[4)]	BBCH 87-92	grain straw	1.4 8.3		7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation CHE 3607, SL (360 g/l Glyphosate,analytical method: DFG No. 405 (HPLC/FPD),LOQ grain: 0.03 mg/kg, LOQ straw: 0,05 mg/kg,max. sample storage: 15 months, RIP9501293, RIP9501294
IF-93/04568-01; AS/1896/CN; trial 3; C United Kingdom (UK) SO634338 Hereford 1994-03-22	Marinka	1) 1991-10-26 (planting) 2) 3)	0.72	200	0.36	1992-07-16[4)]	BBCH 87-92	grain straw	1.9 12.5		7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation CHE 3607, SL (360 g/l Glyphosate) + Frigate, LI 004698-00 (800 g/l Tallow amine ethoxylate),analytical method: DFG No. 405 (HPLC/FPD),LOQ grain: 0.03 mg/kg, LOQ straw: 0,05 mg/kg,max. sample storage: 15 months, RIP9501293, RIP9501294

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
IF-93/04568-01; AS/1896/CN; trial 3; D United Kingdom (UK) SO634338 Hereford 1994-03-22	Marinka	1) 1991-10-26 (planting) 2) 3)	0.36	200	0.18	1992-07-16[4)]	BBCH 87-92	grain straw	0.90 7.2		7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation CHE 3607, SL (360 g/l Glyphosate) + Frigate, LI 004698-00 (800 g/l Tallow amine ethoxylate),analytical method: DFG No. 405 (HPLC/FPD),LOQ grain: 0.03 mg/kg, LOQ straw: 0,05 mg/kg,max. sample storage: 15 months, RIP9501293, RIP9501294

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.19 Barley (Southern Europe)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : August 2000

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : (MON 2139) Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Indoors / outdoors : Outdoors (European South)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30815 98-GLY-06-B/F, R 8144 BD2/C FR- 26750 St. Paul-les-Romans 05/1999	Deborah	1) 07.03.1998 (sowing) 2) – 3) 13.07.1998	2.4	220	1.08	06.07.1998	BBCH 89	grain straw	18 15 14 116 102 61	0.07 0.17 0.20 0.75 2.0 1.9	0 7 15 0 7 15	analytical method: (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Replicate to MLL 30815 98-GLY-06-B/F, R 8144 BD2/D RIP2000-1243

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 680 g/kg
Formulation (e.g. WP) : WG
Commercial product (name) : MON 14420
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : August 2000

Indoors / outdoors : Outdoors (European South)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid(AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30815 98-GLY-06-B/F, R 8144 BD2/D FR- 26750 St. Paul-les-Romans 05/1999	Deborah	1) 07.03.1998 (sowing) 2) – 3) 13.07.1998	2.4	220	1.08	06.07.1998	BBCH 89	grain straw	21 12 9.9 98 102 69	0.08 0.16 0.16 0.63 2.2 2.4	0 9 14 0 9 14	analytical method: (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Replicate to MLL 30815 98-GLY-06-B/F, R 8144 BD2/C RIP2000-1243

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 650 g/l
Formulation (e.g. WP) : SG
Commercial product (name) : MON 78568
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2005-04-21

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 DR1, plot D France 47120 Duras 2002-06-13	Intro	1) 2000-10-28 (sowing) 2) 3)	2.1	295	0.72	2001-06-15[4)]	BBCH 89	grain straw	4.4 11.0	0.080 0.19	6 6	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 6 months for grain, 8 months for straw RIP2005-200
R A1157, trial A1157 TL2, plot D France 31870 Lagardelle sur Leze 2002-06-13	Nevada	1) 2001-02 (sowing) 2) 3)	2.2	310	0.72	2001-07-10[4)]	BBCH 87-89	grain straw	2.7 20.4	0.050 0.65	6 6	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 5 months for grain, 7 months for straw RIP2005-200

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 540 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : MON 78273
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2005-04-21

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 DR1, plot C France 47120 Duras 2002-06-13	Intro	1) 2000-10-28 (sowing) 2) 3)	2.2	309	0.72	2001-06-15[4)]	BBCH 89	grain straw	4.2 19.9	0.070 0.33	6 6	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 6 months for grain, 8 months for straw RIP2005-200
R A1157, trial A1157 TL2, plot C France 31870 Lagardelle sur Leze 2002-06-13	Nevada	1) 2001-02 (sowing) 2) 3)	2.2	300	0.72	2001-07-10[4)]	BBCH 87-89	grain straw	2.7 14.9	<0.05 0.36	6 6	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 5 months for grain, 7 months for straw RIP2005-200

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Spring Barley

Submission date : 2005-04-21

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 DR1, plot B France 47120 Duras 2002-06-13	Intro	1) 2000-10-28 (sowing) 2) 3)	2.2	301	0.72	2001-06-15[4)]	BBCH 89	grain straw	3.8 21.5	0.070 0.28	6 6	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 6 months for grain, 8 months for straw RIP2005-200
R A1157, trial A1157 TL2, plot B France 31870 Lagardelle sur Leze 2002-06-13	Nevada	1) 2001-02 (sowing) 2) 3)	2.1	287	0.72	2001-07-10[4)]	BBCH 87-89	grain straw	2.8 23.5	<0.05 0.40	6 6	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 5 months for grain, 7 months for straw RIP2005-200

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : (MON 2139) Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : August 2000

Indoors / outdoors : Outdoors (European South)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30815, 98-GLY-06-B/F, R 8144 DR2/C FR- 47120 Duras 05/1999	Express	1) 21.10.1997 (sowing) 2) – 3) 26.06.1998	2.1	191	1.1	19.06.1998	BBCH 88-89	grain straw	21 5.2 2.6 63 28 12	0.08 0.06 <0.05 0.24 0.60 0.65	0 9 14 0 9 14	analytical method: (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 9 months Replicate to MLL 30815, 98-GLY-06-B/F, R 8144 DR2/D RIP2000-1243

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 680 g/kg
Formulation (e.g. WP) : WG
Commercial product (name) : MON 14420
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : August 2000

Indoors / outdoors : Outdoors (European South)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30815, 98-GLY-06-B/F, R 8144 DR2/D FR- 47120 Duras 05/1999	Express	1) 21.10.1997 (sowing) 2) – 3) 26.06.1998	2.0	191	1.0	19.06.1998	BBCH 88-89	grain straw	13 6.7 4.7 70 40 31	<0.05 0.06 0.06 0.34 0.60 1.0	0 9 14 0 9 14	analytical method: (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 9 months Replicate to MLL 30815, 98-GLY-06-B/F, R 8144 DR2/C RIP2000-1243

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : (MON 2139) Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : 28.11.2001

Indoors / outdoors : Outdoors (European South)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 31337, 99-GLY-07-B/F, trial 9075 DR2/C FR-47120 Duras 05/2000	Intro	1) 22.10.1998 2) – 3) 28.06.1999	2.2	177	1.2	21.06.1999	BBCH 89	grain straw	10 8.5 3.0 185 40 22	<0.05 0.07 <0.05 1.8 0.98 0.62	0 7 14 0 7 14	analytical method: DFG method 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Replicate to MLL 31337 99-GLY-07-B/F, trial 9075 DR2/D RIP2002-650
MLL 31337, 99-GLY-07-B/F, trial 9075 BD2/C FR-26750 St. Paul les Romans 05/2000	Platine	1) 14.10.1998 2) – 3) 25.06.1999	2.2	179	1.2	18.06.1999	BBCH 89	grain straw	19 19 15 112 61 26	0.09 0.20 0.22 1.7 1.2 0.97	0 7 14 0 7 14	analytical method: DFG method 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Replicate to MLL 31337 99-GLY-07-B/F, trial 9075 BD2/D RIP2002-650

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 450 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : MON 78294
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Winter Barley

Submission date : 28.11.2001

Indoors / outdoors : Outdoors (European South)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 31337 99-GLY-07-B/F, trial 9075 DR2/D FR-47120 Duras 05/2000	Intro	1) 22.10.1998 2) – 3) 28.06.1999	2.3	182	1.2	21.06.1999	BBCH 89	grain straw	19 5.4 7.1 161 59 26	<0.05 <0.05 0.07 1.2 0.99 0.71	0 7 14 0 7 14	analytical method: DFG method 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Replicate to MLL 31337 99-GLY-07-B/F, trial 9075 DR2/C RIP2002-650
MLL 31337 99-GLY-07-B/F, trial 9075 BD2/D FR-26750 St. Paul les Romans 05/2000	Platine	1) 14.10.1998 2) – 3) 25.06.1999	2.2	180	1.2	18.06.1999	BBCH 89	grain straw	9.2 19 12 120 71 28	<0.05 0.19 0.20 1.5 1.6 1.1	0 7 14 0 7 14	analytical method: DFG method 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Replicate to MLL 31337 99-GLY-07-B/F, trial 9075 BD2/C RIP2002-650

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.20 Oats

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Active ingredient : Glyphosate (Isopropylamin-salt)
Crop / crop group : Oat

Submission date : July 1986

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Amino-methyl-phosphonic-acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)			(d)	(e)
HI D-4535 Metten 11/1986	Flämings- krone	1) – 2) – 3) 26.08.1979	1.8	400	0.45	12.08.1979	BBCH 85	ears grain straw	21 30 14 7.4 5.7 4.4 64 9.6 7.4	<0.05 0.19 0.28 0.19 0.17 0.17 0.19 0.18 0.15	0 3 7 10 14 21 0 10 21	analytical method: HPLC, LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501276
MOD 80-D/15 D-6480 Steinau-Klesberg 11/1986	not reported	1) 21.04.1980 2) – 3) –	1.8	400	0.45	23.08.1980	BBCH 85	grain straw	6.1 3.1 2.1 1.2 1.4 141 27 21 24	0.08 0.06 <0.05 <0.05 <0.05 1.4 0.34 0.36 0.34	0 3 7 14 21 0 3 7 14	analytical method: HPLC, LOQ: 0.05 mg/kg, max. sample storage: 2 months ASB2009-5341

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)			(d)	(e)
CM 80-D-63 /64-47 C80.100 72 b) D-6501 Schwabenheim 14.11.1980	no data (lodged grain)	not reported	1.8	400	0.45	11.08.1980	BBCH 85	grain straw	2.0 5.5 5.5 4.3 2.6 60 18 8.1 11 9.1	<0.05 <0.05 <0.05 <0.05 <0.05 0.07 0.64 0.15 0.22 0.06	0 3 7 14 21 0 3 7 14 21	analytical method: no data, LOQ: 0.05 mg/kg, max. sample storage: 2 months ASB2009-5340
001639 D-8073 Koesching 04.10.1985	Pirol	1) 04.05.1981 2) – 3) 26.08.1981	1.8	400	0.45	12.08.1981	BBCH 91	grain	6.8 6.1 4.0 8.0 3.8 9.8	0.09 0.06 0.08 0.10 0.05 0.13	0 3 5 7 10 14	analytical method: HPLC, LOQ: 0.05 mg/kg, max. sample storage: 4 months ASB2009-5713
003188 D-3087 Bordenau 04.10.1985	Leander	1) 08.03.1984 2) – 3) 29.09.1984	1.1	300	0.36	29.08.1984	BBCH 91	grain	3.2 4.5 2.9 0.85 0.42	0.07 <0.05 <0.05 0.07 0.05	0 3 7 10 14	analytical method: HPLC, LOQ: 0.05 mg/kg, max. sample storage: 3 months ASB2009-5710
003188 D-3087 Bordenau 04.10.1985	Leander	1) 08.03.1984 2) – 3) 29.09.1984	1.8	300	0.60	29.08.1984	BBCH 91	grain	3.6 9.5 5.4 1.8 0.46	0.05 0.10 0.08 0.08 <0.05	0 3 7 10 14	analytical method: HPLC, LOQ: 0.05 mg/kg, max. sample storage: 3 months ASB2009-5710
003149 D-6461 Lanzingen 04.10.1985	Erbgraf	1) 26.03.1982 2) – 3) –	1.1	400	0.28	27.07.1982	BBCH 91	grain	15 16 15 15 15	0.10 0.29 0.25 0.29 0.28	0 3 7 10 14	analytical method: HPLC, LOQ: 0.05 mg/kg, max. sample storage: 5 months

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl							
	(a)	(b)				(c)		(a)			(d)	(e)
												ASB2009-5712
003150 D-6461 Lanzingen 04.10.1985	Erbgraf	1) 26.03.1982 2) – 3) –	1.8	400	0.45	27.07.1982	BBCH 91	grain	26 26 20 21 21	0.24 0.47 0.33 0.39 0.32	0 3 7 10 14	analytical method: HPLC, LOQ: 0.05 mg/kg, max. sample storage: 5 months ASB2009-5711

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup **002389-00**
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate (Isopropylamin-salt)
Crop / crop group : Oat

Submission date : 01.09.1995

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)			(d)	(e)
report MLL 30037, A 36, trial RC/79/2 GB-Durham 23.10.1979	Oberon	1) – (sowing) 2) -- 3) --	1.4	200	0.70	04.09.1979	BBCH 79	grain straw	4.4 29.6	<0.05 0.14	7 7	analytical method: GC-FPD), LOQ's: 0.05 mg/kg RIP9501230
			2.9	200	1.4	04.09.1979	BBCH 79	grain straw	12.4 113	0.12 0.37	7 7	

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) :
Applicant : Cheminova A/S

Active ingredient : Glyphosate
Crop / crop group : Oats

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
IF-93/04575-01; AS/1907/CN; T1 United Kingdom SK448233 Disworth / Leicestershire 1994-07-06	Aintree	1) 1991-10-01 (planting) 2) 3)	1.4	200	0.72	1992-07-30[4)]	BBCH 89-92	grain straw whole plant	4.0 82.7 84.4 128 50.9		5 5 0 1 3	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with CHE 3607, SL (360 g/l Glyphosate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: whole plant 0,04mg/kg, grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501299, RIP9501300

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
IF-93/04575-01; AS/1907/CN; T2 United Kingdom SK448233 Disworth / Leices-tershire 1994-07-06	Aintree	1) 1991-10-01 (planting) 2) 3)	0.72	200	0.36	1992-07-30[4)]	BBCH 89-92	grain straw whole plant	1.8 49.5 45.3 51.0 28.6		5 5 0 1 3	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with CHE 3607, SL (360g/l Glypho-sate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: whole plant 0,04mg/kg, grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501299, RIP9501300
IF-93/04575-01; AS/1907/CN; T3 United Kingdom SK448233 Disworth / Leices-tershire 1994-07-06	Aintree	1) 1991-10-01 (planting) 2) 3)	0.72	200	0.36	1992-07-30[4)]	BBCH 89-92	grain straw whole plant	1.6 51.7 45.8 52.8 38.2		5 5 0 1 3	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with tankmix CHE 3607, SL /Frigate, ADJ (360g/l Glyphosate, 800g/l Tallow amine ethox-ylate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: whole plant 0,04mg/kg, grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501299, RIP9501300

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
IF-93/04575-01; AS/1907/CN; T4 United Kingdom SK448233 Disworth / Leices-tershire 1994-07-06	Aintree	1) 1991-10-01 (planting) 2) 3)	0.36	200	0.18	1992-07-30[4)]	BBCH 89-92	grain straw whole plant	2.0 24.8 19.5 22.3 16.8		5 5 0 1 3	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with tankmix CHE 3607, SL /Frigate, ADJ (360g/l Glyphosate, 800g/l Tallow amine ethox-ylate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: whole plant 0,04mg/kg, grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501299, RIP9501300

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : ROUNDUP
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Oats

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, SU 82/10 RP, 11 United Kingdom Buckingham 1991-12-01	Pennal	1) 1982 (planting) 2) 3)	1.4	200	0.72	1982-07-30[4)]		grain straw	1.2 1.3 1.2 8.2 8.8 7.7	<0.2 <0.2 <0.2 <0.4 <0.4 <0.4	12 12 12 12 12 12	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, SU 82/10 RP, 12 United Kingdom Skelton 1991-12-01	Maris Quest	1) 1982 (planting) 2) 3)	1.4	200	0.72	1982-07-26[4)]		grain straw	8.2 8.3 7.8 52.8 43.7 50.8	<0.2 0.27 0.20[5)] 0.52 0.43 0.50	7 7 7 7 7 7 7	4) spraying 5) LOQ: 0.4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate), analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2 mg/kg, straw 0,4 mg/kg, max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, SU 82/10 RP, 13 United Kingdom Cottenham 1991-12-01	Trafalgar	1) 1982 (planting) 2) 3)	1.4	200	0.72	1982-08-14[4)]		grain straw	6.2 6.4 5.5 24.9 21.1 28.9	<0.2 <0.2 <0.2[5)] 0.42 0.48 0.71	7 7 7 7 7 7 7	4) spraying 5) LOQ: 0.4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01, treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, SU 82/10 RP, 14 United Kingdom Edinburgh 1991-12-01	Maris Tabard	1) 1982 (planting) 2) 3)	1.4	200	0.72	1982-09-15[4)]		grain straw	3.2 3.2 3.7 27.7 25.5 52.0	<0.2 <0.2 <0.2[5)] 0.64 0.73 1.6	7 7 7 7 7 7 7	4) spraying 5) LOQ: 0.4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01, treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate), analytical method: (HPLC/FPD, HPLC/OPA), LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2 mg/kg, straw 0,4 mg/kg, max. sample storage: no data, ASB2009-5491, RIP9501249

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) :
Applicant : Cheminova A/S

Active ingredient : Glyphosate
Crop / crop group : Oats

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8 Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
IF-93/04569-01; AS/1897/CN; 01; T1 United Kingdom SK448233 Disworth, Leicestershire 1994-03-01	Aintree	1) 1992-10-01 (planting) 2) 3)	1.4	200	0.72	1992-07-30[4)]	BBCH 89-92	grain straw	4.6 110	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with CHE 3607, SL (360g/l Glyphosate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501297, RIP9501298
IF-93/04569-01; AS/1897/CN; 01; T2 United Kingdom SK448233 Disworth, Leicestershire 1994-03-01	Aintree	1) 1992-10-01 (planting) 2) 3)	0.72	200	0.36	1992-07-30[4)]	BBCH 89-92	grain straw	2.2 36.7	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with CHE 3607, SL (360g/l lypho-sate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501297, RIP9501298

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
IF-93/04569-01; AS/1897/CN; 01; T3 United Kingdom SK448233 Disworth, Leicestershire 1994-03-01	Aintree	1) 1992-10-01 (planting) 2) 3)	0.72	200	0.36	1992-07-30[4)]	BBCH 89-92	grain straw	2.3 42.3	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with tankmix CHE 3607, SL /Frigate, ADJ (360g/l Glyphosate, 800g/l Tallow amine ethoxylate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501297, RIP9501298
IF-93/04569-01; AS/1897/CN; 01; T4 United Kingdom SK448233 Disworth, Leicestershire 1994-03-01	Aintree	1) 1992-10-01 (planting) 2) 3)	0.36	200	0.18	1992-07-30[4)]	BBCH 89-92				4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with tankmix CHE 3607, SL /Frigate, ADJ (360g/l Glyphosate, 800g/l Tallow amine ethoxylate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501297, RIP9501298
IF-93/04569-01; AS/1897/CN; 02; T1 United Kingdom SK892714 Wintringham, Yorkshire 1994-03-01	Image	1) 1991-09-28 (planting) 2) 3)	1.4	200	0.72	1992-07-28[4)]	BBCH 89	grain straw	1.1 66.4	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with CHE 3607, SL (360g/l Glyphosate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501297, RIP9501298

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
IF-93/04569-01; AS/1897/CN; 02; T2 United Kingdom SK892714 Wintringham, Yorkshire 1994-03-01	Image	1) 1991-09-28 (planting) 2) 3)	0.72	200	0.36	1992-07-28[4)]	BBCH 89	grain straw	1.1 54.6	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with CHE 3607, SL (360g/l Glyphosate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501297, RIP9501298
IF-93/04569-01; AS/1897/CN; 02; T3 United Kingdom SK892714 Wintringham, Yorkshire 1994-03-01	Image	1) 1991-09-28 (planting) 2) 3)	0.72	200	0.36	1992-07-28[4)]	BBCH 89	grain straw	0.63 48.1	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with tankmix CHE 3607, SL /Frigate, ADJ (360g/l Glyphosate, 800g/l Tallow amine ethoxylate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501297, RIP9501298
IF-93/04569-01; AS/1897/CN; 02; T4 United Kingdom SK892714 Wintringham, Yorkshire 1994-03-01	Image	1) 1991-09-28 (planting) 2) 3)	0.36	200	0.18	1992-07-28[4)]	BBCH 89	grain straw	0.79 32.2	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with tankmix CHE 3607, SL /Frigate, ADJ (360g/l Glyphosate, 800g/l Tallow amine ethoxylate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501297, RIP9501298

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
IF-93/04569-01; AS/1897/CN; 03; T1 United Kingdom SO637334 Hereford 1994-03-01	Aintree	1) 1991-10-15 (planting) 2) 3)	1.4	200	0.72	1992-07-23[4)]	BBCH 87-92	grain straw	7.9 38.5	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with CHE 3607, SL (360g/l Glyphosate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501297, RIP9501298
IF-93/04569-01; AS/1897/CN; 03; T3 United Kingdom SO637334 Hereford 1994-03-01	Aintree	1) 1991-10-15 (planting) 2) 3)	0.72	200	0.36	1992-07-23[4)]	BBCH 87-92	grain straw	1.0 7.4	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with tankmix CHE 3607, SL /Frigate, ADJ (360g/l Glyphosate, 800g/l Tallow amine ethoxylate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501297, RIP9501298
IF-93/04569-01; AS/1897/CN; 03; T4 United Kingdom SO637334 Hereford 1994-03-01	Aintree	1) 1991-10-15 (planting) 2) 3)	0.36	200	0.18	1992-07-23[4)]	BBCH 87-92	grain straw	4.5 19.6	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with tankmix CHE 3607, SL /Frigate, ADJ (360g/l Glyphosate, 800g/l Tallow amine ethoxylate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501297, RIP9501298

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
IF-93/04569-01; AS/1897/CN; 03;T2 United Kingdom SO637334 Hereford 1994-03-01	Aintree	1) 1991-10-15 (planting) 2) 3)	0.72	200	0.36	1992-07-23[4)]	BBCH 87-92	grain straw	<0.03 0.17	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with CHE 3607, SL (360g/l Glyphosate),analytical method: DFG No. 405 (HPLC/FPD),LOQ: grain 0.03mg/kg, straw 0.05mg/kg,max. sample storage: 17 months, RIP9501297, RIP9501298

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : Soluble concentrate (SL)
Commercial product (name) : Roundup
Applicant : Monsanto Europe S.A./N.V.

Active ingredient : glyphosate
Crop / crop group : Oats
Crop Code : AVESS

Submission date : 2007-10-11

Indoors / Outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30.150, DK84-30, dk8430030, 1 Decline trial Denmark (DK) Jylland 1985-07-26	Selma	1) 2) 3)	1.08	200	0.54	1984-08-20[4)]	BBCH	grain	4.0 5.1 4.9 2.1 3.6	0 4 7 10 15	4) spraying analytical method: HPLC (HPLC), LOQ: 0.05 mg/kg grain, 0.2 mg/kg straw RIP9501275
MLL 30.150, DK84-30, dk8430030, 2 Decline trial Denmark (DK) Jylland 1985-07-26	Selma	1) 2) 3)	2.16	200	1.1	1984-08-20[4)]	BBCH	grain	5.4 9.1 5.3 5.1 3.6	0 4 7 10 15	4) spraying analytical method: HPLC (HPLC), LOQ: 0.05 mg/kg grain, 0.2 mg/kg straw RIP9501275

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30.150, DK84-30, dk8430031, 1 Decline trial Denmark (DK) Fyn 1985-07-26	Selma	1) 2) 3)	1.08	200	0.54	1984-08-14[4)]	BBCH	grain	4.5 6.1 7.1 8.2 8.6	0 4 7 10 15	4) spraying analytical method: HPLC (HPLC), LOQ: 0.05 mg/kg grain, 0.2 mg/kg straw RIP9501275
MLL 30.150, DK84-30, dk8430031, 2 Decline trial Denmark (DK) Fyn 1985-07-26	Selma	1) 2) 3)	2.16	200	1.1	1984-08-14[4)]	BBCH	grain	6.3 19.3 17.1 18.4 17.3	0 4 7 10 15	4) spraying analytical method: HPLC (HPLC), LOQ: 0.05 mg/kg grain, 0.2 mg/kg straw RIP9501275
MLL 30.150, DK84-30, dk8430032, 1 Decline trial Denmark (DK) Fyn 1985-07-26	Selma	1) 2) 3)	1.08	200	0.54	1984-08-13[4)]	BBCH	grain	3.0 7.3 13.3 8.1 14.5	0 4 7 10 15	4) spraying analytical method: HPLC (HPLC), LOQ: 0.05 mg/kg grain, 0.2 mg/kg straw RIP9501275

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30.150, DK84-30, dk8430032, 1 Decline trial Denmark (DK) Fyn 1985-07-26	Selma	1) 2) 3)	2.16	200	1.1	1984-08-13[4)]	BBCH	grain	5.8 16.6 18.2 17.0 21.4	0 4 7 10 15	4) spraying analytical method: HPLC (HPLC), LOQ: 0.05 mg/kg grain, 0.2 mg/kg straw RIP9501275

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 480 g/L
Formulation (e.g. WP) : Soluble concentrate (SL)
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : glyphosate isopropyl amin salt
Crop / crop group : Oats
Crop Code : AVESS

Submission date : 1983-03-08

Indoors / Outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 AMPA (Aminomethylphosphonsäure)
8.2 glyphosate

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.071 ! MLL 30071 ! A 36, CPH 80/3, 01 Reverse decline trial United Kingdom (UK) Winchburgh 1981-10-28	Astor	1) 2) 3)	0.72			1980-09-03[4)]	BBCH 86	grain hull groats	0.080 <0.050 <0.050	1.8 2.0 0.90	36 36 36	4) spraying analytical method: not reported (not reported), max. sample storage time in month(s): 13 RIP9501239 RIP9501256 (amendment)
MLL 30.071 ! MLL 30071 ! A 36, CPH 80/3, 02 Reverse decline trial United Kingdom (UK) Winchburgh 1981-10-28	Astor	1) 2) 3)	0.72			1980-09-11[4)]	BBCH 86	grain hull groats	<0.050 0.050 <0.050	0.80 6.6 0.20	28 28 28	4) spraying analytical method: not reported (not reported), max. sample storage time in month(s): 13 RIP9501239 RIP9501256 (amendment)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.071 ! MLL 30071 ! A 36, CPH 80/3, 03 Reverse decline trial United Kingdom (UK) Winchburgh 1981-10-28	Astor	1) 2) 3)	1.44			1980-09-03[4)]	BBCH 86	grain hull groats	0.19 0.10 0.13	4.3 3.9 3.0	36 36 36	4) spraying analytical method: not reported (not reported), max. sample storage time in month(s): 13 RIP9501239 RIP9501256 (amendment)
MLL 30.071 ! MLL 30071 ! A 36, CPH 80/3, 04 Reverse decline trial United Kingdom (UK) Winchburgh 1981-10-28	Astor	1) 2) 3)	1.44			1980-09-11[4)]	BBCH 86	grain hull groats	<0.050 <0.050 <0.050	1.4 6.3 0.80	28 28 28	4) spraying analytical method: not reported (not reported), max. sample storage time in month(s): 13 RIP9501239 RIP9501256 (amendment)
MLL 30.071 ! MLL 30071 ! A 36, CPH 80/3, 05 Reverse decline trial United Kingdom (UK) Winchburgh 1981-10-28	Astor	1) 2) 3)	2.88			1980-09-03[4)]	BBCH 86	grain hull groats	0.18 0.20 0.13	6.0 11.1 3.3	36 36 36	4) spraying analytical method: not reported (not reported), max. sample storage time in month(s): 13 RIP9501239 RIP9501256 (amendment)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.071 ! MLL 30071 ! A 36, CPH 80/3, 06 Reverse decline trial United Kingdom (UK) Winchburgh 1981-10-28	Astor	1) 2) 3)	2.88			1980-09-11[4)]	BBCH 86	grain hull groats	<0.050 0.10 <0.050	1.9 3.5 0.50	28 28 28	4) spraying analytical method: not reported (not reported), max. sample storage time in month(s): 13 RIP9501239 RIP9501256 (amendment)
MLL 30.071 ! MLL 30071 ! A 36, CPH 80/1, 01 Reverse decline trial United Kingdom (UK) Wintringham 1981-10-28	Peniarth	1) 2) 3)	1.44	200	0.72	1980-08-13[4)]	BBCH 86	grain hull straw groats	<0.050 0.090 0.11 0.11 0.15 0.30 0.25 0.29 0.28 0.050	6.5 4.2 5.1 5.1 5.6 19.4 17.6 21.3 21.6 1.4	7 7 7 7 7 7 7 7 7 7	4) spraying analytical method: not reported (not reported), max. sample storage time in month(s): 15 RIP9501239 RIP9501256 (amendment)
MLL 30.071 ! MLL 30071 ! A 36, CPH 80/1, 02 Reverse decline trial United Kingdom (UK) Wintringham 1981-10-28	Peniarth	1) 2) 3)	1.44	400	0.36	1980-08-13[4)]	BBCH 86	grain hull straw groats	0.13 0.17 0.060 0.10 0.40 0.29 0.22 0.32 0.36 0.13	6.3 4.6 2.6 3.7 17.8 24.9 20.2 19.7 25.4 4.0	7 7 7 7 7 7 7 7 7 7	4) spraying analytical method: not reported (not reported), max. sample storage time in month(s): 15 RIP9501239 RIP9501256 (amendment)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.071 ! MLL 30071 ! A 36, CPH 80/1, 03 Reverse decline trial United Kingdom (UK) Wintringham 1981-10-28	Peniarth	1) 2) 3)	2.88	200	1.4	1980-08-13[4)]	BBCH 86	grain hull straw groats	0.16 0.20 0.25 0.26 0.34 0.24 0.26 0.39 0.36 0.050	8.7 9.2 14.8 12.5 14.2 27.5 26.2 29.4 30.8 1.1	7 7 7 7 7 7 7 7 7 7	4) spraying analytical method: not reported (not reported), max. sample storage time in month(s): 15 RIP9501239 RIP9501256 (amendment)
MLL 30.071 ! MLL 30071 ! A 36, CPH 80/1, 04 Reverse decline trial United Kingdom (UK) Wintringham 1981-10-28	Peniarth	1) 2) 3)	2.88	400	0.72	1980-08-13[4)]	BBCH 86	grain hull straw groats	0.14 0.17 0.24 0.16 0.25 0.36 0.40 0.55 0.34 0.10	5.2 7.6 10.2 5.0 9.1 22.4 31.8 43.8 32.1 1.1	7 7 7 7 7 7 7 7 7 7	4) spraying analytical method: not reported (not reported), max. sample storage time in month(s): 15 RIP9501239 RIP9501256 (amendment)

Remarks: (a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.21 Rye

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup (SAG 539 00)
Applicant : Cheminova A/S

Active ingredient : Glyphosate
Crop / crop group : Winter Rye

Submission date : 1993-12-06

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA), calculated as Glyphosate

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
FRG-0066/R198/90, SAG 12537, processing Germany 6000 Frankfurt-Bergen/Enkheim 1992-10-22	Gepard	1) 1989-10-07 (sowing) 2) 3) 1990-07-27	1.8	400	0.45	1990-07-13[4)]	BBCH 75	grain	2.9 2.3 2.9 4.1 4.7 36	<0.05 0.09 0.15 0.28 0.31 0.27	0 3 7 10 14 21	4) spraying analytical method: DFG method 405 (HPLC) conversion factor AMPA to Glyphosate: 1.523,
								straw	18 25	0.55 0.90	14 21	
								whole meal bread	2.4	0.16	21	Replicate to FRG-0065/R195/90, SAG 12538
								bran	6.0	0.18	21	RIP9501327 & RIP9501328
								flour	12	0.42	21	
								middlings	2.5	0.12	21	
								whole-meal flour	0.37	0.05	21	

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
FRG-0066/R199-90, SAG 12510, processing Germany 8892 Haslangbreit 1992-10-22	Danko	1) 1989-10-22 (sowing) 2) 1990-06-02 - 1990-06-10 3) 1990-07-30	1.8	400	0.45	1990-07-09[4)]	BBCH 85	ears of grain grain straw whole meal bread bran flour middlings whole-meal flour	3.8 26 32 26 16 18 31 15 14 24 2.0 21 16	0.26 0.50 0.78 0.99 0.54 0.68 0.86 0.68 0.42 0.34 0.12 0.50 0.27	0 3 7 10 14 21 14 21 21 21 21 21 21	4) spraying analytical method: DFG method 405 (HPLC) conversion factor AMPA to Glyphosate: 1.523 Replicate to FRG-0065/R195/90, SAG 12509 RIP9501327 & RIP9501328

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : GLYFOS (CHE 03690 H)
Applicant : Cheminova A/S

Active ingredient : Glyphosate
Crop / crop group : Winter Rye

Submission date : 1993-12-06

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA), calculated as Glyphosate

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
FRG-0065/R194-90, SAG 12538, processing Germany 6000 Frankfurt-Bergen/Enkheim 1992-10-22	Gepard	1) 1989-10-07 (sowing) 2) 3) 1990-07-27	1.8	400	0.45	1990-07-13[4)]	BBCH 75	grain straw bran flour middlings whole-meal flour whole-meal bread	2.8 3.4 3.9 4.2 5.4 4.0 16 39 6.7 2.2 5.9 4.5 1.9	<0.05 0.08 0.1 0.17 0.31 0.25 0.57 1.5 0.21 0.25 0.21 0.22 0.12	0 3 7 10 14 21 14 21 21 21 21 21 21	4) spraying analytical method: DFG method 405 (HPLC) conversion factor AMPA to Glyphosate: 1.523 Replicate to FRG-0065/R195/90, SAG 12537 RIP9501327 & RIP9501328

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
FRG-0065/R195/90, SAG 12509, processing Germany 8892 Haslangbreit 1992-10-22	Danko	1) 1989-10-22 (sowing) 2) 1990-06-02 - 1990-06-10 3) 1990-07-30	1.8	400	0.45	1990-07-09[4)]	BBCH 85	ears of grain grain straw bran flour middlings whole-meal flour whole-meal bread	29 24 21 23 17 5.0 18.5 5.5 24 7.5 39 22 13	0.22 0.36 0.78 0.57 0.46 0.05 0.57 0.26 0.4 0.2 0.65 0.69 0.34	0 3 7 10 14 21 14 21 21 21 21 21 21	4) spraying analytical method: DFG method 405 (HPLC) conversion factor AMPA to Glyphosate: 1.523 Replicate to FRG-0065/R195/90, SAG 12510 RIP9501327 & RIP9501328

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : Soluble concentrate (SL)
Commercial product (name) : Roundup
Applicant : Monsanto Europe S.A./N.V.

Active ingredient : glyphosate
Crop / crop group : Rye
Crop Code : SECCE

Submission date : 2007-10-11

Indoors / Outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30.150, DK84-29, dk84329025, 1 Decline trial Denmark (DK) Fyn 1985-07-26	Petkus II	1) 2) 3)	1.08	200	0.54	1984-08-09[4)]		grain straw	3.1 2.4 2.2 2.0 2.1 1.3	0 4 7 10 15 0	4) spraying analytical method: HPLC (HPLC), LOQ: 0.05 mg/kg grain, 0.2 mg/kg straw RIP9501275
MLL 30.150, DK84-29, dk84329025, 2 Decline trial Denmark (DK) Fyn 1985-07-26	Petkus II	1) 2) 3)	2.2	200	1.1	1984-08-09[4)]		grain straw	5.2 5.9 3.5 5.3 5.4 263	0 4 7 10 15 0	4) spraying analytical method: HPLC (HPLC), LOQ: 0.05 mg/kg grain, 0.2 mg/kg straw RIP9501275

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30.150, DK84-29, dk8429026, 1 Decline trial Denmark (DK) Jylland 1985-07-26	Petkus II	1) 2) 3)	1.1	200	0.55	1984-08-10[4)]		grain straw	1.3 1.6 1.6 1.2 1.6 198	0 3 6 9 14 0	4) spraying analytical method: HPLC (HPLC), LOQ: 0.05 mg/kg grain, 0.2 mg/kg straw RIP9501275
MLL 30.150, DK84-29, dk8429026, 2 Decline trial Denmark (DK) Jylland 1985-07-26	Petkus II	1) 2) 3)	2.2	200	1.1	1984-08-10[4)]		grain straw	1.7 2.8 1.9 2.9 2.3 279	0 3 6 9 14 0	4) spraying analytical method: HPLC (HPLC), LOQ: 0.05 mg/kg grain, 0.2 mg/kg straw RIP9501275
MLL 30.150, DK84-29, dk8429027, 1 Decline trial Denmark (DK) Jylland 1985-07-26	Petkus II	1) 2) 3)	2.2	200	1.1	1984-08-09[4)]		grain straw	4.6 1.7 1.3 1.6 1.5 193	0 4 7 10 15 0	4) spraying analytical method: HPLC (HPLC), LOQ: 0.05 mg/kg grain, 0.2 mg/kg straw RIP9501275

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
MLL 30.150, DK84-29, dk8429027, 2 Decline trial Denmark (DK) Jylland 1985-07-26	Petkus II	1) 2) 3)	2.2	200	1.1	1984-08-09[4)]		grain straw	4.2 3.2 2.8 3.1 2.8 362	0 4 7 10 15 0	4) spraying analytical method: HPLC (HPLC), LOQ: 0.05 mg/kg grain, 0.2 mg/kg straw RIP9501275

Remarks: (a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.22 Wheat (Northern Europe)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate (Isopropylamin-salt)
Crop / crop group : Spring Wheat

Submission date : 01.09.1995

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)			(d)	(e)
report MLL 30037, A 36, trial A/79/2 GB- Yelden, Bedshire 23.10.1979	Highbury	1) – (sowing) 2) -- 3) --	1.4	200	0.70	06.09.1979	BBCH 91	grain straw	control 0.5 0.9 control 116 111	control <0.05 <0.05 control 0.23 0.50	 7 7	analytical method: GC-FPD), LOQ's: 0.05 mg/kg RIP9501230
			2.9	200	1.4	06.09.1979	BBCH 91	grain straw	control 0.5 0.9 control 116 272	control <0.05 <0.05 control 0.23 0.85	 7 7	

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i.	(g/kg or g/l)	:	360 g/l	Active ingredient	: Glyphosate
Formulation	(e.g. WP)	:	SL	Crop / crop group	: Spring Soft Wheat
Commercial product	(name)	:	Roundup		
Applicant		:	Monsanto Agrar Deutschland GmbH	Submission date	: 2012-11-01
				Indoors / outdoors	: Outdoors (European North)
				Other a.i. in formulation (content and common name)	:
				Residues calculated as	: 8.1 Glyphosate 8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A1; CP 67573; UK/73/11, part 1 United Kingdom (UK) Clare 1974-01-07	Maris Dove	1) (planting) 2) 3) 1973-08-24	2.2			1973-03-07[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	170 170	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 16 months, RIP9501209

1	2	3	4			5	6	7	8.1	8.2	9	10
Report No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A1; CP 67573; UK/73/11, part 2 United Kingdom (UK) Clare 1974-01-07	Maris Dove	1) (planting) 2) 3) 1973-08-24	4.5			1973-03-07[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	170 170	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 16 months, RIP9501209
REPORT A1; CP 67573; UK/73/3; part 1 United Kingdom (UK) Spalding 1974-01-07	West Desprez	1) 1973-02-21 (planting) 2) 3) 1973-08-23	2.2			1973-02-26[4)]	BBCH 00	grain straw	<0.05 0.050	<0.05 <0.05	178 178	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 17 months RIP9501209

1	2	3	4			5	6	7	8.1	8.2	9	10
Report No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A1; CP 67573; UK/73/3; part 2 United Kingdom (UK) Spalding 1974-01-07	West Desprez	1) 1973-02-21 (planting) 2) 3) 1973-08-23	4.5			1973-02-26[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	178 178	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 17 months RIP9501209
REPORT A1; CP 67573; UK/73/4; part 1 United Kingdom (UK) Amber Hill 1974-01-07	Maris Ranger	1) 1973-02-02 (planting) 2) 3) 1973-08-16	2.2			1973-02-24[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	173 173	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 17 months RIP9501209

1	2	3	4			5	6	7	8.1	8.2	9	10
Report No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A1; CP 67573; UK/73/4; part 2 United Kingdom (UK) Amber Hill 1974-01-07	Maris Ranger	1) 1973-02-02 (planting) 2) 3) 1973-08-16	4.5			1973-02-24[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	173 173	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 17 months RIP9501209
REPORT A1; CP 67573; UK/73/5; part 1 United Kingdom (UK) Swineshead 1974-01-07	Cappelle Desprez	1) 1973-01-30 (planting) 2) 3) 1973-08-16	2.2			1973-02-24[4)]	BBCH 00	straw	<0.05	<0.05	173	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 17 months;no data for grain samples due to sample loss during shipment RIP9501209

1	2	3	4			5	6	7	8.1	8.2	9	10
Report No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A1; CP 67573; UK/73/5; part 2 United Kingdom (UK) Swineshead 1974-01-07	Cappelle Desprez	1) 1973-01-30 (planting) 2) 3) 1973-08-16	4.5			1973-02-24[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	173 173	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 17 months; RIP9501209
REPORT A1; CP 67573; UK/73/6; part 1 United Kingdom (UK) Holbeach 1974-01-07	Cappelle Desprez	1) 1973-02-02 (planting) 2) 3) 1973-08-15	2.2			1973-02-24[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	180 180	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 17 months RIP9501209

1	2	3	4			5	6	7	8.1	8.2	9	10
Report No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A1; CP 67573; UK/73/6; part 2 United Kingdom (UK) Holbeach 1974-01-07	Cappelle Desprez	1) 1973-02-02 (planting) 2) 3) 1973-08-15	4.5			1973-02-24[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	180 180	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 17 months RIP9501209
REPORT A1; CP 67573; UK/73/7, part 1 United Kingdom (UK) Kinton 1974-01-07	Maris Dove	1) (planting) 2) 3) 1973-08-24	2.2			1973-03-16[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 0.050	161 161	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 16 months, RIP9501209

1	2	3	4			5	6	7	8.1	8.2	9	10
Report No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A1; CP 67573; UK/73/7, part 2 United Kingdom (UK) Kinton 1974-01-07	Maris Dove	1) (planting) 2) 3) 1973-08-24	4.5			1973-03-16[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 0.050	161 161	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 16 months, RIP9501209
REPORT A1; CP 67573; UK/73/9, part 1 United Kingdom (UK) Lutton 1974-01-07	Kolibri	1) (planting) 2) 3) 1973-09-07	2.2			1973-03-07[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	184 184	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 16 months, RIP9501209

1	2	3	4			5	6	7	8.1	8.2	9	10
Report No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A1; CP 67573; UK/73/9, part 2 United Kingdom (UK) Lutton 1974-01-07	Kolibri	1) (planting) 2) 3) 1973-09-07	4.5			1973-03-07[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	184 184	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 16 months, RIP9501209

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : (MON 2139) Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Winter Wheat

Submission date : 30.05.2000

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30815, 98-GLY-06 B/F L 05, Trt 2 BE- 5380 Franc- Waret 05/1999	Rialto	1) 21.10.1997 (sowing) 2) – 3) 12.08.1998	2.2	200	1.1	05.08.1998	BBCH 94	grain straw	2.5 1.4 1.7 156 198 103	0.05 <0.05 0.12 0.67 2.36 2.22	0 7 14 0 7 14	analytical method: XA001, LLN-E/S-ASOP-051 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 6 months Replicate to MLL 30815, 98-GLY-06 B/F L 05, Trt 3 RIP2000-1243
MLL 30815, 98-GLY-06 B/F R 8144 OB1/C FR- 29830 Ploudalmezau 05/1999	Tremie	1) 06.12.1997 (sowing) 2) – 3) 12.08.1998	2.2	200	1.1	05.08.1998	BBCH 89	grain straw	2.2 4.0 3.1 95 79 90	<0.05 <0.05 0.05 0.54 1.4 3.2	0 7 14 0 7 14	analytical method: XA001, LLN-E/S-ASOP-051 (HPLC-Fluorescence), LOQ's: 0.05 mg/kg, max. sample storage: 6 months Replicate to MLL 30815, 98-GLY-06 B/F R 8144 OB1/D RIP2000-1243

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30815, 98-GLY-06 B/F R 8144 CT1/ C FR- 36110 Levroux 05/1999	Oracle	1) 19.10.1997 (sowing) 2) – 3) 22.07.1998	2.2	206	1.1	13.07.1998	BBCH 87	grain straw	2.0 0.16 0.16 139 7.5 9.8	<0.05 <0.05 <0.05 0.75 0.12 0.24	0 9 14 0 9 14	analytical method: XA001, LLN-E/S-ASOP-051 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 7 months Replicate to MLL 30815, 98-GLY-06 B/F R 8144 CT1/ D RIP2000-1243
MLL 30815, 98-GLY-06 B/F R 8144 AL1/C FR- 68740 Fessenheim 05/1999	Sideral	1) 25.10.1997 (sowing) 2) – 3) 15.07.1998	2.2	200	1.1	08.07.1998	BBCH 89	grain straw	6.3 2.3 3.1 95 64 59	0.05 <0.05 <0.05 0.41 0.61 0.71	0 7 14 0 7 14	analytical method: XA001, LLN-E/S-ASOP-051 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 7 months Replicate to MLL 30815, 98-GLY-06 B/F R 8144 AL1/D RIP2000-1243

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 680 g/kg
Formulation (e.g. WP) : WG
Commercial product (name) : (MON 14420)
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Winter Wheat

Submission date : August 2000

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30815, 98-GLY-06 B/F L 05, Trt 3 BE- 5380 Franc- Waret 05/1999	Rialto	1) 21.10.1997 (sowing) 2) – 3) 12.08.1998	2.2	200	1.1	05.08.1998	BBCH 94	grain straw	1.6 1.4 1.1 111 151 152	<0.05 <0.05 0.12 0.84 2.4 3.2	0 7 14 0 7 14	analytical method: XA001, LLN-E/S-ASOP-051 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 6 months Replicate to MLL 30815, 98-GLY-06 B/F L 05, Trt 2 RIP2000-1243
MLL 30815, R 8144 OB1/ D FR- 29830 Ploudalmezau 05/1999	Tremie	1) 06.12.1997 (sowing) 2) – 3) 12.08.1998	2.2	198	1.1	05.08.1998	BBCH 89	grain straw	1.1 3.7 2.3 88 80 67	<0.05 0.05 0.05 0.55 1.5 1.8	0 7 14 0 7 14	analytical method: XA001, LLN-E/S-ASOP-051 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 6 months Replicate to MLL 30815, 98-GLY-06 B/F R 8144 OB1/C RIP2000-1243

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30815, R 8144 CT1/ D FR- 36110 Levroux 05/1999	Oracle	1) 19.10.1997 (sowing) 2) – 3) 22.07.1998	2.2	202	1.1	13.07.1998	BBCH 87	grain straw	1.7 0.16 0.15 139 5.6 20	<0.05 <0.05 <0.05 0.78 0.08 0.89	0 9 14 0 9 14	analytical method: XA001, LLN-E/S-ASOP-051 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 7 months Replicate to MLL 30815, 98-GLY-06 B/F R 8144 CT1/ C RIP2000-1243
MLL 30815, R 8144 AL1/ D FR- 68740 Fessenheim 05/1999	Sideral	1) 25.10.1997 (sowing) 2) – 3) 15.07.1998	2.2	201	1.1	08.07.1998	BBCH 89	grain straw	12 3.6 3.8 91 77 59	0.05 0.06 <0.05 0.40 0.55 0.68	0 7 14 0 7 14	analytical method: XA001, LLN-E/S-ASOP-051 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 7 months Replicate to MLL 30815, 98-GLY-06 B/F R 8144 AL1/C RIP2000-1243

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Active ingredient : Glyphosate
Crop / crop group : Winter Wheat

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Submission date : 29.11.2000

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : YF11357
Applicant : Syngenta Agro GmbH

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : PMG-Anion (Glyphosate)

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl						
	(a)	(b)				(c)		(a)		(d)	(e)
RJ2910B, AF/4681/ZE/1 GB- Norton, Worcestershire 18.10.2000	Equinox	1) 15.10.1998 (sowing) 2) – 3) 05.08.1999	2.1	301	0.70	29.07.1999	BBCH 87-89	grain straw	1.2 23	7 7	analytical method: RR-92-042B RES2 (GC-MSD), LOQ: 0.05 mg/kg, max. sample storage: 5 months, *blank value:* *straw 0.27 mg/kg* RIP2000-2130
RJ2910B, AF/4681/ZE/2 GB- Wilson, Derbyshire 18.10.2000	Rialto	1) 21.09.1998 (sowing) 2) – 3) 05.08.1999	2.1	299	0.70	29.07.1999	BBCH 87	grain straw	1.1 27	7 7	analytical method: RR-92-042B RES2 (GC-MSD), LOQ: 0.05 mg/kg, max. sample storage: 5 months, *blank values:* *grain 0.11 mg/kg* *straw 0.37 mg/kg* RIP2000-2130

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl						
	(a)	(b)				(c)		(a)		(d)	(e)
RJ2910B, AF/4681/ZE/3 FR- 45300 Audeville, Loiret 18.10.2000	Courtot	1) 08.11.1998 (sowing) 2) – 3) 29.07.1999	2.1	304	0.69	22.07.1999	BBCH 89	grain straw	1.7 68	7 7	analytical method: RR-92-042B RES2 (GC-MSD), LOQ: 0.05 mg/kg, max. sample storage: 5 months RIP2000-2130
RJ2910B, AF/4681/ZE/4 FR- 77320 Coupigny, Seine-et-Marne 18.10.2000	Isengrain	1) 12.10.1998 (sowing) 2) – 3) 21.07.1999	2.1	305	0.69	14.07.1999	BBCH 87	grain straw	1.1 109	7 7	analytical method: RR-92-042B RES2 (GC-MSD), LOQ: 0.05 mg/kg, max. sample storage: 5 months, *blank value: straw 0.14 mg/kg* RIP2000-2130

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 356 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : MADRIGAL (356 g/l Glyphosate)
Applicant : Inventus

Active ingredient : Glyphosate
Crop / crop group : Winter Wheat

Submission date : 06.08.2002

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :

Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
97 H CP HX P/A, 97 H CP HX P03 FR-37390 Chanceaux sur Choisille Tours 2002-06-07	Texeu	1)17.10.1996 (sowing) 2) -- 3) 24.07.1997	2.1	148	1.4	17.07.1997	BBCH 89	grain straw	0.71 5.3	0.02 <0.05	7 7	analytical method: HPLC-FLD, LOQ's grain: 0.02 mg/kg, LOQ's straw: 0.05 mg/kg, max. sample storage: no data ASB2009-3524 (field part) ASB2009-3522 (analytical part)
97 H CP HX P/A, 97 H CP HX P04 FR-41330 Villefrancoeur Tours (N-FR) 2002-06-07	Sideral	1) 24.10.1996 (sowing) 2) -- 3) 22.07.1997	2.3	158	1.5	15.07.1997	BBCH 89	grain straw	0.33 17.3	<0.02 0.23	7 7	analytical method: HPLC, LOQ's grain: 0.02 mg/kg, LOQ's straw: 0.05 mg/kg, max. sample storage: no data ASB2009-3524 (field part) ASB2009-3522 (analytical part)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : GLIFOGARDE
Applicant : Inventus

Active ingredient : Glyphosate
Crop / crop group : Winter Wheat

Submission date : 06.08.2002

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
94 H CP HX , 94 H CP HX 01 FR-41330 Villefrancoeur Loir et Cher 1994-11-14	Rossini	1) 20.10.1993 (sowing) 2) -- 3) 20.07.1997	2.2	200	1.1	13.07.1994	BBCH 89	grain straw	0.05 26.9	<0.05 1.3	7 7	analytical method: HPLC-FLD, LOQ's grain: 0.05 mg/kg, LOQ's straw: 0.5 mg/kg, max. sample storage: 4 months RIP2002-1448 (field part) ASB2009-3523 (analytical part)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : (MON 2139) Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Winter Wheat

Submission date : 28.11.2001

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 31337, 99-GLY-07-B/F, TRT 1 BE-5380 Franc-Waret 15.07.2001	Soissons	1) 21.10.1998 (sowing) 2) -- 3) 02.08.1999	2.3	214	1.1	26.07.1999	BBCH 97	grain straw	4.7 2.9 3.4 135 88 79	<0.05 0.05 0.07 1.5 1.4 1.3	0 7 14 0 7 14	bridging study analytical method: DFG 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 6 months Replicate to MLL 31337, 99-GLY-07-B/F, TRT 3 RIP2002-650
MLL 31337, 9075 AL1 Plot C FR- 68740 Fessenheim (N-FR) 15.07.2001	Camp Remy	1) 00.11.1998 (sowing) 2) -- 3) 22.07.1999	2.1	173	1.2	15.07.1999	BBCH 89	grain straw	4.6 0.40 0.30 44 13 7.8	<0.05 <0.05 <0.05 0.55 0.38 0.20	0 7 14 0 7 14	bridging study analytical method: DFG 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 7 months Replicate to MLL 31337, 9075 AL1 Plot D RIP2002-650

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 31337, 9075 AN1 Plot C FR- 36110 Battigny (N-FR) 15.07.2001	Camp Remy	1) 23.10.1998 (sowing) 2) -- 3) 22.07.1999	2.2	178	1.2	15.07.1999	BBCH 87	grain straw	6.0 3.4 3.3 84 25 21	<0.05 <0.05 <0.05 0.97 0.41 0.39	0 7 14 0 7 14	bridging study analytical method: DFG 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 7 months Replicate to MLL 31337, 9075 AN1 Plot D RIP2002-650
MLL 31337, 9075 OB1 Plot C FR- 29830 Ploudalmézeau (N-FR) 15.07.2001	Cyrano	1) 23.11.1998 (sowing) 2) -- 3)02.08.1999	2.3	183	1.2	26.07.1999	BBCH 89	grain straw	1.6 3.9 0.70 106 91 16	<0.05 <0.05 <0.05 0.99 1.9 1.3	0 7 14 0 7 14	bridging study analytical method: DFG 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 6.5 months Replicate to MLL 31337, 9075 OB1 Plot D RIP2002-650

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 450 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : MON 78294
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Winter Wheat

Submission date : June 2002

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 31337 99-GLY-07-B/F Trt 3 BE- 5380 Franc-Waret 15.07.2001	Soissons	1) 21.10.1998 (planting) 2) -- 3) 02.08.1999	2.1	198	1.1	26.07.1999	BBCH 97	grain straw	0.91 2.2 3.6 147 103 60	<0.05 <0.05 0.06 1.4 1.4 1.2	0 7 14 0 7 14	bridging study analytical method: DFG 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 6 months Replicate to MLL 31337, 99-GLY-07-B/F, TRT 1 RIP2002-650
MLL 31337 9075 AL1 Plot D FR- 68740 Fessenheim (N-FR) 15.07.2001	Camp Remy	1) 00.11.1998 (sowing) 2) -- 3) 22.07.1999	2.1	169	1.2	15.07.1999	BBCH 89	grain straw	2.6 0.99 0.55 57 24 13	<0.05 0.05 <0.05 0.60 0.56 0.31	0 7 14 0 7 14	bridging study analytical method: DFG 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 7 months Replicate to MLL 31337, 9075 AL1 Plot C RIP2002-650

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 31337 9075 AN1 Plot D FR- 36110 Battigny (N-FR) 15.07.2001	Camp Remy	1) 23.10.1998 (sowing) 2) -- 3) 22.07.1999	2.1	167	1.2	15.07.1999	BBCH 87	grain straw	5.3 3.7 6.3 53 16 18	<0.05 0.07 0.14 0.48 0.27 0.40	0 7 14 0 7 14	bridging study analytical method: DFG 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 7 months Replicate to MLL 31337, 9075 AN1 Plot C RIP2002-650
MLL 31337, 9075 OB1 Plot D FR- 29830 Ploudalmézeau (N-FR) 15.07.2001	Cyrano	1) 23.11.1998 (sowing) 2) -- 3) 02.08.1999	2.2	182	1.2	26.07.1999	BBCH 89	grain straw	1.2 3.5 0.54 113 107 15	<0.05 <0.05 <0.05 1.3 2.8 1.0	0 7 14 0 7 14	bridging study analytical method: DFG 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 6.5 months Replicate to MLL 31337, 9075 OB1 Plot C RIP2002-650

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360~~355.7~~ g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup (SAG 539 00)
Applicant : Cheminova A/S

Active ingredient : Glyphosate
Crop / crop group : Winter Wheat

Submission date : 1993-12-06

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA), calculated as Glyphosate

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
FRG-0066/ R-196-90, SAG 12536, processing Germany 6331 Solms-Oberbiel/Wetzlar 1992-10-22	Urban	1) 1989-10-26 (sowing) 2) 3) 1990-08-10	1.8	400	0.45	1990-07-20[4)]	BBCH 85	grain	0.70 0.67 0.90 0.45 1.6 0.74	<0.05 <0.05 <0.05 <0.05 0.07 <0.05	0 3 7 10 14 21	4) spraying analytical method: DFG method 405 (HPLC-FLD), LOQ's grain, meal, bread, flour: 0.03 mg/kg, LOQ's straw, middlings, semolina bran: 0.05 mg/kg, max. sample storage: 14 months conversion factor AMPA to Glyphosate: 1.523 Replicate to FRG-0065/R192-90, SAG 12535 ASB2009-6583 (report form), RIP9501328 (analytical report)
								straw	29 18	0.67 0.71	14 21	
								bran	0.71	<0.05	21	
								flour	0.09	<0.05	21	
								whole-meal flour	0.40	<0.05	21	
								whole-meal bread	0.25	<0.05	21	
								middlings	0.24	<0.05	21	
								semolina bran	1.0	<0.05	21	
								semolina	0.12	<0.05	21	

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
FRG-0066/R197-90, SAG 12492 Germany 2361 Bad Segeberg 1992-10-22	Kraka	1) 1989-09-28 (sowing) 2) 3) 1990-08-12	1.78	400	0.44	1990-07-17[4)]	BBCH 85	ears of grain grain straw	14 12 11 6.7 9.8 3.7 8.5 5.5	0.14 0.65 0.89 0.64 1.0 0.89 0.48 0.43	0 3 7 10 14 26 14 26	4) spraying analytical method: DFG method 405 (HPLC- FLD), LOQ's ear, grain: 0.03 mg/kg, straw: 0.05 mg/kg, max. sample storage: 15 months conversion factor AMPA to Glyphosate: 1.523 Replicate to FRG-0065/R193-90, SAG 12491 ASB2009-6581 (report form) RIP9501328 (analytical report)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : GLYFOS (CHE 03690 H)
Applicant : Cheminova A/S

Active ingredient : Glyphosate
Crop / crop group : Winter Wheat

Submission date : 1993-12-06

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA), calculated as Glyphosate

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
FRG-0065/R192-90, SAG 12535, processing Germany 6331 Solms-Oberbiel/Wetzlar 1992-10-22	Urban	1) 1989-10-26 (sowing) 2) 3) 1990-08-10	1.8	400	0.45	1990-07-20[4)]	BBCH 85	grain	0.71 0.61 0.61 0.51 1.4 0.64	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05	0 3 7 10 14 21	4) spraying analytical method: DFG method 405 (HPLC-FLD), LOQ's grain, meal, bread, flour: 0.03 mg/kg, LOQ's straw, middlings, semolina bran: 0.05 mg/kg, max. sample storage: 12 months
								straw	26 15	0.80 0.57	14 21	
								bran	0.83	<0.05	21	
								flour	0.05	<0.05	21	conversion factor AMPA to Glyphosate: 1.523
								whole-meal flour	1.1	<0.05	21	
								whole-meal bread	0.25		21	Replicate to FRG-0066/ R-196-90, SAG 12536
								middlings	0.57	<0.05	21	
								semolina bran	1.4	<0.05	21	ASB2009-6582 (report form) RIP9501327 (final report)
								semolina	0.09	<0.05	21	

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
FRG-0065/R193-90, SAG 12491 Germany 2361 Bad Segeberg 1992-10-22	Kraka	1) 1989-09-28 (sowing) 2) 3) 1990-08-12	1.8	400	0.45	1990-07-17[4)]	BBCH 85	ears of grain grain straw	23 19 19 8.8 7.4 15 12 4.9	0.15 0.68 1.1 0.55 0.60 0.73 0.73 0.44	0 3 7 10 14 26 14 26	4) spraying analytical method: DFG method 405 (HPLC- FLD), LOQ's ear, grain: 0.03 mg/kg, straw: 0.05 mg/kg, max. sample storage: 12 months conversion factor AMPA to Glyphosate: 1.523 Replicate to FRG-0066/R197-90, SAG 12492 ASB2009-6580 (report form), RIP9501327 (final report)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate (Isopropylamin-salt)
Crop / crop group : Winter Wheat

Submission date : 01.09.1995

Indoors / outdoors : Outdoors (European North)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)			(d)	(e)
report MLL 30037, A 36, trial RR 78/1B GB-Thirsk, North Yorkshire 30.01.1980	Mega	1) – (sowing) 2) -- 3) --	1.4	200	0.70	25.08.1978	BBCH 80	grain straw	1.8 14.1	<0.05 0.30	11 11	analytical method: GC-FPD), LOQ's: 0.05 mg/kg RIP9501230
			2.2	200	1.1	25.08.1978	BBCH 80	grain straw	2.2 19.7	<0.05 0.10	11 11	
report MLL 30037, A 36, trial RR 78/1A GB-Thirsk, North Yorkshire 30.01.1980	Mega	1) – (sowing) 2) -- 3) --	1.4	200	0.70	01.09.1978	BBCH 80	grain straw	1.0 25.4	<0.05 0.20	4 4	analytical method: GC-FPD), LOQ's: 0.05 mg/kg RIP9501230
			2.2	200	1.1	01.09.1978	BBCH 80	grain straw	3.3 44.9	<0.05 0.30	4 4	
report MLL 30037, A 36, trial A/78/2 GB-Sleaford-Lincoln 30.01.1980	Aton	1) – (sowing) 2) -- 3) --	1.4	225	0.62	21.08.1978	BBCH 87	grain straw	4.0 20.4	0.07 0.43	15 15	analytical method: GC-FPD), LOQ's: 0.05 mg/kg RIP9501230
			2.2	225	0.98	21.08.1978	BBCH 87	grain straw	4.8 43.8	0.11 0.41	15 15	

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)			(d)	(e)
report MLL 30037, A 36, trial A/78/3 GB-Wisbeck, Cambridge 30.01.1980	Maris Hobbitt	1) – (sowing) 2) -- 3) --	1.4	225	0.62	23.08.1978	BBCH 92	grain straw	0.8 17.4	<0.05 0.38	21 21	analytical method: GC-FPD), LOQ's: 0.05 mg/kg RIP9501230
			2.2	225	0.98	23.08.1978	BBCH 92	grain straw	1.4 34.8	<0.05 0.72	21 21	
report MLL 30037, A 36, trial A/79/1 GB-Boston, Lincoln 30.01.1980	Marris Huntsman	1) – (sowing) 2) -- 3) --	1.4	200	0.70	28.08.1979	BBCH 89	grain straw	3.2 56.4	<0.05 0.55	7 7	analytical method: GC-FPD), LOQ's: 0.05 mg/kg RIP9501230
			2.9	200	1.5	28.08.1979	BBCH 89	grain straw	7.9 145	<0.05 0.98	7 7	

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 650 g/l
Formulation (e.g. WP) : SG
Commercial product (name) : MON 78568
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2005-04-21

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA
(Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 AL1, plot D France 68740 Schaeferhoff-Fessenheim 2002-06-13	Soissons	1) 2000-10-25 (sowing) 2) 3)	2.1	294	0.72	2001-07-10[4)]	BBCH 87	grain straw	0.050 1.5	<0.05 0.060	6 6	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ's: 0.05 mg/kg, max. sample storage: 5 months for grain, 7 months for straw Replicate to R A1157, trial A1157 AL1, plot B & R A1157, trial A1157 AL1, plot C RIP2005-200

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 AN1, plot D France 67160 Seebach 2002-06-13	Aztec	1) 2000-10-15 (sowing) 2) 3)	2.2	302	0.72	2001-07-05[4)]	BBCH 87	grain straw	0.15 1.5	<0.05 <0.05	6 6	4) spraying analytical methods: 405,pp 229-238, and MON XA001 (HPLC/FLD), LOQ's: 0.05 mg/kg, max. sample storage: 5 months Replicate to R A1157, trial A1157 AN1, plot B & R A1157, trial A1157 AN1, plot C RIP2005-200
R A1157, trial A1157 BP1, plot D France 45170 Frapuy-Attray 2002-06-13	Isengrain	1) 2000-10-29 (sowing) 2) 3)	2.2	301	0.72	2001-07-12[4)]	BBCH 87	grain straw	0.070 1.3	<0.05 0.060	8 8	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ's: 0.05 mg/kg, max. sample storage: 5 months Replicate to R A1157, trial A1157 BP1, plot B & R A1157, trial A1157 BP1, plot C RIP2005-200

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 CT1, plot D France 36110 Bois Clair-Levroux 2002-06-13	Levis	1) 2000-10-26 (sowing) 2) 3)	2.2	304	0.72	2001-07-16[4)]	BBCH 87	grain straw	0.16 14.7	<0.05 0.64	7 7	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ's: 0.05 mg/kg, max. sample storage: 5 months Replicate to R A1157, trial A1157 CT1, plot B & R A1157, trial A1157 CT1, plot C RIP2005-200

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 540 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : MON 78273
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2005-04-21

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA
(Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 AL1, plot C France 68740 Schaeferhoff-Fessenheim 2002-06-13	Soissons	1) 2000-10-25 (sowing) 2) 3)	2.3	313	0.72	2001-07-10[4)]	BBCH 87	grain straw	0.070 2.2	<0.05 0.080	6 6	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ's: 0.05 mg/kg, max. sample storage: 5 months for grain, 7 months for straw Replicate to R A1157, trial A1157 AL1, plot B & R A1157, trial A1157 AL1, plot D RIP2005-200

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 AN1, plot C France 67160 Seebach 2002-06-13	Aztec	1) 2000-10-15 (sowing) 2) 3)	2.2	297	0.73	2001-07-05[4)]	BBCH 87	grain straw	0.27 1.9	<0.05 <0.05	6 6	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ's: 0.05 mg/kg, max. sample storage: 5 months Replicate to R A1157, trial A1157 AN1, plot B & R A1157, trial A1157 AN1, plot D RIP2005-200
R A1157, trial A1157 BP1, plot C France 45170 Frapuy-Attray 2002-06-13	Isengrain	1) 2000-10-29 (sowing) 2) 3)	2.2	300	0.72	2001-07-12[4)]	BBCH 87	grain straw	0.10 1.5	<0.05 0.060	8 8	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ's: 0.05 mg/kg, max. sample storage: 5 months Replicate to R A1157, trial A1157 BP1, plot B & R A1157, trial A1157 BP1, plot D RIP2005-200

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 CT1, plot C France 36110 Bois Clair-Levroux 2002-06-13	Levis	1) 2000-10-26 (sowing) 2) 3)	2.2	310	0.72	2001-07-16[4)]	BBCH 87	grain straw	0.24 17.7	<0.05 0.91	7 7	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ's: 0.05 mg/kg, max. sample storage: 5 months Replicate to R A1157, trial A1157 CT1, plot B & R A1157, trial A1157 CT1, plot D RIP2005-200

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2005-04-21

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 AL1, plot B France 68740 Schaeferhoff-Fessenheim 2002-06-13	Soissons	1) 2000-10-25 (sowing) 2) 3)	2.2	308	0.72	2001-07-10[4)]	BBCH 87	grain straw	<0.05 2.7	<0.05 0.060	6 6	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 5 months for grain, 7 months for straw Replicate to R A1157, trial A1157 AL1, plot D & R A1157, trial A1157 AL1, plot C RIP2005-200

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 AN1, plot B France 67160 Seebach 2002-06-13	Aztec	1) 2000-10-15 (sowing) 2) 3)	2.2	311	0.72	2001-07-05[4)]	BBCH 87	grain straw	0.33 2.4	<0.05 <0.05	6 6	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 5 months Replicate to R A1157, trial A1157 AN1, plot D & R A1157, trial A1157 AN1, plot C RIP2005-200
R A1157, trial A1157 BP1, plot B France 45170 Frapuy-Attray 2002-06-13	Isengrain	1) 2000-10-29 (sowing) 2) 3)	2.2	312	0.72	2001-07-12[4)]	BBCH 87	grain straw	0.15 1.5	<0.05 <0.05	8 8	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 5 months Replicate to R A1157, trial A1157 BP1, plot D & R A1157, trial A1157 BP1, plot C RIP2005-200

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 CT1, plot B France 36110 Bois Clair-Levroux 2002-06-13	Levis	1) 2000-10-26 (sowing) 2) 3)	2.1	296	0.72	2001-07-16[4)]	BBCH 87	grain straw	0.17 11.7	<0.05 0.42	7 7	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 5 months Replicate to R A1157, trial A1157 CT1, plot D & R A1157, trial A1157 CT1, plot C RIP2005-200

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Cheminova A/S

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2010-10-20

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
IF-96/07067-00, trial F-96-1, plot3 France 67960 Entzheim 1997-06-23	Sideral	1) 1995-10-17 (sowing) 2) 1996-05-28 - 1996-06-26 3) 1996-07-22	2.2	206	1.1	1996-07-17[4)]	BBCH 92	grain straw	7.4 5.2 6.4 92.7 69.3 72.5	7 7 7 7 7 7	4) spraying analytical method: DFG-method No.405, (HPLC/FLD), LOQ: 0.05 mg/kg straw, LOQ: 0.03 mg/kg grain, replicate field samples, max. sample storage: 4 months for grain, 7 months for straw Side-by-side trials with IF-96/07067-00, trial F-96-1, plot2 & 4 Plot 2 selected as critical case ASB2010-14804

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
IF-96/07067-00, trial F-96-2, plot3 France 57170 Hilsprich 1997-06-23	Carolas	1) 1995-09-20 (sowing) 2) 1996-06-05 - 1996-06-30 3) 1996-08-05	2.1	201	1.1	1996-07-24[4)]	BBCH 91-92	grain straw	control 0.22 0.13 0.20 treated 3.8 1.7 1.7 43.0 47.7 47.4	 7 7 7 7 7 7 7 7 7	4) spraying analytical method: DFG-method No.405, (HPLC/FLD), LOQ: 0.05 mg/kg straw, LOQ: 0.03 mg/kg grain, replicate field samples, max. sample storage: 4 months for grain, 5 months for straw Side-by-side trials with IF-96/07067-00, trial F-96-2, plot 2 & 4 Plot 3 selected as critical case ASB2010-14804

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : GLYFOS
Applicant : Cheminova A/S

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2010-10-20

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
IF-96/07067-00, trial F-96-1, plot 2 France 67960 Entzheim 1997-06-23	Sideral	1) 1995-10-17 (sowing) 2) 1996-05-28 - 1996-06-26 3) 1996-07-22	2.1	203	1.1	1996-07-17[4)]	BBCH 92	grain straw	7.2 10.6 10.7 95.1 84.3 110	7 7 7 7 7 7	4) spraying analytical method: DFG-method No.405, (HPLC/FLD), LOQ: 0.05 mg/kg straw, LOQ: 0.03 mg/kg grain, replicate field samples, max. sample storage: 4 months for grain, 5 months for straw Side-by-side trials with IF-96/07067-00, trial F-96-1, plot3 & 4 Plot 2 selected as critical case ASB2010-14804

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
IF-96/07067-00, trial F-96-1, plot 4 France 67960 Entzheim 1997-06-23	Sideral	1) 1995-10-17 (sowing) 2) 1996-05-28 - 1996-06-26 3) 1996-07-22	1.6	203	0.79	1996-07-17[4)]	BBCH 92	grain straw	5.0 4.8 4.5 62.9 68.4 49.7	7 7 7 7 7 7	4) spraying analytical method: DFG-method No.405, (HPLC/FLD), LOQ: 0.05 mg/kg straw, LOQ: 0.03 mg/kg grain, replicate field samples, max. sample storage: 4 months for grain, 7 months for straw Side-by-side trials with IF-96/07067-00, trial F-96-1, plot2 & 3 Plot 2 selected as critical case ASB2010-14804
IF-96/07067-00, trial F-96-2, plot 2 France 57170 Hilsprich 1997-06-23	Carolas	1) 1995-09-20 (sowing) 2) 1996-06-05 - 1996-06-30 3) 1996-08-05	2.0	192	1.0	1996-07-24[4)]	BBCH 91-92	grain straw	control 0.22 0.13 0.20 treated 2.2 2.7 2.0 47.9 39.9 36.1	 7 7 7 7 7 7 7 7 7	4) spraying analytical method: DFG-method No.405, (HPLC/FLD), LOQ: 0.05 mg/kg straw, LOQ: 0.03 mg/kg grain, replicate field samples, max. sample storage: 4 months for grain, 5 months for straw Side-by-side trials with IF-96/07067-00, trial F-96-2, plot 3 & 4 Plot 3 selected as critical case ASB2010-14804

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
IF-96/07067-00, trial F-96-2, plot 4 France 57170 Hilsprich 1997-06-23	Carolas	1) 1995-09-20 (sowing) 2) 1996-06-05 - 1996-06-30 3) 1996-08-05	1.5	188	0.79	1996-07-24[4)]	BBCH 91-92	grain

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 420 g/kg
Formulation (e.g. WP) : WSG
Commercial product (name) : MON 44068
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30320, 92-GLY-01, trial AS/1934/MO/1, plot 3, 1 United Kingdom SE 683 158 Thome, Humberside 1993-06-01	Mercia	1) 1991-09-23 (sowing) 2) 3)	1.5	200	0.74	1992-07-29[4)]	BBCH 87-90	grain straw	1.1 0.20 63.0 33.0	<0.05 <0.05 0.30 0.40	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 10 months RIP9501291
MLL 30320, 92-GLY-01, trial AS/1934/MO/1, plot 3, 2 United Kingdom SE 683 158 Thome, Humberside 1993-06-01	Mercia	1) 1991-09-23 (sowing) 2) 3)	2.9	200	1.5	1992-07-29[4)]	BBCH 87-90	grain straw	1.7 1.3 140 190	<0.05 <0.05 0.70 2.3	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 10 months RIP9501291

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30320, 92-GLY-01, trial AS/1934/MO/2, plot 3, 1 United Kingdom SK 408 245 Wilson, Derbyshire 1993-06-01	Riband	1) 1991-10-09 (sowing) 2) 3)	1.5	200	0.74	1992-08-04[4)]	BBCH 91	grain straw	1.8 0.20 61.0 12.0	<0.05 <0.05 0.30 0.20	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 9 months RIP9501291
MLL 30320, 92-GLY-01, trial AS/1934/MO/2, plot 3, 2 United Kingdom SK 408 245 Wilson, Derbyshire 1993-06-01	Riband	1) 1991-10-09 (sowing) 2) 3)	2.9	200	1.5	1992-08-04[4)]	BBCH 91	grain straw	3.7 0.20 150 22.0	<0.05 <0.05 0.70 0.50	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 9 months RIP9501291
MLL 30320, 92-GLY-01, trial AS/1934/MO/3, plot 3, 1 United Kingdom TL 792 148 Terling, Essex 1993-06-01	Hereward	1) 1991-10-23 (sowing) 2) 3)	1.5	200	0.74	1992-07-29[4)]	BBCH 90	grain straw	1.2 2.1 82.0 96.0	<0.05 <0.05 0.40 0.80	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 10 months RIP9501291

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30320, 92-GLY-01, trial AS/1934/MO/3, plot 3, 2 United Kingdom TL 792 148 Terling, Essex 1993-06-01	Hereward	1) 1991-10-23 (sowing) 2) 3)	2.9	200	1.5	1992-07-29[4)]	BBCH 90	grain straw	2.3 3.3 150 240	<0.05 <0.05 0.80 2.2	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 10 months RIP9501291
MLL 30320, 92-GLY-01, trial AS/1934/MO/4, plot 3, 1 United Kingdom TQ 988 967 Burnham, Essex 1993-06-01	Hereward	1) 1991-10-04 (sowing) 2) 3)	1.5	200	0.74	1992-07-29[4)]	BBCH 90	grain straw	1.0 1.2 95.0 90.0	<0.05 <0.05 0.50 1.6	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 10 months RIP9501291
MLL 30320, 92-GLY-01, trial AS/1934/MO/4, plot 3, 2 United Kingdom TQ 988 967 Burnham, Essex 1993-06-01	Hereward	1) 1991-10-04 (sowing) 2) 3)	2.9	200	1.5	1992-07-29[4)]	BBCH 90	grain straw	2.1 1.5 210 160	<0.05 <0.05 1.2 2.5	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 10 months RIP9501291

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30320, 92-GLY-01, trial AS/1934/MO/1, plot 2, 1 United Kingdom SE 683 158 Thome, Humberside 1993-06-01	Mercia	1) 1991-09-23 (sowing) 2) 3)	1.4	200	0.72	1992-07-29[4)]	BBCH 87-90	grain straw	1.4 0.30 46.0 36.0	<0.05 <0.05 0.20 0.50	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 10 months RIP9501291
MLL 30320, 92-GLY-01, trial AS/1934/MO/1, plot 2, 2 United Kingdom SE 683 158 Thome, Humberside 1993-06-01	Mercia	1) 1991-09-23 (sowing) 2) 3)	2.9	200	1.4	1992-07-29[4)]	BBCH 87-90	grain straw	1.1 1.2 120 150	<0.05 <0.05 0.50 1.1	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 10 months RIP9501291

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30320, 92-GLY-01, trial AS/1934/MO/2, plot 2, 1 United Kingdom SK 408 245 Wilson, Derbyshire 1993-06-01	Riband	1) 1991-10-09 (sowing) 2) 3)	1.4	200	0.72	1992-08-04[4)]	BBCH 91	grain straw	2.0 0.20 70.0 12.0	<0.05 <0.05 0.30 0.40	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 9 months RIP9501291
MLL 30320, 92-GLY-01, trial AS/1934/MO/2, plot 2, 2 United Kingdom SK 408 245 Wilson, Derbyshire 1993-06-01	Riband	1) 1991-10-09 (sowing) 2) 3)	2.9	200	1.4	1992-08-04[4)]	BBCH 91	grain straw	7.4 0.40 140 22.0	<0.05 <0.05 0.50 0.50	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 9 months RIP9501291
MLL 30320, 92-GLY-01, trial AS/1934/MO/3, plot 2, 1 United Kingdom TL 792 148 Terling, Essex 1993-06-01	Hereward	1) 1991-10-23 (sowing) 2) 3)	1.4	200	0.72	1992-07-29[4)]	BBCH 90	grain straw	0.80 1.0 76.0 88.0	<0.05 <0.05 0.20 0.80	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 10 months RIP9501291

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30320, 92-GLY-01, trial AS/1934/MO/3, plot 2, 2 United Kingdom TL 792 148 Terling, Essex 1993-06-01	Hereward	1) 1991-10-23 (sowing) 2) 3)	2.9	200	1.4	1992-07-29[4)]	BBCH 90	grain straw	1.6 3.3 130 260	<0.05 <0.05 0.50 1.9	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 10 months RIP9501291
MLL 30320, 92-GLY-01, trial AS/1934/MO/4, plot 2, 1 United Kingdom TQ 988 967 Burnham, Essex 1993-06-01	Hereward	1) 1991-10-04 (sowing) 2) 3)	1.4	200	0.72	1992-07-29[4)]	BBCH 90	grain straw	1.8 2.0 93.0 88.0	<0.05 <0.05 0.40 1.2	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 10 months RIP9501291
MLL 30320, 92-GLY-01, trial AS/1934/MO/4, plot 2, 2 United Kingdom (UK) TQ 988 967 Burnham, Essex 1993-06-01	Hereward	1) 1991-10-04 (sowing) 2) 3)	2.9	200	1.4	1992-07-29[4)]	BBCH 90	grain straw	1.7 3.4 150 160	<0.05 <0.05 0.60 2.4	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 10 months RIP9501291

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : MON 52276
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30320, 92-GLY-01, trial AS/1934/MO/1, plot 1, 1 United Kingdom SE 683 158 Thome, Humber-side 1993-06-01	Mercia	1) 1991-09-23 (sowing) 2) 3)	1.4	200	0.72	1992-07-29[4)]	BBCH 87-90	grain straw	1.1 0.30 53.0 41.0	<0.05 <0.05 0.20 0.50	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 10 months RIP9501291
MLL 30320, 92-GLY-01, trial AS/1934/MO/1, plot 1, 2 United Kingdom SE 683 158 Thome, Humber-side 1993-06-01	Mercia	1) 1991-09-23 (sowing) 2) 3)	2.9	200	1.4	1992-07-29[4)]	BBCH 87-90	grain straw	2.3 1.0 93.0 81.0	<0.05 <0.05 0.50 0.90	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 10 months RIP9501291

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30320, 92-GLY-01, trial AS/1934/MO/2, plot 1, 1 United Kingdom SK 408 245 Wilson, Derby-shire 1993-06-01	Riband	1) 1991-10-09 (sowing) 2) 3)	1.4	200	0.72	1992-08-04[4)]	BBCH 91	grain straw	2.9 0.10 58.0 12.0	<0.05 <0.05 0.30 0.40	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 9 months RIP9501291
MLL 30320, 92-GLY-01, trial AS/1934/MO/2, plot 1, 2 United Kingdom SK 408 245 Wilson, Derby-shire 1993-06-01	Riband	1) 1991-10-09 (sowing) 2) 3)	2.9	200	1.4	1992-08-04[4)]	BBCH 91	grain straw	4.7 0.40 100 24.0	<0.05 <0.05 0.50 0.60	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 9 months RIP9501291
MLL 30320, 92-GLY-01, trial AS/1934/MO/3, plot 1, 1 United Kingdom TL 792 148 Terling, Essex 1993-06-01	Hereward	1) 1991-10-23 (sowing) 2) 3)	1.4	200	0.72	1992-07-29[4)]	BBCH 90	grain straw	1.8 1.0 66.0 230	<0.05 <0.05 0.30 1.5	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 10 months RIP9501291

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30320, 92-GLY-01, trial AS/1934/MO/3, plot 1, 2 United Kingdom TL 792 148 Terling, Essex 1993-06-01	Hereward	1) 1991-10-23 (sowing) 2) 3)	2.9	200	1.4	1992-07-29[4)]	BBCH 90	grain straw	2.1 2.2 140 190	<0.05 <0.05 0.70 1.5	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 10 months RIP9501291
MLL 30320, 92-GLY-01, trial AS/1934/MO/4, plot 1, 1 United Kingdom TQ 988 967 Burnham, Essex 1993-06-01	Hereward	1) 1991-10-04 (sowing) 2) 3)	1.4	200	0.72	1992-07-29[4)]	BBCH 90	grain straw	1.1 0.80 68.0 80.0	<0.05 <0.05 0.40 1.3	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 10 months RIP9501291
MLL 30320, 92-GLY-01, trial AS/1934/MO/4, plot 1, 2 United Kingdom TQ 988 967 Burnham, Essex 1993-06-01	Hereward	1) 1991-10-04 (sowing) 2) 3)	2.9	200	1.4	1992-07-29[4)]	BBCH 90	grain straw	1.3 2.4 150 150	<0.05 <0.05 0.80 2.0	0 7 0 7	4) spraying analytical method: XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, residues corrected for recoverys, max. sample storage: 10 months RIP9501291

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Submission date : 1995-09-01

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : GLYFOS
Applicant : Cheminova A/S

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
AS/1898/CN, trial AS/1898/CN/1, plot 2 United Kingdom SK 497 235 East Midlands 1993-01-29	Hereward	1) 1991-10-21 (sowing) 2) 3)	1.4	200	0.72	1992-07-30[4)]	BBCH 92	grain straw	0.36 33.1	7 7	4) spraying analytical method: DFG 405 (HPLC-FLD), LOQ grain. 0.03 mg/kg, LOQ straw: 0.05 mg/kg, max. sample storage: 14 months, metabolite AM-PA not analysed RIP9501302 (analytical part) RIP9501301 (field part)
AS/1898/CN, trial AS/1898/CN/1, plot 3 United Kingdom SK 497 235 East Midlands 1993-01-29	Hereward	1) 1991-10-21 (sowing) 2) 3)	0.72	200	0.36	1992-07-30[4)]	BBCH 92	grain straw	0.30 14.3	7 7	4) spraying analytical method: DFG 405 (HPLC-FLD), LOQ grain. 0.03 mg/kg, LOQ straw: 0.05 mg/kg, max. sample storage: 14 months, metabolite AM-PA not analysed RIP9501302 (analytical part) RIP9501301 (field part)

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
AS/1898/CN, trial AS/1898/CN/2, plot 2 United Kingdom SE 684 158 Thorne, Humber-side 1993-01-29	Mercia	1) 1991-09-23 (sowing) 2) 3)	1.4	200	0.72	1992-07-29[4)]	BBCH 87-89	grain straw	0.36 25.4	7 7	4) spraying analytical method: DFG 405 (HPLC-FLD), LOQ grain. 0.03 mg/kg, LOQ straw: 0.05 mg/kg, max. sample storage: 14 months, metabolite AM-PA not analysed RIP9501302 (analytical part) RIP9501301 (field part)
AS/1898/CN, trial AS/1898/CN/2, plot 3 United Kingdom SE 684 158 Thorne, Humber-side 1993-01-29	Mercia	1) 1991-09-23 (sowing) 2) 3)	0.72	200	0.36	1992-07-29[4)]	BBCH 87-89	grain straw	0.67 26.8	7 7	4) spraying analytical method: DFG 405 (HPLC-FLD), LOQ grain. 0.03 mg/kg, LOQ straw: 0.05 mg/kg, max. sample storage: 14 months, metabolite AM-PA not analysed RIP9501302 (analytical part) RIP9501301 (field part)
AS/1898/CN, trial AS/1898/CN/3, plot 2 United Kingdom TQ 988 967 Dammerwick, Essex 1993-01-29	Hereward	1) 1991-10-04 (sowing) 2) 3)	1.4	200	0.72	1992-07-29[4)]	BBCH 89	grain straw	1.1 25.6	7 7	4) spraying analytical method: DFG 405 (HPLC-FLD), LOQ grain. 0.03 mg/kg, LOQ straw: 0.05 mg/kg, max. sample storage: 14 months, metabolite AM-PA not analysed RIP9501302 (analytical part) RIP9501301 (field part)

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
AS/1898/CN, trial AS/1898/CN/3, plot 3 United Kingdom TQ 988 967 Dammerwick, Essex 1993-01-29	Hereward	1) 1991-10-04 (sowing) 2) 3)	0.72	200	0.36	1992-07-29[4)]	BBCH 89	grain straw	0.42 53.1	7 7	4) spraying analytical method: DFG 405 (HPLC-FLD), LOQ grain. 0.03 mg/kg, LOQ straw: 0.05 mg/kg, max. sample storage: 14 months, metabolite AM-PA not analysed RIP9501302 (analytical part) RIP9501301 (field part)
AS/1906/CN, trial AS/1906/CN/1, plot 2 United Kingdom SK 409 245 Derbyshire 1993-01-29	Riband	1) 1991-10-09 (sowing) 2) 3)	1.4	200	0.72	1992-08-04[4)]	BBCH 91	whole plant grain straw	43.6 35.6 41.5 0.080 5.7	0 1 3 7 7	4) spraying analytical method: DFG 405 (HPLC-FLD), LOQ whole plant: 0.04 mg/kg, LOQ grain:0.03 mg/kg, LOQ straw: 0.05 mg/kg, max. sample storage: 17 months, metabolite AMPA not analysed RIP9501304 (analytical part) RIP9501303 (field part)
AS/1906/CN, trial AS/1906/CN/1, plot 3 United Kingdom SK 409 245 Derbyshire 1993-01-29	Riband	1) 1991-10-09 (sowing) 2) 3)	0.72	200	0.36	1992-08-04[4)]	BBCH 91	whole plant grain straw	26.5 23.6 30.1 0.030 4.6	0 1 3 7 7	4) spraying analytical method: DFG 405 (HPLC-FLD), LOQ whole plant: 0.04 mg/kg, LOQ grain: 0.03 mg/kg, LOQ straw: 0.05 mg/kg, max. sample storage: 17 months, metabolite AMPA not analysed RIP9501304 (analytical part) RIP9501303 (field part)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : GLYFOS + FRIGATE
Applicant : Cheminova A/S

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : Glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
AS/1898/CN, trial AS/1898/CN/1, plot 4 United Kingdom SK 497 235 East Midlands 1993-01-29	Hereward	1) 1991-10-21 (sowing) 2) 3)	0.72	200	0.36	1992-07-30[4)]	BBCH 92	grain straw	0.17 16.4	7 7	4) spraying analytical method: DFG 405 (HPLC-FLD), LOQ grain. 0.03 mg/kg, LOQ straw: 0.05 mg/kg, max. sample storage: 14 months, metabolite AMPA and Tallow amine ethoxylate not analysed RIP9501302 (analytical part) RIP9501301 (field part)
AS/1898/CN, trial AS/1898/CN/1, plot 5 United Kingdom SK 497 235 East Midlands 1993-01-29	Hereward	1) 1991-10-21 (sowing) 2) 3)	0.36	200	0.18	1992-07-30[4)]	BBCH 92	grain straw	0.13 8.9	7 7	4) spraying analytical method: DFG 405 (HPLC-FLD), LOQ grain. 0.03 mg/kg, LOQ straw: 0.05 mg/kg, max. sample storage: 14 months, metabolite AMPA and Tallow amine ethoxylate not analysed RIP9501302 (analytical part) RIP9501301 (field part)

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
AS/1898/CN, trial AS/1898/CN/2, plot 4 United Kingdom SE 684 158 Thorne, Humber-side 1993-01-29	Mercia	1) 1991-09-23 (sowing) 2) 3)	0.72	200	0.36	1992-07-29[4)]	BBCH 87-90	grain straw	0.15 21.6	7 7	4) spraying analytical method: DFG 405 (HPLC-FLD), LOQ grain. 0.03 mg/kg, LOQ straw: 0.05 mg/kg, max. sample storage: 14 months, metabolite AMPA and Tallow amine ethoxylate not analysed RIP9501302 (analytical part) RIP9501301 (field part)
AS/1898/CN, trial AS/1898/CN/2, plot 5 United Kingdom SE 684 158 Thorne, Humber-side 1993-01-29	Mercia	1) 1991-09-23 (sowing) 2) 3)	0.36	200	0.18	1992-07-29[4)]	BBCH 87-90	grain straw	0.22 10.1	7 7	4) spraying analytical method: DFG 405 (HPLC-FLD), LOQ grain. 0.03 mg/kg, LOQ straw: 0.05 mg/kg, max. sample storage: 14 months, metabolite AMPA and Tallow amine ethoxylate not analysed RIP9501302 (analytical part) RIP9501301 (field part)
AS/1898/CN, trial AS/1898/CN/3, plot 4 United Kingdom TQ 988 967 Dammerwick, Essex 1993-01-29	Hereward	1) 1991-10-04 (sowing) 2) 3)	0.72	200	0.36	1992-07-29[4)]	BBCH 90	grain straw	0.38 38.9	7 7	4) spraying analytical method: DFG 405 (HPLC-FLD), LOQ grain. 0.03 mg/kg, LOQ straw: 0.05 mg/kg, max. sample storage: 14 months, metabolite AMPA and Tallow amine ethoxylate not analysed RIP9501302 (analytical part) RIP9501301 (field part)

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
AS/1898/CN, trial AS/1898/CN/3, plot 5 United Kingdom TQ 988 967 Dammerwick, Essex 1993-01-29	Hereward	1) 1991-10-04 (sowing) 2) 3)	0.36	200	0.18	1992-07-29[4)]	BBCH 90	grain straw	0.15 13.9	7 7	4) spraying analytical method: DFG 405 (HPLC-FLD), LOQ grain. 0.03 mg/kg, LOQ straw: 0.05 mg/kg, max. sample storage: 14 months, metabolite AMPA and Tallow amine ethoxylate not analysed RIP9501302 (analytical part) RIP9501301 (field part)
AS/1906/CN, trial AS/1906/CN/1, plot 4 United Kingdom SK 409 245 Derbyshire 1993-01-29	Riband	1) 1991-10-09 (sowing) 2) 3)	0.72	200	0.36	1992-08-04[4)]	BBCH 91	whole plant grain straw	11.1 12.5 14.5 0.050 1.9	0 1 3 7 7	4) spraying analytical method: DFG 405 (HPLC-FLD), LOQ whole plant:0.04 mg/kg, LOQ grain: 0.03 mg/kg, LOQ straw: 0.05 mg/kg, max. sample storage: 17 months, metabolite AMPA and Tallow amine ethoxylate not analysed RIP9501304 (analytical part) RIP9501303 (field part)

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
AS/1906/CN, trial AS/1906/CN/1, plot 5 United Kingdom SK 409 245 Derbyshire 1993-01-29	Riband	1) 1991-10-09 (sowing) 2) 3)	0.36	200	0.18	1992-08-04[4)]	BBCH 91	whole plant grain straw	12.5 12.0 6.0 0.040 2.2	0 1 3 7 7	4) spraying analytical method: DFG 405 (HPLC-FLD), LOQ whole plant:0.04 mg/kg, LOQ grain: 0.03 mg/kg, LOQ straw: 0.05 mg/kg, max. sample storage: 17 months, metabolite AMPA and Tallow amine ethoxylate not analysed RIP9501304 (analytical part) RIP9501303 (field part)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.069, A 36, trial 30053, plot 1, processing United Kingdom Holbeach Hurn, Lincs 1981-10-28	Bounty	1) 1980 (sowing) 2) 3) 1980-09-02	1.4			1980-08-26[4)]	BBCH 92	grain grain, RAC bran flour bread offal	 0.50 1.7 <0.05 <0.05 0.90	<0.05	7 7 7 7 7 7	4) spraying analytical method: Reg. Sec. 180.364-1977, LO-Q's: 0.05 mg/kg AMPA, 0,1 mg/kg grain, residues corrected for recoverys, max. sample storage: no data No information on field or processing conditions. Study not considered acceptable. RIP9501237, RIP9501256

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.069, A 36, trial 30053, plot 2, processing United Kingdom Holbeach Hurn, Lincs 1981-10-28	Bounty	1) 1980 (sowing) 2) 3) 1980-09-02	4.3			1980-08-26[4)]	BBCH 92	grain grain, RAC bran flour bread offal	 1.1 1.9 0.10 0.070 2.7	<0.05	7 7 7 7 7 7	4) spraying analytical method: Reg. Sec. 180.364-1977, LO-Q's: 0.05 mg/kg AMPA, 0,1 mg/kg grain, residues corrected for recoverys, max. sample storage: no data No information on field or processing conditions. Study not considered acceptable. RIP9501237, RIP9501256
MLL 30.069, A 36, trial 30054, plot 1, processing United Kingdom Peterborough, Cambs 1981-10-28	Armada	1) 1980 (sowing) 2) 3) 1980-09-03	1.4			1980-08-27[4)]	BBCH 92	grain grain, RAC bran flour bread offal	 0.30 0.50 <0.05 <0.05 0.30	<0.05	7 7 7 7 7 7	4) spraying analytical method: Reg. Sec. 180.364-1977, LO-Q's: 0.05 mg/kg AMPA, 0,1 mg/kg grain, residues corrected for recoverys, max. sample storage: no data No information on field or processing conditions. Study not considered acceptable. RIP9501237, RIP9501256

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.069, A 36, trial 30054, plot 2, processing United Kingdom Peterborough, Cambs 1981-10-28	Armada	1) 1980 (sowing) 2) 3) 1980-09-03	4.3			1980-08-27[4)]	BBCH 92	grain grain, RAC bran flour bread offal	 1.0 1.7 0.10 0.060 1.5	<0.05	7 7 7 7 7 7	4) spraying analytical method: Reg. Sec. 180.364-1977, LO-Q's: 0.05 mg/kg AMPA, 0,1 mg/kg grain, residues corrected for recoverys, max. sample storage: no data No information on field or processing conditions. Study not considered acceptable. RIP9501237, RIP9501256
MLL 30.069, A 36, trial 30055, plot 1, processing United Kingdom Swineshead, Lincs 1981-10-28	Hustler	1) 1980 (sowing) 2) 3) 1980-09-04	1.4			1980-08-28[4)]	BBCH 92	grain grain, RAC bran flour bread offal	 0.90 1.7 0.10 0.050 1.3	<0.05	7 7 7 7 7 7	4) spraying analytical method: Reg. Sec. 180.364-1977, LO-Q's: 0.05 mg/kg AMPA, 0,1 mg/kg grain, residues corrected for recoverys, max. sample storage: no data No information on field or processing conditions. Study not considered acceptable. RIP9501237, RIP9501256

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.069, A 36, trial 30055, plot 2, processing United Kingdom Swineshead, Lincs 1981-10-28	Hustler	1) 1980 (sowing) 2) 3) 1980-09-04	4.3			1980-08-28[4)]	BBCH 92	grain grain, RAC bran flour bread offal	 1.6 3.4 0.35 0.15 4.9	<0.05	7 7 7 7 7 7	4) spraying analytical method: Reg. Sec. 180.364-1977, LO-Q's: 0.05 mg/kg AMPA, 0,1 mg/kg grain, residues corrected for recoverys, max. sample storage: no data No information on field or processing conditions. Study not considered acceptable. RIP9501237, RIP9501256

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 28 JANV 78, plot 1 France 28310 Janville 1980-01-25	Tom Clair	1) 1978 (sowing) 2) 3) 1978-08-16	1.8			1978-08-07[4)]	BBCH 89	grain	0.50	<0.05	9	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 14 months Side-by-side trials with plot 2 & 3, Plot 2 selected as critical case RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 28 JANV 78, plot 2 France 28310 Janville 1980-01-25	Tom Clair	1) 1978 (sowing) 2) 3) 1978-08-16	2.7			1978-08-07[4)]	BBCH 89	grain	0.70	<0.05	9	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 14 months Side-by-side trials with plot 1 & 3, Plot 2 selected as critical case RIP9501231
MLL 30046, A 36, trial 28 JANV 78, plot 3 France 28310 Janville 1980-01-25	Tom Clair	1) 1978 (sowing) 2) 3) 1978-08-16	3.6			1978-08-07[4)]	BBCH 89	grain	0.80	<0.05	9	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 14 months Side-by-side trials with plot 1 & 2, Plot 2 selected as critical case RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 1 France 41310 Le Haut Bourg, Authon 1980-01-25	Top	1) 1979 (sowing) 2) 3) 1979-08-20	2.1	500	0.43	1979-08-03[4)]	BBCH 87	grain straw	0.63 15.8	<0.05 0.31	17 17	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Decline plots withs MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 1, 1, 2, & 3 Plot 3 selected as critical case RIP9501231
MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 1, 1 France 41310 Le Haut Bourg, Authon 1980-01-25	Top	1) 1979 (sowing) 2) 3) 1979-08-27	2.1	500	0.43	1979-08-03[4)]	BBCH 87	grain straw	0.16 5.8	<0.05 0.30	24 24	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Decline plots withs MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 1, 1, 2, & 3 Plot 3 selected as critical case RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 1, 2 France 41310 Le Haut Bourg, Authon 1980-01-25	Top	1) 1979 (sowing) 2) 3) 1979-08-03	2.1	500	0.43	1979-08-03[4)]	BBCH 87	grain straw	1.8 216	<0.05 1.1	0 0	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Decline plots withs MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 1, 1, 2, & 3 Plot 3 selected as critical case RIP9501231
MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 1, 3 France 41310 Le Haut Bourg, Authon 1980-01-25	Top	1) 1979 (sowing) 2) 3) 1979-08-13	2.1	500	0.43	1979-08-03[4)]	BBCH 87	grain straw	0.85 19.1	<0.05 0.18	10 10	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Decline plots withs MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 1, 1, 2, & 3 Plot 3 selected as critical case RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 2 France 41310 Le Haut Bourg, Authon 1980-01-25	Top	1) 1979 (sowing) 2) 3) 1979-08-20	4.3	500	0.86	1979-08-03[4)]	BBCH 87	grain straw	1.2 25.0	<0.05 0.42	17 17	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Decline plots with MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 2, 1, 1, 2 & 3 RIP9501231
MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 2, 1 France 41310 Le Haut Bourg, Authon 1980-01-25	Top	1) 1979 (sowing) 2) 3) 1979-08-27	4.3	500	0.86	1979-08-03[4)]	BBCH 87	grain straw	0.31 15.7	<0.05 0.54	24 24	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Decline plots with MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 2, 1, 1, 2 & 3 RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 2, 2 France 41310 Le Haut Bourg, Authon 1980-01-25	Top	1) 1979 (sowing) 2) 3) 1979-08-03	4.3	500	0.86	1979-08-03[4)]	BBCH 87	grain straw	2.8 309	<0.05 1.5	0 0	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Decline plots with MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 2, 1, 1, 2 & 3 RIP9501231
MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 2, 3 France 41310 Le Haut Bourg, Authon 1980-01-25	Top	1) 1979 (sowing) 2) 3) 1979-08-13	4.3	500	0.86	1979-08-03[4)]	BBCH 87	grain straw	1.7 27.8	<0.05 0.31	10 10	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Decline plots with MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 2, 1, 1, 2 & 3 RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 3 France 41310 Le Haut Bourg, Authon 1980-01-25	Top	1) 1979 (sowing) 2) 3) 1979-08-20	8.6	500	1.7	1979-08-03[4)]	BBCH 87	grain straw	1.2 47.0	<0.05 0.66	17 17	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Decline plots with MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 3, 1, 1, 2 & 3 RIP9501231
MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 3, 1 France 41310 Le Haut Bourg, Authon 1980-01-25	Top	1) 1979 (sowing) 2) 3) 1979-08-27	8.6	500	1.7	1979-08-03[4)]	BBCH 87	grain straw	1.0 40.4	<0.05 1.1	24 24	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Decline plots with MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 3, 1, 1, 2 & 3 RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 3, 2 France 41310 Le Haut Bourg, Authon 1980-01-25	Top	1) 1979 (sowing) 2) 3) 1979-08-03	8.6	500	1.7	1979-08-03[4)]	BBCH 87	grain straw	5.4 928	<0.05 3.7	0 0	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Decline plots with MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 3, 1, 1, 2 & 3 RIP9501231
MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 3, 3 France 41310 Le Haut Bourg, Authon 1980-01-25	Top	1) 1979 (sowing) 2) 3) 1979-08-13	8.6	500	1.7	1979-08-03[4)]	BBCH 87	grain straw	2.1 52.8	<0.05 0.69	10 10	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months Decline plots with MLL 30046, A 36, trial 41 AUTH 79, 1 WBH, plot 3, 1, 1, 2 & 3 RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 41 AUTH 79, 3 WBH, plot 1 France 41310 Le Haut Bourg, Authon 1980-01-25	Top	1) 1979 (sowing) 2) 3) 1979-08-03	2.1	500	0.43	1979-07-17[4)]	BBCH 87	straw	42.0	0.43	17	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231
MLL 30046, A 36, trial 41 AUTH 79, 3 WBH, plot 2 France 41310 Le Haut Bourg, Authon 1980-01-25	Top	1) 1979 (sowing) 2) 3) 1979-08-03	4.3	500	0.86	1979-07-17[4)]	BBCH 87	straw	74.0	0.52	17	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231
MLL 30046, A 36, trial 41 AUTH 79, 3 WBH, plot 3 France 41310 Le Haut Bourg, Authon 1980-01-25	Top	1) 1979 (sowing) 2) 3) 1979-08-03	8.6	500	1.7	1979-07-17[4)]	BBCH 87	straw	192	1.3	17	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 45 SULL 78, plot 1 France 45600 Sully 1980-01-25	Courteau	1) 1978 (sowing) 2) 3) 1978-08-18	2.5	450	0.56	1978-07-28[4)]	BBCH 89	grain	0.60	0.070	21	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 14 months RIP9501231
MLL 30046, A 36, trial 45 SULL 78, plot 2 France 45600 Sully 1980-01-25	Courteau	1) 1978 (sowing) 2) 3) 1978-08-18	3.2	450	0.72	1978-07-28[4)]	BBCH 89	grain	0.70	0.050	21	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 14 months RIP9501231
MLL 30046, A 36, trial 45 SULL 78, plot 3 France 45600 Sully 1980-01-25	Courteau	1) 1978 (sowing) 2) 3) 1978-08-18	3.9	450	0.88	1978-07-28[4)]	BBCH 89	grain	1.2	0.15	21	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 14 months RIP9501231
MLL 30046, A 36, trial 51 CHAM 78, plot 1 France 51480 Champlat 1980-01-25	Talent	1) 1978 (sowing) 2) 3) 1978-08-16	3.6			1978-08-01[4)]	BBCH 89	grain	2.0	<0.05	15	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 14 months RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 51 CHAM 78, plot 2 France 51480 Champlat 1980-01-25	Talent	1) 1978 (sowing) 2) 3) 1978-08-16	4.3			1978-08-01[4)]	BBCH 89	grain	4.2	<0.05	15	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 14 months RIP9501231
MLL 30046, A 36, trial 60 BELL 79, 1 WBH, plot 1, processing France 60 Plessis Belleville 1980-01-25	Lutin	1) 1979 (sowing) 2) 3) 1979-08-13	2.1			1979-07-18[4)]	BBCH 87	grain, RAC bran flour	0.50 0.59 0.36	<0.05 <0.05 <0.05	26 26 26	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231, RIP9501256
MLL 30046, A 36, trial 60 BELL 79, 1 WBH, plot 2, processing France 60 Plessis Belleville 1980-01-25	Lutin	1) 1979 (sowing) 2) 3) 1979-08-13	4.3			1979-07-18[4)]	BBCH 87	grain, RAC bran flour	1.2 2.4 0.64	<0.05 <0.05 <0.05	26 26 26	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231, RIP9501256

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 60 BELL 79, 1 WBH, plot 3, processing France 60 Plessis Belleville 1980-01-25	Lutin	1) 1979 (sowing) 2) 3) 1979-08-13	8.6			1979-07-18[4)]	BBCH 87	grain, RAC bran flour	2.2 4.4 1.2	<0.05 <0.05 <0.05	26 26 26	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231, RIP9501256
MLL 30046, A 36, trial 60 IVRY 78, plot 1 France 60173 Ivry le Temple 1980-01-25	Talent	1) 1978 (sowing) 2) 3) 1978-08-22	2.5	400	0.63	1978-08-07[4)]	BBCH 92	grain	0.70	<0.05	15	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 14 months RIP9501231
MLL 30046, A 36, trial 60 IVRY 78, plot 2 France 60173 Ivry le Temple 1980-01-25	Talent	1) 1978 (sowing) 2) 3) 1978-08-22	3.2	400	0.81	1978-08-07[4)]	BBCH 92	grain	0.20	<0.05	15	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 14 months RIP9501231
MLL 30046, A 36, trial 60 JUST 79 - 1, 3 WBH, plot 1 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-07-18	2.1	375	0.57	1979-07-18[4)]	BBCH 87	straw	245	1.1	0	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 3 months RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 60 JUST 79 - 1, 3 WBH, plot 1, 1 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-07-24	2.1	375	0.57	1979-07-18[4)]	BBCH 87	straw	117	0.20	6	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 3 months RIP9501231
MLL 30046, A 36, trial 60 JUST 79 - 1, 3 WBH, plot 1, 2 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-04	2.1	375	0.57	1979-07-18[4)]	BBCH 87	straw	31.4	0.28	17	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231
MLL 30046, A 36, trial 60 JUST 79 - 1, 3 WBH, plot 1, 3 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-11	2.1	375	0.57	1979-07-18[4)]	BBCH 87	straw	14.5	0.14	24	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231
MLL 30046, A 36, trial 60 JUST 79 - 1, 3 WBH, plot 2 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-07-18	4.3	375	1.1	1979-07-18[4)]	BBCH 87	straw	540	1.8	0	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 3 months RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 60 JUST 79 - 1, 3 WBH, plot 2, 1 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-07-24	4.3	375	1.1	1979-07-18[4)]	BBCH 87	straw	130	0.74	6	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 3 months RIP9501231
MLL 30046, A 36, trial 60 JUST 79 - 1, 3 WBH, plot 2, 2 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-04	4.3	375	1.1	1979-07-18[4)]	BBCH 87	straw	79.2	0.54	17	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231
MLL 30046, A 36, trial 60 JUST 79 - 1, 3 WBH, plot 2, 3 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-11	4.3	375	1.1	1979-07-18[4)]	BBCH 87	straw	34.2	0.37	24	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231
MLL 30046, A 36, trial 60 JUST 79 - 1, 3 WBH, plot 3 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-07-18	8.6	375	2.3	1979-07-18[4)]	BBCH 87	straw	784	2.3	0	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 3 months RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 60 JUST 79 - 1, 3 WBH, plot 3, 1 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-07-24	8.6	375	2.3	1979-07-18[4)]	BBCH 87	straw	352	1.9	6	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 3 months RIP9501231
MLL 30046, A 36, trial 60 JUST 79 - 1, 3 WBH, plot 3, 2 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-04	8.6	375	2.3	1979-07-18[4)]	BBCH 87	straw	140	0.78	17	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231
MLL 30046, A 36, trial 60 JUST 79 - 1, 3 WBH, plot 3, 3 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-11	8.6	375	2.3	1979-07-18[4)]	BBCH 87	straw	83.0	0.81	24	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231
MLL 30046, A 36, trial 60 JUST 79, 1 WBH 3, plot 1, pro-cessing France 60 Plessier St.Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-13	2.1	375	0.57	1979-08-06[4)]	BBCH 87	grain, RAC bran flour	0.75 1.4 0.47	<0.05 <0.05 <0.05	7 7 7	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231, RIP9501256

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 60 JUST 79, 1 WBH 3, plot 2, processing France 60 Plessier St.Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-13	4.3	375	1.1	1979-08-06[4)]	BBCH 87	grain, RAC bran flour	1.3 2.4 1.0	<0.05 <0.05 <0.05	7 7 7	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231, RIP9501256
MLL 30046, A 36, trial 60 JUST 79, 1 WBH 3, plot 3, processing France 60 Plessier St.Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-13	8.6	375	2.3	1979-08-06[4)]	BBCH 87	grain, RAC bran flour	3.4 4.4 2.0	<0.05 <0.05 <0.05	7 7 7	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231, RIP9501256
MLL 30046, A 36, trial 60 JUST 79-3, 1 WBH, plot 1 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-06	2.1	375	0.57	1979-08-06[4)]	BBCH 87	grain straw	1.7 186	<0.05 0.43	0 0	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 60 JUST 79-3, 1 WBH, plot 1, 1 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-13	2.1	375	0.57	1979-08-06[4)]	BBCH 87	grain straw	0.75 27.4	<0.05 0.41	7 7	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231
MLL 30046, A 36, trial 60 JUST 79-3, 1 WBH, plot 1, 2 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-21	2.1	375	0.57	1979-08-06[4)]	BBCH 87	grain straw	0.46 15.1	<0.05 0.21	15 15	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231
MLL 30046, A 36, trial 60 JUST 79-3, 1 WBH, plot 1, 3 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-24	2.1	375	0.57	1979-08-06[4)]	BBCH 87	grain straw	0.30 8.4	<0.05 0.090	18 18	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231
MLL 30046, A 36, trial 60 JUST 79-3, 1 WBH, plot 2 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-06	4.3	375	1.1	1979-08-06[4)]	BBCH 87	grain straw	2.4 448	<0.05 0.90	0 0	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 60 JUST 79-3, 1 WBH, plot 2, 1 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-13	4.3	375	1.1	1979-08-06[4)]	BBCH 87	grain straw	1.3 55.4	<0.05 0.68	7 7	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231
MLL 30046, A 36, trial 60 JUST 79-3, 1 WBH, plot 2, 2 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-21	4.3	375	1.1	1979-08-06[4)]	BBCH 87	grain straw	0.71 30.5	<0.05 0.72	15 15	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231
MLL 30046, A 36, trial 60 JUST 79-3, 1 WBH, plot 2, 3 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-24	4.3	375	1.1	1979-08-06[4)]	BBCH 87	grain straw	0.45 17.9	<0.05 0.27	18 18	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231
MLL 30046, A 36, trial 60 JUST 79-3, 1 WBH, plot 3 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-06	8.6	375	2.3	1979-08-06[4)]	BBCH 87	grain straw	4.4 980	<0.05 1.6	0 0	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 60 JUST 79-3, 1 WBH, plot 3, 1 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-13	8.6	375	2.3	1979-08-06[4)]	BBCH 87	grain straw	3.4 80.0	<0.05 0.90	7 7	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231
MLL 30046, A 36, trial 60 JUST 79-3, 1 WBH, plot 3, 2 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-21	8.6	375	2.3	1979-08-06[4)]	BBCH 87	grain straw	2.3 69.4	<0.05 0.80	15 15	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231
MLL 30046, A 36, trial 60 JUST 79-3, 1 WBH, plot 3, 3 France 60130 Plessier-St-Just 1980-01-25	Roazon	1) 1979 (sowing) 2) 3) 1979-08-24	8.6	375	2.3	1979-08-06[4)]	BBCH 87	grain straw	1.2 47.6	<0.05 0.47	18 18	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 60 PLES 78, plot 1 France 60130 Le Plessier 1980-01-25	Capitole	1) 1978 (sowing) 2) 3) 1978-08-23	2.1			1978-08-11[4)]	BBCH 87	grain	3.9	<0.05	12	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 14 months Side-by-side plots 1 to 5. Only single value selected for evaluation. RIP9501231
MLL 30046, A 36, trial 60 PLES 78, plot 2 France 60130 Le Plessier 1980-01-25	Capitole	1) 1978 (sowing) 2) 3) 1978-08-23	2.5			1978-08-11[4)]	BBCH 87	grain	4.7	0.11	12	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 14 months Side-by-side plots 1 to 5. Only single value selected for evaluation. RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 60 PLES 78, plot 3 France 60130 Le Plessier 1980-01-25	Capitole	1) 1978 (sowing) 2) 3) 1978-08-23	3.2			1978-08-11[4)]	BBCH 87	grain	3.0	<0.05	12	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 14 months Side-by-side plots 1 to 5. Only single value selected for evaluation. RIP9501231
MLL 30046, A 36, trial 60 PLES 78, plot 4 France 60130 Le Plessier 1980-01-25	Capitole	1) 1978 (sowing) 2) 3) 1978-08-23	4.3			1978-08-11[4)]	BBCH 87	grain	8.0	0.080	12	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 14 months Side-by-side plots 1 to 5. Only single value selected for evaluation. RIP9501231

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 60 PLES 78, plot 5 France 60130 Le Plessier 1980-01-25	Capitole	1) 1978 (sowing) 2) 3) 1978-08-23	8.6			1978-08-11[4)]	BBCH 87	grain	17.7	0.16	12	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 14 months Side-by-side plots 1 to 5. Only single value selected for evaluation. RIP9501231
MLL 30046, A 36, trial 60 REMY 79, 1 WBH, plot 1, processing France 60130 Remy 1980-01-25	Talent	1) 1979 (sowing) 2) 3) 1979-08-09	2.1			1979-07-31[4)]	BBCH 87	grain, RAC bran flour	0.67 1.1 0.48	<0.05 <0.05 <0.05	9 9 9	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231, RIP9501256
MLL 30046, A 36, trial 60 REMY 79, 1 WBH, plot 2, processing France 60130 Remy 1980-01-25	Talent	1) 1979 (sowing) 2) 3) 1979-08-09	4.3			1979-07-31[4)]	BBCH 87	grain, RAC bran flour	1.6 2.7 0.90	<0.05 <0.05 <0.05	9 9 9	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231, RIP9501256

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30046, A 36, trial 60 REMY 79, 1 WBH, plot 3, processing France 60130 Remy 1980-01-25	Talent	1) 1979 (sowing) 2) 3) 1979-08-09	8.6			1979-07-31[4)]	BBCH 87	grain, RAC bran flour	2.7 7.4 2.5	<0.05 <0.05 <0.05	9 9 9	4) spraying analytical method: not reported (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 2 months RIP9501231, RIP9501256

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : MON52276
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06D01; T1 France 71 Boyer / Tournus 1991-12-01	Campremy	1) 1991 (planting) 2) 3)	1.1	200	0.54	1991-07-18[4)]	BBCH 87	grain straw	0.40 36.0	<0.05 0.030[5)]	7 7	4) spraying 5) LOQ: 0.05mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON52276, SL~~SC~~ (360g/l Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months, Replicate plot to MLL 30281; 91-GLY-01; 06D01; T1 RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06D01; T2 France 71 Boyer / Tournus 1991-12-01	Campremy	1) 1991 (planting) 2) 3)	2.2	200	1.1	1991-07-18[4)]	BBCH 87	grain straw	0.80 70.0	<0.05 0.70	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON52276, SL~~SC~~ (360g/l Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months, Replicate plot to MLL 30281; 91-GLY-01; 06D01; T3, T4 & T5 RIP9501287
MLL 30281; 91-GLY-01; 06D01; T5 France 71 Boyer / Tournus 1991-12-01	Campremy	1) 1991 (planting) 2) 3)	2.2	200	1.1	1991-07-18[4)]	BBCH 87	grain straw	0.70 63.0	<0.05 0.50	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months, Replicate plot to MLL 30281; 91-GLY-01; 06D01; T2, T3 & T4 RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06D02; T1 France 77 La Plache / Cely 1991-12-01	Pernel	1) 1991 (planting) 2) 3)	1.1	200	0.54	1991-07-14[4)]	BBCH 87	grain straw	0.30 50.0	<0.05 0.70	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON52276, SL~~SC~~ (360g/l Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months, Replicate plot to MLL 30281; 91-GLY-01; 06D02; T3 RIP9501287
MLL 30281; 91-GLY-01; 06D02; T2 France 77 La Plache / Cely 1991-12-01	Pernel	1) 1991 (planting) 2) 3)	2.2	200	1.1	1991-07-14[4)]	BBCH 87	grain straw	0.60 87.0	<0.05 1.6	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON52276, SL~~SC~~ (360g/l Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months, Replicate plot to MLL 30281; 91-GLY-01; 06D02; T4 & T5 RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06D02; T5 France 77 Planche / Cely 1991-12-01	Pernel	1) 1991 (planting) 2) 3)	2.2	200	1.1	1991-07-14[4)]	BBCH 87	grain straw	1.1 120	<0.05 2.2	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months, Replicate plot to MLL 30281; 91-GLY-01; 06D02; T2 & T4 RIP9501287
MLL 30281; 91-GLY-01; 06D03; T1 France 77 Secqueville / Caen 1991-12-01	Thesee	1) 1991 (planting) 2) 3)	1.1	200	0.54	1991-08-10[4)]	BBCH 87	grain straw	0.70 42.0	<0.05 0.80	10 10	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON52276, SL~~SC~~ (360g/l Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 4 months Replicate plot to MLL 30281; 91-GLY-01; 06D03; T3 RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06D03; T2 France 77 Secqueville / Caen 1991-12-01	Thesee	1) 1991 (planting) 2) 3)	2.2	200	1.1	1991-08-10[4)]	BBCH 87	grain straw	2.1 85.0	0.050 1.8	10 10	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON52276, SL~~SC~~ (360g/l Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 4 months, Replicate plot to MLL 30281; 91-GLY-01; 06D03; T4 & T5 RIP9501287
MLL 30281; 91-GLY-01; 06D03; T5 France 77 Secqueville / Caen 1991-12-01	Thesee	1) 1991 (planting) 2) 3)	2.2	200	1.1	1991-08-10[4)]	BBCH 87	grain straw	1.4 71.0	<0.05 1.6	10 10	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 4 months, Replicate plot to MLL 30281; 91-GLY-01; 06D03; T2 & T4 RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06D04; T1 France 02 Montigny sur Credy / Laon 1991-12-01	Slepner	1) 1991 (planting) 2) 3)	1.1	200	0.54	1991-08-09[4)]	BBCH 87	grain straw	0.50 56.0	<0.05 0.60	10 10	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON52276, SL~~SC~~ (360g/l Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 4 months, Replicate plot to MLL 30281; 91-GLY-01; 06D04; T3 RIP9501287
MLL 30281; 91-GLY-01; 06D04; T2 France 02 Montigny sur Credy / Laon 1991-12-01	Slepner	1) 1991 (planting) 2) 3)	2.2	200	1.1	1991-08-09[4)]	BBCH 87	grain straw	0.90 130	<0.05 1.4	10 10	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON52276, SL~~SC~~ (360g/l Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 4 months, Replicate plot to MLL 30281; 91-GLY-01; 06D04; T4 & T5 RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06D04; T5 France 02 Montigny sur Credy / Laon 1991-12-01	Slepner	1) 1991 (planting) 2) 3)	2.2	200	1.1	1991-08-09[4)]	BBCH 87	grain straw	0.80 70.0	<0.05 0.70	10 10	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glypho-sate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 4 months, Replicate plot to MLL 30281; 91-GLY-01; 06D04; T2 & T4 RIP9501287

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 420 g/kg
Formulation (e.g. WP) : WG
Commercial product (name) : MON44068
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06D01; T3 France 71 Boyer / Tournus 1991-12-01	Campremy	1) 1991 (planting) 2) 3)	1.1	200	0.53	1991-07-18[4)]	BBCH 87	grain straw	0.30 28.0	<0.05 0.30	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON44068, WG (420g/kg Glyphosate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months, Replicate plot to MLL 30281; 91-GLY-01; 06D01; T1 RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06D01; T4 France 71 Boyer / Tour-nus 1991-12-01	Campremy	1) 1991 (planting) 2) 3)	2.1	200	1.1	1991-07-18[4)]	BBCH 87	grain straw	0.60 57.0	<0.05 0.70	7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON44068, WG (420g/kg Glypho-sate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months, Replicate plot to MLL 30281; 91-GLY-01; 06D01; T2 & T5 RIP9501287
MLL 30281; 91-GLY-01; 06D02; T3 France 77 Planche / Cely 1991-12-01	Pernel	1) 1991 (planting) 2) 3)	1.1	200	0.53	1991-07-14	BBCH 87	grain straw	0.20 74.0	<0.05 1.7	3 3	submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON44068, WG (420g/kg Glypho-sate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months, Replicate plot to MLL 30281; 91-GLY-01; 06D02; T1 RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06D02; T4 France 77 Planche / Cely 1991-12-01	Pernel	1) 1991 (planting) 2) 3)	2.1	200	1.1	1991-07-14	BBCH 87	grain straw	0.40 79.0	<0.05 1.6	3 3	submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON44068, WG (420g/kg Glypho-sate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 5 months, Replicate plot to MLL 30281; 91-GLY-01; 06D02; T2 & T5 RIP9501287
MLL 30281; 91-GLY-01; 06D03; T3 France 77 Secqueville / Caen 1991-12-01	Thesee	1) 1991 (planting) 2) 3)	1.1	200	0.53	1991-08-10[4)]	BBCH 87	grain straw	0.50 31.0	<0.05 0.80	33 33	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON44068, WG (420g/kg Glypho-sate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 4 months, Replicate plot to MLL 30281; 91-GLY-01; 06D03; T1 RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06D03; T4 France 77 Secqueville / Caen 1991-12-01	Thesee	1) 1991 (planting) 2) 3)	2.1	200	1.1	1991-08-10[4)]	BBCH 87	grain straw	1.7 98.0	<0.05 2.2	10 10	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON44068, WG (420g/kg Glypho-sate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 4 months, Replicate plot to MLL 30281; 91-GLY-01; 06D03; T2 & T5 RIP9501287
MLL 30281; 91-GLY-01; 06D04; T3 France 02 Montigny sur Credy / Laon 1991-12-01	Slepner	1) 1991 (planting) 2) 3)	1.1	200	0.53	1991-08-09[4)]	BBCH 87	grain straw	0.30 33.0	<0.05 0.40	10 10	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON44068, WG (420g/kg Glypho-sate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 4 months, Replicate plot to MLL 30281; 91-GLY-01; 06D04; T1 RIP9501287

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30281; 91-GLY-01; 06D04; T4 France 02 Montigny sur Credy / Laon 1991-12-01	Slepner	1) 1991 (planting) 2) 3)	2.1	200	1.1	1991-08-09[4)]	BBCH 87	grain straw	0.60 87.0	<0.05 1.1	10 10	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON44068, WG (420g/kg Glypho-sate),analytical method: XA001 (HPLC/OPA),LOQ Glyphosate and AMPA for all samples 0,05mg/kg,max. sample storage: 4 months, Replicate plot to MLL 30281; 91-GLY-01; 06D04; T2 & T5 RIP9501287

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2012-11-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
IF-92/11567-01;AGR/RP-H92 / SKG-9255-01, B,	no data	1) (planting) 2) 3) 1992-08-18	1.8			1992-07-28[4)]		grain	0.42 0.58 0.18 0.050	<0.03 0.040 <0.03 <0.03	0 6 14 21	4) spraying submitted to HAG 500 02 H 007385-00/00 at 2011-08-

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
								straw	11.0 3.2	0.84 0.41	14 21	
								bran	0.43	<0.05	21	
								whole-meal flour	0.16	<0.05	21	
								whole-meal bread	0.12	0.080	21	
								flour type 550	<0.05	<0.05	21	
IF-92/11567-01;AGR/RP-H92 / SKG-9255-02, B Germany 3006 Burgwedel 1993-04-06	no data	1) (planting) 2) 3) 1992-08-18	1.8			1992-07-28[4)]		grain	1.0 1.2 0.25 0.080	<0.03 <0.03 <0.03 <0.03	0 6 14 21	4) spraying submitted to HAG 500 02 H 007385-00/00 at 2011-08-11,treated with formulation ROUNDUP 042389-00 (360 g/l Glypho-sate,analytical method: DFG No. 405 (GC-FLD),LOQ grain: 0.03 mg/kg, LOQ straw and processed food samples: 0.05 mg/kg,max. sample storage: 5 months, Side-by-side plot with IF-92/11567-01;AGR/RP-H92 / SKG-9255-02, A ASB2009-6573, RIP9501329, RIP9500134, ASB2011-9181
								straw	14.0 4.1	1.1 0.51	14 21	

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
IF-92/11567-01;AGR/RP-H92 / SKG-9255-03, B Germany 2556 Sanitz 1993-04-06	no data	1) (planting) 2) 3) 1992-07-28	1.8			1992-07-28[4)]		grain straw	0.71 0.54 0.25 0.090 13.0 4.8		0 6 14 21 14 21	4) spraying submitted to HAG 500 02 H 007385-00/00 at 2011-08-11,treated with formulation ROUNDUP 042389-00 (360 g/l Glypho-sate,analytical method: DFG No. 405 (GC-FLD),LOQ grain: 0.03 mg/kg, LOQ straw and processed food samples: 0.05 mg/kg,max. sample storage: 5 months, Side-by-side plot with IF-92/11567-01;AGR/RP-H92 / SKG-9255-03, A ASB2009-6573, RIP9501329, RIP9500134, ASB2011-9181

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
IF-92/11567-01;AGR/RP-H92 / SKG-9255-04, B, processing Germany 1320 Angermünde 1993-04-06	no data	1) (planting) 2) 3) 1992-07-28	1.8			1992-07-28[4)]		grain straw bran whole-meal flour whole-meal bread flour type 550	1.0 0.50 0.24 0.090 11.0 4.0 0.45 0.16 0.14 <0.05	<0.03 <0.03 <0.03 <0.03 0.85 0.53 <0.05 <0.05 <0.05 <0.05	0 6 14 21 14 21 21 21 21 21	4) spraying submitted to HAG 500 02 H 007385-00/00 at 2011-08-11,treated with formulation ROUNDUP 042389-00 (360 g/l Glyphosate,analytical method: DFG No. 405 (GC-FLD),LOQ grain: 0.03 mg/kg, LOQ straw and processed food samples: 0.05 mg/kg,max. sample storage: 5 months, Side-by-side plot with IF-92/11567-01;AGR/RP-H92 / SKG-9255-04, A ASB2009-6573, RIP9501329, RIP9500134, ASB2011-9181

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : GLYFOS
Applicant : Cheminova A/S

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2012-11-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
IF-92/11567-01;AGR/RP-H92 / SKG-9255-01, A, processing Germany 4180 Goch-Nierswalde 1993-04-06	no data	1) (planting) 2) 3) 1992-08-18	1.8			1992-07-28[4)]		grain straw bran whole-meal flour whole-meal bread flour type 550	0.53 1.1 0.28 0.040 5.8 1.2 0.36 0.13 0.12 <0.05	<0.03 <0.03 <0.03 <0.03 0.41 0.17 <0.05 <0.05 0.080 <0.05	0 6 14 21 14 21 21 21 21 21	4) spraying submitted to HAG 500 02 H 007385-00/00 at 2011-08-11,treated with formulation GLYFOS 004162-00 (360 g/l Glyphosate,analytical method: DFG No. 405 (GC-FLD),LOQ grain: 0.03 mg/kg, LOQ straw and processed food samples: 0.05 mg/kg,max. sample storage: 5 months, Side-by-side plot with IF-92/11567-01;AGR/RP-H92 / SKG-9255-01, B ASB2009-6573, RIP9501329, RIP9500134, ASB2011-9181

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
IF-92/11567-01;AGR/RP-H92 / SKG-9255-02, A Germany 3006 Burgwedel 1993-04-06	no data	1) (planting) 2) 3) 1992-08-18	1.8			1992-07-28[4)]		grain straw	0.23 0.26 0.20 0.030 8.7 2.3	<0.03 <0.03 <0.03 <0.03 0.59 0.38	0 6 14 21 14 21	4) spraying submitted to HAG 500 02 H 007385-00/00 at 2011-08-11,treated with formulation GLYFOS 004162-00 (360 g/l Glyphosate,analytical method: DFG No. 405 (GC-FLD),LOQ grain: 0.03 mg/kg, LOQ straw and processed food samples: 0.05 mg/kg,max. sample storage: 5 months, Side-by-side plot with IF-92/11567-01;AGR/RP-H92 / SKG-9255-02, B ASB2009-6573, RIP9501329, RIP9500134, ASB2011-9181

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
IF-92/11567-01;AGR/RP-H92 / SKG-9255-03, A Germany 2556 Sanitz 1993-04-06	no data	1) (planting) 2) 3) 1992-07-28	1.8			1992-07-28[4)]		grain straw	0.29 0.62 0.26 0.050 8.2 2.7	<0.03 <0.03 <0.03 <0.03 0.54 <0.05	0 6 14 21 14 21	4) spraying submitted to HAG 500 02 H 007385-00/00 at 2011-08-11,treated with formulation GLYFOS 004162-00 (360 g/l Glyphosate,analytical method: DFG No. 405 (GC-FLD),LOQ grain: 0.03 mg/kg, LOQ straw and processed food samples: 0.05 mg/kg,max. sample storage: 5 months, Side-by-side plot with IF-92/11567-01;AGR/RP-H92 / SKG-9255-03, B ASB2009-6573, RIP9501329, RIP9500134, ASB2011-9181

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
IF-92/11567-01;AGR/RP-H92 / SKG-9255-04, A, processing Germany 1320 Angermünde 1993-04-06	no data	1) (planting) 2) 3) 1992-07-28	1.8			1992-07-28[4)]		grain straw bran whole-meal flour whole-meal bread flour type 550	0.49 0.97 0.20 0.050 7.9 2.9 0.25 0.16 0.11 <0.05	<0.03 <0.03 <0.03 <0.03 0.52 0.31 <0.05 <0.05 <0.05 <0.05	0 6 14 21 14 21 21 21 21 21	4) spraying submitted to HAG 500 02 H 007385-00/00 at 2011-08-11,treated with formulation GLYFOS 004162-00 (360 g/l Glyphosate,analytical method: DFG No. 405 (GC-FLD),LOQ grain: 0.03 mg/kg, LOQ straw and processed food samples: 0.05 mg/kg,max. sample storage: 5 months, Side-by-side plot with IF-92/11567-01;AGR/RP-H92 / SKG-9255-04, B ASB2009-6573, RIP9501329, RIP9500134, ASB2011-9181

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2012-11-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A1; CP 67573; G/R/W/21 Germany Giessen-Krofdorf 1974-01-07	Jubilar	1) (sowing) 2) 3) 1974-08-22	2.2			1973-09-24[4]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	332 332	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 5 months, RIP9501209

1	2	3	4			5	6	7	8.1	8.2	9	10
Report No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A1; CP 67573; G/R/W/26 Germany Hohenheim 1974-01-07	Jubilar	1) (sowing) 2) - 3) 1974-08-15	2.2			1973-10-08	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	325 325	pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 5 months RIP9501209
REPORT A1; CP 67573; G/R/W/3 Germany Göttingen 1974-01-07	no data	1) (planting) 2) - 3) 1974-08-17	2.2			1973-09-06[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	345 345	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 5 months RIP9501209

1	2	3	4			5	6	7	8.1	8.2	9	10
Report No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A1; CP 67573; SE Sweden Kungsangin-Ultuna 1974-01-07	no data	1) 1973-09-13 (planting) 2) 3) 1974-09-10	2.1			1973-08-29[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	377 377	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: no data, RIP9501209
REPORT A1; CP 67573; UK/73/10; part 1 United Kingdom (UK) Bozeat 1974-01-07	Cappelle Desprez	1) (planting) 2) 3) 1973-09-05	2.2			1972-10-03[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	337 337	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 16 months RIP9501209

1	2	3	4			5	6	7	8.1	8.2	9	10
Report No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
REPORT A1; CP 67573; UK/73/10; part 2 United Kingdom (UK) Bozeat 1974-01-07	Cappelle Desprez	1) (planting) 2) 3) 1973-09-05	4.5			1972-10-03[4)]	BBCH 00	grain straw	<0.05 <0.05	<0.05 <0.05	337 337	4) spraying pre-seed application,submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with formulation Roundup, (360 g/l Glyphosate, 480 g/l Glyphosate isopropylamine salt) = Roundup 042389-00,analytical method: not reported (GLC/FPD),LOQ: 0.05 mg/kg,mind the low recovery values!,max. sample storage: 16 months RJP9501209

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : ROUNDUP
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, SU 82/10 RP, 01 United Kingdom (UK) Debden 1991-12-01	Norman	1) 1982 (planting) 2) 3)	1.4	200	0.72	1982-08-07[4)]		grain straw	0.60 0.90 0.50 41.0 50.9 48.4	<0.2 <0.2 <0.2 0.70 <0.4 0.42	7 7 7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, SU 82/10 RP, 02 United Kingdom (UK) Debden 1991-12-01	Norman	1) 1982 (planting) 2) 3)	1.4	200	0.72	1982-08-07[4)]		grain straw	0.50 0.50 0.60 40.6 51.7 48.0	<0.2 <0.2 <0.2 <0.4 1.3 0.44	7 7 7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249
MLL 30.087; MLL 30087; A 36; P III, SU 82/10 RP, 03 United Kingdom (UK) Tadcaster 1991-12-01	Bounty	1) 1982 (planting) 2) 3)	1.4	200	0.72	1982-08-12[4)]		grain straw	<0.4 <0.4 <0.4 7.1 6.2 5.7	<0.2 <0.2 <0.2 <0.4 <0.4 <0.4	7 7 7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, SU 82/10 RP, 04 United Kingdom (UK) Edinburgh 1991-12-01	Bounty	1) 1982 (planting) 2) 3)	1.4	200	0.72	1982-08-31[4)]		grain straw	<0.4 <0.4 <0.4 6.7 4.8 10.2		8 8 8 8 8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249
MLL 30.087; MLL 30087; A 36; P III, SU 82/10 RP, 05 United Kingdom (UK) Chisledon 1991-12-01	Bounty	1) 1982 (planting) 2) 3)	1.4	200	0.72	1982-08-08[4)]		grain straw	<0.4 <0.4 0.40 20.5 15.8 18.1	<0.2 <0.2 <0.2 <0.4 0.53 <0.4	8 8 8 8 8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-13, 01 United Kingdom (UK) Cocksmill 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	0.72	20	3.6	1982-07-27[4)]		grain	0.60	<0.1	6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249
MLL 30.087; MLL 30087; A 36; P III, 82-164-13, 02 United Kingdom (UK) Cocksmill 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	0.72	40	1.8	1982-07-27[4)]		grain	0.30[5)]	<0.1	6	4) spraying 5) LOQ: 0.4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-13, 03 United Kingdom (UK) Cocksmill 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	0.72	100	0.72	1982-07-27[4)]		grain	0.20[5)]	<0.1	6	4) spraying 5) LOQ: 0.4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249
MLL 30.087; MLL 30087; A 36; P III, 82-164-13, 04; processing United Kingdom (UK) Cocksmill 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	0.72	200	0.36	1982-07-27[4)]		grain grain, RAC flour bread	0.40 <0.4 <0.4 <0.4	<0.1 <0.1 <0.2 <0.2	6 6 6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-13, 05 United Kingdom (UK) Cocksmill 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	1.4	20	7.2	1982-07-27[4)]		grain	0.40	<0.1	6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249
MLL 30.087; MLL 30087; A 36; P III, 82-164-13, 06 United Kingdom (UK) Cocksmill 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	1.4	40	3.6	1982-07-27[4)]		grain	0.70	<0.1	6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-13, 07 United Kingdom (UK) Cocksmill 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	1.4	100	1.4	1982-07-27[4)]		grain	0.30[5)]	<0.1	6	4) spraying 5) LOQ: 0.4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249
MLL 30.087; MLL 30087; A 36; P III, 82-164-13, 08; processing United Kingdom (UK) Cocksmill 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	1.4	200	0.72	1982-07-27[4)]		grain grain, RAC flour bread	2.5 0.46 <0.4 <0.4	<0.1 <0.1 <0.2 <0.2	6 6 6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-13, 09 United Kingdom (UK) Cocksmill 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	2.9	20	14	1982-07-27[4)]		grain	0.90	<0.1	6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249
MLL 30.087; MLL 30087; A 36; P III, 82-164-13, 10 United Kingdom (UK) Cocksmill 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	2.9	40	7.2	1982-07-27[4)]		grain	1.1	<0.1	6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-13, 11 United Kingdom (UK) Cocksmill 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	2.9	100	2.9	1982-07-27[4)]		grain	0.80	<0.1	6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249
MLL 30.087; MLL 30087; A 36; P III, 82-164-13, 12; processing United Kingdom (UK) Cocksmill 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	2.9	200	1.4	1982-07-27[4)]		grain grain, RAC flour bread	0.90 0.90 <0.4 <0.4	<0.1 <0.1 <0.2 <0.2	6 6 6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-14, 01 United King-dom (UK) Foxearth 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	0.72	20	3.6	1982-08-03[4)]		grain	0.20[5)]	<0.1	6	4) spraying 5) LOQ: 0.4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-14, 02 United King-dom (UK) Foxearth 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	0.72	40	1.8	1982-08-03[4)]		grain	0.20[5)]	<0.1	6	4) spraying 5) LOQ: 0.4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-14, 03 United King-dom (UK) Foxearth 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	0.72	100	0.72	1982-08-03[4)]		grain	0.20[5)]	<0.1	6	4) spraying 5) LOQ: 0.4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-14, 04, processing United Kingdom (UK) Foxearth 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	0.72	200	0.36	1982-08-03[4)]		grain grain, RAC flour bread	0.10[5)] <0.4 <0.4 <0.4	<0.1 <0.1 <0.2 <0.2	6 6 6 6	4) spraying 5) LOQ: 0.4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-14, 05 United King-dom (UK) Foxearth 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	1.4	20	7.2	1982-08-03[4)]		grain	0.20[5)]	<0.1	6	4) spraying 5) LOQ: 0.4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-14, 06 United King-dom (UK) Foxearth 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	1.4	40	3.6	1982-08-03[4)]		grain	0.20[5)]	<0.2	6	4) spraying 5) LOQ: 0.4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-14, 07 United King-dom (UK) Foxearth 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	1.4	100	1.4	1982-08-03[4)]		grain	0.30[5)]	<0.1	6	4) spraying 5) LOQ: 0.4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-14, 08, processing United Kingdom (UK) Foxearth 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	1.4	200	0.72	1982-08-03[4)]		grain grain, RAC flour bread	0.40 <0.4 <0.4 <0.4	0.060[5)] <0.1 <0.2 <0.2	6 6 6 6	4) spraying 5) LOQ: 0.1mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249
MLL 30.087; MLL 30087; A 36; P III, 82-164-14, 09 United Kingdom (UK) Foxearth 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	0.72	20	3.6	1982-08-03[4)]		grain	0.60	<0.1	6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-14, 10 United Kingdom (UK) Foxearth 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	0.72	40	1.8	1982-08-03[4)]		grain	0.70	<0.1	6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249
MLL 30.087; MLL 30087; A 36; P III, 82-164-14, 11 United Kingdom (UK) Foxearth 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	0.72	100	0.72	1982-08-03[4)]		grain	0.50	0.080[5)]	6	4) spraying 5) LOQ: 0.1mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-14, 12, processing United Kingdom (UK) Foxearth 1991-12-01	Avalon	1) 1982 (planting) 2) 3)	0.72	200	0.36	1982-08-03[4)]		grain grain, RAC flour bread	0.70 0.62 0.41 <0.4	<0.06[5)] <0.1 <0.2 <0.2	6 6 6 6	4) spraying 5) LOQ: 0.1mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1 mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-19, 01 United Kingdom (UK) Doddington 1991-12-01	Kador	1) 1982 (planting) 2) 3)	0.72	20	3.6	1982-08-20[4)]		grain	0.20[5)]	<0.1	7	4) spraying 5) LOQ: 0.4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249
MLL 30.087; MLL 30087; A 36; P III, 82-164-19, 02 United Kingdom (UK) Doddington 1991-12-01	Kador	1) 1982 (planting) 2) 3)	0.72	40	1.8	1982-08-20[4)]		grain	0.40	<0.1	7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-19, 03 United Kingdom (UK) Doddington 1991-12-01	Kador	1) 1982 (planting) 2) 3)	0.72	100	0.72	1982-08-20[4)]		grain	0.30[5)]	<0.1	7	4) spraying 5) LOQ: 0.4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-19, 04, processing United Kingdom (UK) Diddington 1991-12-01	Kador	1) 1982 (planting) 2) 3)	0.72	200	0.36	1982-08-20[4)]		grain grain, RAC flour bread	0.30[5)] <0.4 <0.4 <0.4	<0.1 <0.1 <0.2 <0.2	7 7 7 7	4) spraying 5) LOQ: 0.4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249
MLL 30.087; MLL 30087; A 36; P III, 82-164-19, 05 United Kingdom (UK) Doddington 1991-12-01	Kador	1) 1982 (planting) 2) 3)	1.4	20	7.2	1982-08-20[4)]		grain	0.40	<0.1	7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-19, 06 United Kingdom (UK) Doddington 1991-12-01	Kador	1) 1982 (planting) 2) 3)	1.4	40	3.6	1982-08-20[4)]		grain	0.50	0.050[5)]	7	4) spraying 5) LOQ: 0.1mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-19, 07 United Kingdom (UK) Doddington 1991-12-01	Kador	1) 1982 (planting) 2) 3)	1.4	100	1.4	1982-08-20[4)]		grain	0.20[5)]	<0.1	7	4) spraying 5) LOQ: 0.4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249
MLL 30.087; MLL 30087; A 36; P III, 82-164-19, 08, processing United Kingdom (UK) Diddington 1991-12-01	Kador	1) 1982 (planting) 2) 3)	1.4	200	0.72	1982-08-20[4)]		grain grain, RAC flour bread	0.50 0.42 0.40 <0.4	<0.1 <0.1 <0.2 <0.2	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-19, 09 United Kingdom (UK) Doddington 1991-12-01	Kador	1) 1982 (planting) 2) 3)	2.9	20	14	1982-08-20[4)]		grain	0.80	0.070[5)]	7	4) spraying 5) LOQ: 0.1mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-19, 10 United Kingdom (UK) Doddington 1991-12-01	Kador	1) 1982 (planting) 2) 3)	2.9	40	7.2	1982-08-20[4)]		grain	0.80	0.060[5)]	7	4) spraying 5) LOQ: 0.1mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.087; MLL 30087; A 36; P III, 82-164-19, 11 United Kingdom (UK) Doddington 1991-12-01	Kador	1) 1982 (planting) 2) 3)	2.9	100	2.9	1982-08-20[4)]		grain	1.1	0.050[5)]	7	4) spraying 5) LOQ: 0.1mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249
MLL 30.087; MLL 30087; A 36; P III, 82-164-19, 12, processing United Kingdom (UK) Diddington 1991-12-01	Kador	1) 1982 (planting) 2) 3)	2.9	200	1.4	1982-08-20[4)]		grain grain, RAC flour bread	0.60 0.83 0.70 0.43	<0.1 <0.1 <0.2 <0.2	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/FPD, HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, flour 0.4mg/kg, wholemeal bread 0.4mg/kg,LOQ AMPA: grain 0.1mg/kg, flour 0.2mg/kg, wholemeal bread 0.2mg/kg,max. sample storage: no data, ASB2009-5491, RIP9501249

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : ROUNDUP
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 01 United Kingdom (UK) Dunbar - East Lothians 1983-09-11	Armada	1) (planting) 2) 3)	0.36	20	1.8	1982-08-23[4)]		grain straw	0.50 <0.4 7.1	<0.2 <0.2 <0.4	8 8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 02 United Kingdom (UK) Dunbar - East Lothians 1983-09-11	Armada	1) (planting) 2) 3)	0.36	200	0.18	1982-08-23[4)]		grain straw	<0.4 <0.4 8.0	<0.2 <0.2 <0.4	8 8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 03 United Kingdom (UK) Dunbar - East Lothians 1983-09-11	Armada	1) (planting) 2) 3)	0.36	20	1.8	1982-08-23[4)]		grain straw	0.40 <0.4 7.4	<0.2 <0.2 <0.4	8 8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (+ Frigate) (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 04 United Kingdom (UK) Dunbar - East Lothians 1983-09-11	Armada	1) (planting) 2) 3)	0.36	200	0.18	1982-08-23[4)]		grain straw	<0.4 0.60 8.4	<0.2 <0.2 <0.4	8 8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (+ Frigate) (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 05 United Kingdom (UK) Dunbar - East Lothians 1983-09-11	Armada	1) (planting) 2) 3)	0.54	20	2.7	1982-08-23[4)]		grain straw	0.50 0.50 8.6	<0.2 <0.2 <0.4	8 8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 06 United Kingdom (UK) Dunbar - East Lothians 1983-09-11	Armada	1) (planting) 2) 3)	0.54	20	2.7	1982-08-23[4)]		grain straw	<0.4 0.50 11.3	<0.2 <0.2 <0.4	8 8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (+ Frigate) (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 07 United Kingdom (UK) Dunbar - East Lothians 1983-09-11	Armada	1) (planting) 2) 3)	0.72	20	3.6	1982-08-23[4)]		grain straw	0.80 0.50 15.2	<0.2 <0.2 0.40	8 8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 08 United Kingdom (UK) Dunbar - East Lothians 1983-09-11	Armada	1) (planting) 2) 3)	0.72	200	0.36	1982-08-23[4)]		grain straw	0.40 0.80 17.3	<0.2 <0.2 <0.4	8 8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 09 United Kingdom (UK) Dunbar - East Lothians 1983-09-11	Armada	1) (planting) 2) 3)	0.72	20	3.6	1982-08-23[4)]		grain	0.70 1.3 0.96	<0.2 <0.2 30.7	8 8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (+ Frigate) (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; SU 82/13/RP; 10 United Kingdom (UK) Dunbar - East Lothians 1983-09-11	Armada	1) (planting) 2) 3)	0.72	200	0.36	1982-08-23[4)]		grain straw	0.60 0.50 12.9	<0.2 <0.2 <0.4	8 8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (+ Frigate) (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; 82-164-13 / SU 82/11RP; 01 United Kingdom (UK) Cooksmill Green - Essex 1983-09-11	Avalon	1) (planting) 2) 3)	0.72	100	0.72	1982-07-27[4)]		grain	0.50 0.40	<0.2 <0.2	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; 82-164-13 / SU 82/11RP; 02 United Kingdom (UK) Cooksmill Green - Essex 1983-09-11	Avalon	1) (planting) 2) 3)	0.72	200	0.36	1982-07-27[4)]		grain	0.60 <0.4 <0.2[5)] 0.40	<0.2 <0.2 <0.2 <0.2	6 6 6 6	4) spraying 5) LOQ: 0.4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; 82-164-13 / SU 82/11RP; 03 United Kingdom (UK) Cooksmill Green - Essex 1983-09-11	Avalon	1) (planting) 2) 3)	1.4	100	1.4	1982-07-27[4)]		grain	1.0 1.1 0.60 4.1	<0.2 <0.2 <0.2 <0.2	6 6 6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; 82-164-13 / SU 82/11RP; 04 United Kingdom (UK) Cooksmill Green - Essex 1983-09-11	Avalon	1) (planting) 2) 3)	1.4	200	0.72	1982-07-27[4)]		grain	0.40 0.70 <0.4 2.3	<0.2 <0.2 <0.2 <0.2	6 6 6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; 82-164-13 / SU 82/11RP; 05 United Kingdom (UK) Cooksmill Green - Essex 1983-09-11	Avalon	1) (planting) 2) 3)	2.9	100	2.9	1982-07-27[4)]		grain	1.3 2.0	<0.2 <0.2	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; 82-164-13 / SU 82/11RP; 06 United Kingdom (UK) Cooksmill Green - Essex 1983-09-11	Avalon	1) (planting) 2) 3)	2.9	200	1.4	1982-07-27[4)]		grain	0.90 1.1 0.80 0.90	<0.2 <0.2 <0.2 <0.2	6 6 6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; 82-164-14 / SU 82/11+12/RP; 01 United Kingdom (UK) Foxearth - Suffolk 1983-09-11	Avalon	1) (planting) 2) 3)	0.72	20	3.6	1982-08-03[4)]		grain	<0.4 <0.4	<0.2 <0.2	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; 82-164-14 / SU 82/11+12/RP; 02 United Kingdom (UK) Foxearth - Suffolk 1983-09-11	Avalon	1) (planting) 2) 3)	0.72	40	1.8	1982-08-03[4)]		grain	<0.4 <0.4	<0.2 <0.2	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; 82-164-14 / SU 82/11+12/RP; 03 United Kingdom (UK) Foxearth - Suffolk 1983-09-11	Avalon	1) (planting) 2) 3)	0.72	100	0.72	1982-08-03[4)]		grain	<0.4 <0.4	<0.2 <0.2	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; 82-164-14 / SU 82/11+12/RP; 04 United Kingdom (UK) Foxearth - Suffolk 1983-09-11	Avalon	1) (planting) 2) 3)	0.72	200	0.36	1982-08-03[4)]		grain	<0.4 <0.4 <0.4 <0.4	<0.2 <0.2 <0.2 <0.2	6 6 6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; 82-164-14 / SU 82/11+12/RP; 05 United Kingdom (UK) Foxearth - Suffolk 1983-09-11	Avalon	1) (planting) 2) 3)	1.4	20	7.2	1982-08-03[4)]		grain	<0.4 <0.4	<0.2 <0.2	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; 82-164-14 / SU 82/11+12/RP; 06 United Kingdom (UK) Foxearth - Suffolk 1983-09-11	Avalon	1) (planting) 2) 3)	1.4	40	3.6	1982-08-03[4)]		grain	<0.4 0.40	<0.2 <0.2	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; 82-164-14 / SU 82/11+12/RP; 07 United Kingdom (UK) Foxearth - Suffolk 1983-09-11	Avalon	1) (planting) 2) 3)	1.4	100	1.4	1982-08-03[4)]		grain	<0.4 <0.4 0.50 0.70	<0.2 <0.2 <0.2 <0.2	6 6 6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; 82-164-14 / SU 82/11+12/RP; 08 United Kingdom (UK) Foxearth - Suffolk 1983-09-11	Avalon	1) (planting) 2) 3)	1.4	200	0.72	1982-08-03[4)]		grain	<0.4 <0.4 <0.4 0.40	<0.2 <0.2 <0.2 <0.2	6 6 6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; 82-164-14 / SU 82/11+12/RP; 09 United Kingdom (UK) Foxearth - Suffolk 1983-09-11	Avalon	1) (planting) 2) 3)	2.9	20	14	1982-08-03[4)]		grain	<0.4 0.60	<0.2 <0.2	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; 82-164-14 / SU 82/11+12/RP; 10 United Kingdom (UK) Foxearth - Suffolk 1983-09-11	Avalon	1) (planting) 2) 3)	2.9	40	7.2	1982-08-03[4)]		grain	<0.4 <0.4	<0.2 <0.2	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; 82-164-14 / SU 82/11+12/RP; 11 United Kingdom (UK) Foxearth - Suffolk 1983-09-11	Avalon	1) (planting) 2) 3)	2.9	100	2.9	1982-08-03[4)]		grain	0.50 0.60	<0.2 <0.2	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; 82-164-14 / SU 82/11+12/RP; 12 United Kingdom (UK) Foxearth - Suffolk 1983-09-11	Avalon	1) (planting) 2) 3)	2.9	200	1.4	1982-08-03[4)]		grain	0.60 0.70 0.50 0.70	<0.2 <0.2 <0.2 <0.2	6 6 6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; 82-164-19 / SU 82/11+12/RP; 01 United Kingdom (UK) Doddington - Cambs 1983-09-11	Kador	1) (planting) 2) 3)	0.72	20	3.6	1982-08-20[4)]		grain straw	<0.4 1.3 20.1 51.2	<0.2 <0.2 0.54 <0.4	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; 82-164-19 / SU 82/11+12/RP; 02 United Kingdom (UK) Doddington - Cambs 1983-09-11	Kador	1) (planting) 2) 3)	0.72	40	1.8	1982-08-20[4)]		grain straw	0.40 <0.4 11.2 <4.0	<0.2 <0.2 <0.4 <0.4	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; 82-164-19 / SU 82/11+12/RP; 03 United Kingdom (UK) Doddington - Cambs 1983-09-11	Kador	1) (planting) 2) 3)	0.72	100	0.72	1982-08-20[4)]		grain straw	<0.4 0.40 13.6 20.6	<0.2 <0.2 <0.4 0.45	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; 82-164-19 / SU 82/11+12/RP; 04 United Kingdom (UK) Doddington - Cambs 1983-09-11	Kador	1) (planting) 2) 3)	0.72	200	0.36	1982-08-20[4)]		grain straw	<0.2[5)] 0.40 <0.3[5)] <0.3[5)] 10.6 14.2	<0.2 <0.2 <0.2 <0.2 <0.4 <0.4	7 7 7 7 7 7	4) spraying 5) LOQ: 0.4mg/kg submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; 82-164-19 / SU 82/11+12/RP; 05 United Kingdom (UK) Doddington - Cambs 1983-09-11	Kador	1) (planting) 2) 3)	1.4	20	7.2	1982-08-20[4)]		grain straw	0.90 0.50 58.7 21.5	<0.2 <0.2 1.1 0.58	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; 82-164-19 / SU 82/11+12/RP; 06 United Kingdom (UK) Doddington - Cambs 1983-09-11	Kador	1) (planting) 2) 3)	1.4	40	3.6	1982-08-20[4)]		grain straw	0.70 0.60 38.0 23.3	<0.2 <0.2 0.79 <0.4	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; 82-164-19 / SU 82/11+12/RP; 07 United Kingdom (UK) Doddington - Cambs 1983-09-11	Kador	1) (planting) 2) 3)	1.4	100	1.4	1982-08-20[4)]		grain straw	<0.4 <0.4 0.40 <0.4 16.5 23.1	<0.2 <0.2 <0.2 <0.2 0.46 0.56	7 7 7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; 82-164-19 / SU 82/11+12/RP; 08 United Kingdom (UK) Doddington - Cambs 1983-09-11	Kador	1) (planting) 2) 3)	1.4	200	0.72	1982-08-20[4)]		grain straw	<0.4 0.50 <0.4 0.50 17.7 32.1	<0.2 <0.2 <0.2 <0.2 <0.4 0.77	7 7 7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; 82-164-19 / SU 82/11+12/RP; 09 United Kingdom (UK) Doddington - Cambs 1983-09-11	Kador	1) (planting) 2) 3)	2.9	20	14	1982-08-20[4)]		grain straw	0.70 0.60 37.0 44.6	<0.2 <0.2 0.78 0.72	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; 82-164-19 / SU 82/11+12/RP; 10 United Kingdom (UK) Doddington - Cambs 1983-09-11	Kador	1) (planting) 2) 3)	2.9	40	7.2	1982-08-20[4)]		grain straw	0.40 0.50 35.0 23.2	<0.2 <0.2 0.64 0.74	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269
MLL 30.112; MLL 30112; 55; 82-164-19 / SU 82/11+12/RP; 11 United Kingdom (UK) Doddington - Cambs 1983-09-11	Kador	1) (planting) 2) 3)	2.9	100	2.9	1982-08-20[4)]		grain straw	0.40 0.70 47.1 50.9	<0.2 <0.2 0.77 0.71	7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.112; MLL 30112; 55; 82-164-19 / SU 82/11+12/RP; 12 United Kingdom (UK) Doddington - Cambs 1983-09-11	Kador	1) (planting) 2) 3)	2.9	200	1.4	1982-08-20[4)]		grain straw	0.80 0.80 1.1 0.60 46.6 47.8	<0.2 <0.2 <0.2 <0.2 0.70 1.2	7 7 7 7 7 7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: (HPLC/OPA),LOQ Glyphosate: grain 0,4 mg/kg, straw 4mg/kg,LOQ AMPA: grain 0.2mg/kg, straw 0,4 mg/kg,max. sample storage: no data,analytical report RIP9501249, RIP9501269

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 485 g/l
Formulation (e.g. WP) : SC
Commercial product (name) : RUP Four80
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87/283/11; 11 United Kingdom (UK) Cluny 1988-02-05	Norman	1) (planting) 2) 3)	1.6	200	0.80	1987-09-02[4)]		grain	<0.4	<0.4	7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with RUP Four80, SC (480g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data Side-by-side trials: MLL 30.200; 87/283/11; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87/283/11; 12 United Kingdom (UK) Cluny 1988-02-05	Norman	1) (planting) 2) 3)	2.0	200	1.0	1987-09-02[4)]		grain	<0.4	<0.4	7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with RUP Four80, SC (480g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87/283/11; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 400 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : MON14478
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87-RS-0S-04; 01 United Kingdom (UK) Cottenham, Cambs 1988-02-05	Avalon	1) (planting) 2) 3)	0.60	200	0.30	1987-08-28[4)]		grain straw	0.50 37.0	<0.4 <0.4	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87-RS-0S-04; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87-RS-0S-04; 02 United Kingdom (UK) Cottenham, Cambs 1988-02-05	Avalon	1) (planting) 2) 3)	0.80	200	0.40	1987-08-28[4)]		grain straw	0.60 51.0	<0.4 <0.4	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87-RS-0S-04; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87-RS-0S-04; 03 United Kingdom (UK) Cottenham, Cambs 1988-02-05	Avalon	1) (planting) 2) 3)	1.0	200	0.50	1987-08-28[4)]		grain straw	1.0 45.0	<0.4 <0.4	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87-RS-0S-04; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87-RS-0S-04; 04 United Kingdom (UK) Cottenham, Cambs 1988-02-05	Avalon	1) (planting) 2) 3)	1.6	200	0.80	1987-08-28[4)]		grain straw	1.2 86.0	<0.4 <0.4	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87-RS-0S-04; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87-RS-0S-04; 05 United Kingdom (UK) Cottenham, Cambs 1988-02-05	Avalon	1) (planting) 2) 3)	2.0	200	1.0	1987-08-28[4)]		grain straw	2.6 140	<0.4 <0.4	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87-RS-0S-04; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87-RS-0S-04; 06 United Kingdom (UK) Cottenham, Cambs 1988-02-05	Avalon	1) (planting) 2) 3)	0.60	200	0.30	1987-08-28[4)]		grain straw	0.50 34.0	<0.4 <0.4	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87-RS-0S-04; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87-RS-0S-04; 07 United Kingdom (UK) Cottenham, Cambs 1988-02-05	Avalon	1) (planting) 2) 3)	0.80	200	0.40	1987-08-28[4)]		grain straw	0.80 39.0	<0.4 <0.4	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87-RS-0S-04; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87-RS-0S-04; 08 United Kingdom (UK) Cottenham, Cambs 1988-02-05	Avalon	1) (planting) 2) 3)	1.0	200	0.50	1987-08-28[4)]		grain straw	1.1 48.0	<0.4 <0.4	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87-RS-0S-04; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87-RS-0S-04; 09 United Kingdom (UK) Cottenham, Cambs 1988-02-05	Avalon	1) (planting) 2) 3)	1.6	200	0.80	1987-08-28[4)]		grain straw	1.7 110	<0.4 <0.4	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87-RS-0S-04; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87-RS-0S-04; 10 United Kingdom (UK) Cottenham, Cambs 1988-02-05	Avalon	1) (planting) 2) 3)	2.0	200	1.0	1987-08-28[4)]		grain straw	1.5 130	<0.4 <0.4	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87-RS-0S-04; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87-RS-0S-04; 11 United Kingdom (UK) Cottenham, Cambs 1988-02-05	Avalon	1) (planting) 2) 3)	1.6	200	0.80	1987-08-28[4)]		grain straw	1.6 100	<0.4 <0.4	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87-RS-0S-04; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87-RS-0S-04; 12 United Kingdom (UK) Cottenham, Cambs 1988-02-05	Avalon	1) (planting) 2) 3)	2.0	200	1.0	1987-08-28[4)]		grain straw	1.7 120	<0.4 <0.4	6 6	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87-RS-0S-04; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87/283/11; 01 United Kingdom (UK) Cluny 1988-02-05	Norman	1) (planting) 2) 3)	0.60	200	0.30	1987-09-02[4)]		grain	<0.4	<0.4	7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87/283/11; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87/283/11; 02 United Kingdom (UK) Cluny 1988-02-05	Norman	1) (planting) 2) 3)	1.0	200	0.50	1987-09-02[4)]		grain	<0.4	<0.4	7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87/283/11; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87/283/11; 03 United Kingdom (UK) Cluny 1988-02-05	Norman	1) (planting) 2) 3)	1.6	200	0.80	1987-09-02[4)]		grain	0.40	<0.4	7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87/283/11; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87/283/11; 04 United Kingdom (UK) Cluny 1988-02-05	Norman	1) (planting) 2) 3)	2.0	200	1.0	1987-09-02[4)]		grain	0.70	<0.4	7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87/283/11; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87/283/11; 05 United Kingdom (UK) Cluny 1988-02-05	Norman	1) (planting) 2) 3)	0.60	200	0.30	1987-09-02[4)]		grain	<0.4	<0.4	7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87/283/11; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87/283/11; 06 United Kingdom (UK) Cluny 1988-02-05	Norman	1) (planting) 2) 3)	1.0	200	0.50	1987-09-02[4)]		grain	0.40	<0.4	7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87/283/11; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87/283/11; 07 United Kingdom (UK) Cluny 1988-02-05	Norman	1) (planting) 2) 3)	1.6	200	0.80	1987-09-02[4)]		grain	0.40	<0.4	7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87/283/11; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.200; 87/283/11; 08 United Kingdom (UK) Cluny 1988-02-05	Norman	1) (planting) 2) 3)	2.0	200	1.0	1987-09-02[4)]		grain	0.40	<0.4	7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87/283/11; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : ROUNDUP
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2012-10-31

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid), calculated as Glyphosate
8.3 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	8.3	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl								
	(a)	(b)				(c)		(a)				(d)	(e)
MLL 30.200; 87/283/11; 09 United Kingdom (UK) Cluny 1988-02-05	Norman	1) (planting) 2) 3)	0.72	200	0.36	1987-09-02[4)]		grain	<0.4		<0.4	7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87/283/11; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

1	2	3	4			5	6	7	8.1	8.2	8.3	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl								
	(a)	(b)				(c)		(a)				(d)	(e)
MLL 30.200; 87/283/11; 10 United Kingdom (UK) Cluny 1988-02-05	Norman	1) (planting) 2) 3)	1.4	200	0.72	1987-09-02[4)]		grain	<0.4		<0.4	7	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with ROUNDUP, SL~~SC~~ (360g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.4mg/kg, straw 1.6mg/kg,LOQ AMPA grain and straw 0.4mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.200; 87/283/11; 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11 & 12 RIP9501278

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 400 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : MON14478
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2012-11-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-291-009, 01 France 22 Saclay 1988-02-05	Moulin	1) 1986-11-28 (planting) 2) 3)	1.1	200	0.55	1987-08-12		grain straw	2.3 14.0	0.090 <0.4	22 22	submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.205; 80, 87-291-009, 01, 02, 03, 04, 05, 06, 07 & 08 RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-291-009, 02 France 22 Saclay 1988-02-05	Moulin	1) 1986-11-28 (planting) 2) 3)	2.2	200	1.1	1987-08-12		grain straw	2.2 52.0	0.080 0.60	22 22	submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.205; 80, 87-291-009, 01, 02, 03, 04, 05, 06, 07 & 08 RIP9501280
MLL 30.205; 80, 87-291-009, 03 France 22 Saclay 1988-02-05	Moulin	1) 1986-11-28 (planting) 2) 3)	4.4	200	2.2	1987-08-12		grain straw	2.6 79.0	0.10 0.70	22 22	submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.205; 80, 87-291-009, 01, 02, 03, 04, 05, 06, 07 & 08 RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-291-009, 04 France 22 Saclay 1988-02-05	Moulin	1) 1986-11-28 (planting) 2) 3)	8.8	200	4.4	1987-08-12		grain straw	5.5 193	0.16 2.8	22 22	submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glypho-sate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.205; 80, 87-291-009, 01, 02, 03, 04, 05, 06, 07 & 08 RIP9501280
MLL 30.205; 80, 87-291-009, 05 France 22 Saclay 1988-02-05	Moulin	1) 1986-11-28 (planting) 2) 3)	1.1	200	0.55	1987-08-12		grain straw	0.90 20.0	0.10 0.40	22 22	submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.205; 80, 87-291-009, 01, 02, 03, 04, 05, 06, 07 & 08 RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-291-009, 06 France 22 Saclay 1988-02-05	Moulin	1) 1986-11-28 (planting) 2) 3)	2.2	200	1.1	1987-08-12		grain straw	1.3 37.0	0.10 0.60	22 22	submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.205; 80, 87-291-009, 01, 02, 03, 04, 05, 06, 07 & 08 RIP9501280
MLL 30.205; 80, 87-291-009, 07 France 22 Saclay 1988-02-05	Moulin	1) 1986-11-28 (planting) 2) 3)	4.4	200	2.2	1987-08-12		grain straw	2.6 74.0	0.15 2.3	22 22	submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.205; 80, 87-291-009, 01, 02, 03, 04, 05, 06, 07 & 08 RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-291-009, 08 France 22 Saclay 1988-02-05	Moulin	1) 1986-11-28 (planting) 2) 3)	8.8	200	4.4	1987-08-12		grain straw	5.4 213	0.090 3.7	22 22	submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data, Side-by-side trials: MLL 30.205; 80, 87-291-009, 01, 02, 03, 04, 05, 06, 07 & 08 RIP9501280

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 570 g/L
Formulation (e.g. WP) : Soluble concentrate (SL)
Commercial product (name) : MON 0139
Applicant : Monsanto Europe S.A./N.V.

Active ingredient : glyphosate
Crop / crop group : Winter Soft Wheat
Crop Code : TRZAW

Submission date : 1995-09-01

Indoors / Outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 glyphosate
8.2 AMPA (Aminomethylphosphonsäure)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.206, 79, SU83/10/RP, p43 United Kingdom (UK) Chisleton, Wilts. 1988-04-01	Rapier	1) 2) 3)	0.85	300	0.28	1983-07-27[4)]		grain straw	1.8 1.5 8.0 19.0	0.090 0.060 0.080 0.15	7 7 7 7	4) spraying analytical method: not reported (not reported), addition of surfactant 63780 (0.05 %), no controls RIP9501281 ASB2009-2210 ASB2009-2242 ASB2009-2244

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 690 g/L
Formulation (e.g. WP) : Soluble concentrate (SL)
Commercial product (name) : GLISTAR
Applicant : Alkaloida Europe

Active ingredient : glyphosate
Crop / crop group : Winter Soft Wheat
Crop Code : TRZAW

Submission date : 2012-09-25

Indoors / Outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 glyphosate
8.2 AMPA (Aminomethylphosphonsäure)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
RF 2052, 92 H CP AC 02 France (FR) 41 Villefrancoeur 1993-02-15		1) 1991-10-17 (sowing) 2) 3) 1992-07-15	2.2	380	0.58	1992-07-16[4)]	BBCH 83-87	grain straw Controls > LOQ grain straw	0.93 0.15 12.8 15.6 0.15 0.22	<0.050 <0.050 0.98 0.86	22 22 22 22 22 22	4) spraying analytical method: MA 157 (HPLC), LOQ(s): 8.1: 0.05 mg/kg (grain, straw), 8.2: 0.05 mg/kg (grain, straw), max. sample storage time in month(s): 6 Control samples contained significant residues, trial not acceptable. RIP9501345

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
RF 2052, 92 H CP AC 04 France (FR) 02 Orainville 1993-02-15	Sleipner	1) 1991-10-05 (sowing) 2) 3) 1992-07-15	2.2	400	0.55	1992-07-20[4)]	BBCH 92-97	grain straw Controls > LOQ straw straw	35.4 45.4 12.8 15.6 0.22 0.22	0.050 <0.050 0.65 0.68	9 9 9 9 9 9	4) spraying analytical method: MA 157 (HPLC), LOQ(s): 8.1: 0.05 mg/kg (grain, straw), 8.2: 0.05 mg/kg (grain, straw), max. sample storage time in month(s): 5 Control samples contained significant residues, trial not acceptable. In addition straw and grain seem switched. RIP9501345
94035/01-FPWW, 01 Germany (DE) 75177 Pforzheim 1995-04-12	Bussard	1) 1993-11-02 (sowing) 2) 1994-06 3) 1994-08-04	1.8	204	0.88	1994-07-22[4)]	BBCH 89	grain straw	1.5 1.5 93.0 103		6 6 6 6	4) spraying analytical method: DFG method 405 (HPLC), LOQ(s): 8.1: 0.05 mg/kg (grain), 1.5 mg/kg (straw), max. sample storage time in month(s): 7 Replicate plot to 94035/01-FPWW, 02 & 03 RIP9501341

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
94035/01-FPWW, 02 Germany (DE) 75177 Pforzheim 1995-04-12	Bussard	1) 1993-11-02 (sowing) 2) 1994-06 3) 1994-08-04	1.7	197	0.86	1994-07-22[4)]	BBCH 89	grain straw	1.7 1.3 68.0 91.0		6 6 6 6	4) spraying analytical method: DFG method 405 (HPLC), LOQ(s): 8.1: 0.05 mg/kg (grain), 1.5 mg/kg (straw), max. sample storage time in month(s): 7 Replicate plot to 94035/01-FPWW, 02 & 01 RIP9501341
94035/01-FPWW, 04 Germany (DE) 71686 Remseck 1995-04-12	Orestis	1) 1993-10-20 (sowing) 2) 1994-06-10 3) 1994-08-05	1.7	193	0.88	1994-07-21[4)]	BBCH 89	grain straw	1.4 1.6 61.0 58.0		7 7 7 7	4) spraying analytical method: DFG method 405 (HPLC), LOQ(s): 8.1: 0.05 mg/kg (grain), 1.5 mg/kg (straw), max. sample storage time in month(s): 7 Replicate plot to 94035/01-FPWW, 05 & 06 RIP9501341

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
94035/01-FPWW, 05 Germany (DE) 71686 Remseck 1995-04-12	Orestis	1) 1993-10-20 (sowing) 2) 1994-06-10 3) 1994-08-05	1.7	194	0.88	1994-07-21[4)]	BBCH 89	grain straw	0.81 1.4 47.0 45.0		7 7 7 7	4) spraying analytical method: DFG method 405 (HPLC), LOQ(s): 8.1: 0.05 mg/kg (grain), 1.5 mg/kg (straw), max. sample storage time in month(s): 7 Replicate plot to 94035/01-FPWW, 04 & 06 RIP9501341
94035/01-FPWW, 07 Germany (DE) 67590 Monsheim 1995-04-12	Monopol	1) 1993-10-04 (sowing) 2) 1994-06 3) 1994-08	1.8	207	0.87	1994-07-15[4)]	BBCH 89	grain straw	1.5 1.5 34.0 32.0		10 10 10 10	4) spraying analytical method: DFG method 405 (HPLC), LOQ(s): 8.1: 0.05 mg/kg (grain), 1.5 mg/kg (straw), max. sample storage time in month(s): 7 Replicate plot to 94035/01-FPWW, 08 & 09 RIP9501341

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
94035/01-FPWW, 08 Germany (DE) 67590 Monsheim 1995-04-12	Monopol	1) 1993-10-04 (sowing) 2) 1994-06 3) 1994-08	1.7	195	0.87	1994-07-15[4)]	BBCH 89	grain straw	0.23 0.24 33.0 22.0		10 10 10 10	4) spraying analytical method: DFG method 405 (HPLC), LOQ(s): 8.1: 0.05 mg/kg (grain), 1.5 mg/kg (straw), max. sample storage time in month(s): 7 Replicate plot to 94035/01-FPWW, 07 & 09 RIP9501341
94035/01-FPWW, 10 Germany (DE) 37603 Holzminden 1995-04-12	Orestis	1) 1993-10-16 (sowing) 2) 1994-06 3) 1994-08-05	1.7	200	0.85	1994-07-25[4)]	BBCH 89-90	grain straw	0.87 1.3 50.0 53.0		9 9 9 9	4) spraying analytical method: DFG method 405 (HPLC), LOQ(s): 8.1: 0.05 mg/kg (grain), 1.5 mg/kg (straw), max. sample storage time in month(s): 6 Replicate plot to 94035/01-FPWW, 11 & 12 RIP9501341

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
94035/01-FPWW, 11 Germany (DE) 37603 Holzminden 1995-04-12	Orestis	1) 1993-10-16 (sowing) 2) 1994-06 3) 1994-08-05	1.8	205	0.88	1994-07-25[4)]	BBCH 89-90	grain straw	0.34 0.29 58.0 28.0		9 9 9 9	4) spraying analytical method: DFG method 405 (HPLC), LOQ(s): 8.1: 0.05 mg/kg (grain), 1.5 mg/kg (straw), max. sample storage time in month(s): 6 Replicate plot to 94035/01-FPWW, 10 & 12 RIP9501341

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 356 g/L
Formulation (e.g. WP) : Soluble concentrate (SL)
Commercial product (name) : Glistar
Applicant : Alkaloida Europe

Active ingredient : glyphosate
Crop / crop group : Winter Soft Wheat
Crop Code : TRZAW

Submission date : 2012-09-25

Indoors / Outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : glyphosate

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
94035/01-FPWW, 03 Germany (DE) 75177 Pforzheim 1995-04-12	Bussard	1) 1993-11-02 (sowing) 2) 1994-06 3) 1994-08-04	1.8	203	0.89	1994-07-22[4)]	BBCH 89	grain straw	1.6 87.0	6 6	4) spraying analytical method: , LOQ(s): 0.05 mg/kg (grain), 1.5 mg/kg (straw), max. sample storage time in month(s): 7 Replicate plot to 94035/01-FPWW, 02 & 01 RIP9501341

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
94035/01-FPWW, 06 Germany (DE) 71686 Remseck 1995-04-12	Orestis	1) 1993-10-20 (sowing) 2) 1994-06-10 3) 1994-08-05	1.7	188	0.9	1994-07-22[4)]	BBCH 89	grain straw	1.7 48.0	6 6	4) spraying analytical method: , LOQ(s): 0.05 mg/kg (grain), 1.5 mg/kg (straw), max. sample storage time in month(s): 7 Replicate plot to 94035/01-FPWW, 04 & 05 RIP9501341
94035/01-FPWW, 09 Germany (DE) 67590 Monsheim 1995-04-12	Monopol	1) 1993-10-04 (sowing) 2) 1994-06 3) 1994-08	1.7	195	0.87	1994-07-15[4)]	BBCH 89	grain straw	0.24 27.0	10 10	4) spraying analytical method: , LOQ(s): 0.05 mg/kg (grain), 1.5 mg/kg (straw), max. sample storage time in month(s): 7 Replicate plot to 94035/01-FPWW, 07 & 08 RIP9501341

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl						
	(a)	(b)				(c)		(a)		(d)	(e)
94035/01-FPWW, 12 Germany (DE) 37603 Holzminden 1995-04-12	Orestis	1) 1993-10-16 (sowing) 2) 1994-06 3) 1994-08-05	1.8	202	0.89	1994-07-25[4)]	BBCH 89-90	grain straw	0.40 27.0	9 9	4) spraying analytical method: , LOQ(s): 0.05 mg/kg (grain), 1.5 mg/kg (straw), max. sample storage time in month(s): 6 Replicate plot to 94035/01-FPWW, 10 & 11 RIP9501341

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360~~355.7~~ g/L
Formulation (e.g. WP) : Soluble concentrate (SL)
Commercial product (name) : Roundup
Applicant : Monsanto Europe S.A./N.V.

Active ingredient : glyphosate
Crop / crop group : Winter Soft Wheat
Crop Code : TRZAW

Submission date : 1995-09-01

Indoors / Outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 AMPA (Aminomethylphosphonsäure)
8.2 glyphosate

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.206, 79, p28 United Kingdom (UK) Perrysfield, Rushden, Hertz 1988-04-01	Flanders	1) 2) 3)	0.51	300	0.17	1983-08-06[4)]		straw, stacked in field straw, stored	0.68 1.1	81.0 120	9 9	4) spraying analytical method: not reported (not reported), RIP9501281 ASB2009-2210 ASB2009-2242 ASB2009-2244

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.23 Wheat (Southern Europe)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Active ingredient : Glyphosate
Crop / crop group : Winter Wheat

Submission date : August 2000

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : (MON 2139) Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Indoors / outdoors : Outdoors (European South)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30815, 98-GLY-06-B/F, R 8144 DR1/C FR- 47120 Duras 05/1999	Soissons	1) 19.10.1997 (sowing) 2) – 3) 01.07.1998	2.3	208	1.1	24.06.1998	BBCH 87-88	grain straw	4.1 0.12 0.28 39 5.1 15	<0.05 <0.05 <0.05 0.26 <0.05 3.7	0 7 15 0 7 15	analytical method: (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 9 months Side-by-side trial to MLL 30815, 98-GLY-06-B/F, R 8144 DR1/D RIP2000-1243
MLL 30815, 98-GLY-06-B/F, R 8144 BD1/C FR- 26750 St. Paul-les-Romans	Tremie	1) 27.10.1997 (sowing) 2) – 3) 13.07.1998	2.2	199	1.1	03.07.1998	BBCH 87-89	grain straw	4.6 4.9 1.2 67 16 16	<0.05 <0.05 0.05 0.61 0.49 0.67	0 10 18 0 10 18	analytical method: (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8.5 months Side-by-side trial to MLL 30815,

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl							
	(a)	(b)				(c)		(a)			(d)	(e)
05/1999												98-GLY-06-B/F, R 8144 BD1/D RIP2000-1243

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 680 g/kg
Formulation (e.g. WP) : WG
Commercial product (name) : MON 14420
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Winter Wheat

Submission date : August 2000

Indoors / outdoors : Outdoors (European South)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30815, 98-GLY-06-B/F, R 8144 DR1/D FR- 47120 Duras 05/1999	Soissons	1) 19.10.1997 (sowing) 2) – 3) 01.07.1998	2.3	213	1.1	24.06.1998	BBCH 87-88	grain straw	3.0 0.66 0.29 53 13 16	<0.05 <0.05 <0.05 0.41 0.18 0.44	0 7 15 0 7 15	analytical method: (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 9 months Side-by-side trial to MLL 30815, 98-GLY-06-B/F, R 8144 DR1/C RIP2000-1243

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30815, 98-GLY-06-B/F, R 8144 BD1/D FR- 26750 St. Paul-les-Romans 05/1999	Tremie	1) 27.10.1997 (sowing) 2) – 3) 13.07.1998	2.2	204	1.1	03.07.1998	BBCH 87-89	grain straw	4.1 0.66 0.70 86 24 9.7	<0.05 <0.05 0.17 0.75 0.58 0.26	0 10 18 0 10 18	analytical method: (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8.5 months Side-by-side trial to MLL 30815, 98-GLY-06-B/F, R 8144 BD1/C RIP2000-1243

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 356 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Madrigal
Applicant : Logofit (HPQ)

Active ingredient : Glyphosate
Crop / crop group : Winter Wheat

Submission date : October 2002

Indoors / outdoors : Outdoors (European South)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
97 H CP HX P/A 97 H CP HX P01 FR-Toulouse 2002-06-07	Soissons	1) 05.11.1996 2) – 3) 02.07.1997	2.4	230	1.0	25.06.1997	BBCH 89	grain straw	<0.02 <0.05	<0.02 <0.05	7 7	analytical method: HPLC-FLD, LOQ's grain: 0.02 mg/kg, LOQ's straw: 0.05 mg/kg, max. sample storage: 24 months Plot not considered valid, since no residue >LOQ are unlikely for desiccation use. ASB2009-3524 (field part) ASB2009-3522 (analytical part)
97 H CP HX P/A 97 H CP HX P02 FR-Toulouse 2002-06-07	Soissons	1) 15.11.1996 2) – 3) 16.07.1997	2.2	168	1.3	09.07.1997	BBCH 89	grain straw	0.40 3.4	<0.02 <0.05	7 7	analytical method: HPLC-FLD, LOQ's grain: 0.02 mg/kg, LOQ's straw: 0.05 mg/kg, max. sample storage: 24 months ASB2009-3524 (field part) ASB2009-3522 (analytical part)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l

Formulation (e.g. WP) : SL
Commercial product (name) : GLIFOGARDE
Applicant : Inventus

Active ingredient : Glyphosate
Crop / crop group : Winter Wheat

Submission date : 06.08.2002

Indoors / outdoors : Outdoors (European South)

Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
94 H CP HX , 94 H CP HX 02 FR-32 Gers 1994-11-14	Soisson	1) 11.1993 (sowing) 2) -- 3) 20.07.1997	2.2	150	1.5	06.07.1994	BBCH 89	grain straw	0.07 28.0	<0.05 1.1	7 7	analytical method: PG0111 (HPLC-FLD), LOQ's grain: 0.05 mg/kg, LOQ's straw: 0.5 mg/kg, max. sample storage: 4.5 months RIP2002-1448 (field part) ASB2009-3523 (analytical part)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : (MON 2139) Roundup
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Active ingredient : Glyphosate
Crop / crop group : Winter Wheat

Submission date : June 2002

Indoors / outdoors : Outdoors (European South)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 31337, 99-GLY-07-B/F, trial 9075 DR1/C FR- 47120 Duras 05/2000	Isengrain	1) 22.10.1998 (sowing) 2) -- 3) 28.06.1999	2.2	182	1.2	21.06.1999	BBCH 87-89	grain straw	1.8 0.70 0.40 53 25 21	<0.05 <0.05 <0.05 0.55 0.77 0.20	0 7 14 0 7 14	analytical method: DFG m ethod 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Side-by-side trials with MLL 31337, 99-GLY-07-B/F, trial 9075 DR1/D RIP2002-650

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 31337, 99-GLY-07-B/F, trial 9075 BD1/C FR- 26750 St. Paul les Romans 05/2000	Aztec	1) 14.10.1998 (sowing) 2) -- 3) 08.07.1999	2.1	173	1.2	01.07.1999	BBCH 87	grain straw	2.3 0.50 0.40 67 19 19	<0.05 <0.05 <0.05 0.91 0.61 0.58	0 7 14 0 7 14	analytical method: DFG m ethod 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Side-by-side trial with MLL 31337, 99-GLY-07-B/F, trial 9075 BD1/D RIP2002-650

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Active ingredient : Glyphosate
Crop / crop group : Winter Wheat

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Submission date : 28.11.2001

Content of a.i. (g/kg or g/l) : 450 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : MON 78294
Applicant : Monsanto Agrar Deutschland GmbH (MOT)

Indoors / outdoors : Outdoors (European South)
Other a. i. in formulation (common name and content) :
Residues calculated as : 8.1 Glyphosate
8.2 Aminomethyl phosphonic acid (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 31337, 99-GLY-07-B/F, trial 9075 DR1/D FR- 47120 Duras 05/2000	Isengrain	1) 22.10.1998 (sowing) 2) -- 3) 28.06.1999	2.2	178	1.2	21.06.1999	BBCH 87-89	grain straw	2.5 0.90 1.2 61 32 28	<0.05 <0.05 <0.05 0.52 0.72 1.1	0 7 14 0 7 14	analytical method: DFG method 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Side-by-side trials with MLL 31337, 99-GLY-07-B/F, trial 9075 DR1/C RIP2002-650

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	DAT	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 31337, 99-GLY-07-B/F, trial 9075 BD1/D FR- 26750 St. Paul les Romans 05/2000	Aztec	1) 14.10.1998 (sowing) 2) -- 3) 08.07.1999	2.1	173	1.2	01.07.1999	BBCH 87	grain straw	2.4 0.32 0.30 100 25 19	<0.05 <0.05 <0.05 0.86 0.95 0.57	0 7 14 0 7 14	analytical method: DFG method 405 (HPLC-FLD), LOQ's: 0.05 mg/kg, max. sample storage: 8 months Side-by-side trial with MLL 31337, 99-GLY-07-B/F, trial 9075 BD1/C RIP2002-650

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 650 g/l
Formulation (e.g. WP) : SG
Commercial product (name) : MON 78568
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2005-04-21

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA
(Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 IT1, plot D Italy 44032 Contrapo 2002-06-13	Neodur	1) 2000-10-19 (sowing) 2) 3)	2.2	306	0.72	2001-06-23[4)]	BBCH 87	grain straw	0.34 57.9	<0.05 0.88	7 7	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 8 months Side-by-side trial with R A1157, trial A1157 IT1, plot B & C RIP2005-200

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 SA1, plot D France 40700 Ste.Colombe 2002-06-13	Soissons	1) 2000-10-20 (sowing) 2) 3)	2.1	299	0.72	2001-06-22[4)]	BBCH 89	grain straw	0.84 20.3	<0.05 0.60	7 7	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 5 months for grain, 7 months for straw Side-by-side trial with R A1157, trial A1157 SA1, plot B & C RIP2005-200

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 540 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : MON 78273
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2005-04-21

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 IT1, plot C Italy 44032 Contrapo 2002-06-13	Neodur	1) 2000-10-19 (sowing) 2) 3)	2.3	313	0.72	2001-06-23[4)]	BBCH 87	grain straw	0.51 67.7	<0.05 0.46	7 7	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 8 months Side-by-side trial with R A1157, trial A1157 IT1, plot B & D RIP2005-200
R A1157, trial A1157 SA1, plot C France 40700 Ste.Colombe 2002-06-13	Soissons	1) 2000-10-20 (sowing) 2) 3)	2.1	285	0.72	2001-06-22[4)]	BBCH 89	grain straw	0.55 10.9	<0.05 0.35	7 7	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 5 months for grain, 7 months for straw Side-by-side trial with R A1157, trial A1157 SA1, plot B & D RIP2005-200

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2005-04-21

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
R A1157, trial A1157 IT1, plot B Italy 44032 Contrapo 2002-06-13	Neodur	1) 2000-10-19 (sowing) 2) 3)	2.1	287	0.72	2001-06-23[4)]	BBCH 87	grain straw	0.28 40.9	<0.05 0.66	7 7	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 8 months Side-by-side trial with R A1157, trial A1157 IT1, plot C & D RIP2005-200
R A1157, trial A1157 SA1, plot B France 40700 Ste.Colombe 2002-06-13	Soissons	1) 2000-10-20 (sowing) 2) 3)	2.1	294	0.72	2001-06-22[4)]	BBCH 89	grain straw	0.41 16.9	<0.05 0.87	7 7	4) spraying analytical methods: 405,pp 229-238 and MON XA001 (HPLC/FLD), LOQ: 0.05 mg/kg, max. sample storage: 5 months for grain, 7 months for straw Side-by-side trial with R A1157, trial A1157 SA1, plot C & D RIP2005-200

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 400 g/l
Formulation (e.g. WP) : SL~~SC~~
Commercial product (name) : MON14478
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Winter Soft Wheat

Submission date : 2012-11-01

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-246-015, 01 France 31 Saclay 1988-02-05	Fidel	1) 1986-11-06 (planting) 2) 3)	1.1	200	0.55	1987-08-10[4)]		grain straw	<0.8 48.0	<0.08 <0.4	8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data, Side-by-side plots MLL 30.205; 80, 87-246-015, 01, 02, 03, 04, 05, 06, 07 & 08 RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-246-015, 02 France 31 Saclay 1988-02-05	Fidel	1) 1986-11-06 (planting) 2) 3)	2.2	200	1.1	1987-08-10[4)]		grain straw	1.2 96.0	<0.08 0.82	8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data, Side-by-side plots MLL 30.205; 80, 87-246-015, 01, 02, 03, 04, 05, 06, 07 & 08 RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-246-015, 03 France 31 Saclay 1988-02-05	Fidel	1) 1986-11-06 (planting) 2) 3)	4.4	200	2.2	1987-08-10[4)]		grain straw	2.0 146	<0.08 1.1	8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data, Side-by-side plots MLL 30.205; 80, 87-246-015, 01, 02, 03, 04, 05, 06, 07 & 08 RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-246-015, 04 France 31 Saclay 1988-02-05	Fidel	1) 1986-11-06 (planting) 2) 3)	8.8	200	4.4	1987-08-10[4)]		grain straw	3.0 332	<0.08 2.0	8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate),analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data, Side-by-side plots MLL 30.205; 80, 87-246-015, 01, 02, 03, 04, 05, 06, 07 & 08 RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-246-015, 05 France 31 Saclay 1988-02-05	Fidel	1) 1986-11-06 (planting) 2) 3)	1.1	200	0.55	1987-08-10[4)]		grain straw	1.1 62.0	<0.08 0.60	8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data, Side-by-side plots MLL 30.205; 80, 87-246-015, 01, 02, 03, 04, 05, 06, 07 & 08 RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-246-015, 06 France 31 Saclay 1988-02-05	Fidel	1) 1986-11-06 (planting) 2) 3)	2.2	200	1.1	1987-08-10[4)]		grain straw	1.2 100	0.080 0.80	8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data, Side-by-side plots MLL 30.205; 80, 87-246-015, 01, 02, 03, 04, 05, 06, 07 & 08 RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-246-015, 07 France 31 Saclay 1988-02-05	Fidel	1) 1986-11-06 (planting) 2) 3)	4.4	200	2.2	1987-08-10[4)]		grain straw	1.2 142	<0.08 1.0	8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data, Side-by-side plots MLL 30.205; 80, 87-246-015, 01, 02, 03, 04, 05, 06, 07 & 08 RIP9501280

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
MLL 30.205; 80, 87-246-015, 08 France 31 Saclay 1988-02-05	Fidel	1) 1986-11-06 (planting) 2) 3)	8.8	200	4.4	1987-08-10[4)]		grain straw	3.2 296	0.10 1.8	8 8	4) spraying submitted to Glyphosat 004282-00/00 at 1995-09-01,treated with MON14478, SL~~SC~~ (400g/l Glyphosate) + AS,analytical method: AC-004 (HPLC/OPA),LOQ Glyphosate: grain 0.8mg/kg, straw 4mg/kg,LOQ AMPA grain 0.4mg/kg and straw 0.08mg/kg,max. sample storage: no data, Side-by-side plots MLL 30.205; 80, 87-246-015, 01, 02, 03, 04, 05, 06, 07 & 08 RIP9501280

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.18.24 Sugar beets

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l (480 g/l Glyphosate-isopropylamine salt)
Formulation (e.g. WP) : SL
Commercial product (name) : Roundup (MON 2139)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Sugar Beet

Submission date : 1995-09-01

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate (PMG)
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
A74/SB, trial 1+2 United Kingdom Thriplow, Cambs 1975-06-23	Kleine Polybeet K 17	1) 1974 (sowing) 2) 3) 1974-10-18	1.4	no data		1973-10-08[4)]	BBCH 00	leaf, with top beet root	<0.05 <0.05	<0.05 <0.05	375 375	4) spraying stubble application, (pre-seedbed), analytical method: GC-PFD RIP9501210
	Kleine Polybeet K 17	1) 1974 (sowing) 2) 3) 1974-10-18	2.9	no data		1973-10-08[4)]	BBCH 00	leaf, with top beet root	<0.05 <0.05	<0.05 <0.05	375 375	
A74/SB, trial 3+4 United Kingdom Norfolk Agricultural College 1975-06-23	Nomo	1) 1974 (sowing) 2) 3) 1974-10-23	1.8	no data		1974-03-30[4)]	BBCH 00	leaf, with top beet root	<0.05 <0.05	<0.05 <0.05	207 207	4) spraying pre emergence/pre drilling of crop, analytical method: GC-PFD RIP9501210
	Nomo	1) 1974 (sowing) 2) 3) 1974-10-23	2.8	no data		1974-03-30[4)]	BBCH 00	leaf, with top beet root	<0.05 <0.05	<0.05 <0.05	207 207	
A74/SB, trial 5+6 United Kingdom Norfolk	Monotry	1) 1974 (sowing) 2) 3) 1974-10-23	1.8	no data		1974-04-01[4)]	BBCH 01	leaf, with top beet root	<0.05 <0.05	<0.05 <0.05	205 205	4) spraying pre emergence/post drilling of crop,

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
Agricultural College 1975-06-23	Monotry	1) 1974 (sowing) 2) 3) 1974-10-23	2.8	no data		1974-04-01[4)]	BBCH 01	leaf, with top beet root	<0.05 <0.05	<0.05 <0.05	205 205	analytical method: GC-PFD RIP9501210
74/69 Chimac Belgium Kallo 1975-06-23	no data	1) 1974 (sowing) 2) 3) 1974-10-17	1.4	no data		1973-10-11[4)]	BBCH 00	leaf, with top beet root	<0.05 <0.05	<0.05 <0.05	371 371	4) spraying pre emergence/ pre-plant analytical method: GC-PFD RIP9501210

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 178 g/l (240 g/l Glyphosate isopropylamine salt)
Formulation (e.g. WP) : SL
Commercial product (name) : SWING
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Sugar Beet

Submission date : 1989-07-21

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate (PMG)
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
002999 Germany 3017 Pattensen-Reden 1986-11-05	Novadima	1) 1985-04-18 (sowing) 2) 3) 1985-10-02	0.72	300	0.24	1985-04-23[4)]	BBCH 01	plant leaf beet root	<0.02 <0.02 <0.02 <0.02	<0.02 <0.02 <0.02 <0.02	87 120 162 162	4) spraying analytical method: XA001 (HPLC) for both substances, max. sample storage: 11 months ASB2009-2244 RIP9501281
008905 Germany 3017 Pattensen-Oerie 1986-11-05	Hilma	1) 1985-04-19 (sowing) 2) 3) 1985-10-02	0.72	300	0.24	1985-04-22[4)]	BBCH 00	plant leaf beet root	<0.02 <0.02 <0.02 <0.02	<0.02 <0.02 <0.02 <0.02	39 88 163 163	4) spraying analytical method: XA001 (HPLC) for both substances, max. sample storage: 16 months ASB2009-2210 RIP9501281

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
01811 Germany 3002 Blumhof 1986-11-05	Novadima	1) 1985-04-05 (sowing) 2) 3) 1985-10-01	0.72	300	0.24	1985-04-10[4)]	BBCH 01	plant leaf beet root	<0.02 <0.02 <0.02 <0.02 <0.02	<0.02 <0.02 <0.02 <0.02 <0.02	56 86 118 174 174	4) spraying analytical method: XA001 (HPLC) for both substances, max. sample storage: 12 months ASB2009-2242 RIP9501281

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l (480 g/l Glyphosate isopropylamine salt)
Formulation (e.g. WP) : SL
Commercial product (name) : Taifun forte
Applicant : ADAMA Agan Ltd GmbH

Active ingredient : Glyphosate
Crop / crop group : Sugar Beet

Submission date : 2008-07-25

Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate (PMG)
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
FSG-0608, trial 1 Germany 04668 Motterwitz 2007-09-26	Wiebke	1) 2006-05-12 (sowing) 2) 3) 2006-10-09	1.8	296	0.60	2006-05-09[4)]	no data	whole plant leaf, with top beet root	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05	55 76 97 118 153 153	4) spraying broadcast before sowing, analytical method: DFG method 405 (HPLC-FLD) for both substances, max. sample storage: 13 months ASB2008-5609
FSG-0608, trial 2 Germany 04880 Trossin 2007-09-26	Wiebke	1) 2006-05-10 (sowing) 2) 3) 2006-09-28	1.7	288	0.60	2006-05-08[4)]	no data	whole plant leaf, with top beet root	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05	56 77 98 120 143 120 143	4) spraying broadcast before sowing, analytical method: DFG method 405 (HPLC-FLD) for both substances, max. sample storage: 13 months ASB2008-5609

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
FSG-0608, trial 3 Germany 47574 Goch-Nierswalde 2007-09-26	Alabama	1) 2006-04-21 (sowing) 2) 3) 2006-09-21	1.9	207	0.90	2006-04-19[4)]	no data	leaf, with top beet root	<0.05 <0.05	<0.05 <0.05	155 155	4) spraying broadcast before sowing, analytical method: DFG method 405 (HPLC-FLD) for both substances, max. sample storage: 10 months ASB2008-5609
FSG-0608, trial 4 Germany 47574 Goch-Nierswalde 2007-09-26	Felicitas	1) 2006-04-24 (sowing) 2) 3) 2006-09-25	1.8	203	0.90	2006-04-21[4)]	no data	leaf, with top beet root	<0.05 <0.05	<0.05 <0.05	157 157	4) spraying broadcast before sowing, analytical method: DFG method 405 (HPLC-FLD) for both substances, max. sample storage: 10 months ASB2008-5609

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Federal Institute for Risk Assessment, Berlin
Federal Republic of Germany

Content of a.i. (g/kg or g/l) : 360 g/l
Formulation (e.g. WP) : SC
Commercial product (name) : MON 52276, SC (360 g/l Glyphosate)
Applicant : Monsanto Agrar Deutschland GmbH

Active ingredient : Glyphosate
Crop / crop group : Sugar Beet

Submission date : 2012-08-16

Indoors / outdoors : Outdoors (European South)
Other a.i. in formulation (content and common name) :
Residues calculated as : 8.1 Glyphosate
8.2 AMPA (Aminomethyl phosphonic acid)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	Residues (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
S11-00266-01 Spain 42210 Barca, Soria 2012-03-08	Sandrina	1) 2011-03-28 (sowing) 2) 3)	2.2	180	1.2	2011-04-14[4)]	BBCH 01	leaf, with top beet root	<0.05 <0.05	<0.05 <0.05	152 152	4) spraying at least 3 days after seeding and before emergence, analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 5 months, ASB2012-12376
S11-00266-02 Italy 40054 Budrio, Bologna 2012-03-08	Gea	1) 2011-03-12 (sowing) 2) 3)	2.5	207	1.2	2011-03-18[4)]	BBCH 01	leaf, with top beet root	<0.05 <0.05	<0.05 <0.05	144 144	4) spraying at least 3 days after seeding and before emergence, analytical method: AG-ME-1294-01 (LC-MS/MS), LOQ 0.05 mg/kg, max. sample storage: 5 months, ASB2012-12376

Remarks:
(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

Renewal Assessment Report

18 December 2013

Revised
29 January 2015
10 February 2015
31 March 2015

Glyphosate

Volume 3

Annex B.8

Environmental
fate and behaviour

RMS: Germany **Co-RMS: Slovakia**

Version history (Volume 3, chapter B.8)

Date	**Reason for revision**
19 December 2013	Initial Renewal Assessment Report
29 January 2015	Updated following RAR peer review, taking into account the reporting table, the evaluation table and additional data
10 February 2015	Updated taking into account further information from the open literature provided by the applicant (and not listed in the official EFSA Additional information request)
31 March 2015	Updated following discussion in Pesticides Peer Review Meeting 128 (03-05 March 2015)

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B.8 Environmental fate and behaviour

B.8.1 Route and rate of degradation in soil (Annex KIIA 7.1 to KIIA 7.2; KIIIA1 9.1 to KIIIA1 9.2)

B.8.1.1 Route of degradation in soil

B.8.1.1.1 Aerobic degradation

During the first EU review of glyphosate (2001), among the submitted studies, only two route of degradation studies conducted with glyphosate in the laboratory under aerobic conditions were assessed as "acceptable" (Galicia and Morgenroth, 1993, BVL no 1932059; Matla and Vonk, 1993, BVL no 2151389) based on the evaluation criteria and guidance in force at that time. In the study Matla & Vonk (1993, BVL no 2151389), the degradation route of glyphosate was only determined in one soil, in two additional soils only the degradation rate of glyphosate was tested. Study summaries of the studies can be found in the glyphosate Monograph.

Additionally, two route studies with glyphosate-trimesium, McBain (1985, BVL no 1052660) and McBain et al. (1985, BVL no 1052661), were submitted during the first EU review of glyphosate (2001). The route studies were considered acceptable on the evaluation criteria and guidance in force at that time. Study summaries of the studies can be found in the glyphosate-trimesium Monograph.

However, when re-evaluating the previously submitted studies, it was concluded by the RMS that the arguments in the monograph against the study Kesterson & Atkins (1991, BVL no 1932061)/ Honegger (1992, BVL no 2325652) (previously considerd as not acceptable) don't comply to current evaluation criteria anymore. It was argued, that the study had been performed at 25 °C instead of 20 °C. However, the study was performed according to EPA Subdivison N 162-1 and this applied temperature is now considered acceptable by the RMS. Besides, it was argued that the microbial biomass in the soil was not determined. However, number of total colony forming units for aerobic bacteria, actinomyetes and fungi and microbial biomass were determined at the start of the incubations indicating microbial viable soils. Thus, the study is now considered acceptable by the RMS regarding the results of the incubation of glyphosate in the silt loam soil Dupo. The results of the incubation of glyphosate in the sandy loam soil Kickapoo however will not be considered, since the total radioactive recovery was < 90 % for most of the sampling points of this soil.

Since the first EU review of glyphosate (2001), the Glyphosate Task Force (GTF) has conducted a new aerobic soil route study (Ponte, 2010, BVL no 2310242) in order to comply with current guidelines according to the requirements of the new OECD guideline no. 307 (OECD, 2002) for aerobic transformation in soil. The GTF members concluded that a new aerobic soil study is needed since older reviewed studies have deficiencies including low mass balance in one study and chromatographic anomalies in the other studies. In the old studies, one-dimensional Thin Layer Chromatography (TLC) systems were used as the primary analytical tool for quantification of glyphosate soil metabolites. TLC analyses of the soil extracts often showed glyphosate and AMPA in addition to some unidentified radioactive smear that usually started at the origin of the TLC plates and slowly moved as the plate developed in the TLC solvent system utilised in these studies. The study reports characterised these unidentified

fractions as glyphosate and AMPA bound to humic or fulvic acids, co-extracted by the high pH extraction solvent. Since 1993, the year that these studies were conducted, this TLC chromatographic anomaly was consistently confirmed in other glyphosate environmental fate studies. To circumvent the chromatographic artefacts, in recent environmental fate studies, small amounts of EDTA in soil extracts have been routinely employed prior to the TLC analysis in order to reverse the binding of glyphosate and AMPA to natural materials.

A summary of the new aerobic soil metabolism study is included under B.8.1.1.1 (Ponte, 2010, BVL no 2310242).

Additionally, four laboratory aerobic soil route of degradation studies (Dean et al., 1995, BVL no 2310244; Goodyear, 1996, BVL no 2310246; Esser, 1996, BVL no 2310248 and McLaughlin & Schanné, 1996, BVL no 2310250) were also available from Glyphosate Task Force (GTF) members which were not evaluated during the first glyphosate review (2001). These studies are also summarised under B.8.1.1.1 and provide further information on the route of degradation of glyphosate under laboratory aerobic soil conditions.

Finally, an additional laboratory aerobic soil rate of degradation study with glyphosate (Ponte, 2010, BVL no 2310255) was conducted. This study is summarised under B.8.1.2.1. However, since the formation of the metabolite AMPA, of volatiles, CO_2 and bound residues were also measured in this study; the results are also taken into account to define the route of glyphosate degradation under aerobic conditions.

Table B.8.1-1 summarises the guidelines, the laboratory conditions and the soil characteristics of the old route of degradation studies that were considered acceptable in the first glyphosate review, of the newly submitted route of degradation studies of glyphosate and of the rate study Ponte (2010, BVL no 2310255) conducted under aerobic soil conditions.

Table B.8.1-1: Overview of the glyphosate and glyphosate-trimesium laboratory aerobic route of degradation studies[1)]

Reference	Guideline	Temp (°C)	Duration of test	Soil characteristics				
				Soil origin	Soil type	pH	OC (%)	Moisture
McBain, 1985, BVL no 1052660	US EPA Subdivision N, Section 162-1 (1982)	18 – 26.7	376	Sorrento	Loam	6.8	1.8	Field capacity
McBain et al., 1985, BVL no 1052661		18 – 26.7	376	Sorrento	loam	6.8	1.8	Field capacity
Galicia & Morgenroth, 1993 BVL no 1932059	US EPA Section 162-1, BBA Part IV, 4-1	20	364 d	Les Evouettes	Silt loam	6.1[2)]	1.4	40 % MWHC
Kesterson & Atkins, 1991, BVL no 1932061 / Honegger, 1992, BVL no 2325652	US EPA Section 162-1	25	12 month	Dupo	Silt Loam	7.5	0.6	75 % FC
Matla & Vonk, 1993, BVL no 2151389	Dutch Regulations for Biocides	20	100 d	Maasdijk	Sandy Loam	7.5[3)]	1.1	1/3 bar
Dean, 1995, BVL no 2310244	US EPA Section 162-1,	20	180 d	Arrow	Sandy Loam	5.9[4)]	2.2	40 % MWHC

	BBA Part IV, 4-1, Japanese MAFF, Draft Guidelines for 91/414							
Goodyear, 1996, BVL no 2310246	US EPA Section 162-1, Japanese MAFF	25	121 d	Soil A	Loam	5.9[5)]	6.8	75 % of 1/3 bar
		25	121 d	Soil B	Sandy Loam	6.7[5)]	0.7	75 % of 1/3 bar
Esser, 1996, BVL no 2310248	US EPA Section 162-1	25	31 d	Visalia	Sandy Loam	8.3[2)]	0.3	75 % of 1/3 bar
McLaughlin & Schanné, 1996, BVL no 2310250	BBA Part IV, 4-1, FAO guidelines, SETAC (1995)	20	90 d	Speyer 2.1	Sand	5.9[4)]	0.6	45 % MWHC
		20	120 d	Speyer 2.2	Loamy Sand	5.6[4)]	2.3	45 % MWHC
		20 & 10	60 d	Speyer 2.3	Loamy Sand	6.4[4)]	1.2	45 % MWHC
Ponte, 2010, BVL no 2310242	US EPA OPPTS 835.4100, OECD 307	20	132 d	Gartenacker	Loam	7.1[5)]	2.0	pF 2.5
Ponte, 2010, BVL no 2310255	US EPA OPPTS 835.4100, OECD 307	20	70 d	Drusenheim	Loam	7.4[5)]	1.7	pF 2.5
		20	120 d	Pappelacker	Sandy Loam	7.0[5)]	1.9	pF 2.5
		20	120 d	18-Acres	Clay Loam	5.7[5)]	2.5	pF 2.5

1) Temp = temperature; OC= organic carbon; MWHC= maximum water-holding capacity; AMPA= aminomethylphosphonic acid
2) buffer solution unknown
3) buffer solution = KCl
4) buffer solution = $CaCl_2$
5) buffer solution = H_2O

The newly submitted study Esser (1996, BVL no 2310248) and the older studies McBain (1985, BVL no 1052660) and McBain et al. (1985, BVL no 1052661) are not considered acceptable by the RMS, since oxygen was used instead of CO_2-free air for airing the headspace of the test vessels thus creating a best case regarding the supply with oxygen for the soil microbes. Besides, it was decided by the RMS not to include the soil A of the study Goodyear (1996, BVL no 2310246), since the soil exhibits a very high oc-content which does not reflect the usual soil conditions of soils under agricultural use and would not comply with the more recent guideline OECD 307 (for more details see study summaries under B.8.1.1.1).

Table B.8.1-2 and Table B.8.1-3 summarise the main results of the route studies that were considered acceptable by the RMS.

Table B.8.1-2: Overview of the formation of metabolites, CO_2 and bound residues in the glyphosate laboratory aerobic route of degradation studies at 20°C and 25°C

Reference	Soil origin	Soil type	Metabolite AMPA[1)]	CO_2[1)]	Bound residues [1)]
			Max. formation (% of applied dose)	at the end of study (% of applied dose)	
Galicia & Morgenroth, 1993 BVL no 1932059	Les Evouettes	Silt loam	29.3% max. on day 84	41.6	19.8
Kesterson & Atkins, 1991, BVL no 1932061/ Honegger, 1992, BVL no 2325652	Dupo	Silt loam	28.65% max. on day 14	78.3	4.2
Matla & Vonk, 1993, BVL no 2151389	Maasdijk	Sandy Loam	not found	79.6	18.9
Dean, 1995, BVL no 2310244	Arrow	Sandy Loam	27.3 % max on day 120	23.6	9.35
Goodyear, 1996, BVL no 2310246	Soil B	Sandy Loam	21.0 % on day 30	70.6[2)]	12.4
McLaughlin & Schanné, 1996, BVL no 2310250	Speyer 2.1	Sand	50.1 % on day 90	43.0	2.5
	Speyer 2.2	Loamy Sand	42.4 % on day 60	36.5	4.9
	Speyer 2.3	Loamy Sand	32.0 % on day 7	63.4	7.8
Ponte, 2010, BVL no 2310242	Gartenacker	Loam	14.7 % on day 55	60.0	20.7
Ponte, 2010, BVL no 2310255	Drusenheim	Loam	21.2 % max on day 8	62.1	30.4
	Pappelacker	Sandy Loam	29 % max on day 48	54.4	20.15
	18-Acres	Clay Loam	13.3 % on day 91	16.9	43.2

n.d. not determined

1) if replicates were determined, then arithmetic mean of replicates is reported. If more than one analytical method is used in a study, the arithmetic mean of the method measurements is reported.

2) total volatiles

Table B.8.1-3: Overview of the formation of metabolites, CO_2 and bound residues in the glyphosate laboratory aerobic route of degradation study at 10 °C

Reference	Soil origin	Soil type	Metabolite AMPA[1)]	CO_2[1)]	Bound residues [1)]
			Max. formation (% of applied dose)	at the end of study (% of applied dose)	
McLaughlin & Schanné, 1996, BVL no 2310250	Speyer 2.3	Loamy Sand	34.3 % on day 60	48.2	2.4

1) if replicates were determined, then arithmetic mean of replicates is reported. If more than one analytical method is used in a study, the arithmetic mean of the method measurements is reported.

Based on the degradation studies submitted during the first EU review of glyphosate (2001), it was concluded that glyphosate was degraded in soil over time by micro-organisms. The prin-

cipal soil metabolite was aminomethylphosphonic acid (AMPA). The maximum amount of AMPA detected ranged from 15 to 29 % of the total glyphosate applied as reported in the glyphosate Monograph. These studies also established that AMPA is further degraded by soil micro-flora, although at a slower rate than glyphosate. They also demonstrated that 41.6 to 79.6 % of the applied glyphosate was mineralised to CO_2 and 18.9 to 19.8 % bound residues were formed. Volatiles other than CO_2 remained, when measured, at ≤ 0.3 % of AR.

The results of the new studies and the now considered acceptable study of Kesterson & Atkins (1991, BVL no 1932061)/ Honegger (1992, BVL no 2325652) are consistent with the conclusions of the first EU review of glyphosate (2001) and confirm that glyphosate is degraded in soil over time by micro-organisms in the laboratory under aerobic conditions. It is also confirmed that the only significant soil metabolite of glyphosate is AMPA. However, the maximum amount of AMPA detected from all studies ranged from 13.3 to 50.1 % AR. The maximum occurrence of 50.1 % is recommended to be used in further risk assessment.

The studies demonstrate that at 20 and 25° C, 16,9 to 79.6 % AR was mineralized to CO_2 and 2.5 to 43,2 % AR bound residues were formed. At 10 °C, 48.2 % AR CO_2 and 2 % AR bound residues were formed. Several other minor components were also detected in the new aerobic soil metabolism study; however, no single metabolite was detected in an amount greater than 2.3 % of the applied glyphosate. Volatiles other than CO_2 were not detected.

The proposed pathway for the degradation of glyphosate in soil under aerobic conditions is presented in Figure B. 8.1-1.

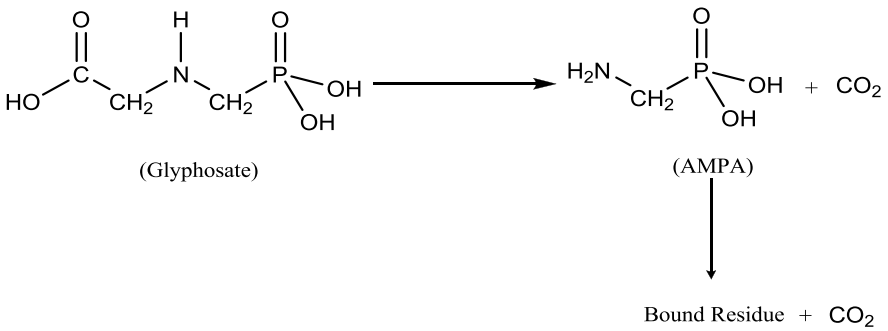


Figure B. 8.1-1: Proposed degradation pathway of glyphosate in soil

During the peer review the applicant was asked to provide information on pH dependency of degradation of glyphosate and AMPA together with discussion of the pH dependence of mineralisation and the likelihood of differential degradation routes depending an pH; see reporting table 4(3) from 02.05.2014.

pH Dependency of Soil DT_{50} (GTF): The data presented in Table B.8.1-2 of glyphosate RAR, Vol 3, Section B.8.1.1.1 summarises the results of all available and accepted route of degradation studies of glyphosate conducted under laboratory aerobic soil conditions. Glyphosate comprises of one basic amino function and three ionizable acidic sites. It has a number of pK_as (2.34, 5.73 and 10.2) and therefore exists as multiple species at most pHs, although the dianion predominates at physiologically typical pH 5-9. Furthermore, the molecule can exist as a zwitterion at most pHs

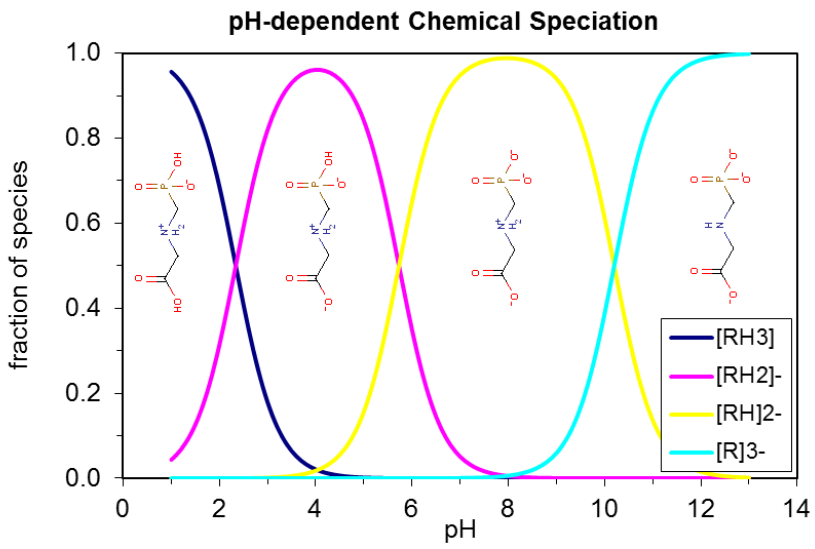


Figure B. 8.1-2: pH dependent chemical specification of glyphosate

Degradation dependency on pH is often due to abiotic degradation processes for most pesticides. However, both glyphosate and AMPA are hydrolytically stable and thus are inert to even extreme pH changes in aqueous solutions. Glyphosate and AMPA degradation in soil is a microbiological process which is generally not sensitive to pH changes.
Table B. 8.1-4 and Figure B. 8.1-3 (shown in next page), indeed indicate a moderate ($r^2 = 0.73$) pH dependency of CO_2 formation from these studies. It is important to mention that these studies were conducted with ^{14}C-labelled glyphosate with the ^{14}C located on the phosphonomethylene carbon of glyphosate. Therefore, the ^{14}C was monitored and accounted for every step throughout the analysis for mass balance purpose. The levels of CO_2 were measured by trapping evolved $^{14}CO_2$ with near quantitative mass balance. Any $^{14}CO_2$ entrain in soil or dissolved in soil pore water would have been detected when soil extracts were analyzed by HPLC. Therefore, the pH dependent solubility of CO_2 in water could be ruled out because of the experimental setup employed and the excellent mass balance generally observed in these aerobic soil degradation studies.

Table B. 8.1-4: Overview of the formation of CO_2 in the glyphosate laboratory aerobic route of degradation studies

Study	Soil	Soil pH (H_2O)*	CO_2 (% of dose)	AMPA (% of dose)
Galicia & Morgenroth, 1993	Les Evouettes	6.1	41.6	29.3
Kesterson & Atkins, 1991	Dupo	7.3	78.3	28.65
Matla & Vonk, 1993	Maasdijk	7.5	79.6	Not found
Dean et al., 1995	Arrow	6.5	23.6	27.3
Goodyear, 1996	Soil B	6.7	70.6	21.0
McLaughlin & Schanné, 1996	Speyer2.1	6.5	43.0	50.1
	Speyer 2.2	6.2	36.50	42.4
	Speyer 2.3	6.9	63.40	32.0
Ponte, 2010b	Gartenacker	7.1	60.0	14.7
	Drusenheim	7.4	62.1	21.2

	Pappelacker	7.0	54.40	29.0
	18-acres	5.7	16.90	13.3

*pH values are reproduced from Table B.8.91 of glyphosate RAR

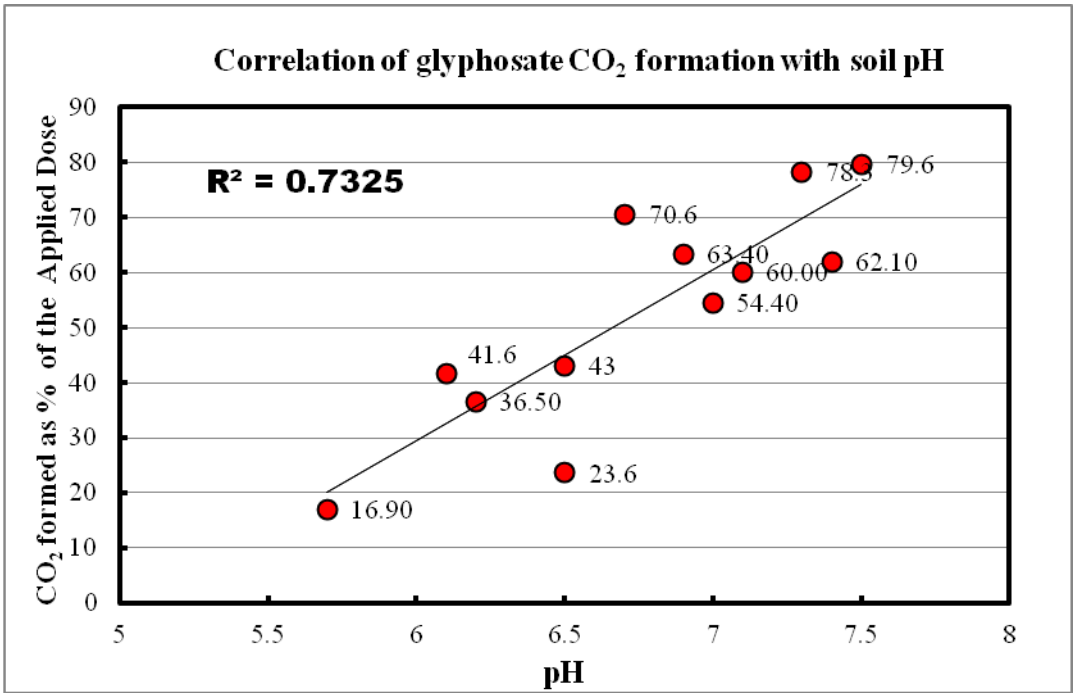


Figure B. 8.1-3: Correlation of glyphosate CO_2 formation with soil pH (H_2O) from the route of degradation studies

The soil pH values and the normalized modeling DT_{50} values for laboratory aerobic soil degradation of glyphosate and AMPA are shown in Table B. 8.1-5.
Figure B. **8.1-4** show the lack of correlation of degradation DT_{50} with soil pH is quite clear from
Figure B. **8.1-4** with the coefficient of determination (r^2) value of 0.2403 as to be expected where degradation is primarily biological rather than chemical. Degradation dependency on pH is often due to abiotic degradation processes for most pesticides. However, both glyphosate and AMPA are hydrolytically stable and thus are inert to even extreme pH changes in aqueous solutions. It is well established that glyphosate or AMPA degradation in soil is a microbiological process which is generally not sensitive to pH changes.
Figure B. **8.1-4** confirms that DT_{50} values for soil degradation of glyphosate are not influenced by soil pH.

Table B. 8.1-5: Overview of glyphosate and AMPA normalized modelling DT_{50} values from Laboratory aerobic degradation studies*

Study	**Soil**	**Soil pH**	**Glyphosate Normalised Modelling DT_{50} (days)**	**AMPA Normalised Modelling DT_{50} (days)**
Ponte, 2010a	Gartenacker	7.1	16.0	119.9
Dean et al., 1995	Arrow	6.5	257.1	-

Goodyear, 1996	Soil B	6.7	6.6	106.2
Galicia & Morgenroth, 1993	Les Evouettes	6.1	43.3	300.9
Matla & Vonk, 1993	Maasdijk	7.5	15.2	-
Ponte, 2010b	Drusenheim	7.4	4.2	36.8
	Pappelacker	7.0	12.0	116.3
	18-acres	5.7	160.5	-
Galicia & Flückiger, 1993 & add. Mamouni, 2002	Speyer 2.2	6.0	45.4	-
	Speyer 2.3	6.9	7.2	70.92
McLaughlin & Schanné, 1996	Speyer 2.1	6.5	19.5	-
	Speyer 2.2	6.2	72.2	-
	Speyer 2.3	6.9	3.76	42.14
Kesterson & Atkins, 1991	Dupo	7.3	3.70	30.5
Runnalls, 1991	Speyer 2.2	6.0	40.6	-
Lewis & Turnbull, 1992	Speyer 2.1	6.9	43.06	230.7

- No statistically reliable fit could be obtained

* DT_{50} value reported in Beedon Manor clay loam soil in glyphosate RAR is related to the TMS fate of glyphosate trimesium. Therefore it was not used for the investigation of pH dependency of glyphosate/AMPA DT_{50}

At first glance there appear to be a weak correlation of soil degradation DT_{50} values of AMPA with soil pH, with r^2 value of 0.62 (Figure B. 8.1-5). However dropping the DT_{50} value of 300.9 days from Galicia & Morgenroth, 1993 study, the correlation coefficient (r^2) for the remaining data is 0.2 (Figure B. 8.1-6), indicating that soil degradation DT_{50} values for AMPA is not influenced by soil pH. As recorded in the commenting table, GTF disagrees with the RMS decision to include data points beyond study duration of 120 days for the Galicia & Morgenroth, 1993 study. Soil microbial biomass clearly declined in the study. As it was noted previously the RMS re-calculated endpoint from Galicia & Morgenroth, 1993 study is clearly an outlier in the modelling endpoint for AMPA suggesting that inclusion of data points beyond 120 days resulted in an endpoint that is not consistent with the weight of evidence. The fact that microbial biomass decreased from beginning to end of studies is likely to have an impact on the overall degradation rates of AMPA. If OECD guideline 307 does not recommend laboratory incubation beyond 120 days because of concern over soil viability, it seems like the results from the biomass determination confirms this concern and gives us a rationale for excluding data beyond 120 days. GTF believes it is justified to drop the Galicia & Morgenroth DT_{50} value since there should be enough doubt in the viability of soil considering the decline in microbial biomass from the beginning to the end of the study was > 60%.

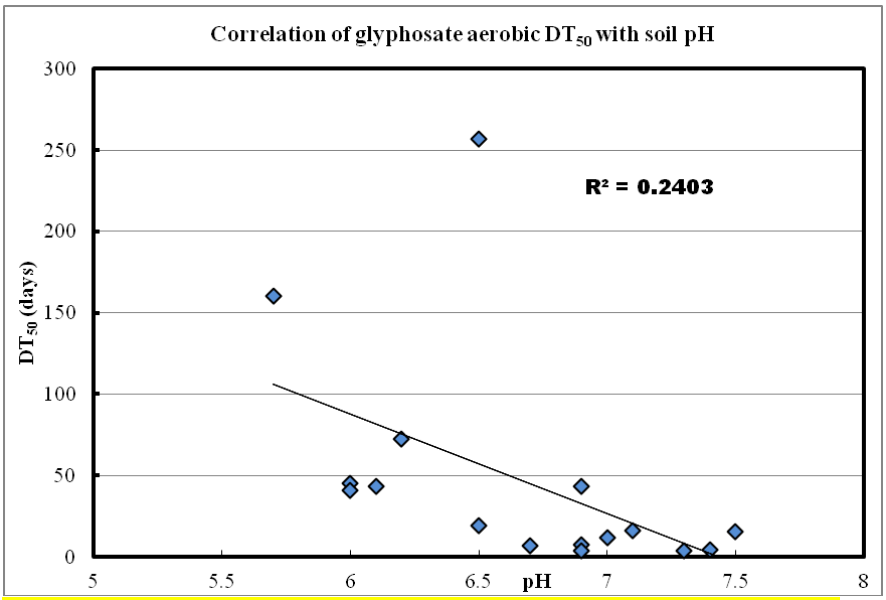


Figure B. 8.1-4: Correlation of Glyphosate DT_{50} with soil pH

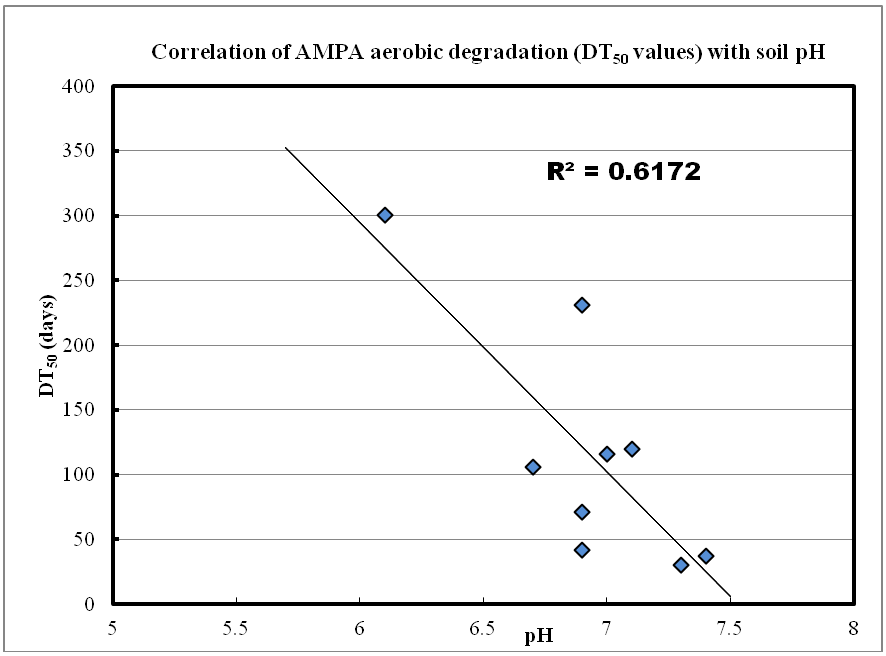


Figure B. 8.1-5: Correlation of AMPA DT_{50} with soil pH (include all data)

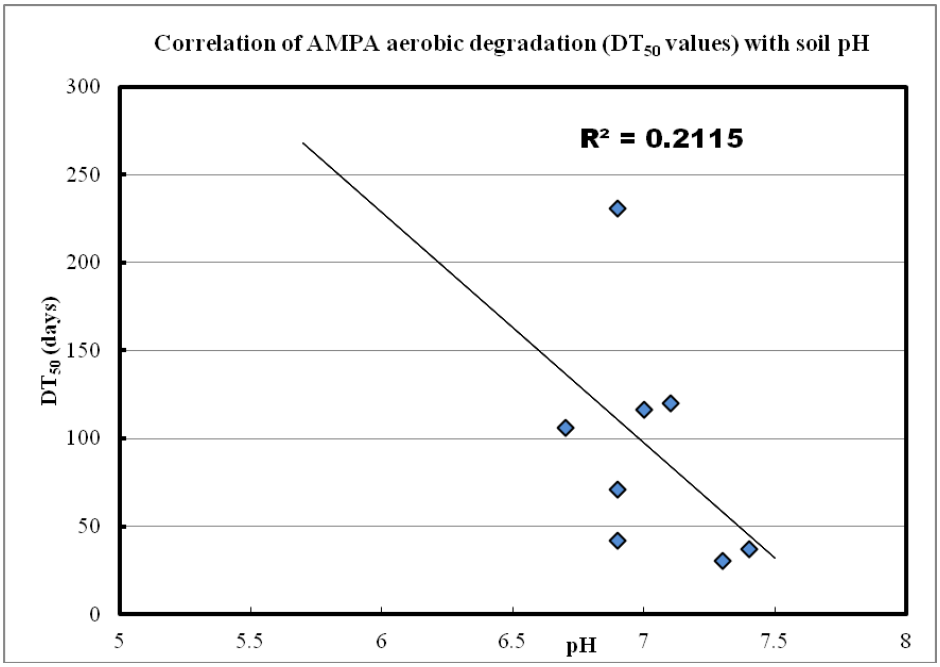


Figure B. 8.1-6: Correlation of AMPA DT_{50} with soil pH (exclude the outlier DT_{50})

Degradation Route(s) of Glyphosate: It has been suggested that the pH dependency of CO_2 formation in the aerobic route of degradation studies of glyphosate may support the notion that the route of degradation of glyphosate differs in various soils depending on the soil pH. In theory, GTF agrees that it is conceivable that differential rate of mineralisation may signify differing glyphosate degradation pathway. However, GTF believes that this possibility is highly unlikely for reasons discussed below.

If glyphosate were to mineralize by different and competing degradation routes, then the expectation is that different degradation products should be detected depending on soil pH. We point out however, that all aerobic route of degradation studies were conducted using ^{14}C-labelled glyphosate with excellent accountability for mass balance. Consequently, we would have detected any potential degradation products other than AMPA in these studies which we did not. The results of route of degradation studies have consistently shown that the only significant soil metabolite of glyphosate was AMPA and no other single metabolite has been detected in an amount greater than 2.3 % of the applied glyphosate.

Figure B. 8.1-7 shows highlights nearly all possible metabolic transformation of glyphosate we could envision and provides the potential degradation product(s). If the site of degradation of glyphosate is through breakage of bond #1, N-methyl AMPA is expected as the degradation product. We are familiar with the chromatographic behavior of N-methyl AMPA since it is an impurity in the technical glyphosate production. N-methyl AMPA is a relatively stable molecule, however, we did not detect significant amount of N-methyl AMPA in any of the study. Therefore this pathway is not a significant route for degradation of glyphosate in the environment.

Degradation of glyphosate via the breakage of bond #2 results in the formation of AMPA and glyoxylic acid. It is well established that glyoxylate in the environment is relatively short lived and can be readily metabolized (Mahler et al,, 1966) via the glyoxylate and citric acid cycles. Since significant amount of AMPA is always detected from glyphosate degradation in

soil, we proposed that the route of degradation depicted via this pathway most likely occurs in the environment. Additionally, isolated soil bacteria *Flavobacterium* sp.(Balthazoar et al, 1986) and *A. atrocyaneus ATCC 13752 (*Pipke, R., and Amrhein, N. 1988a*)* are capable of degrading glyphosate exclusively via AMPA pathway.

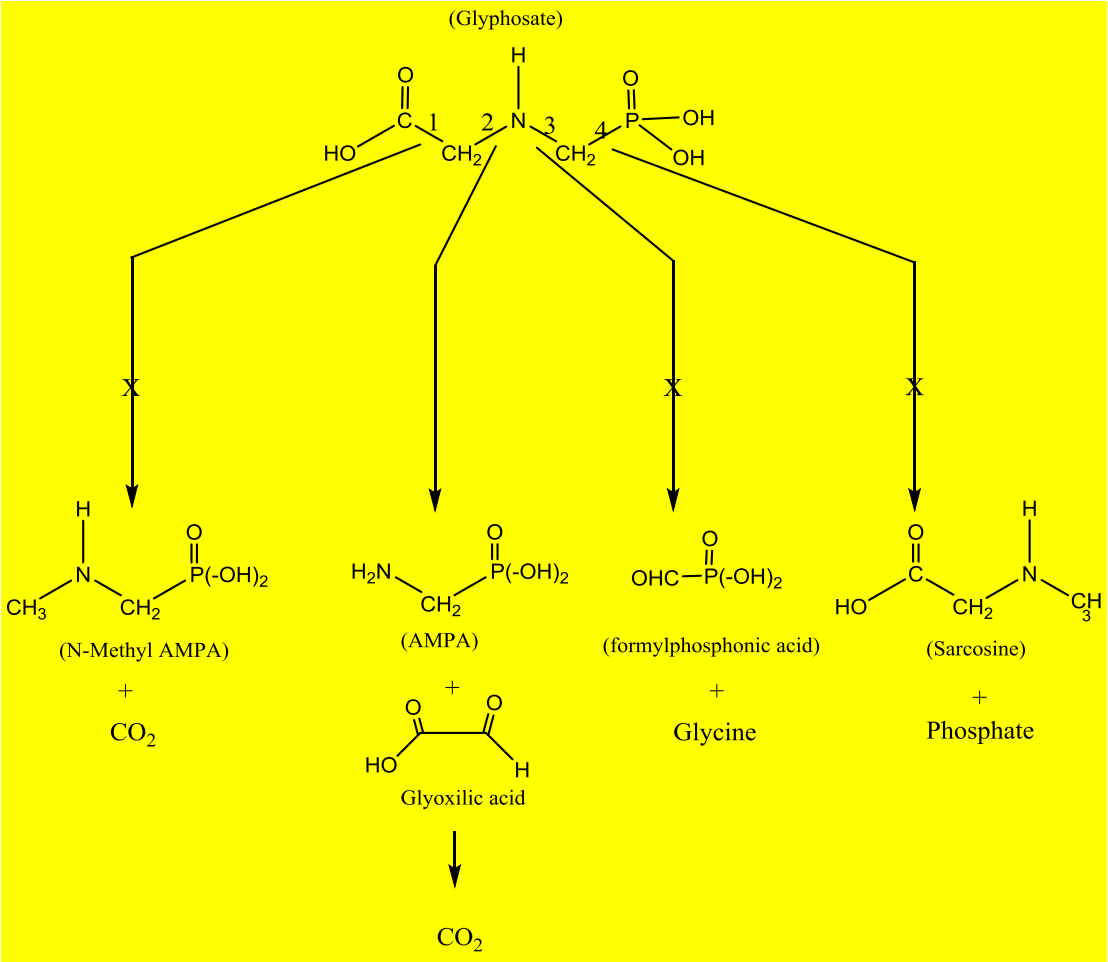


Figure B. 8.1-7: Proposed Degradation pathway of glyphosate

Degradation of glyphosate via the breakage of bond #3 would result in the formation of glycine and formylphosphonic acid which is expected to be transitory and will be most likely oxidized in soil to phosphonoformic acid first and then to $^{14}CO_2$. Glycine should be relatively stable in the environment however, it was not a significant degradation product in the aerobic soil study conducted with the test substance containing the ^{14}C-lable in the glycine portion of glyphosate (Rüppel et al. 1977). Therefore this pathway is most likely not a significant route for degradation of glyphosate in the environment.

Cleavage of the C-P bond of glyphosate (bond #4) to give phosphate and sarcosine, has been observed with isolated soil bacteria in the absence of phosphate (Pipke R, Amrhein N. 1988b). GTF member companies have conducted 10 aerobic route of degradation studies using radiolabeled glyphosate (generally with excellent mass balances) and none of the study has reported sarcosine as a significant degradation product of glyphosate. Lack of sarcosine detection in the GTF studies does not necessarily rule out the sarcosine pathway since one might argue that sarcosine may be labile and conceivably could instantaneously degrade to $^{14}CO_2$ in soil. Based on this assumption one could

postulate that in alkaline soil with higher propensity for mineralization, the sarcosine pathway is the predominant pathway.

Conversely, in acidic soils with less CO_2 production then AMPA pathway should be the predominant pathway. If this was true then there should be a negative correlation between % maximum AMPA detected in each soil (Table B.8.1-2) with the soil pH values. However Figure B. 8.1-8 shows that the maximum AMPA formation is independent of soil pH, invalidating the above postulate.

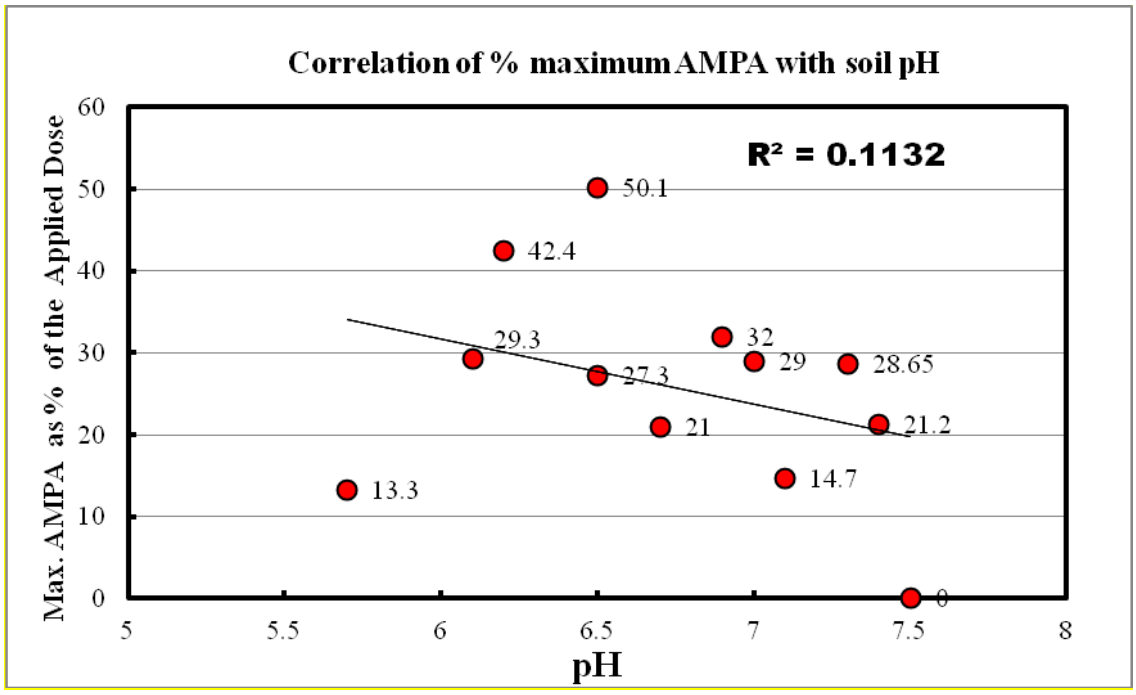


Figure B. 8.1-8: Correlation of % maximum AMPA with soil pH

In conclusion, in terms of environmental regulation and risk assessment, no matter which degradation pathway is occurring, the undeniable fact is that AMPA is the only significant degradation product of glyphosate transformation in soil. Therefore the issue of differing degradation pathway is irrelevant in regulatory terms involving assessing the fate and behavior of glyphosate in the environment and it is only important as an academic interest.

References of GTF statement to pH dependency of degradation:

- Mahler, H. R., Cordes, E. H., "Biological Chemistry", Harper and Row, New York, N.Y.,1966
- Balthazor, T. M., Hallas, L. E. 1986. Glyphosate-degrading microorganisms from industrial activated sludge. Appl. Environ. Microbiol. 51:432-434.
- Pipke, R., and Amrhein, N. 1988a. Degradation of the phosphonate herbicide glyphosate by *Arthrobacter atrocyaneus* ATCC 13752. Appl. Environ. Microbiol. 54:1293-1296
- Rueppel M.L., Brightwell B.B., Schaefer J., Marvel J.T. 1977 Metabolism and degradation of glyphosate in soil and water. J Agric Food Chem 25(3):5 17-528.
- Pipke R, Amrhein N. 1988b. Isolation and characterization of a mutant of *Arthrobacter* sp. strain GLP-1 which utilizes the herbicide glyphosate as its sole source of phosphorus and nitrogen. Appl Environ Microbial 54(11):2868-2870.

RMS Comment:

The argumentation of GTF regarding the pH dependency of degradation rate of glyphosate and AMPA is considered acceptable by the RMS. Additional statistical calculations (Kendall, Pearson correlation coefficient with "R") by RMS support the provided statement of GTF. It is considered, that AMPA is the only significant degradation product of glyphosate transformation in soil. Therefore the issue of differing degradation pathway is irrelevant in regulatory terms and will not be consideredin further risk assessment.

KIIA 7.1.1/1 (Ponte, 2010, BVL no 2310242)

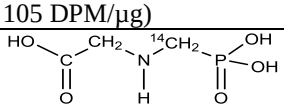
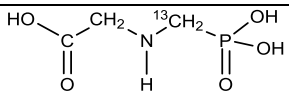
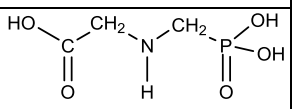
Annex point:	IIA 7.1.1/01
Author(s):	Ponte, M.
Title:	Rate and route of degradation of [^{14}C]-glyphosate in one soil incubated under aerobic conditions
Date:	October 6, 2010
Guideline(s):	U.S. EPA Fate, Transport and Transformation Test Guidelines OPPTS 835.4100, Aerobic Soil Metabolism (adopted October 2008) and OECD Guideline 307 for Testing of Chemicals: Aerobic and Anaerobic Transformation in Soil (adopted April 2002)
Deviations:	In the present study the soil samples treated with [^{14}C]-glyphosate were incubated for up to 132 days. Due to the fact that there was more biomass at the end of the study than in the beginning, the longer incubation time is acceptable.
GLP:	Yes
Acceptability:	Yes

Material and methods

The metabolism of glyphosate, N-(phosphonomethyl)glycine was studied in loam soil (Gartenacker) under aerobic conditions using [^{14}C]-glyphosate. Individual soil samples with moisture contents of 50 ± 10 % of water holding capacity at pF 2.5 were treated with [^{14}C]-glyphosate at a dose rate of 3.8 µg/g soil and incubated in the dark at 20 °C for up to 132 days.

The test materials are characterised in Table B.8.1-6.

Table B.8.1-6: Test materials

	Test material (radiolabelled)	**Test material (radiolabelled)**	**Test material (non-radiolabelled)**
Name	[^{14}C] N-(phosphonomethyl) glycine (glyphosate, specific activity 47 mCi/mmol or 10.28 MBq/mg or 6.17 x 105 DPM/µg)	[^{13}C] N-(phosphonomethyl) glycine (glyphosate, analytical reference standard))	N-(phosphonomethyl)glycine (glyphosate)
Identification			
Purity	HPLC Radiochemical purity: ≥ 96 %	98.0 %	99.8 %
CAS	-	-	1071-83-6

Stability of test compound	The stability of the test substance was confirmed under conditions of the dose administration.	Stored in the refrigerator, expiration date: June 30, 2011	Stored at ambient lab temperature, expiration date: January 31, 2011

Gartenacker field loam soil from Basel, Switzerland was used. Soil was collected from a location with restricted access with no pesticides or organic fertiliser treatments for at least five years prior to collection. Soil physicochemical properties are given in Table B.8.1-7.

Table B.8.1-7: Soil physicochemical properties

Soil origin	**Soil type (USDA)**	**pH [1])**	**CEC (meq/ 100g)**	**OC (%)**	**OM (%)**	**Sand (%)**	**Silt (%)**	**Clay (%)**	**MWHC (%)**	**Moisture (%) at 1/3 bar**	**Bulk density (gm/cc)**	**Bio-mass (µg/g) dry soil**
Basel, Switzer-land	loam	7.1	13.6	2.0	3.5	49	38	13	52.1	21.4	0.91	37.5 (day 0) 71.7 (day 112) 59.2 (day 132)

[1)] pH in 1:1 soil: water ratio

Study design

The degradation of glyphosate in soil was studied following an application rate of 3.8 µg glyphosate/g soil, corresponding to a maximum recommended field rate of 2.88 kg glyphosate/ha, based on a soil density of 1.5 g/cm^3 and a soil depth of 5 cm.
Portions of approximately 63 g of Gartenacker soil (50.00 g dry weight equivalent), adjusted to 50 % ± 10 % of maximum water holding capacity (MWHC) were transferred into incubation bottles. Prior to test substance application, the samples were placed in a constant temperature chamber and allowed to equilibrate in the dark at 20 ± 1 °C under a continuous flow of CO_2-free air for one week.

The dosing solution was prepared by combining an aliquot (7.76 mg) of [^{12}C]-glyphosate standard with an aliquot (8.83 mg) of [^{13}C]-glyphosate standard and an aliquot of [^{14}C]-glyphosate test substance (4.23 mg, 1.2 mCi) in a amber bottle and dissolved in water to a final volume of 54.5 mL. The resulting [^{14}C]-glyphosate treatment solution which had a specific activity of 1.26 × 10^5 DPM/µg was used for dosing. The target dose for all samples was 3.8 µg/g soil.

A total of 28 samples, each containing 50 g of soil (dry weight) and consisting of 4 samples for biomass determination and 24 [^{14}C]-glyphosate treated samples were prepared. Subsequently, samples were connected to the trapping system and maintained at 20 ± 1 °C in the dark, except for the day 0 samples, which were extracted immediately after treatment (time 0). Samples prepared for biomass determination were not dosed with the test substance.
Soil samples were collected immediately after treatment (day 0) and after 3, 6, 10, 20, 34, 55, 90, 112 and 132 days of incubation. At each sampling date, duplicate samples were removed from the constant temperature chamber and then analysed.

The microbial biomass of soil was determined prior to dosing (0 day), during the incubation period (112 days) and at the end of the incubation period (132 days).

Traps for volatiles included an ethylene glycol trap to collect organic volatiles and two 10 % aqueous NaOH traps to collect CO_2. For quantification of volatiles the 10 % NaOH and volatile organic traps were radioassayed directly by Liquid Scintillation Counting (LSC).

Soil samples were extracted with an aliquot (100 mL) of the 0.5 M ammonium hydroxide on a wrist-action shaker for 1 hour followed by centrifugation for 30 minutes at 4000 rpm. The supernatant (soil extract) was decanted and the extraction repeated 2-3 more times with 100 mL of fresh extraction solvent. Prior to analysis, the extracts were pooled and their total volumes and radioactivity measured. The post-extracted soil residues were weighed and aliquots were combusted to determine the levels of unextracted radiocarbon. For the characterisation of post-extraction soil residues, selected post-extracted soil residues were subjected to humic acid/fulvic acid partition following the initial extraction sequence.

Prior to HPLC analysis, soil extracts were concentrated to dryness and re-suspended in pure water by sonication. If concentrated samples appeared cloudy, they were micro centrifuged prior to HPLC analysis. The reconstituted supernatants were analysed by LSC to monitor loss of radioactivity. All samples were extracted on the day of collection, and initial HPLC analysis was performed within 7 days of collection. All samples and standard solutions were frozen (<0 °C) when not in use.

All soil extracts were analysed using cation-exchange HPLC (Transgenomic Glyphosate Analysis Column, 300 x 4.6 mm; flow rate 0.5 mL/min; temperature 40 °C; UV detector 215 nm) eluted with an isocratic solvent [0.005M KH_2PO_4 in HPLC water with 4% methanol (pH 1.80)] for 40 min. Chromatography using an anion-exchange HPLC column (Allsphere SAX, 250 mm x 4.6 mm x 5 µm) eluted with a solvent gradient of 5 mM to 100 mM KH_2PO_4 in HPLC water at pH 2 with methanol (96/4 v/v) were used to confirm the identity of glyphosate and AMPA. The limits of detection for combustion samples were 0.0014 µg/g. The limits of detection for glyphosate and metabolites observed in the HPLC radio chromatogram were 0.003 µg/g.

Results and discussion

Data

Table B.8.1-8: Degradation product distribution over 132 days aerobic incubation of [^{14}C]-glyphosate treated soil (20 °C)

Days after application	% of applied dose							
	Glyphosate	AMPA	D-1[1)]	Others	Bound residues	Organic volatiles	CO_2	Mass balance
0 Rep A	96.6	0.6	0.7	0.1	2.1	NS	NS	100.1
0 Rep B	95.8	0.6	0.4	0.3	2.1	NS	NS	99.2
3 Rep A	71.1	4.3	0.9	0.2	11.8	0.0	9.5	97.8
3 Rep B	69.2	4.6	0.9	0.1	11.7	0.0	9.5	96.0
6 Rep A	58.1	7.0	1.5	0.7	13.4	0.0	16.6	97.3
6 Rep B	56.6	7.2	1.5	0.1	12.9	0.0	15.1	93.4
10 Rep A	44.4	8.2	1.8	0.6	14.2	0.0	22.3	91.5
10 Rep B	43.4	8.0	1.7	0.8	13.6	0.0	23.6	91.2
20 Rep A	33.3	11.0	1.2	0.6	16.8	0.0	34.0	96.8
20 Rep B	29.2	13.7	2.2	0.5	17.2	0.0	33.7	96.5
34 Rep A	17.6	11.5	4.0	2.3	18.8	0.0	43.4	97.6
34 Rep B	18.0	12.7	3.0	1.8	17.2	0.0	40.6	93.3
55 Rep A	10.5	14.9	1.9	1.0	18.1	0.0	48.8	95.2

55 Rep B	9.3	14.5	2.1	1.5	18.1	0.0	46.8	92.3
90 Rep A	4.5	12.1	2.2	0.7	19.9	0.0	55.4	94.7
90 Rep B	4.7	12.3	2.2	0.5	20.8	0.0	52.9	93.4
112 Rep A	3.0	9.9	2.0	0.6	19.7	0.0	58.3	93.6
112 Rep B	3.4	10.2	2.0	0.6	19.7	0.0	54.9	90.8
132 Rep A	2.3	8.8	1.9	1.0	19.1	0.0	60.4	93.5
132 Rep B	2.7	7.8	1.9	1.2	18.7	0.0	59.5	91.7
Average Total % Recovery								94.8
Std. dev. ±								2.8

Rep = Replicate; NS = not sampled

1) Secondary HPLC analysis of the D-1 isolate showed multiple peaks demonstrating the presence of multiple degradates but none represented >1.8 % applied radioactivity.

Mass balance

The material balance was determined as the sum of radiocarbon in soil extracts, volatile traps and residual soil radiocarbon. Radiocarbon mass balance ranged from 90.8 % to 100.1 % and averaged 94.8 ± 2.8 % of the applied dose.

Bound and extractable residues

The amount of extractable radiocarbon steadily declined from 97.6 % AR (applied radioactivity) at time 0 to 13.8 % AR at day 132. Bound residues increased from 2.1% AR at time 0 to a maximum of 20.4 % AR at day 90, and then slightly decreased to 18.9 % AR at day 132. The bound unextracted residues were further characterised by humic acid/fulvic acid partition of selected samples. The results showed that an average of 5.1 % AR partitioned into the humic acid fraction, while 5.9 % AR was associated with the fulvic acids fraction, leaving the majority of the unextracted bound residues associated with the insoluble humin (9.5 % AR).

Volatilisation

For the duration of the study, no organic volatiles were detected. A significant proportion of the parent compound was mineralised and released as radioactive carbon dioxide, which increased to a maximum average of 60.0 % AR at study termination after 132 days of incubation.

Transformation of the parent compound

Under aerobic conditions glyphosate degraded in loam soil, and decreased from 96.2 % AR at day 0 to 57.4 % AR at day 6 and then declined to 2.5 % AR at study termination at day 132.

In addition, the main degradation product AMPA was observed in soil extracts with a maximum of 14.7 % AR (average of 2 replicates) after 55 days of incubation and then declined to 8.3 % AR at day 132. There were other minor degradates present in the soil extracts but none represented >1.8 % AR.

Conclusion

It was found that [^{14}C]-glyphosate degrades in Gartenacker loam soil under aerobic laboratory conditions. The main degradate observed was $^{14}CO_2$ at a maximum amount of 60.0 % AR at the end of the incubation period. The only other significant metabolite observed in soil extracts was AMPA, which represented a maximum of 14.7 % AR at day 55 (average of 2 replicates). No other metabolites are present at of more than 1.8 % of the applied glyphosate.

RMS Comment

According to OECD 307 the rate and pathway studies should normally not exceed 120 days, because thereafter a decrease of the soil microbial activity with time would be expected in an artificial laboratory system isolated from natural replenishment. Longer incubation periods should accompanied by biomass measurements during and at the end of these periods. In the present study the soil samples treated with [^{14}C]-glyphosate were incubated for up to 132 days. Due to the fact that there was more biomass at the end of the study than in the beginning, the longer incubation time is acceptable.

A new kinetic evaluation (Dorn, 2012, BVL no 2315991) according with the guidance of FOCUS (2006, 2001) of the study results is available which is described under B.8.1.2.1. Thus, the DT_{50} and DT_{90} values of glyphosate derived by the study author are not presented here anymore.

KIIA 7.1.1/2 (Goodyear 1996, BVL no 2310246)

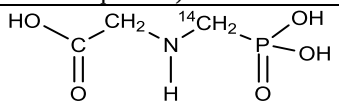
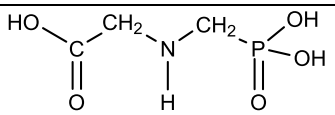
Annex point:	IIA 7.1.1/02
Author(s):	Goodyear, A.
Title:	(^{14}C)-glyphosate: Aerobic Soil Metabolism
Date:	26/4/1995
Guideline(s):	EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 162-1 (October 1982) and Japanese MAFF Guidelines (Jan 1985)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Material and methods

The rate and route of metabolism of (^{14}C)-glyphosate was studied at a dosing rate of 3 mg/kg (equivalent to 3 kg glyphosate acid/ha) under aerobic conditions in a humus volcanic (soil A) and a non-volcanic inorganic soil (soil B). Soil incubations were performed for up to 121 days at 25 ± 2 °C in the dark with a soil moisture of ca. 75 % of moisture holding capacity.

The test materials are characterised in Table B.8.1-9.

Table B.8.1-9: Test material

	Test material (radiolabelled)	**Test material (non-radiolabelled)**
Name	[^{14}C] N-(phosphonomethyl) glycine (glyphosate, specific activity 55 mCi/mmol or 322 µCi/mg or 2.04 GBq/mmol)	N-(phosphonomethyl) glycine (glyphosate)
Structural formula		
CAS No.	1071-83-6	1071-83-6
Purity	99.2 % (by HPLC)	96 %
Storage conditions	Stored in the dark, at ca. -20 °C	Stored refrigerated in the dark

Two soil types were collected, a humus volcanic soil (soil A), obtained from The Japan Association for Advancement of Phyto-Regulators, Iwate Experimental Station, 35 Shizukuishi-

cho, Iwate-gun, Iwate-prefecture, Japan 620-05 and a non-volcanic inorganic soil (soil B), obtained from Fukuoka Prefectural Agricultural Center, Buzen Experimental Station, Nishii-zumi 2-41, Ikuhashi-shi, Fukuoka prefecture, Japan 824. The soil characteristics are presented in Table B.8.1-10.

Table B.8.1-10: Soil characteristics

Soil characteristic	**Soil A humus volcanic**	**Soil B non-volcanic inorganic**
Soil Classification (USDA)	Loam	Sandy loam
Sand (USDA characterisation, %)	46.0	68.3
Silt (USDA characterisation, %)	44.9	16.6
Clay (USDA characterisation, %)	9.1	15.2
Organic carbon (%)	6.8	0.7
Organic matter (%)	11.7	1.2
Cation exchange capacity (meq/100 g)	65.9	11.7
pH in KCl	5.5	6.1
pH in H_2O	5.9	6.7
Microbial biomass (µg C per g soil)[2)]	442 (pre-study) 546 (post study)	214 (pre-study) 229 (post study)
Maximum water holding capacity at 0.33 bar (% w/w of dry soil)	72.1	14.2
Moisture (%) at 1/3 bar	17.6	12.4
2) Determined at Euro Laboratories (Sandy, Bedfordshire, England); all other parameters determined at Levington Agriculture Ltd (Ipswich, Suffolk, England)		

The soil samples were sieved through a 2 mm mesh sieve and stored in the dark, at 4 ± 2 °C for a period of 76 days prior to dispensing into the study units. Following dispensing, the units were maintained at 25 ± 2 °C for an acclimation period of 5 days prior to application of the test compound.

Study design

Twenty four samples (25 g dry weight equivalent for soils) of each of the two soil types were adjusted to a soil moisture content of ca 75 % of 0.33 bar moisture holding capacity. Then, soils were treated with [^{14}C]-glyphosate at a rate of 3 mg/kg (equivalent to to 3 kg glyphosate acid/ha) and incubated for a period of 121 days. A further four control units for each soil type were treated with the non-radiolabelled glyphosate treatment solution.

During incubation, the moist carbon-dioxide free air was pulled into each soil unit and then passed through a series of trapping reagents attached to collect polar volatile (ethandiol), non-polar volatiles (2 % paraffin in xylene) and CO_2 (two 0.1M sodium hydroxide traps).

Soils extracted immediately after test compound application produced poor procedural recoveries. As a consequence, duplicate units of each soil type were re-dispensed and treated with [^{14}C]-glyphosate.

Duplicate sample units were removed for analysis at intervals of 0 (immediately after treatment), 1, 3, 7, 14, 30, 63, 90, 121 days after test compound application. The control units were used to determine the biomass at the termination of the study.

After sampling, duplicate soil samples each were extracted consecutively four times by shaking in the presence of 0.5 M aqueous ammonia solution (140 mL in total). The liquid extracts were separated from the soils by centrifugation, pooled, and radioactivity was determined by LSC. Remaining solid residues were further extracted by shaking once with ~100 mL of acetone and the solvent extract was similarly separated by centrifugation and analysed by LSC. The remaining solid soil residues were dried and the radioactivity present determined by LSC

following combustion. The limit of detection for liquid scintillation counting data was taken as 1.5 times the background radioactivity, determined by counting similar volumes of blank solvent, in the same batch as the samples.
The ammonia extract from each soil was analysed without further preparation, using TLC and HPLC chromatography. Non-radiolabelled glyphosate and its metabolite AMPA were co-chromatographed with each sample.
Further characterisation of glyphosate and AMPA in soil extracts was performed by using mass spectrometry. Three samples were analysed (soil A, 0 hour, replicate C; soil A, 60 day, replicate B; soil B, 7 day, replicate A).
The unextracted residues remaining in post-extracted samples from both soil types, incubated for 1, 14 and 90 days, were exhaustively extracted to determine the distribution of radioactivity between humin, humic acids and fulvic acids. In addition the fulvic acid fractions were partitioned against dichloromethane to distinguish between organic and aqueous soluble fulvic acids.
The presence of CO_2 in sodium hydroxide traps was confirmed by precipitation of insoluble barium carbonate following the addition of barium chloride solution. Typically, a sub-sample (ca 1 g) was removed from the two traps and combined for each sample in respective centrifuge tubes. A saturated solution of barium chloride was added drop wise to each tube until no precipitation formed. The tubes were then centrifuged and aliquots of the resulting supernatant were removed and the radioactivity present quantified by LSC.

Results and discussion

Data

The recovery of the radioactivity and the distribution of glyphosate and its metabolites in both soils are given in Table B.8.1-11, Table B.8.1-12 and Table B.8.1-13.

Table B.8.1-11: Recovery of applied radioactivity from soil A and B (% of applied dose)

Time (day)	Replicate	Soil A humus volcanic			Soil B non-volcanic inorganic		
		Bound residues	CO_2	Mass balance	Bound residues	CO_2	Mass balance
0	A	65.1	NA	99.0	3.5	NA	100.3
	B	63.1	NA	98.9	3.6	NA	99.2
1	A	72.2	0.5	101.1	11.1	19.3	100.3
	B	71.1	0.4	99.8	11.9	19.0	98.9
3	A	68.8	0.9	98.3	14.3	31.6	101.0
	B	70.8	0.9	98.6	14.7	30.1	98.8
7	A	69.9	1.5	98.8	14.6	40.8	98.8
	B	72.3	2.2	101.3	15.1	38.8	97.2
14	A	71.7	2.1	98.7	15.3	51.2	101.8
	B	73.5	2.2	100.3	16.0	45.7	98.1
30	A	77.5	3.0	100.8	13.0	58.9	102.5
	B	78.2	3.0	100.7	12.6	60.2	101.9
63	A	72.6	3.7	96.3	12.6	68.2	101.7
	B	74.3	4.0	99.8	12.1	68.7	102.0
90	A	80.3	4.5	105.0	12.3	70.4	101.4
	B	82.6	4.4	106.6	12.3	70.7	101.1

121	A	76.6	4.5	99.8	11.7	73.4	101.9
	B	77.2	4.8	100.9	13.1	67.7	100.3

Table B.8.1-12: Distribution of glyphosate and its metabolites in soil A -TLC and HPLC analysis (% of applied dose)

Time (day)	Replicate	Soil A humus volcanic					
		TLC analysis			HPLC analysis		
		Glyphosate	AMPA	Unknowns	Glyphosate	AMPA	Unknowns
0	A	32.0	0.9	ND	30.5	3.2	ND
	B	33.3	1.2	ND	33.7	2.0	ND
1	A	27.1	0.5	ND	25.3	2.3	ND
	B	26.6	0.6	ND	26.7	1.2	ND
3	A	27.2	0.8	ND	27.5	1.0	ND
	B	25.6	0.6	ND	25.7	0.8	ND
7	A	25.9	0.6	ND	26.0	1.2	ND
	B	24.7	0.9	ND	25.3	1.1	ND
14	A	22.8	0.8	ND	24.0	0.7	ND
	B	23.0	0.6	ND	23.8	0.7	ND
30	A	18.0	1.0	ND	19.2	0.8	ND
	B	17.6	0.9	ND	18.6	0.7	ND
63	A	18.0	0.9	ND	18.6	1.2	ND
	B	19.0	1.1	ND	19.9	1.5	ND
90	A	17.9	1.2	ND	19.0	1.1	ND
	B	17.2	1.2	ND	18.7	0.9	ND
121	A	14.5	1.7	1.7	17.2	1.3	ND
	B	15.3	1.6	1.2	17.7	1.1	ND

ND = Not detected (defined as less than 0.05 % of applied radioactivity)

Table B.8.1-13: Distribution of glyphosate and its metabolites in soil A -TLC and HPLC analysis (% of applied dose)

Time (day)	Replicate	Soil B non-volcanic inorganic					
		TLC analysis			HPLC analysis		
		Glyphosate	AMPA	Unknowns	Glyphosate	AMPA	Unknowns
0	A	93.6	2.7	ND	92.8	3.6	ND
	B	92.4	2.5	ND	92.9	2.3	ND
1	A	56.2	12.7	ND	44.7	20.6	2.7
	B	55.3	11.6	ND	45.6	19.1	1.7
3	A	38.8	15.7	ND	34.4	17.4	2.7
	B	38.7	14.9	ND	34.0	17.3	2.0
7	A	21.4	18.9	2.1	18.6	22.6	1.9
	B	20.5	19.4	2.6	18.6	22.1	2.3
14	A	11.2	21.2	2.4	11.4	19.7	3.1
	B	13.6	19.5	2.4	13.5	19.7	3.0
30	A	6.6	20.8	2.3	6.0	21.9	2.3

	B	5.7	20.0	2.5	5.0	21.3	2.0
63	A	2.2	15.6	2.0	13.3	7.3	ND
	B	2.1	16.0	1.9	2.6	16.3	1.3
90	A	1.3	14.5	1.5	2.9	14.4	1.4
	B	1.5	13.3	1.6	2.6	13.9	1.1
121	A	1.9	10.8	3.3	1.8	13.0	1.6
	B	2.1	13.5	3.4	2.0	15.8	1.4

ND = Not detected (defined as less than 0.05 % of applied radioactivity)

Mass balance

The mass balance was calculated from soil extractable radioactivity, soil combusted residue and volatile products. In both soil types a mean recovery of greater than 98.0 % of the applied radioactivity was found for each treatment group at each sampling interval.

Bound and extractable residues

In soil A, the major proportion of the radioactivity applied was detected as unextracted residue. Immediately after application, a mean level of 64.1 % of the AR was observed in the unextracted fraction. During incubation this figure increased, reaching a mean of 76.9 % of AR after 121 days.

In soil B, a mean of 96.2 % of the radioactivity was extractable and a mean of 3.6 % AR remained as unextracted residue. Throughout the incubation period, the amounts of unextractable radioactivity increased, reaching a mean level of 12.4 % AR after 121 days.

The unextracted residues from each soil type at selected sampling intervals were fractionated into humin, humic acids and fulvic acids. A significant proportion of the residue in each soil type remained unextracted (humin fraction) with the remainder distributed between humic and fulvic acids fractions. In soil A, the majority of radioactivity was present in the humic acid fraction; however, losses of radioactivity were observed which appeared to be associated with material bound to fulvic acids. In soil B, the major proportion of radioactivity was present in the fulvic acid fraction, however, no losses of radioactivity were observed.

Volatilisation

In soil A, the levels of formed CO_2 were low, with a maximum mean level of 4.6 % of the applied radioactivity observed after 121 days of incubation. The formation of CO_2 from soil B was high with a mean of 19.1 % AR after 1 day to a mean of 70.6 % AR after 121 days. No other volatile degradation products were detected.

Transformation of the parent compound

Throughout the incubation period, the majority of radioactivity detected in soil A was attributed to glyphosate. The overall amount of glyphosate declined during the incubation period, however, the results altered only in relation to the declining amounts of radioactivity extracted. Immediately after application of radiolabelled test compound, mean levels of 32.6 % of AR (TLC) and 32.1 % of AR (HPLC) were detected as glyphosate. After 121 days incubation, the amount of glyphosate declined to mean levels of 14.9 % of AR (TLC) and 17.5 % of AR (HPLC). The remainder of the radioactivity was attributed to the metabolite AMPA, which remained virtually constant throughout the incubation period at values ranging between 0.5 % and 1.6 % AR of AMPA detected by TLC, and 0.7 % and 2.6 % AR of AMPA detected by HPLC. No unidentified metabolites were detected.

In soil B, the amount of applied radioactivity detected as glyphosate declined rapidly during the incubation period with corresponding increases in AMPA. Immediately after application of radiolabelled test compound, mean levels of 93.0 % (TLC) and 92.9 % (HPLC) of the applied

radioactivity were observed as glyphosate. Afterwards, the amounts of glyphosate declined to mean levels of 2.0 % of AR (TLC) and 1.9 % of AR (HPLC) after 121 days. The amount of AMPA initially increased from a mean of 2.6 % of AR (TLC) and 3.0 % of AR (HPLC) at the beginning of the test up to a mean of 20.4 % of AR (TLC) and 21.6 % of AR (HPLC) after 30 days of incubation. Thereafter, the amount of AMPA decreased to mean levels of 12.1 % of AR (TLC) and 14.4 % of AR (HPLC) after 121 days of incubation. In addition in the soil B samples, a total of three unidentified metabolites were also observed by TLC and HPLC methods, with total amounts not exceeding 3.5 % of AR in the test system. The level of each individual unidentified metabolite was typically less than 2 % of AR detected by TLC and HPLC methods.

Conclusion

The rate and route of metabolism of (^{14}C)-glyphosate was studied under aerobic conditions in a humus volcanic (soil A) and a non-volcanic inorganic soil (soil B).

In soil A, glyphosate decreased from 32.6 % of AR (TLC) and 32.1 % of AR (HPLC) at the beginning of the study to 14.9 % of AR (TLC) and 17.5 % of AR (HPLC). Maximum amount of the metabolite AMPA of 1.6 % AR (TLC) and 2.6 % AR (HPLC) were formed at the end of the study. CO_2 formation was low, with a maximum mean level of 4.6 % of AR, while high amounts of 76.9 % of AR of unextractable residues were measured after 121 days.

In soil B, glyphosate decreased from 93.0 % (TLC) and 92.9 % (HPLC) at the beginning of the study to 2.0 % of AR (TLC) and 1.9 % of AR (HPLC). Maximum amount of the metabolite AMPA of 20.4 % of AR (TLC) and 21.6 % of AR (HPLC) were formed after 30 days of incubation with subsequent decline. CO_2 formation was high, with a maximum mean level of 70.6 % AR after 121 days, while 12.4 % of AR AR of unextractable residues were measured after 121 days.

RMS Comment

The study was performed according to guideline and is considered acceptable by the RMS. However, the soil A has a very high organic carbon content of 6.8 % and is not typical for soils under agricultural use. Since the use of such a soil is not recommended by the more recent guideline OECD 307 (April 2002), the results of this soil A will not be used for further assessment.

A new kinetic evaluation (Dorn, 2012, BVL no 2315991) according with the guidance of FOCUS (2006, 2001) of the study results is available which is described under B.8.1.2.1. Thus, the DT_{50} and DT_{90} values of glyphosate derived by the study author are not presented here anymore.

KIIA 7.1.1/3 (McLaugin & Schanné, 1996, BVL no 2310250)

Annex point:	II7 7.1.1/03
Author(s):	McLaughlin, S. Schanné, C.
Title:	[^{14}C]-Glyphosate: determination of soil degradation, biotransformation and metabolism under aerobic conditions
Date:	14/06/1996
Guideline(s):	SETAC- Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, 1995; annex of FAO revised guidelines on environmental criteria for the registration of pesticides, and on the BBA Guideline Part IV, 4-1
Deviations:	None

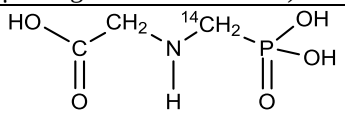
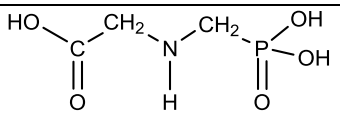
GLP: Yes
Acceptability: Yes

Material and methods

The aerobic degradation of (^{14}C)-glyphosate was investigated at a dosing rate of 3.11 mg/kg (equivalent to 2.3 kg glyphosate /ha) at 20 °C in one sand soil (Speyer 2.1) and two loamy sand soils (Speyer 2.2 and Speyer 2.3) and additionally at 10 °C in one loamy sand soil (Speyer 2.3.). Soil incubations were performed up to 120 days at a soil moisture of 40 % MWHC.

The test materials are characterised in Table B.8.1-14.

Table B.8.1-14: Test material

	Test material (radiolabelled)	**Test material (non-radiolabelled)**
Name	[^{14}C] N-(phosphonomethyl)glycine, (glyphosate, specific activity 316 µCi/mg or 54.0 mCi/mmol)	N-(phosphonomethyl) glycine (glyphosate)
Structural formula		
CAS No.	1071-83-6	1071-83-6
Purity	99.2% (by HPLC)	99.2%
Stability/ Storage conditions	Proven by HPLC	Storage at 4 °C

The fresh field soils from Germany Speyer 2.1 (sand), Speyer 2.2 (loamy sand, high in organic carbon) and Speyer 2.3 (loam sand, low in organic carbon) were used. The soil characteristics are presented in Table B.8.1-15.

Table B.8.1-15: Soil characteristics

Parameter	**Speyer 2.1**	**Speyer 2.2**	**Speyer 2.3**
Soil Classification (DIN)	Sand	Loamy Sand	Loamy Sand
Sand (% >63 µm)	88.4	81.2	60.9
Silt (%, 2 to 63 µm)	9.8	13.4	29.6
Clay (%, <2 µm)	1.9	5.5	9.5
Organic carbon (%)	0.62	2.32	1.22
Cation exchange capacity (meq/100g dry soil)	5.0	10.9	10.2
pH in 0.01 M $CaCl_2$	5.9	5.6	6.4
MWHC (g/100 g dry soil)	31	48	39
Pre-study Microbial biomass (µg C per g dry soil)	90	71	89 (20 °C) 89 (10 °C)
Post-study Microbial biomass (µg C per g dry soil)	210	246	173 (20 °C) 123 (10 °C)

The soil samples were sieved through a 2 mm mesh sieve and stored under test conditions in closed plastic boxes.

Study design

Twelve samples (100 g dry weight equivalent) of each soil and temperature combination were adjusted to a target soil moisture content of 45 ± 2% of maximum water holding capacity. Soil moisture was checked every 7 to 14 days throughout the study and loss of water was compensated by adding demineralised water followed by thorough mixing of the soil. The temperature was kept constant at a target temperature of 20 °C ± 2 °C (Speyer 2.1, Speyer 2.2 and Speyer 2.3) and at 10 °C ± 2 °C (Speyer 2.3).
[^{14}C]-glyphosate, was incorporated into the soils at 3.11 mg/kg dry soil. This corresponds to a field rate of 2.3 kg/ha assuming penetration of glyphosate into the top 5 cm layer of soil and a bulk density of 1.5 g/cm^3.
Six untreated samples of each soil and temperature combination were incubated under the same temperature and moisture conditions as the treated samples for determination of microbial biomass at the end of the study.
During incubation, moist carbon-dioxide free air was pulled into each soil unit and then passed through a series of trapping reagents attached to collect organic volatiles (ethylenglycol) and CO_2 (0.5M sodium hydroxide).
Samples were taken immediately after dosing and after 1, 2, 4, 7, 15, 29, 60 days (Speyer 2.3, 20 °C and 10 °C) and in addition after 90 days (Speyer 2.1) and after 120 days (Speyer 2.2).

Soil samples were extracted consecutively 3 times with 125 mL portions of 0.35 M H_3PO_4/0.09 M $CaCl_2$ per 50 g dry weight soil. Soils were extracted at room temperature at a soil to solvent ratio of approximately 1:2.5 (w:v). This was done by shaking the samples in an overhead shaker at about 60 rpm. After centrifugation of each individual extract, extraction efficiency was determined by LSC. After exhaustive solvent extraction, extracts were pooled and extraction efficiency was determined. Extracts were analysed qualitatively and quantitatively by HPLC via direct injection.
Non-extractable radioactivity was measured by post-extraction combustion followed by radioassay.
The presence of CO_2 in sodium hydroxide traps was confirmed by precipitation of insoluble barium carbonate following the addition of barium chloride solution.

Results and discussion

Data

The recovery of the radioactivity and the distribution of glyphosate and its metabolites in the soils Speyer 2.1, 2.2 and 2.3 at 20 °C are given in Table B.8.1-16, Table B.8.1-17 and Table B.8.1-18. The recovery of the radioactivity and the distribution of glyphosate and its metabolites in the soils Speyer 2.3 at 10°C is given in Table B.8.1-19.

Table B.8.1-16: Material balance and degradation product pattern of [^{14}C]-glyphosate in soil Speyer 2.1 at 20 °C

Radioactive residues (%)		Incubation time (days)								
		0	1	2	4	7	15	29	60	90
Volatiles	CO_2	ND	5.1	7.4	12.8	17.9	23.2	32.4	39.4	43.0
	Organic volatiles	ND	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Total	ND	5.1	7.4	12.8	17.9	23.2	32.4	39.4	43.0
Extracta-bles	Glyphosate	96.0	84.8	74.3	59.2	53.9	38.2	21.0	8.5	2.2
	AMPA	1.3	12.1	12.9	25.1	27.3	27.5	37.9	42.3	50.1
	Total	97.2	97.0	87.2	84.4	81.2	65.7	58.9	50.8	52.3
Bound Residues		0.5	0.7	1.0	1.1	1.7	1.7	2.0	1.9	2.5
Mass Balance		97.7	102.7	95.6	98.2	100.8	90.7	93.3	92.1	97.8

Table B.8.1-17: Material balance and degradation product pattern of [^{14}C]-glyphosate in soil Speyer 2.2 at 20 °C

Radioactive residues (%)		Incubation time (days)									
		0	1	2	4	7	15	29	60	90	120
Volatiles	CO_2	ND	2.8	3.8	7.2	10.5	16.3	22.5	30.6	33.9	36.5
	Organic volatiles	ND	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Total	ND	2.8	3.8	7.2	10.5	16.3	22.5	30.6	33.9	36.5
Extractables	Glyphosate	99.2	96.1	84.2	77.1	71.8	60.3	41.7	26.7	25.9	19.0
	AMPA	3.7	4.3	7.9	12.9	15.7	21.0	34.5	42.4	39.0	40.9
	Total	102.9	100.5	92.1	89.9	87.5	81.3	76.2	69.1	64.9	59.9
Bound Residues		0.9	0.9	1.2	1.2	1.7	1.6	2.2	1.6	3.7	4.9
Mass Balance		103.8	104.1	97.0	98.3	99.7	99.1	100.9	101.4	102.4	101.2

Table B.8.1-18: Material balance and degradation product pattern of [^{14}C]-glyphosate in soil Speyer 2.3 at 20 °C

Radioactive residues (%)		Incubation time (days)							
		0	1	2	4	7	15	29	60
Volatiles	CO_2	ND	12.9	18.6	30.5	38.1	48.2	55.4	63.4
	Organic volatiles	ND	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Total	ND	12.9	18.6	30.5	38.1	48.2	55.4	63.4
Extractables	Glyphosate	91.1	76.2	63.9	34.2	18.4	13.3	<0.1	3.0
	AMPA	7.0	13.0	27.0	25.7	32.0	25.3	31.1	18.5
	Total	98.1	89.3	90.9	60.0	50.4	38.6	31.1	21.5
Bound Residues		1.0	2.0	2.8	2.3	3.6	4.1	4.4	7.8
Mass Balance		98.1	104.2	112.3	92.8	92.2	91.0	90.9	92.7

Table B.8.1-19: Material balance and degradation product pattern of [^{14}C]-glyphosate in soil Speyer 2.3 at 10 °C

Radioactive residues (%)		Incubation time (days)							
		0	1	2	4	7	15	29	60
Volatiles	CO_2	ND	4.8	6.6	12.6	18.9	30.0	40.4	48.2
	Organic volatiles	ND	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Total	ND	4.8	6.6	12.6	18.9	30.0	40.4	48.2
Extractables	Glyphosate	93.6	87.3	80.0	62.2	54.9	35.9	21.7	7.5
	AMPA	4.7	8.7	9.2	19.3	22.1	25.8	28.7	34.3
	Total	98.3	96.1	89.2	81.6	76.9	61.7	50.4	41.8
Bound Residues		1.1	1.5	1.6	1.7	2.3	3.6	3.9	2.4
Mass Balance		99.3	102.4	97.4	95.9	98.2	95.3	94.7	92.4

Mass balance
The total recovery level under aerobic conditions ranged from 90.7 %.to 112.3 % AR in Speyer 2.1, Speyer 2.2 and Speyer 2.3 soil at 20 °C, respectively, and from 92.4 % to 102.4 % AR in Speyer 2.3 soil incubated at 10 °C.

Bound and extractable residues
At begin of the study, almost complete extraction of radioactivity was observed with 97.2 % to 102.9 % of AR in the extracts. Thereafter, a decrease of extractable radioactivity was observed in all soils. At the end of the experiments, extractable radioactivity accounted for 52.3 % of AR (Speyer 2.1 soil, day 90), 59.9 % of AR (Speyer 2.2 soil, day 120), 21.5 % of AR (Speyer 2.3 soil incubated at 20 °C, day 60) and 41.8 % of AR (Speyer 2.3 soil incubated at 10 °C, day 60). Non-extractable radioactivity accounted for 2.5 % of AR (Speyer 2.1 soil), 4.9 % of AR (Speyer 2.2 soil), 7.8 % of AR (Speyer 2.3 soil incubated at 20 °C), and 2.4 % of AR (Speyer 2.3 soil incubated at 10 °C) at the end of the studies.

Volatilisation
High amounts of CO_2 were formed in all soils throughout the study. Total mineralisation during sample incubation at 20 °C were 63.4 % of AR within 60 days in the Speyer 2.3 soil, 43.0 % of AR within 90 days in the Speyer 2.1 soil and 36.5 % of AR within 120 days in the Speyer 2.2 soil. AT 10 °C, mineralisation of glyphosate in the Speyer 2.3 soil was slightly delayed with 48.2 % of AR CO_2 production after 60 days. Organic volatiles contributed less than 0.1 % of AR in all soils and 20 °C and at 10 °C.

Transformation of the parent compound
Throughout the incubation period at 20 °C, glyphosate declined quickly in all soils with 2.2 % of AR after 90 days in the soil Speyer 2.1, 19.0 % of AR after 120 days in the soil Speyer 2.2 and 3.0 % of AR after 60 days in the soil Speyer 2.3 at the end of the study. At 10 °C, the decrease of glyphosate was slightly delayed with 7.5 % of AR still in the soil Speyer 2.3 at the end of the study after 60 days.
The soil metabolite AMPA was formed in concentrations >10 % of AR in all soils independent of the incubation temperature. In the soils Speyer 2.1 and Speyer 2.2, the formation of AMPA increased until the end of the incubation period reaching maxima of 50.1% and 40.9 % of AR after day 90 and 120, respectively. In the soil Speyer 2.3, AMPA reached a maximum of 32.0 % of AR after day 7 with a subsequent slow decrease to 18.5 % of AR after day 60 at 20 °C. At 10 °C, similar concentrations of AMPA was measured with 34 % of AR after

60 day at the end of the study without a decrease throughout the incubation period. No other soil metabolites were detected in the HPLC radiochromatograms.

Conclusion
The degradation rate of (^{14}C)-glyphosate was studied under aerobic conditions at 20 °C in three soils one sand soil (Speyer 2.1) and two loamy sand soils (Speyer 2.2 and Speyer 2.3). Additionally, one incubation at 10 °C was performed in the loamy sand soil Speyer 2.3.
Main degradation products of glyphosate were CO_2 and the soil metabolite AMPA. Between 36.5 % and 63.4 % of applied radioactivity was mineralised to CO_2, depending on soil type and incubation temperature. AMPA was characterised as the major radioactive component in soil extracts and found to be present at levels ranging from 18.5 % to 50.1 % of the applied glyphosate. Soil bound residues amounted to maximum of 7.8 % of AR.

RMS Comment
The study was performed according to guideline and is considered acceptable by the RMS.

A new kinetic evaluation (Dorn, 2012, BVL no 2315991) according with the guidance of FOCUS (2006, 2001) of the study results is available which is described under B.8.1.2.1. Thus, the DT_{50} and DT_{90} values of glyphosate derived by the study author are not presented here anymore.

KIIA 7.1.1/4 (Esser, 1996, BVL no 2310248)

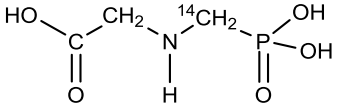
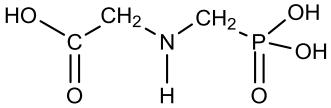
Annex point:	IIA 7.1.1/04
Author(s):	Esser, T.
Title:	[P-Methylene-^{14}C]glyphosate acid: aerobic soil metabolism
Date:	11/07/1996
Guideline(s):	EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 162-1 (October 1982)
Deviations:	None
GLP:	Yes
Acceptability:	No -the incubation was performed under an atmophere of pure oxygen instead of CO_2 free air thus possibly accelerating glyphosate degradation

Material and methods
The rate and route of metabolism of [^{14}C]-glyphosate acid was studied under aerobic conditions in the dark in a sandy loam soil (Visalia) over a period of 31 days at 24.9 ± 0.8 °C and a soil moisture of 75 % of the water holding capacity at 1/3 bar. Glyphosate was applied at a rate of 4.74 ± 0.15 ppm, equivalent to 4 lb/acre.

The test materials are characterised in Table B.8.1-20.

Table B.8.1-20: Test material

	Test material (radiolabelled)	**Test material (non-radiolabelled)**
Name	[^{14}C] N-(phosphonomethyl)glycine (glyphosate acid (PMG), specific	N-(phosphonomethyl) glycine (glyphosate)

	activity 42.7 mCi/mmol or 561000 dpm/µg)	
Structural formula		
CAS No.	1071-83-6	1071-83-6
Purity	97.4 % (by HPLC)	99.2 %
Stability	After its use in the study the stability of the test substance under the conditions of administration was determined by HPLC (Purity 97.22 %). The test substance was stored in a freezer at < 0 °C	The reference substance was stored in a freezer at < 0 °C. The stability was periodically examined by HPLC over the course of the study and the reference standard was found to be stable.

A sandy loam soil from Visalia, CA was used. The soil characteristics are presented in Table B.8.1-21.

Table B.8.1-21: Soil characteristics

Parameter	**Visalia**
Soil Classification	Sandy Loam
Sand (%)	71.2
Silt (%)	20.0
Clay (%)	8.8
Organic carbon (%)	0.35
Organic matter (%)	0.60
Cation exchange capacity (meq/100g dry soil)	6.14
pH (buffer soultion unknown)	8.3
Maximum water holding capacity at 0.33 bar (%)	11.92
Bacteria (Tryptic Soy Agar, x 106 CFU per g soil)1)	10.5 (pre-study) 5.0 (end of study, dosed soil)

1) CFU = Colony forming units. In addition to aerobic bacteria, the CFUs were enumerated for actinomycetes and funghi

The sandy loam soil was collected on the 12th of May 1994 at ZENECA's Western Regional Technical Centre (Visalia, CA). The soil samples were sieved through a 2 mm mesh sieve and stored at about 8 °C in an incubator until the experimental start of the study on the 4th of December 1995.

Study design

Following weighing of the soil into biometer test flasks (50 g dry weight equivalent), the units were maintained unsealed at 25 ± 1.0 °C for an acclimation period of 5 days to dry the soils below 75 % of field moisture capacity. Then, the flasks were reweighed, and the necessary volume of deionised water was added individually to each flask just before application to achieve 75 % of water holding capacity at 1/3 bar.

Twenty-four biometer flasks were prepared, including duplicate samples for each sampling date and two extra vessels. [^{14}C]-glyphosate was applied at a test substance concentration of 4.74 ± 0.15 ppm. The flasks were sealed, shaken by hand to mix the dosed soil and, with the exception of the 0 time flasks, placed in an incubator maintained at 25.0 ± 1.0 °C in the dark. The incubated flasks were equipped with a tube containing a foam plug for the trapping of

organic volatiles and connected to a sidearm containing an aqueous 10 % KOH solution (40 mL) to trap $^{14}CO_2$. Each flask was connected via a thistle tube to an oxygen cylinder.
Duplicate sample units were removed for analysis at intervals of 0 (immediately after treatment), 1, 2, 3, 4, 8, 11, 14, 18, 24 and 31 days after application.
The total volumes of the 10 % KOH solutions were measured and aliquots were radioassayed by LSC. Each foam plug was extracted with 20 mL dichloromethane and aliquots thereof were radioassayed.
The soil samples were transferred into centrifuge bottles using 1 M aqueous KH_2PO_4 solution and the pH was adjusted to about 2.0 with concentrated phosphoric acid. The bottles were shaken and the liquid extract was separated from the soils by centrifugation and filtered (0.45 µm). The soil residue was extracted twice more with the same extraction solvent in the same manner. The supernatants of the three extraction steps were combined, the volumes were measured and the radioactivity was determined by LSC. Aliquots were analysed by HPLC without further preparation. The samples taken on day 8 were subjected to a second extraction procedure since the first extraction procedure did not remove the expected amount of radioactivity possibly due to use of the wrong phosphate buffer strength in the extraction solvent.
To minimise losses of CO_2 that may be entrained in the soil, the sampling procedure was modified from day 4 onwards. A magnetic stirring bar was inserted into the flask, the stop cock replaced by a septum, the thistle tube replaced by a septum and the flask connected to additional 10 % KOH traps. The traps were connected to a water aspirator pump. A portion of the extraction buffer was injected into the flask via the septum, while stirring and purging the headspace through the 10 % KOH traps using the water aspirator pump. The trap volumes were measured and aliquots taken for radioassay by LSC.
The presence of CO_2 in the KOH trapping solutions was confirmed by the precipitation of insoluble barium carbonate using the samples taken on day 11. Therefore the radioactivity in the KOH trapping solution was analysed by LSC prior and after overnight precipitation with saturated barium chloride solution. The difference between the radiocarbon in the solution before and after precipitation was calculated to determine the amount of radiocarbon precipitated.
The remaining post-extraction solid soil residues cobusted with subsequent LSC to determine the levels of unextracted radiocarbon.
The combined extracts were analysed by HPLC to determine the amounts of glyphosate and its degradates. Glyphosate and radiolabelled degradates were detected by fraction collection with subsequent LSC and chromatogram reconstruction. Unlabeled reference standards were detected by a refractive index detector.
Analysis of extracts by TLC was performed using pre-coated TLC plates containing fluorescent indicator. After elution, the plates were scanned with an Ambis Radioanalytic Imaging System. The non-radiolabelled reference standards were visualised using a 2 % ninhydrin solution in ethanol. The radioactive spots were than matched against the ninhydrin trace of the standards.
The viability of the soil was evaluated prior to the experimental start date and at the end of the study by enumerating the total colony forming units (CFU) of (1) aerobic bacteria, (2) actinomycetes and (3) funghi.

Results and discussion

Data

The material balance (extraction and recovery of the radioactivity) and the distribution of glyphosate and its degradation products in the soil Visalia are given in Table B.8.1-22 and Table B.8.1-23.

Table B.8.1-22: Recovery of applied radioactivity from soil Visalia (% of applied dose)

Time (day)	Replicate	Material Balance (% of applied dose)			
		Radiocarbon in Soil Extract	Radiocarbon in Post-extracted Soil	$^{14}CO_2$ 1)	Total Recovery
0	A	94.9	1.9	n.d. 2)	96.8
	B	94.7	2.1	n.d.	96.7
1	A	60.1	6.6	27.5	94.2
	B	63.7	7.1	19.5	90.6
2	A	46.1	5.8	38.8	90.7
	B	46.1	5.5	38.5	90.1
3	A	43.4	5.5	43.8	92.7
	B	43.4	5.0	42.2	90.6
4	A	40.0	5.3	49.5	94.8
	B	37.4	5.3	53.7	96.3
8	A	30.6 3)	7.5 4)	55.3	93.3
	B	31.3 3)	7.4 4)	54.8	93.4
11	A	36.2	6.0	58.5	100.7
	B	33.2	6.0	52.2	91.4
14	A	25.8	5.7	56.6	88.1
	B	30.1	5.9	47.3	83.4
18	A	22.3	5.9	17.0	45.3 5)
	B	22.8	6.5	63.9	93.2
24	A	25.9	7.0	63.4	96.3
	B	23.3	6.2	67.0	96.5
31	A	23.1	5.9	67.2	96.2
	B	22.5	5.9	61.4	89.7

1) Radiocarbon found in dichloromethane extracts of foam plugs was considered insignificant (radiocarbon in aliquots < 2 x background)
2) Not determined
3) Total of 2 extractions (% of applied dose): Rep A: 23.1 % +7.5 % = 30.6 %, Rep B: 24.2 % + 7.1 % = 31.3 %
4) After second extraction
5) Loss of KOH trapping solution of replicate A during work up at sampling (possibly into foam plug). This replicate was not used for the calculation of averaged recovery

Table B.8.1-23: Recovery of applied radioactivity from soil Visalia (% of applied dose)

Time (day)	Replicate	Results of HPLC analysis (% of applied dose)			
		Glyphosate	AMPA	Degradate 1	Others
0	A	92.9	1.6	0.0	0.4
	B	93.1	1.6	0.0	0.0
1	A	47.7	12.4	0.0	0.0
	B	52.2	10.9	0.5	0.1
3	A	26.7	18.8	0.3	0.3
	B	26.6	18.8	0.6	0.1
4	A	21.3	21.2	0.8	0.1
	B	21.1	21.4	0.8	0.1
8	A	16.6	22.5	0.8	0.1
	B	12.8	23.4	0.9	0.4
11	A	5.2/2.1[1)]	16.9/5.1[1)]	0.9/0.2[1)]	0.0/0.0[1)]
	B	5.0/2.0[1)]	18.4 /4.9[1)]	0.6/0.2[1)]	0.1/0.0[1)]
14	A	11.2	24.0	0.9	0.1
	B	7.2	24.8	1.1	0.1
18	A	3.3	21.4	1.0	0.1
	B	8.4	20.5	0.9	0.2
24	A	1.9	19.5	0.9	0.0
	B	2.0	20.0	0.9	0.0
31	A	2.0	22.4	1.4	0.0
	B	1.5	20.6	1.2	0.0

1) Two extractions performed. The first extraction probably with diluted phosphate buffer solution and the second with full strength buffer solution

Mass balance

The mean of the total radiocarbon recovery of the duplicate samples ranged from 90.4 % to 96.4 % of AR with the exception of day 14 where only 85.8 % could be recovered. The mass balances obtained for the individual flasks ranged from 90.1 to 100.7 % of AR with exception of day 14 with 88.1 % and 83.4 % AR and one replicate of day 31 with 89.7 % of AR. The losses were possibly due to the leaking of volatile $^{14}CO_2$.

At day 18, a substantial volume of the replicate A KOH solution was lost during work-up at sampling (possibly into the foam plug). This replicate was not used in the calculation of the total recovery.

Bound and extractable residues

Extractable radiocarbon from soil decreased from 94.8 % of AR at day 0 to 22.8 % AR at the end of the study (mean of duplicates). Non extractable residues in the soil increased from 2.0 % of the applied dose at day 0 to a maximum of 7.5 % at day 8 and then decreased to 5.9 % by the end of the study.

Volatilisation

High amounts of CO_2 were formed throughout the study. $^{14}CO_2$ reached a maximum of 65.2 % of the applied dose at day 24 and accounted for 64.3 % at day 31.

Transformation of the parent compound

The amount of the test substance decreased from 93.0 % of the applied dose at day 0 to 1.3 % of the applied dose at the end of the study on day 31 (means of duplicate samples). The major

degradation product detected in the soil extracts was the metabolite AMPA which reached a maximum of 24.4 % at day 11 and remained relatively constant until the end of the study with 20.2 % of AR at day 31. No other significant metabolites ≥ 10 % of the applied dose were detected. An unidentified degradation product with a HPLC retention time of about 6 min. (Degradate 1) reached 1.3 % of the applied dose at day 24 and represented 1.2 % of the applied dose at day 31.

Conclusion

The degradation rate of (^{14}C)-glyphosate was studied over a period of 31 days under aerobic conditions at 25 °C in one sandy loam soil (Visalia).
Glyphosate declined to less than 50 % of the applied radioactivity by the second day of incubation only with 2.0 % at the end at day 31. High amounts of $^{14}CO_2$ were produced with 64.3 % of AR at the end of the study. The major degradation product detected in the soil extracts was AMPA which reached a maximum of 24.4 % at day 11 and remained relatively constant with 20.2 % of AR at day 31. No other significant metabolites ≥ 10 % of the applied dose were detected. An unidentified degradation product reached 1.3 % of the applied dose at day 24. Unextracted radiocarbon reached 7.5 % of the applied dose at day 8 and decreased to 5.9 % towards the end of the study.

RMS Comment

The DT_{50} and DT_{90} values of glyphosate derived by the study author are not presented here anymore since a new kinetic evaluation (Dorn, 2012, BVL no 2315991) according with the guidance of FOCUS (2006, 2001) of the study results is available, which is described under B.8.1.2.1.

However, oxygen was used instead of CO_2-free air for airing the headspace of the test vessels thus creating a best case regarding the supply with oxygen for the soil microbes and possibly accelerating glyphosate degradation. Thus, the study is not considered acceptable by the RMS and the derived DT_{50} and DT_{90} values will not be used for persistence calculations or modelling.

KIIA 7.1.1/5 (Dean, 1995, BVL no 2310244)

Annex point:	IIA 7.1.1/ 05
Author(s):	Dean, G. M.
Title:	Rate and route of degradation of [^{14}C]glyphosate in one soil incubated under aerobic conditions, report no: SNY 333/951445
Date:	01/12/1995
Guideline(s):	Japanese MAFF, US EPA pesticide Assessment Guidelines, Subdivision N, 162-1, German BBA Guidelines for the Official Testing of Plant Protection Products; Part IV, 4-1, Stage 1 and Draft Guidelines Concerning the Inclusion of Active Substances in Annex I to Council Directive 91/414/EEC part 7.1.1.2.
Deviations:	None
GLP:	Yes
Acceptability:	Yes

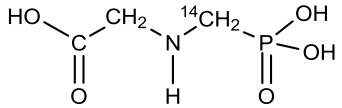
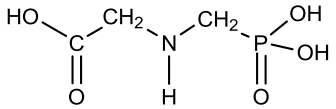
Material and methods

The degradation of glyphosate under aerobic conditions was investigated for 180 days in a sandy loam soil (Arrow) at 20 ± 2 °C in the dark, maintaining a moisture content of 40% of

maximum water holding capacity. Glyphosate was applied at a nominal rate of 10 mg/kg dry weight soil.

The test materials are characterised in Table B.8.1-24.

Table B.8.1-24: Test material

	Test material (radiolabelled)	**Test material (non-radiolabelled)**
Name	[^{14}C] N-(phosphonomethyl)glycine (glyphosate, specific activity 327.7 µCi/mg, 56 mCi/mmol)	N-(phosphonomethyl) glycine (glyphosate)
Structural formula		
CAS No.	1071-83-6	1071-83-6
Purity	Radiochemical purity > 99%	100%
Stability	Stored at 4 °C in the absence of light and air	Stored at < -20°C in the dark, expiry date: 13th of Nov 1996

A sandy loam soil (Soil Series Name Arrow, No. 534, Reference 1 HRC code 1/395A) was supplied by the Soil Survey and Land Research Centre, Shardlow, Derby, UK. The soil characteristics are presented in Table B.8.1-25.

Table B.8.1-25: Soil characteristics

Parameter	**Arrow**
Soil origin	Shardlow, Derby, UK
Soil Classification (ADAS)	Sandy Loam
Sand (% >63 µm)	68.61
Silt (%, 2 to 63 µm)	19.22
Clay (%, <2 µm)	12.18
Organic carbon (%)	2.2
Organic matter (%)	3.8
Cation exchange capacity (meq/100g dry soil)	10.0
pH in 01M $CaCl_2$	5.9
Maximum water holding capacity (%)	37.95
Biomass (µ/g) dry soil	337 (day 0) 337 (day 120) 256 (day 217)

Study design

Samples of sieved soil (58.91 g, equivalent to 50 g soil on a dry weight basis) were weighted into glass vessels and were adjusted to a soil moisture of slightly wetter than 40 % of its maximum water holding capacity. The vessels were incubated for one week prior to test substance application under aerobic conditions in the test apparatus at 20 ± 2 °C.

[^{14}C]-glyphosate was applied to 26 test soil samples at a test substance concentration of of 9.7 mg/kg soil, dry weight. Subsequently, the soils were gently mixed to ensure adequate incorporation and the soil was re-moistened with distilled water to 40 % of its maximum water holding capacity. Six additional vessels were treated with non-radiolabelled glyphosate at a

application rate 10 mg/kg soil, dry weight. Six further vessels were not treated with any test substance and used for determination of biomass.
The test vessels were connected to a trapping system with three consecutively attached traps for organic volatiles (trap 1: ethyl digol) and $^{14}CO_2$ (trap 2: 1 M aqueous potassium hydroxide + phenolphthalein indicator and trap 3: ethanolamine). A polyurethane foam in a lid connector contained in a storage column was used to trap neutral, volatile organic compounds. The use of polyurethane foam plugs were discontinued after 60 days as no radioactivity was being trapped in them.
The test systems were incubated for 180 days in darkness at a mean temperature of 19.9 ± 0.9 °C and a gas flow of approximately 60 mL/min.
Duplicate soil samplings were taken for analysis immediately and 3, 7, 14, 30, 60, 90, 120 and 180 days after test substance application. Soil samples for determination of microbial biomass were taken on the day of application (day 0), 120 days after application and shortly after the end of the test period (217 days).
The polyurethane foam bungs removed at sampling (Day 0 to Day 60 inclusive) were individually extracted with an aqueous solution containing ammonium hydroxide (0.25 M) and potassium dihydrogen phosphate (0.1 M) and aliquots of the extracts were radioassayed using LSC. Aliquots of the trapping solvent/solution were radioassayed.
Duplicate soil samples were analysed separately at each sampling time. Each soil sample was extracted with an aqueous solution containing ammonium hydroxide (0.25 M) and potassium dihydrogen phosphate (0.1 M). Samples were placed in an ultrasonic bath at ambient temperature for 15 minutes and afterwards shaken for 15 minutes at ambient temperature. The extraction procedure was repeated two more times. Additionally, the soil samples were extracted with 100 mL of the same aqueous extraction solution by sonication at 50 °C for 60 minutes and afterwards shaken for 15 minutes at ambient temperature.
After each extraction step the solvent was separated by centrifugation and the radioactivity of the extracts were determined via radioassay. The soil residue was allowed to air dry and the post-extracted soil residue were combusted and taken for radioassay.
Prior to analysis by HPLC and TLC, the soil extracts were pooled together (10 % by volume) and then cleaned-up by passing the combined extracts through strong cation exchange solid phase extraction cleanup. The soil extracts were eluted from the resin with 0.5 M hydrochloric acid (6 mL), concentrated and reconstituted in 5 mM potassium dihydrogen orthophosphate.

Results and discussion

Data

The material balance (extraction and recovery of the radioactivity) and the distribution of glyphosate and its degradation products in the soil Arrow are given in Table B.8.1-26.

Table B.8.1-26: Material balance (extraction and recovery of radioactivity) from soil Arrow and distribution of glyphosate and its degradation products (% of applied dose)

Time (day)	Replicate	Polar compounds	Glyphosate	AMPA	Others[1)]	Bound Resiudes	CO_2	Mass balance
0	A	0.6	92.6	1.0	0.7	2.7	ns	97.6
	B	0.8	91.2	1.1	1.2	2.4	ns	96.6
3	A	0.7	87.0	3.9	0.6	5.9	2.3	100.3
	B	0.8	82.2	3.1	0.6	5.3	2.1	93.6
7	A	1.4	74.0	6.9	1.1	9.0	4.2	96.5
	B	1.5	73.9	6.6	0.9	8.2	4.3	95.3
14	A	1.8	64.2	10.4	0.8	7.6	7.1	91.4
	B	1.2	69.5	8.3	1.1	8.4	7.0	94.8
30	A	2.6	54.0	14.4	1.2	7.6	11.7	91.3
	B	3.0	54.6	13.7	1.4	9.0	11.0	92.6
60	A	3.3	41.1	22.1	1.4	9.6	16.6	94.1
	B	3.0	38.4	22.3	1.4	9.7	15.8	90.4
90	A	2.7	32.5	27.5	0.8	11.3	19.4	94.2
	B	2.3	35.5	25.4	0.8	10.5	18.7	93.1
120	A	2.8	28.1	28.0	0.2	10.0	21.3	90.4
	B	3.0	29.0	26.6	0.4	11.8	20.7	91.5
180	A	4.0	26.5	25.8	0.8	9.4	23.9	90.4
	B	3.9	27.6	25.3	0.6	9.3	23.3	90.0

ns= not sample
1) radioactivity distributed through regions of the chromatogram other than those specified and which did not contain any discrete radioactive peaks

Mass balance
Total recoveries of radioactivity from Arrow soil system after glyphosate treatment were in the range 90.0 – 100.3 %.

Bound and extractable residues
Extractable radioactivity declined from initial 94.6 % AR to 57.3 % AR after 180 days (mean of duplicates). Non-extractable radioactivity initially increased to 8.6 % AR after 7 days and then remained at about this level for up to 180 days where 9.4 % AR were measured.

Volatilisation
Radioactivity was only trapped in the 1 M potassium hydroxide trap and was identified as ^{14}C-CO_2. was formed by precipitiaon with barium chloride. Maximum amount of 23.6 % of AR CO_2 (mean of duplicate samples) were formed after 180 days.

Transformation of the parent compound
After application, glyphosate declined from 91.9 % AR immediately after application to 54.3 % after 30 days and then followed by a slower decline to 27.1 % AR after 180 days of incubation. The main degradation product was characterised as aminomethylphosphonic acid (AMPA) in soil. AMPA accounted for a mean maximum of 27.3 % AR after 120 days, slightly declining to 25.6 % AR at study termination.
On additinal metabolites described by polar compounds was detected, which accounted for a mean maximum of 4 % AR at 180 days after application.

Conclusion

The degradation rate of (^{14}C)-glyphosate was studied over a period of 180 days under aerobic conditions at 20 °C in one sandy loam soil (Arrow).

Glyphosate declined glyphosate declined from 91.9 % AR immediately after application to 54.3 % after 30 days and then followed by a slower decline to 27.1 % AR after 180 days of incubation. Maximum amounts of 23.6 % of AR CO_2 (mean of duplicate samples) were formed after 180 days, while non-extractable radioactivity increased to 9.4 % AR.

The only metabolite in significant concentrations observed in soil extracts was AMPA, which represented a mean maximum of 27.3 % AR after 120 days, slightly declining to 25.6 % AR at study termination. No other metabolites in concentrations above 4 % of AR was observed.

RMS Comment

The study was performed according to guideline and is considered acceptable by the RMS.

A new kinetic evaluation (Dorn, 2012, BVL no 2315991) according with the guidance of FOCUS (2006, 2001) of the study results is available, which is described under B.8.1.2.1. Thus, the DT_{50} and DT_{90} values of glyphosate derived by the study author are not presented here anymore.

KIIA 7.1.1/6 (Kesterson and Atkins, 1991, BVL no 2147433)

Annex point:	IIA 7.1.1/06
Author(s):	Kesterson, L. and Atkins, R.H.
Title:	Aerobic metabolism of [^{14}C]glyphosate in sandy loam and silt loam soils with biometer flask
Date:	January 2, 1991
Guideline(s):	EPA Pesticide Assessment Guidelines Subdivision N, Section 162-1
Deviations:	None
GLP:	Yes
Acceptability:	Partly. The study is considered acceptable by the RMS regarding the results of the incubation of glyphosate in the silt loam soil Dupo. The results of the incubation of glyphosate in the sandy loam soil Kickapoo will not be considered, since the total radioactive recovery was < 90 % for most of the sampling points of this soil.

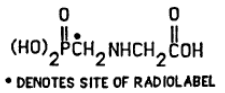
Material and methods

The metabolism of glyphosate was studied in Kickapoo sandy loam and Dupo silt loam under aerobic conditions using [^{14}C]-glyphosate. Soil samples with moisture contents of 75 % of field capacity were treated with [^{14}C]-glyphosate at a dose rate of 4.0 ppm and incubated in the dark at 25 °C for up to 12 months.

The test material is characterised in the following table.

Table B.8.1-27: Test materials

	Test material (radiolabelled)
Name	[^{14}C]glyphosate (specific activity - 3.98 mCi/mmol)

Identification	
Purity	98.8 %
CAS	-
Stability of test compound	Glyphosate was relatively stable under the storage conditions.

The Kickapoo sandy loam soil used for the study was obtained from Madison County, Kentucky. The Dupo silt loam soil was provided by Monsanto Agricultural Company from St. Charles County, Missouri. Soil physicochemical properties are given in the following table.

Table B.8.1-28: Soil physicochemical properties

Soil origin	Soil type (USDA)	pH	CEC (meq/100g)	OM (%)	Sand (%)	Silt (%)	Clay (%)	Field Capacity (%) at 1/3 bar
Kickapoo, USA	sandy loam	7.3	9.0	2.8	68	22	10	21.0
Dupo, USA	Silt loam	7.5	10.7	1.0	24	68	8	18.0

Soil viability was evaluated before study initiation enumerating the total colony forming units (CFU) of aerobic bacteria, actinomycetes and fungi. Aerobic bacteria were enumerated on trypticase soy agar, actinomycetes on actinomycetes isolation agar and fungi on potato dextrose agar. Soil samples were serially diluted in sterile water from 10^{-1} to 10^{-6}. Aliquots (1.0 ml) from the 10^{-3} to 10^{-6} dilutions were then plated in duplicate on nypticase soy agar and actinomycetes isolation agar, and aliquots (1.0 ml) from the 10^{-3} to 10^{-6} dilutions were then plated in duplicate on potato dextrose agar. The culture plates were incubated at 35°C for 1 and 2 days to detect aerobic bacteria and actinomycetes, respectively, and at 25°C for 2 days to detect fungi.

Study design

The test system consisted of test solution and Kickapoo sandy loam or Dupo silt loam soil in each 500 ml biometer flask. Flasks were autoclaved at 15 psi and 121°C for 30 minutes prior to addition of soil. Sterile flasks containing 50 g of soil were autoclaved twice at 15 psi and 121°C for 1 hour. The biometer flasks have a side arm to which was added 50 ml N NaOH, and a side arm connector, in which was placed a polyurethane foam plug. Equilibrium was established in the biometer flasks by way of the humidified oxygen passed through the system which maintained a positive pressure on flasks to accommodate pressure differences realized by the adsorption of $^{14}CO_2$ into the 1N NaOH upon its formation. Flasks were maintained in a dark incubator at 25.0 ± 0.1°C.

Soil was air dried and passed through a 2 mm screen prior to use. Field capacity for the Kickapoo sandy loam and Dupo silt loam was determined. An initial sample of soil was dried to determine moisture content. The appropriate soil weight/water weight ratio for maintenance of 75% of field capacity was determined from these data.

A solution of [^{14}C]glyphosate was prepared in sterile (autoclaved), deionized water such that 4.8 µCi were contained in 2 ml. This solution (2 ml) was evenly applied to 50 g (dry weight) soil contained in each flask. Appropriate volumes of sterile (autoclaved), deionized water were added to moisten the soil to 75% of field capacity. The final concentration of

[^{14}C]glyphosate in soil was 4.0 ppm based on the dry soil weight. Flasks were then stoppered and maintained in total darkness in an incubator maintained at 25.0 ± 0.1°C.

The biometer flasks have a side arm to which was added 50 ml 1N NaOH to trap CO_2. A pre-extracted (acetone) polyurethane foam plug was placed in the side arm connector to trap volatile organic compounds. The NaOH solutions and foam plugs were removed and replaced, if appropriate, at sampling through Month 4, and then NaOH solutions were replaced monthly. Following Month 4, foam plugs were removed at the time the flask was sampled. The foam plugs were extracted with methanol. The extracts and the extracted foam plugs were radio assayed directly.

Duplicate flasks for each soil type were sampled at the following times: 0, 1, 3, 7 and 14 days and 1, 2, 3, 4, 6, 9 and 12 months. Duplicate sterile flasks for each soil type were sampled at the following times: 1, 3 and 6 months. The ^{14}C-residues in soils were extracted and characterized.

At each sampling time soil, NaOH and foam plug were removed from each flask. A subsample of each soil sample was weighed into a Teflon® centrifuge tube and 10 ml[2] of 0.5 N KOH was added. Tubes were then placed on a wrist action shaker for approximately 20 minutes then centrifuged at approximately 10.000 rpm for approximately 10 minutes. Supernatants were decanted and the procedure was repeated twice for a total of three extractions. The extracts were pooled, their volumes were measured and they were radio assayed. Extracted soil for the following samples was allowed to air dry for combustion analysis: Dupo silt loam soil, Day 0B, Day 1A and Day 3A; Kickapoo sandy loam, Day 1B. To remove more tightly bound residues, the remaining samples were extracted once with 1 N KOH using the same procedures. All supernatants were pooled with previous extracts, extract volumes were measured and aliquots were taken for radio assay. Extracted soil was then allowed to air dry prior to combustion analysis. The soil extracts were stored at approximately 4°C until clean-up and analysis by HPLC. Since a suitable analysis method for the soil extracts was not developed until later in the study, extracts were stored for up to 14 months prior to clean-up. Extracts were radio assayed after storage to demonstrate that no losses of radiocarbon occurred during storage.

The total radiocarbon present in each soil sample was determined prior to and following extraction by combustion of five aliquots (approximately 500 mg each) with a Harvey Biological Sample Oxidizer and subsequent radio assay by LSC. Samples were re-homogenized and re-combusted if the relative standard deviation of replicates exceeded 15%. The detection limit was twice background.

The radiochemical purity of [^{14}C]glyphosate was determined by injecting stock solution directly onto a modular liquid chromatograph consisting of a Spectra-Physics SP 8700XR ternary pump equipped with a Whatman Partisil SAX column. The eluting compounds were detected by fraction collection with an ISCO Cygnet Fraction Collector and subsequent radio assay. This system was used to check dosing solution purity and to confirm the HPLC characterization of samples. This system was not suitable for repeated analyses of soil extracts due to poor peak shape and reproducibility, probably due to the soil matrix. A second HPLC system was later developed for analysis of soil extracts after clean-up step. The "cleaned-up" extracts (100 - 150 µL) were injected directly onto a modular liquid chromatograph consisting

of a Spectra-Physics SP 8700 XR ternary pump equipped with a Bio-Rad Glyphosate Analysis Column. The eluting compounds were detected by fraction collection with an ISCO Cygnet Fraction Collector for subsequent radio assay.

Results and discussion

Data

Table B.8.1-29: Accountability of radiocarbon in Kickapoo sandy loam soil

Days/months after application	Volatile traps [%] NaOH	Extract	Extracted soil [%]	Total recovered [%]
0 d Rep A	n.a.	68.1	3.2	71.3
0 d Rep B	n.a.	70.6	3.7	74.3
1 d Rep A	23.5	54.2	4.9	82.6
1 d Rep B	24.6	62.5	7.7	94.8
3 d Rep A	33.6	47.8	7.8	89.3
3 d Rep B	38.3	45.7	5.7	89.9
7 d Rep A	38.8	40.1	7.1	86.0
7 d Rep B	44.4	39.0	6.0	89.4
14 d Rep A	47.2	30.3	9.5	87.0
14 d Rep B	47.3	38.6	6.5	92.4
1 months Rep A	61.0	22.0	7.5	91.5
1 months Rep B	54.1	28.2	7.9	90.2
2 months Rep A	59.8	19.2	8.5	87.6
2 months Rep B	59.1	17.9	6.1	83.1
3 months Rep A	68.7	14.4	7.8	91.0
3 months Rep B	67.5	12.8	9.4	89.6
4 months Rep A	64.0	11.5	8.1	83.6
4 months Rep B	67.1	11.6	8.6	87.4
5 months Rep A	66.8	9.5	11.9	88.2
5 months Rep B	72.5	9.1	5.6	87.3
9 months Rep A	69.6	6.6	6.1	82.3
9 months Rep B	74.0	6.6	6.9	87.5
12 months Rep A	71.0	6.7	7.8	85.4
12 months Rep B	70.5	6.3	7.0	83.9

Table B.8.1-30: Accountability of radiocarbon in Dupo silt loam soil

Days/months after application	Volatile traps [%] NaOH	Extract	Extracted soil [%]	Total recovered [%]
0 d Rep A	n.a.	81.3	2.7	84.0
0 d Rep B	n.a.	97.9	5.4	103.2
1 d Rep A	16.9	75.5	7.0	99.5
1 d Rep B	16.3	67.9	3.0	87.1
3 d Rep A	32.2	65.2	7.0	104.4
3 d Rep B	32.8	48.1	3.9	84.8
7 d Rep A	36.9	45.5	5.0	87.4
7 d Rep B	38.4	38.6	4.4	81.4
14 d Rep A	46.5	39.9	7.8	94.1
14 d Rep B	47.2	39.6	6.8	93.6
1 months Rep A	58.7	28.2	5.7	92.6
1 months Rep B	57.4	25.8	5.2	88.4
2 months Rep A	65.2	21.4	7.0	93.5
2 months Rep B	63.8	18.7	4.7	87.2
3 months Rep A	71.7	11.4	6.2	89.3
3 months Rep B	72.1	15.7	5.9	93.6

4 months Rep A	76.4	8.8	4.7	89.8
4 months Rep B	75.4	11.9	5.4	92.8
5 months Rep A	79.7	6.9	5.0	91.7
5 months Rep B	80.4	7.2	5.2	92.9
9 months Rep A	81.9	5.4	5.3	92.6
9 months Rep B	83.8	5.4	4.9	94.1
12 months Rep A	78.0	4.4	3.7	86.1
12 months Rep B	78.6	4.9	4.7	88.3

Table B.8.1-31: Quantitative characterization of [14C]glyphosate residues found in extracts from Kickapoo sandy loam samples incubated under aerobic conditions

Days/months after application	Glyphosate	AMPA	Unknown A	Unknown B	Unknown C	Others[1]	% of applied radio-carbon analyzed
0 d Rep A	50.6	12.9	1.8	2.0	n.d.	n.d.	68.1
0 d Rep B	44.4	19.0	2.5	2.6	n.d.	0.8	70.6
1 d Rep A	32.8	21.6	2.7	2.5	n.d.	n.d.	59.9
1 d Rep B	32.0	25.0	2.5	2.3	n.d.	0.7	62.5
3 d Rep A	18.9	31.1	2.8	0.7	n.d.	n.d.	53.1
3 d Rep B	15.9	20.0	2.1	2.4	n.d.	0.4	41.1
7 d Rep A	10.6	28.5	3.5	3.6	n.d.	n.d.	47.6
7 d Rep B	7.7	18.0	2.4	2.4	n.d.	n.d.	31.1
14 d Rep A	5.4	23.9	2.2	1.2	n.d.	0.4	33.5
14 d Rep B	6.4	28.6	1.8	1.3	n.d.	n.d.	38.6
1 months Rep A	2.8	17.3	2.3	2.4	0.6	1.3	26.7
1 months Rep B	2.8	17.0	2.7	2.2	n.d.	n.d.	25.0
2 months Rep A	1.6	9.9	2.9	1.8	0.3	n.d.	16.6
2 months Rep B	2.1	12.1	3.0	2.0	n.d.	0.5	19.7
3 months Rep A	2.0	7.7	1.7	0.8	0.2	0.2	12.3
3 months Rep B	1.1	5.5	2.0	1.3	0.3	0.1	10.8
4 months Rep A	2.1	5.4	2.6	1.0	0.4	0.1	11.7
4 months Rep B	1.3	4.1	2.4	1.4	n.d.	0.3	9.6
5 months Rep A	1.9	3.7	2.3	0.9	0.3	n.d.	9.3
5 months Rep B	0.8	3.5	1.9	1.0	0.3	n.d.	7.5
9 months Rep A	0.9	2.8	2.0	1.2	0.4	0.2	7.6
9 months Rep B	0.5	0.1	1.4	0.7	0.1	0.1	4.6
12 months Rep A	0.5	1.7	1.7	0.9	0.2	n.d.	5.,0
12 months Rep B	0.6	2.1	1.3	0.5	0.2	0.1	4.8

1) Unknown products, none of which exceeded 1 % of the applied radiocarbon unless noted.

Table B.8.1-32: Quantitative characterization of [14C]glyphosate residues found in extracts from Dupo silt loam samples incubated under aerobic conditions

Days/months after application	Glyphosate	AMPA	Unknown A	Unknown B	Unknown C	Others[1]	% of applied radio-carbon analyzed
0 d Rep A	64.3	14.3	1.4	0.9	n.d.	n.d.	81.3
0 d Rep B	82.2	18.7	0.9	0.9	n.d.	4.6	108.5
1 d Rep A	49.7	22.0	2.0	0.9	n.d.	n.d.	75.5

1 d Rep B	61.5	19.1	2.4	1.0	n.d.	n.d.	84.3
3 d Rep A	27.3	26.3	1.7	0.9	n.d.	1.0	57.2
3 d Rep B	23.9	21.7	1.3	1.2	n.d.	n.d.	48.1
7 d Rep A	13.9	28.0	1.7	1.3	n.d.	n.d.	45.5
7 d Rep B	11.7	23.4	1.6	1.3	n.d.	n.d.	38.6
14 d Rep A	7.0	34.6	1.4	0.6	n.d.	n.d.	45.2
14 d Rep B	5.6	22.7	2.8	3.1	n.d.	n.d.	35.0
1 months Rep A	2.7	22.3	1.8	0.8	n.d.	n.d.	28.2
1 months Rep B	3.3	26.3	2.2	1.3	n.d.	n.d.	33.2
2 months Rep A	1.5	14.7	1.6	0.9	n.d.	n.d.	19.0
2 months Rep B	1.5	14.9	1.4	0.7	n.d.	n.d.	18.9
3 months Rep A	1.7	10.0	1.7	1.0	n.d.	n.d.	14.6
3 months Rep B	1.4	11.4	1.7	0.9	n.d.	n.d.	15.7
4 months Rep A	1.1	5.1	1.6	1.0	0.1	n.d.	8.8
4 months Rep B	1.3	8.8	1.8	0.9	0.2	0.6	14.0
5 months Rep A	0.7	2.8	0.8	0.4	0.2	0.1	5.0
5 months Rep B	0.7	4.3	1.3	0.7	0.1	0.1	7.2
9 months Rep A	0.8	2.1	1.0	0.4	0.1	n.d.	4.5
9 months Rep B	0.6	2.2	1.1	0.6	0.1	n.d.	4.6
12 months Rep A	0.4	1.6	1.7	0.8	0.2	n.d.	4.6
12 months Rep B	0.7	1.5	0.9	0.3	0.2	n.d.	3.6

1) Unknown products, none of which exceeded 1 % of the applied radiocarbon unless noted.

Mass balance

The total recovery of applied radiocarbon throughout the definitive study was 86.5 ± 5.3% on Kickapoo sandy loam soil and 91.4 ± 5.6% on Dupo silt loam soil. Total recovery for the day 0 samples for the Kickapoo sandy loam soil is low (mean 72.8% of applied radiocarbon) probably due to some anomaly during sample harvest or extraction. For sterile samples, no greater than 9.5% of the applied radiocarbon remained on extracted Kickapoo sandy loam and Dupo silt loam soils. The total recoveries of applied radiocarbon were 100.0 ± 1.5 and 102.0 ± 5.3% for the Kickapoo sandy loam and Dupo silt loam, respectively.

Volatilisation

In the definitive study, radiolabelled CO_2 accounted for 70.8 and 78.3% (mean) of the applied radiocarbon in the Month 12 samples for the Kickapoo sandy loam and Dupo silt loam soils, respectively. In the sterile samples, radiolabelled CO_2 accounted for 42.5 and 38.2% of applied radiocarbon in the Month 6 samples for Kickapoo sandy loam and Dupo silt loam soils, respectively. The slightly low material balance in several instances suggests that actual amounts of $^{14}CO_2$ produced in the study were probably somewhat higher. Radiolabelled volatiles other than CO_2 accounted for less than 0.1 % of the applied radiocarbon in all samples.

Transformation of the parent compound

Under aerobic conditions degradation of glyphosate was rapid. The definitive study determined that only 0.6 and 0.5% (mean) of the applied radiocarbon remained as [^{14}C]glyphosate in the Kickapoo sandy loam and the Dupo silt loam soil Month 12 samples, respectively.

The major degradate in the soil extracts, [^{14}C]aminomethylphosphonic acid (AMPA) accounted for 1.9 and 1.6% of the applied radiocarbon in the Month 12 samples of the Kickapoo sandy loam and Dupo silt loam soils, respectively. Several unknown metabolites were detected in the soil extracts from the definitive study. These were characterized by HPLC. No unknown compounds were detected in the soil extracts of the sterile samples. One unknown metabolite (Unknown A), eluting at approximately 4 minutes, never exceeded 3.5% of the ap-

plied radiocarbon in any sample. Another metabolite (Unknown B), eluting at approximately 7 - 10 minutes, never exceeded 3.6% of the applied radiocarbon. Several other minor unknowns were detected; however, no other single unknown exceeded 1.5% of the applied radiocarbon.

Conclusion

It was found that [^{14}C]-glyphosate degrades in Kickapoo sandy loam and Dupo silt loam soils under aerobic laboratory conditions. The primary degradation products in both soils are CO_2 and aminomethylphosphonic acid (AMPA). Several low-level unidentified metabolites are also produced. However, none of these unidentified products constitute greater than 3.6% of the initial glyphosate concentration and, therefore, are considered insignificant. The proposed metabolic pathway involved the initial conversion of glyphosate to AMPA, followed by further metabolism of AMPA to CO_2.

RMS Comment

In the monograph the study was considered as not acceptable. It was argued, that the study had been performed at 25 °C instead of 20 °C. However, the study was performed according to EPA Subdivision N 162-1 and this applied temperature is now considered acceptable by the RMS. Besides, it was argued that the microbial biomass in the soil was not determined. However, number of total colony forming units for aerobic bacteria, actinomyetes and fungi and microbial biomass were determined at the start of the incubations indicating microbial viable soils. Thus, the study is now considered acceptable by the RMS regarding the results of the incubation of glyphosate in the silt loam soil Dupo. The results of the incubation of glyphosate in the sandy loam soil Kickapoo will not be considered, since the total radioactive recovery was < 90 % for most of the sampling points of this soil.

A new kinetic evaluation of the study results (Dupo silt loam soil) according to FOCUS (2006, 2001) was performed by the RMS (see B.8.1.2.1). Thus, the DT_{50} and DT_{90} values of glyphosate derived by the study author are not presented here anymore.

KIIA 7.1.1/7 (Honegger, 1992, BVL no 2147496)

Annex point:	IIA 7.1.1/07
Author(s):	Honegger, J.L.
Title:	Review of the aerobic metabolism of [14C]-Glyphosate in soil. Addendum to Monsanto Report No PTRL 368
Date:	January, 1992
Guideline(s):	-
Deviations:	-
GLP:	No (review does not contain laboratory work)
Acceptability:	Yes

Study summary

In Honegger (1992) the aerobic soil metabolism study of Kesterson and Atkins (1991) "Aerobic metabolism of [^{14}C]glyphosate in sandy loam and silt loam soils with biometer flask" are summarised. Criteria with respect to storage, pesticide treatment and experimental details are described. Furthermore, the DT_{50} and DT_{90} values derived from Kesterson and Atkins (1991) and from field soil dissipation studies are compared. It was concluded that the results of these field dissipation studies are consistent with the results of the soil metabolism study and indicate that glyphosate and AMPA readily degrade in the soil.

RMS Comment
The study can generally be accepted.

Due to kinetic re-evaluation of the study results of Kesterson and Atkins (1991) and the field study results the comparison of DT_{50} and DT_{90} performed by Honegger (1991) is no longer relevant and therefore, not presented here anymore.

B.8.1.1.2 Anaerobic degradation

One laboratory study on anaerobic route of degradation (Keirs & Mackie, 2000, BVL no 1939595) was considered acceptable during the 2001 EU glyphosate review. The study Keirs & Mackie (2000, BVL no 1939595) conducted under the old SETAC anaerobic soil test guideline showed that glyphosate degradation was negligible under anoxic anaerobic conditions. The main degradation product observed was AMPA. The results from this study were summarized in an Addendum III to the glyphosate Monograph (Addendum to the monograph – volume 3, 11th Dec. 1998, Part A, Glyphosate 16th October 2000). In addition, an anaerobic route of degradation study McBain (1987, BVL no 1031560) conducted with ^{14}C-glyphosate trimesium (labelled in the Carboxymethylamonimethylphosphonic acid moiety) was reviewed and the results from this study were summarized in the glyphosate–trimesium Monograph. This study was conducted with an initial aerobic aging period equivalent to one half-life (3 days) followed by 63 days of incubation under flooded anaerobic conditions and showed substantial mineralization of glyphosate–trimesium to carbon dioxide.

Since the EU review, a new laboratory anaerobic degradation study (Lowrie et al., 2003, BVL no 2310253) with glyphosate has been performed by one GTF member in support of national applications. The study was conducted according to the requirements of the new OECD guideline no. 307 (OECD, 2002) for anaerobic transformation in soil. In the new study the route and rate of degradation of [^{14}C]-glyphosate was investigated in a flooded sandy loam soil at a nominal temperature of 20 °C maintained under anaerobic conditions following an aerobic aging period equivalent to one half-life. Glyphosate was degraded rapidly during the aerobic aging period of the study, and the aerobic soil DT_{50} was determined to be 10 days. Following 10 days aerobic incubation, unchanged glyphosate accounted for 55 % of the applied radioactivity. The release of $^{14}CO_2$ was observed during the aerobic phase of the study and accounted for 12 % of the applied radioactivity during the 10-day aerobic incubation. After the incubation under aerobic conditions for 10 days (pre-determined aerobic half life), the soil samples were flooded with distilled water and the aeration system was switched to moist nitrogen in order to achieve anaerobic conditions. Upon initiation of anaerobic conditions, the rate of degradation was observed to slow down significantly. Levels of glyphosate in soil decreased to 33 % of the applied radioactivity after 120 days incubation under anaerobic conditions. Levels of AMPA, the most significant degradation product, increased to 30 % of applied dose after 84 days and subsequently declined to 28 % of the dose after 120 days of anaerobic incubation. Radioactivity associated with the surface (distilled) water, following flooding, was observed to increase slowly from < 1 % applied after 1 hour to 5 % after 28 days. By 120 days of anaerobic incubation, levels of radioactivity associated with the surface water accounted for 7 % of the applied radioactivity. The principle component in the surface water was glyphosate, with smaller amounts of AMPA also detected. The anaerobic DT_{50}, calculated over a period of 120 days of anaerobic incubation was established as 142 and 205 days for the soil and total system (soil/water compartments), respectively. The metabolite

distribution resulting from the degradation of glyphosate in soil was similar under both aerobic and anaerobic conditions.

Two additional anaerobic soil route and rate of degradation studies (McEwen, 2004; Knoch, 2003) which were not evaluated during the first glyphosate EU evaluation (2001) have been made available from GTF members. According to the applicant, these studies were not conducted exactly according to the requirements of the new OECD Guideline 307 (OECD, 2002) for anaerobic transformation in soil. Therefore the dossier of the notifier for glyphosate considers these studies as supplementary studies. ~~It has to be noted that the studies of McEwen (2004) and Knoch (2003) were not presented to the RMS and therefore could not be evaluated. However, results are in line with the available studies -although the DT_{50} values are slightly smaller than in the available studies – and therefore are plausible.~~

Table B.8.1-33 summarizes the results of both old and new route of degradation studies of glyphosate conducted under anaerobic soil conditions. The results of the three new studies demonstrate that glyphosate degrades under anaerobic conditions although at a slower rate than under aerobic conditions. The results from the studies conducted by McBain (1987, BVL no 1031560), Knoch (2003), Lowrie et al. (2003, BVL no 2310253) and McEwen (2004) are in contrast to the results obtained from the anaerobic degradation of glyphosate by Keirs & Mackie (2000, BVL no 1939595) conducted under the old SETAC anaerobic soil test guideline. The Keirs & Mackie (2000, BVL no 1939595) study examined the degradation of glyphosate when applied directly to sandy loam topsoil that had been flooded and converted to anaerobic conditions during a pre-incubation period of 39 days. The redox potential at the time of glyphosate application was extremely low, around –400 mV, indicating complete anoxic conditions. Under the anoxic conditions established for the study, glyphosate did not degrade significantly. The SETAC anaerobic soil test guideline method uses a completely anaerobic/anoxic system, which does not reflect the circumstances of the use of glyphosate for weed control. Prolonged and fully anaerobic conditions are expected to be rare throughout the surface soil zone where glyphosate occurs due to its limited mobility in soil. The new testing guideline (OECD Guideline 307, 2002) reflects more realistic anaerobic conditions found in the arable cropping environment, which are held under an aerobic/anaerobic gradient.

Table B.8.1-33: Overview of the glyphosate laboratory anaerobic degradation studies

Reference		Guideline	Application rate (µg/g)	Temp (°C)	Duration of test (days)	Soil characteristics			Major metabolites (% of dose)		Remarks
						Soil type	pH	OC (%)	AMPA (% max)	CO_2 (% max)	
Studies from the 2001 evaluation	Keirs & Mackie, 2000, BVL no 1939595	SETAC, 1995	5	20	120	Loamy sand	5.8	1.7	10.07	0.87	No significant degradation
	McBain, 1987, BVL no 1031560	40 CFR Part 160	30	23	66	Loamy sand	6.9	1.3[1]	0.5	45.42	No DT_{50} value was calculated
Studies **not** reviewed in the 2001 evaluation	Knoch, 2003	SETAC, 1995	5.8	20	120	Silt loam	5.1	1.2	19.5	20.1	DT_{50}: 31 days (anaerobic) [2]
	McEwen, 2004	SETAC	20	20	120	Sandy loam	6.5	1.0	44.2	25.70	DT_{50}: 19.3 days (anaerobic)[2]
New study	Lowrie et al., 2003, BVL no 2310253	OECD 307	5.22	20	120	Sandy loam	5.9	1.8	30.2	13.42	DT_{50}: 142 days (anaerobic)

nd = not determined
1) calculated from organic matter content (OC (%) = OM (%)/1.724)
2) not confirmed to be valid

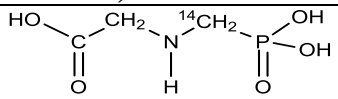
KIIA 7.1.2/1 (Lowrie et al., 2003, BVL no 2310253)

Annex point:	KIIA 7.1.2/1
Author(s):	Lowrie, C., Clayton, M.A., Paterson, K.
Title:	The degradation of [14C]-glyphosate in soil under anaerobic conditions
Date:	July 08, 2003
Guideline(s):	OECD Guideline 307 for Testing of Chemicals: Aerobic and Anaerobic Transformation in Soil (adopted April 2002)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Material and methods

The test material is characterised in Table B.8.1-34.

Table B.8.1-34: Test materials

	Test material (radiolabelled)
Name	N-(phosphonomethyl) glycine (glyphosate, specific activity 38.79 µCi/mg or 6.56 mCi/mmol)
Identification	
Purity	Radiochemical purity 97.28% (HPLC)
CAS	-
Stability of test compound	Stored at - 20 °C in the dark

The soil used for the study, a sandy loam soil was supplied by Landlook, Midlands, UK from a site with no pesticides or organic fertiliser treatments for at least five years prior to collection. The soil was collected from the upper 20 cm layer of a grassland site by removing surface vegetation and collecting the top soil. Soil was sieved (2mm) prior to use on the study. Soil physicochemical properties are given in Table B.8.1-35.

Table B.8.1-35: Soil physicochemical properties

Soil origin	**Soil type (USDA)**	**pH[1)]**	**CEC (meq/100g)**	**OC (%)**	**Sand (%)**	**Silt (%)**	**Clay (%)**	**Moisture (%) at 1/3 bar**	**Biomass (mg C/ 100 g) dry soil**
Landlook, UK	Sandy loam	5.9	15.7	1.8	69.43	18.85	11.72	16.0	32.4

1) pH in 1:5 in 1 M KCl

Study design

A total of 22 soil samples (ca. 50 g oven dry equivalent) were prepared. The moisture content of each soil sample was adjusted to ca 50 % maximum water holding capacity and maintained at this level throughout the aerobic phase of the study. Soil samples were pre-incubated under aerobic conditions at 20 ± 2 °C for 8 days prior to application for an acclimation period.

The dosing solution was prepared by combining an aliquot of non-labelled glyphosate standard with an aliquot of [^{14}C]-glyphosate test substance dissolved in water. The resulting [^{14}C]-glyphosate treatment solution which had a specific activity of 29.96 µCi/mg was used for dosing. Treatment solution was applied drop-wise to the surface of the soil with the radioactive application of 7.82 µCi, equivalent to an application rate of 5.22 mg/kg (dry weight equivalent).

Following test material application, the samples were re-connected to the continuous air flow system and incubated under aerobic conditions at 20 ± 2 °C in the dark for a period of 10 days after application of glyphosate (pre-determined aerobic half life). The gas mixture leaving each flask was passed through four traps, the first one acting as a safety trap, the second one contained ethanediol to trap non-specific organic volatiles and the final 2 traps contained 2 M sodium hydroxide to trap liberated $^{14}CO_2$.

Following removal of day 10 aerobic incubates, all remaining soil samples were then flooded by the addition of approximately 100 ml milli-Q water to give a depth of 1-3 cm. A stream of moist nitrogen was then introduced to the test system. The samples were maintained under anaerobic conditions at 20 ± 2 °C in the dark for a period of 120 days post-flooding.

Two additional samples were prepared for the in situ redox measurements during the anaerobic phase. The measured redox potential indicates that the test system achieved anaerobic conditions 14 days post flooding.

During the aerobic phase (10 days), duplicate soil samples were taken for analysis at zero time (immediately post-application) and at the pre-determined aerobic soil half-life (10 days after application).

Duplicate soil samples were removed and analysed at 1 h, and 3, 7, 14, 28, 56, 84 and 120 days post-flooding.

For the aerobic phase of the study, soil samples were transferred into centrifuge bottles and extracted three times with 0.5 M ammonium hydroxide (100 mL) using an end-over-end shaker for a period of approximately one hour. After shaking, the extract was separated from the residue by centrifugation (2200 g, 30 min) and the radioactivity in the supernatant was determined by liquid scintillation counting. The quantitative distribution of radiolabelled components in the combined soil extracts was determined using ion exchange HPLC.

For the anaerobic phase of the study, the surface water was separated from soils by decanting. The remaining soil was transferred into centrifuge bottles and extracted with 0.5 M ammonium hydroxide (3 x ca 100 mL) using an end-over-end shaker for approximately one hour. Following shaking, the extract was separated from the residue by centrifugation (2200 g; ca. 30 min) and the radioactive content of the supernatant determined by liquid scintillation counting. The combined soil extracts from each sampling were subjected to chromatographic analysis (HPLC and TLC). Surface waters containing > 5 % of applied radioactivity were also subjected to chromatographic analysis (HPLC and TLC). Following extraction, the radioactivity remaining in the post-extracted soil was determined by combustion analysis.

The distribution of radioactivity in organic matter in selected post-extracted soil samples was determined. Each sample was extracted by shaking in 0.5 M sodium hydroxide (ca 100 mL)

for about one hour. The extracts were separated by centrifugation from soil residues and the radioactive content of the soil (humin) was determined by combustion analysis. The extract was adjusted to ca pH 1 using concentrated hydrochloric acid, to precipitate the humic acid fraction. The extract was centrifuged and the supernatant, containing the fulvic acid fraction, was removed and aliquots were submitted for liquid scintillation counting. Radioactivity associated with the humic acid fraction was quantified by dissolving directly in scintillation fluid.

Prior to chromatographic analysis for each individual soil sample, an aliquot of each extract was combined. All combined soil extracts and surface water samples containing > 5 % of the applied radioactivity were analysed using HPLC. A Hewlett-Packard 1050 series HPLC, equipped with an autosampler and an UV detector (set at 200 nm), was connected to a Spherisorb S5 SAX HPLC column (25 cm x 10 mm; 5 µm; Waters) and a Packard Flo-one Series radioactivity monitor (Series A-100, Model A140; liquid scintillant Ultima-FloTMM). The mobile phase started with 100 % of solvent A [5 mM potassium dihydrogen phosphate (adjusted to ca pH 2 using concentrated phosphoric acid): methanol (96:4, v/v)] for 10 minutes followed by a linear increase to 100 % solvent B [100 mM Potassium dihydrogen phosphate (adjusted to ca pH 2 using concentrated phosphoric acid): methanol (96:4, v/v)] within 30 minutes at a flow rate of 3 ml/min by using a linear gradient programme.

For TLC analyses, aliquots of selected sample extracts and surface waters were applied to Polygram Ionex-25 SA-Na TLC plates which were subsequently developed in 0.01 M potassium dihydrogen phosphate (adjusted to ca pH 2 with concentrated phosphoric acid): methanol (9:1, v/v). The solvent was allowed to develop to a height of 170 mm. Non-radiolabelled glyphosate and AMPA prepared in Milli-Q grade water were chromatographed at each sample. Following chromatography, the areas of radioactivity present on TLC plates were quantified using a Molecular Dynamics phosphor imager or a Fuji FLA5000 phosphor imager. Standards were visualised using ninhydrin spray reagent.

For combustion analyses, Cellulose powder and Combustaid® (ca 100 µL) were added to triplicate portions of air-dried soil residues (ca 0.3 g) prior to combustion in oxygen using a Packard Sample Oxidiser, Model 307. The combusted products were absorbed in Carbo-Sorb®, mixed with Permafluor®E+ and the radioactivity determined by liquid scintillation counting.

All extract aliquots, surface water aliquots, trap solution aliquots and apparatus wash aliquots were added directly to scintillates and submitted for liquid scintillation counting. All radioassays were performed in duplicate. Radioactivity was quantified using a liquid scintillation analyser (Packard 1600TR or Packard 2100TR), with automatic quench correction by external standard-channels ratio. Each individual sample was counted for 5 min.

A limit of reliable determination of 30 dpm above background has been instituted in these laboratories.

Results and discussion

Data

Table B.8.1-36: Recovery of applied radioactivity from a sandy loam soil following single application of [^{14}C]-glyphosate under aerobic followed by anaerobic conditions

Time point	Study phase	Sample	% of Applied Radioactivity						
			Surface water	Soil extract	$^{14}CO_2$ 1)	^{14}C Volatiles 2)	Non-extractable residue	Apparatus wash	Mass Balance
Zero time	Aerobic phase	Rep A	NS	107.42	NS	NS	2.33	NS	109.75
		Rep B	NS	106.08	NS	NS	2.45	NS	108.53
Day 10		Rep A	NS	75.59	12.37	0.01	15.11	0.01 3)	103.09
		Rep B	NS	76.66	12.50	ND	14.78	0.01 3)	103.95
1 h	Anaerobic phase	Rep A	0.49	76.96	12.49	ND	12.65	0.05	102.64
		Rep B	0.49	78.18	12.46	ND	13.21	0.02 3)	104.36
Day 3		Rep A	1.20	72.76	13.13	0.01	16.38	0.01 3)	103.49
		Rep B	1.47	74.61	12.97	0.01	16.73	0.01 3)	105.80
Day 7		Rep A	0.97	71.36	12.14	ND	17.68	0.02 3)	102.17
		Rep B	1.17	70.46	1.64 4)	ND	17.88	0.01 3)	91.16
Day 14		Rep A	1.82	67.30	13.08	ND	20.22	0.01 3)	102.43
		Rep B	2.06	66.94	13.32	0.01	21.05	0.01 3)	103.39
Day 28		Rep A	5.90	65.25	13.40	0.01	20.57	0.01 3)	105.14
		Rep B	4.36	67.61	13.44	0.01	20.41	ND	105.83
Day 56		Rep A	5.41	62.15	12.18	0.01	24.74	0.01 3)	104.50
		Rep B	5.48	63.06	1.73 4)	0.01	24.52	0.01 3)	94.81
Day 84		Rep A	5.72	62.19	12.52	0.52	22.02	0.03 3)	103.00
		Rep B	6.37	61.73	13.01	0.27	22.53	0.01 3)	103.92
Day 120		Rep A	6.31	61.55	11.89	0.28	22.54	ND	102.57
		Rep B	6.78	60.36	13.13	0.29	22.49	0.01 3)	103.06

1) trapped with 2M sodium hydroxide
2) non-specific organic volatiles: trapped with ethanediol
3) results calculated from data less than 30 dpm above background
4) low recoveries of $^{14}CO_2$ are probably caused by a leak in the flow-through apparatus
NS = no sample, ND = not detected

Table B.8.1-37: Degradation product distribution in soil extracts, surface water and in total following single application of [^{14}C]-glyphosate under anaerobic conditions

Time point	Study phase	Sample	% of applied radioactivity											
			Soil extracts				Surface water				Total			
			Glyphosate	AMPA	Unknown B	Unknown C	Glyphosate	AMPA	Unknown B	Unknown C	Glyphosate	AMPA	Unknown B	Unknown C
Zero time	Aero-bic phase	Rep A	104.41	3.01	ND	ND	NS	NS	NS	NS	104.41	3.01	ND	ND
		Rep B	101.20	4.88	ND	ND	NS	NS	NS	NS	101.20	4.88	ND	ND
Day 10		Rep A	55.85	18.92	0.82	ND	NS	NS	NS	NS	55.85	18.92	0.82	ND
		Rep B	54.05	19.38	ND	3.23	NS	NS	NS	NS	54.05	19.38	ND	3.23
1 h	An-aerobic phase	Rep A	57.50	19.46	ND	ND	NP	NP	NP	NP	57.99[1)]	19.46	ND	ND
		Rep B	56.99	21.19	ND	ND	NP	NP	NP	NP	57.48[1)]	21.19	ND	ND
Day 3		Rep A	53.35	19.41	ND	ND	NP	NP	NP	NP	54.55[1)]	19.41	ND	ND
		Rep B	54.85	19.76	ND	ND	NP	NP	NP	NP	56.32[1)]	19.76	ND	ND
Day 7		Rep A	53.08	18.28	ND	ND	NP	NP	NP	NP	54.05[1)]	18.28	ND	ND
		Rep B	51.84	18.62	ND	ND	NP	NP	NP	NP	53.01[1)]	18.62	ND	ND
Day 14		Rep A	46.38	20.92	ND	ND	NP	NP	NP	NP	48.20[1)]	20.92	ND	ND
		Rep B	46.44	20.49	ND	ND	NP	NP	NP	NP	48.50[1)]	20.49	ND	ND
Day 28		Rep A	39.06	26.19	ND	ND	5.90	ND	ND	ND	44.96	26.19	ND	ND
		Rep B	42.37	25.24	ND	ND	NP	NP	NP	NP	46.73[1)]	25.24	ND	ND
Day 56		Rep A	31.59	30.56	ND	ND	5.41	ND	ND	ND	37.00	30.56	ND	ND
		Rep B	40.52	22.54	ND	ND	5.48	ND	ND	ND	46.00	22.54	ND	ND
Day 84		Rep A	32.85	29.34	ND	ND	5.46	0.26	ND	ND	38.31	29.60	ND	ND
		Rep B	31.74	29.99	ND	ND	5.47	0.90	ND	ND	37.21	30.89	ND	ND
Day 120		Rep A	31.62	29.93	ND	ND	6.31	ND	ND	ND	37.93	29.93	ND	ND
		Rep B	33.40	26.96	ND	ND	6.78	ND	ND	ND	40.18	26.96	ND	ND

NS = no sample, ND = not detected, NP = not profiled as <5 % applied radioactivity in sample

1) Radioactivity in surface water for these samples accounted for <5 % applied activity. It was assumed to be glyphosate and was included in total.

Mass balance
The mean total recoveries of applied radioactivity from a sand loam soil incubated for 10 days, during the aerobic phase of the study were in a range of ca. 104 % to 109 %.

The mean recoveries of applied radioactivity under anaerobic conditions incubated subsequently for up to 120 days were in the range of ca. 97 to ca 105 %.

Bound and extractable residues
Extractable radioactivity accounted for 107 % of applied radioactivity at zero time (aerobic phase), declining to 63 % after 56 days post flooding (anaerobic phase), remaining relatively constant. Radioactivity associated with surface water following flooding was observed to increase slowly from < 1 % applied after 1 hour to 5 % after 28 days. At day 120 levels of radioactivity associated with the surface water accounted for 7 % of applied radioactivity.

Non-extractable residue increased from a minimum of 2 % of applied radioactivity at zero time to a maximum of 25 % after 56 days. At day 120, levels recovered as non-extractable residues accounted for 23 % of applied radioactivity.

Volatilisation
$^{14}CO_2$ was observed during the aerobic phase of the study and accounted for 12 % of applied radioactivity in the majority of study samples prior to flooding. Production of $^{14}CO_2$ after initiation of anaerobic conditions decreased to levels of less than 2 % of applied radioactivity for the remainder of incubation. Radioactivity associated to ethanediol trap (non-specific volatiles) and apparatus washings accounted for < 0.1 % of applied radioactivity.

Transformation of the parent compound
At zero time (aerobic), levels of glyphosate in soil extracts were quantified and subsequently declined to 55 % after 10 days of aerobic incubation. Upon initiation of anaerobic conditions, levels of glyphosate accounted for 57 % (1 hour post-flooding) applied radioactivity and decreased to 33 % after 120 days of incubation under anaerobic conditions.

The rate of degradation of glyphosate was determined under anaerobic conditions by linear regression analysis, assuming first order kinetics. The following equation was used in the calculation:

$$DT_{50} = \frac{\ln 2}{K_{dc}}$$

Where K_{dc} is the first order rate content.

The DT_{50} for glyphosate incubated under anaerobic conditions at 20 ± 2 °C was calculated based on radioactivity present as glyphosate in the soil compartment and radioactivity present as glyphosate in the test system (soil and surface water) and was determined as 142 and 205 days, respectively. The coefficient of regression (R^2) was 0.86 and 0.83 for soil compartment and test system, respectively.

It should be noted that the DT_{50} for the study was calculated from the sum of the soil extracts and surface waters (ie. whole system) based on the following assumption. Prior to day 28 no HPLC or TLC analysis of surface waters was conducted since there was less than 5 % applied

radioactivity in the samples. It is assumed that since only glyphosate was present in all subsequent surface waters (with the exception of day 84) that samples collected prior to day 28 only contained glyphosate. Therefore the % applied radioactivity associated with surface waters prior to day 28 was added to the half-life calculation as glyphosate.

The most significant degradation product detected was AMPA. At zero time AMPA accounted for 4 % applied radioactivity, increasing to a maximum of 30 % after 84 days and subsequently declining to 28 % after 120 days of incubation. In addition to glyphosate and AMPA, two further unknown components were detected in the 10 day aerobic samples (designated Unknown B and C). These components were only detected in one replicate and at one sampling interval, each individually accounting for < 3 % of applied radioactivity.

Conclusion

Glyphosate was degraded rapidly during the aerobic period of the study; the DT_{50} was 10 days following first order kinetics. The only significant metabolites detected were AMPA and $^{14}CO_2$. Following initiation of anaerobic conditions, the rate of degradation was observed to slow down significantly. The anaerobic DT_{50} was calculated based on radioactivity present as glyphosate in the soil compartment and radioactivity present as glyphosate in the test system (soil and surface water) over a period of 120 days and was determined as 142 and 205 days for soil and test system (water and soil extracts) compartments, respectively. During the anaerobic phase, liberation of $^{14}CO_2$ was significantly reduced when compared to the aerobic ageing period and AMPA was the only significant metabolite.

RMS Comment

The study is considered acceptable by the RMS.

KIIA 7.1.2/2 (Knoch, 2003, BVL no 2716557)

Annex point:	KIIA 7.1.2/2
Author(s):	E. Knoch
Title:	Route and Rate of Anaerobic Soil Degradation of Glyphosate According to SETAC, Part 1, 1.2 (March 1995).
Date:	February 7th 2003
Guideline(s):	SETAC, "Procedures for assessing the environmental fate and ecotoxicity of pesticides", Part 1, 1.2 (March 1995)
Deviations:	No
GLP:	Yes
Acceptability:	No

Material and methods

Test item: ^{14}C-glyphosate

The test system is designed to represent the upper layer of the soil profile that has become waterlogged of flooded due to heavy rain or some other event. The test system is composed of the test item placed in the soil surface in an all glass flow-through system. (See illustration below)

The soil (2-5% organic matter (1.2 to 2.9% organic carbon), pH 5.5 – 7.5, and 10-25% clay) will be characterized according to GLP regulations (Table B. 8.1-38).

Amounts of conditioned soil, equivalent to 100 g dry weight will be bottled into 1000 ml all glass metabolism flasks. Bottled soil will be flooded by addition of reagent water (water column height of about 2 cm, equivalent to approximately 180 ml water) and maintained under dynamic atmosphere of nitrogen gas, in the dark, at 20 °C±2°C. The flooded soil will be pre-incubated for 30 days to reach anaerobic equilibrium, based on measurable variables (redox potential and pH value of water and soil, oxygen concentration of water). After establishment of anaerobic conditions (water and soil), the application of the test item will be made to the surface water.
Each flooded soil will be treated at the maximum field rate of 4.32 kg a.s./ha. The target rate of 5.76 mg a.s./kg soil (dry weight) was calculated by assuming a penetration depth of the test item in the soil of 5 cm and a soil density of 1.5 g/cm^3.
Metabolism flasks will be placed in a constant temperature room at 20 °C, with a temperature control of ±2 °C and incubated in the dark. The temperature will be recorded continuously. Specimen will be weighed approximately every two weeks to ensure that a water column height of about 2 cm is being maintained. If the water level drops more than 10 % below this value (in weight equivalents), oxygen free reagent water, degassed by nitrogen inlet, will be added until the desired water level is obtained.
Flooded soil specimen will be assayed at the following times : zero-time (initial value), 6 h, 1 d, 7 d, 14 d, 30 d, 60 d, 90 d, and 120 days after fortification. Aliquots from the volatile traps will be radioassayed at each sampling point (excluding 0-time) or at 14 day intervals. The traps will be assayed directly by adding aliquots of the trapping solution into liquid scintillation cocktail and counting by liquid scintillation counting (LSC). Specimen from sodium hydroxide traps will be checked for CO_2 saturation on a weekly basis. If greater than 5 % of the applied dose (on a per sample basis) is cumulatively trapped in the organic traps, the identification of the degradate(s) will be attempted (development of chromatographic procedures). If any radioactivity is cumulatively trapped in the sodium hydroxide traps (≥2 % of the applied dose), identification of CO_2 will be performed by precipitation of $BaBO_3$ using baring chloride.
For analysis, the soil and water from each metabolism flask will be separated. For that purpose, the water will be withdrawn from the soil as quantitavely as possible by decantation. Particulates at the water/soil interface will be removed by filtration, if necessary. Thereafter soil and water will be analysed separately.
The soil will be transferred quantitavely into 750 mL centrifuge vessels and extracted using 1 M NH_3. The ratio of extraction solvent and soil will be approximately 1:1 (volume : soil dry weight). The sequential extractability of radioactivity will be checked by analysis (liquid scintillation couting) of each individual extract. After exhaustive extraction, the remaining soil bound residues will be assayed by combustion of soil subsamples. The soil of one sampling interval will be selected for further characterization of residual soil radioactivity (F. J. Stevenson, "Humus chemistry, genesis, composition, reactions", John Wiley & Sons, 443 p (1982), reflected by Standard Operating Procedure (SOP) M 160 of Institut Fresenius Group).
The radioactivity of the water phase will be assayed directly by adding aliquots of the water into liquid scintillation cocktail and counting by liquid scintillation counting. Depending on the radioactivity level the water phase will be subjected directly to appropriate chromatographic procedures.
For the determination of aged microbial activity at the end of the study (J. P. E. Anderson and K. H. Domsch, "A physiological method for the quantitative measurement of microbial biomass in soils", Soil Biol. Biochem. Vol. 10, pp. 215 to 221(Pergamon Press Ltd. 1978), reflected by Standard Operating Procedure (SOP) M 018 of Institut Fresenius Group), unlabeled glyphosate ([^{12}C]-glyphosate) will be used.

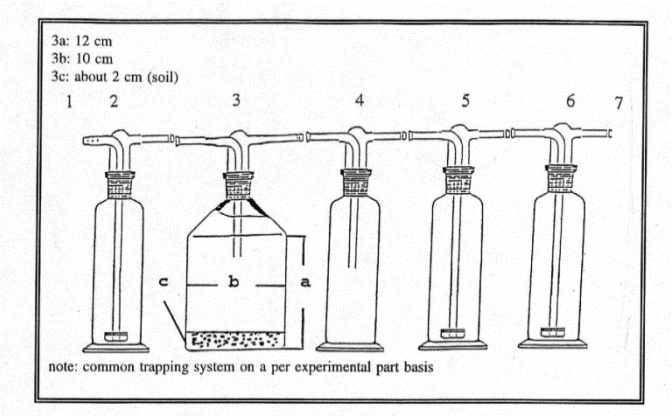


Figure B. 8.1-9: Illustration of the Experimental Design - Flow through system

1 Absorption tube (NaOH) for CO^2 with saturation indication (grey → blue)
2 Gas washing bottle with 50 mL reagent water to moisten the incoming nitrogen gas
3 Metabolism flask (1000 mL conical shoulder bottle, inner diameter: 10 cm)
4 Security bottle
5 Gas washing bottle containing 50 mL 2N NaOH (with saturation indication by cresol red) to absorb CO_2 from soil respiration and $14CO_2$ from the mineralization of the test item. The trap will be installed in duplicate.
6 Gas washing bottle containing 50 mL 2-methoxy ethanol to trap organic volatiles. The trap will be installed in duplicate.
a) Flow meter, not continuously installed, to measure the air flow
b) Dynamic nitrogen gas of a flow rate of about 10 -15 mL/min
c) a common trapping system will be used for the experimental setup

Table B. 8.1-38: Soil properties

Parameters	Soil
Site location	Hofheim, Germany
Soil characteristics :	
Maximum water holding capacity (%)	43.0
Organic carbon (% C)	1.24
pH (H_2O)	6.06
pH ($CaCl_2$)	5.10
Cation exchange capacity (meq./100 g)	13.5
Density (g/l)	1119
% $CaCO_3$	<0.1
Soil type (according to USDA)	
Particle size analysis (mm) USDA :	
Clay (<0.002 mm)	17.8
Silt (0.002 – 0.050 mm)	52.3
Sand (0.050 – 2.00 mm)	29.9

Findings

Recovery and stability of applied radioactivity onto soil and in water :
The total mean recovery of ^{14}C-glyphosate was 97.5 % of the applied radioactivity in soil and 102.6 % in water. Glyphosate was demonstrated to be stable during the extraction method.
The radioactive mass-balance and metabolic pattern are presented in the tables hereafter.

Table B. 8.1-39: a Mass balance from zero time to day 120

Hofheim soil	Incubation time								
	Zero time	6 h	1 d	7 d	14 d	32 d	60 d	90 d	120 d
Water, total (%AR)	96.5	63.8	43.6	37.3	20.9	7.8	6.2	4.1	5.8
Soil ambient extractables, (%AR)	1.9	26.8	40.9	39	53.2	52.6	49.85	43.95	34.3
Soil unextractables, (%AR)	0.6	5.4	9.2	20.8	23.6	31.05	26.8	31.6	36.1
Soil, total (%AR)	2.4	32.2	50.1	59.8	76.7	83.65	76.65	75.55	70.4
CO_2 (%AR)	n.p.	0.1	0.2	0.6	1.1	6.0	15.3	19.1	20.1
Volatiles (%AR)	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Mass balance (%AR)	98.9	96.1	93.9	97.7	98.7	97.4	98.15	98.75	96.2

n.p.: not performed.

The total radioactivity in water decreased up to 4 % until day 90. The total radioactivity increased in soil extractables up to 50 % within day 14, remained at approximately the same level up to day 60 and started to decrease to about 44 % at day 90 and to 34 % at day 120. In soil unextractables, the total radioactivity increased to about 30 % until day 32 and remained stable up to day 120. Radioactivity in CO_2 increased from 1 % to 20 % from day 14 to day 120.

Table B. 8.1-40: Radioactivity distribution in water from day 0 to day 120

Hofheim water	Incubation time								
Rt (min) HPLC	0 h	6 h	1 day	7 days	14 days	32 days	60 days	90 days	120 days
Parent (%AR)	92.9	58.8	41.7	35	18.3	6.6	5.45	2.0	2.2
AMPA	2.4	2.6	3.7	1.4	1.3	0.5	0.8	1.3	3.1
All unknown	1.2	2.4	1.1	1.0	1.4	0.7	nd	1	0.6
Largest unknown	1.2	1.3	0.7	1.0	0.6	0.6	nd	0.8	0.6

nd : not detected

In water glyphosate remained the main compound and decreased by factor 15 within 32 days and stayed at the same level at day 60. It decreased to about 2 % till day 120. The level of AMPA was at its highest value at day 1 (3.7 %) and did not exceed 1 % at day 60. Unknown metabolites were detected at very low level (all unknown < 2.5 %).

Table B. 8.1-41: Radioactivity distribution in soil from day 0 to day 120

Hofheim soil	Incubation time								
Rt (min) HPLC	0 h	6 h	1 day	7 days	14 days	32 days	60 days	90 days	120 days
Parent (%AR)	np	21.4	33.4	30.7	43	42.9	30.85	24.7	13.2
AMPA	np	5.4	7.5	8.3	8.2	7.6	19	19.3	18.3
All unknown	np	nd	nd	nd	2.0	2.2	nd	nd	3.0
Largest unknown	np	nd	nd	nd	1.2	1.3	nd	nd	2.2

np : not performed
nd : not detected

In soil glyphosate remained the main compound within 90 days with an increase from 20 % to about 43 % until day 14 and a decrease up to 69 % from day 14 to day 120. AMPA appeared as the major metabolite in soil increasing to about 8 % within 32 days. A very important increase of AMPA from 8 % to 19 % between day 32 and day 60 can be noticed. Unknown metabolites were found at a very low level not exceeding 3 %.
The DT_{50} glyphosate in the system soil and water was calculated to be 31 days.

RMS Comment
No information on the calculation of the DT_{50} was provided, the study mentiones that this calculation will be provided by an amendment that was not presented to the reviewer. As the study was not performed according to OECD Guideline 307, it is anyway considered as supplementary information and no further calculations were performed.

KIIA 7.1.2/3 (McEwen A., 2004, BVL no 2716555)

Annex point: KIIA 7.1.2/3
Author(s): McEwen A.

Title:	[^{14}C] -Glyphosate: Anaerobic Soil Metabolism (Rate and Route of Degradation in a Sandy Loam Soil)
Date:	July 19th 2014
Guideline(s):	Guidelines concerning the inclusion of Active Substances in Annex I to EEC Council Directive 91/4141EEC as amended by Commission Directive 96/68/EC. Society of Environmental Toxicology and Chemistry (SETAC - EU-ROPE).
Deviations:	None
GLP:	Yes
Acceptability:	No

Material and methods

The metabolism and fate of glyphosate was studied in anaerobic sandy loam soil system collected from Manningtree, Essex. Anaerobicity was achieved by flooding the soil with Milli-Q grade water to a depth of 3 cm over the soil surface and then purging the soil with oxygen-free nitrogen. The ^{14}C-labelled test substance was added to each test vessel containing acclimatised soll at a rate of 4.8 mg/kg (approximately equivalent to an agricultural use rate of 3.6 kg/ha). The test vessels were incubated in the dark at a mean temperature of 20 ± 2 °C. Soil samples were taken for analysis at appropriate time intervals up to 120 days after test substance application. The soil and water in each test system were analysed separately. The soil was extracted with aqueous solvents and the proportions of the test substance and its major degradation products in water and soil were determined. Radiolabelled volatile metabolites and CO_2 were trapped and quantifled. The test substance and major degradation products in water and in soil extracts were identified by comparison with reference substances. Radiolabelled degradation products, which constitute more than 10 % of the applied radioactivily at any time, ware characterised wherever possible.

A single test soil was obtained for the study. Soil was obtained immediately prior to the start of the study from AgroChemex, Manningtree, Essex. Shipment to BioDynamics took place on the day of collection. On receipt the soil was air-dried and was passed through a 2 mm sleve. Characterisation and analysis of the soil was conducted as aseparate GLP-compliant sludy by the Soil Survey & Land Research Centre, Silsoe, UK. The water content of the sieved soil was determined at BioDynamics prior to the establishment of the soil flasks.
The microbial biomass of the soil was measured by the fumigation/extraction method of Vance et al (Soil Biol. and Biochem. 19, 703-707 (1987) and Wu et al (Soil Biol. and Biochem. 22, 1167-1169 (1990)). The work was performed as aseparate GLP-compliant study by Natural Resource Management Ltd. (Table B. 8.1-42)

Table B. 8.1-42: Physical and chemical characteristics of Manningtree A soil

Parameter[a]	Manningtree A
% Sand	66
% Silt	26
% Clay	8
Textural class (MAFF)	Sandy loam

Organic carbon (%)	1.0
pH	6.5
Calion exchange capacity (mEq/1 00g)	7.9
Bulk density (g cm^{-3}')	1.4
Maximum water holding capacity (% m/m)	36.4
Water holding capacity (0.33 bar) (% m/m)	18.2
Water content (% dry weight)*	4.6
Biomass (mg C/100g)[b]	
Day 0	16.55
Day 120	18.49

a Analysis performed at Soil Survey & Land Research Centre, Silsoe
b Analysis performed at Natural Resource Management Ltd
* Measured at BioDynamics

Individual soil systems containing about 50 g soil on a dry weight basis (52.3 g soil wet weight) and 70 ml of MiIIi-Q grade water (to a depth of 3 cm) contained in cylindrical glass vessels of 250 ml capacity.
The weight of each test vessel, contalnlng water and soil, was measured before being incorporated into separate gas-flow system arranged in a series as folIows:

Pre-test system

a) Dreschel bottle (with sintered stem for uniform gas dispersion) containing water to humidify the air-flow.

Test system

b) Test vessel, contalning the soil test system
The end of the glass tube bringing air into the test vessel was positloned just below the water surface.

Post-test system

c) Empty Dreschel bottle (to prevent transfer of trapping solutions into the test vessel in the event of pump malfunction);
d) Dreschel bottle contalning ethyl digol (to trap organic volatile compounds);
e) Dreschel bottle contalning 1 M aqueous potassium hydroxide solution with phenolphthalein Indlcator (to trap $^{14}C0_2$);
f) Dreschel bottle containlng ethanolamine/2-ethoxyethanol (1:3, v/v) (backup $^{14}C0_2$ trap)

During the acclimatisation phase (section 2.4.2) all traps except the humidifying bottle (a) were empty.

Duplicate vessels treated with the 14C-test substance were taken for analysis at zero time and on or about the following times after test substance application: 1. 3, 7, 14, 30, 60, 90 and 120 days. Trapping media was taken for analysis and replaced with fresh media at each sampling

interval then at about weekly intervals during the first month of the study and about every ten days thereafter.

In the analytical phase, the soil and water in each test vessel ware separated by decanting the water from the test vessel. Soil was then extracted as folIows:
Extract 1: Soil was shaken for 2 hours at room temperature with ca 100 ml of 0.5M ammonium hydroxide. The extract solution was separated from the soil solids by centrifugation and the total extract volume recorded. Duplicate aliquots were taken for LSC analysis.
Extract2-3: As extract 1
Extract 4-5:As extract 1 using 1 M hydrochloric acid.

Radioactivity in sam pies was quantified directly by liquid scintillation counting (LSC). Soil residues were air-dried and analysed by eombustion/LSC. Thin-Iayer chromatography was carried out on pre-Iayered cellulose TLC plates, layer thickness 0.25 mm.

Results

Recovery of radloaetivity from soil

Field rate
Recovery of radioactivity during the course of the study was generally in Ihe range 90.5 - 98.0 % applied radioactivity except Day 14 where the recovery was 88.4 % AR. Radioactivity in the water decreased from 97.2 % AR at Day 0 to 5.5 % AR at 120 days whereas radioactivity in the extracts increased from 0.8 % AR at Day 0 to 54.6 % AR at 120 days. No radioactivity was detected in the residue at Day 0 but by Day 120 unextractable radioactivity had increased to 10.6 % AR. Analysis of a representative soil sample showed that the majority of the bound residue *(ca* 65 %) was associated with the fulvic acid fraction. The humin fraction accounted for ca 2 % and the humic acid fraction accounted for ca 30 %. Volalile radioactivity (associated with air traps) increased from 0.5 % AR at Day 1 (no sample at zerotime) to 25.7 % AR at 120 days. The majority of the volatile radioactivity was associated with potassium hydroxide trapping solutions. This indicated that the volatile radioactivity was present as carbon dioxide. This was confirmed by precipitation of the potassium hydroxide solutions with barium chloride. Radioactivity in the solution following precipitation was below twice background indicating radioactivity was present as ^{14}C-carbonate.

Exaggerated rate
Recovery of radioactivity was in the range 89.5 - 98.2 % applied radioactivity. Radioactivity in the water remained at a fairly constant level of approximately 12 %AR. Radioactivity in the soil extracts also remained at a constant level of ca 70 % AR. Unextractable radioactivity accounted for about 5 % AR. Volatile radioactivity generally accounted for 2 %AR or lass (vessel MA40 accounted for 5.6 % AR). As was observed at the field rate, the volatile radioactivity was predominantly associated with the potassium hydroxide traps and was confirmed by precipitation with barium chloride.

Chromatographie analysis

Water

Analysis of water samples at Day 0 by HPLC showed that water contained predominantly glyphosate. Glyphosate in Day 0 samples accounted for 93.2 % AR. This had decreased by Day 30 to 5.6 % AR and was 3.6 % AR by 120 days. Correspondingly there was an increase in the amount of aminomethylphosphonic acid (AMPA) in the water from 1.8 % AR at Day 0 to 5.3 % AR at 90 days decreasing to 1.1 % AR at 120 days. At Day 7 a third unidenlified peak was detected. This accounted for 2.4 % AR at Day 7 and 0.3 % AR by 120 days. It accounted for no more than 2.5 % AR during the course of the study.

Soil extracts
Analysis of soil extracts at Day 0 by HPLC showed that soil contained mainly AMPA, 0.6 % AR. Glyphosate in these samples accounted for 0.1 % AR. The proportion of glyphosate in the soil extracts increased over the course of the study reaching a maximum at 30 days (34.9 % AR). Glyphosate then decreased down to 1.7 %AR at 120 days post application. The proportion AMPA, the major metabolite of glyphosate, increased from 0.6 %AR at Day 0 to 13.0 % AR at Day 1 to 23.1 %AR at Day 7. The proportion of AMPA then remained at ca 20 %AR unlil Day 90 followed by a rapid increase to 44.2 %AR at 120 days post application. A third more polar unidentified peak was also detected. This accounted for a maximum of 14.9 % AR at 90 days post application.

DT_{50} and DT_{90} values
DT_{50} and DT_{90} values have been calculated for glyphosate in both water end soil. In water glyphosate has DT_{50} and DT_{90} values of 15.1 and 49.9 days respectively. The corresponding values in soil are 19.3 and 64.1 days respectively.

Table B. 8.1-43: After applicalion of [^{14}C]-glyphosale. recovery of radioactivily was as folIows

Day	Water	Extracts	Residue	Air traps	Total recovery
0	97.2	0.8	< 0.1	No sample	98.0
1	63.3	28.3	1.7	0.5	93.7
3	40.2	46.8	4.5	0.3	91 .8
7	29.7	56.5	6.6	0.3	93.0
14	17.0	62.9	5.4	3.2	88.4
30	10.8	59.9	8.9	11.0	90.5
60	8.1	55.2	11.4	16.7	91.4
91	7.9	59.6	8.3	18.5	94.2
120	5.5	54.6	10.6	25.7	96.3

Results expressed as % applied radioactivity

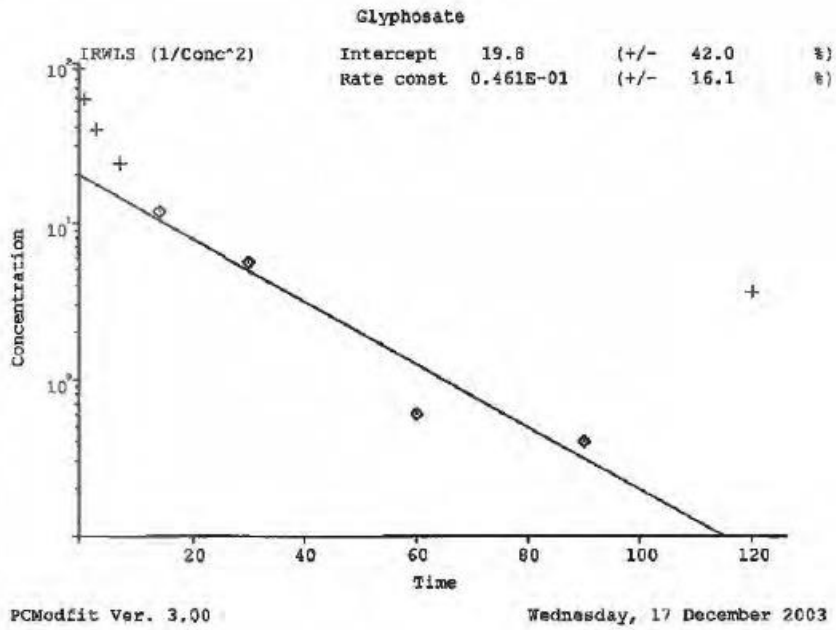
Analysis of the soil water showed that glyphosate was the major component, decreasing from 93.2 % AR at Day 0 to 0.4 % AR at Day 90. Correspondingly AMPA (the major metabolile of Glyphosate) increased from 1.8 %AR at Day 0 to 5.3 % AR by Day 90. A minor, more polar melabolile was also observed which accounted for no more than 2.5 % AR (Day 30).

Analysis of soll extracts showed that the proportion of glyphosate increased for 0.1 % AR at Day 0 to a maximum of 34.9 %AR at Day 30 and then decreased to 1.7 % AR at 120 days. Radioactivity associalеd wilh AMPA remained at a constant level of approximately 20 % AR between Day 7 and Day 90 and then increased to 44.2 %AR at Day 120. The polar peak detected in water was also detected and this accounted for a maximum of 14.9 % AR at Day 90.

Table B. 8.1-44: DT_{50} and DT_{90} values for glyphosale in anaerobic soil and water were also deterrnined.

Parameter (days)	Water	Soil
DT_{50}	15.1	19.3
DT_{90}	49.9	64.1

Water



Soil

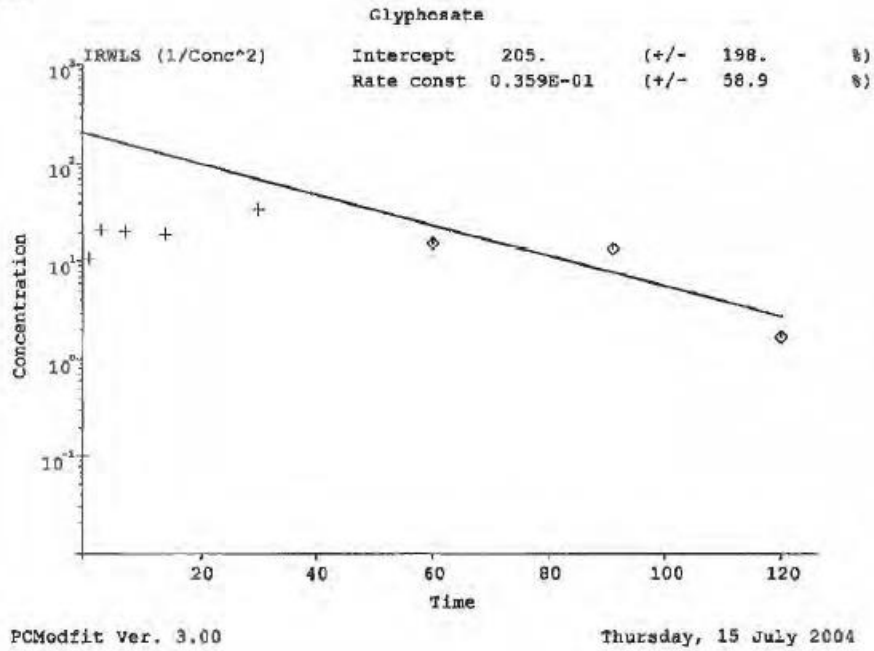


Figure B. 8.1-10: Caalculations of the degradation rates

RMS Comment

Degradation rates were not calculated according to the current guidance. As the study was not performed according to OECD Guideline 307, it is anyway considered as supplementary information and no further calculations were performed.

B.8.1.1.3 Soil photolysis

In the 2001 EU evaluation of glyphosate, several soil photolysis studies (Rueppel and Brightwell (1972, BVL no 1932065); Van Dijk and Burri (1993, BVL no 1932050); Shepler and McGovern (1989, BVL no 1932096) were reviewed and considered acceptable by the RMS. The study summaries can be found in the glyphosate monograph. Additionally, one soil photolysis study with glyphosate trimesium (McGahen, 1983, BVL no 1052662) was reviewed and the results from this study were summarized in the glyphosate–trimesium monograph. In a summary of the study results, the 2001 glyphosate EU evaluation concluded that:

> "Although some differences are found between the various soil photolysis studies, taken together, the results of these soil photolysis studies show that the photolytic degradation of glyphosate on soil surfaces to AMPA is a slow process and is, at most, a very minor pathway for the degradation of glyphosate in soil."

Within the peer review process (evaluation table, data requirement 4.3) the applicant was asked to submit the new soil photolysis studies of Esser (1996), which is evaluated by the RMS below. The results described in Esser (1996) are in line with the results from the 2001 EU evaluation. A summary of the accepted photolysis studies from the 2001 EU evaluation and Esser (1996) is presented in Table B.8.1-45.

~~Therefore, no new soil photolysis studies were submitted by the applicant in the renewal dossier. A summary of the accepted photolysis studies from the 2001 EU evaluation is presented in Table B.8.1-45. It has to be noted that the study of Esser (1996) was not presented to the RMS and therefore could not be evaluated. However, the results described in the dossier of the Notifier are in line with the available studies and therefore are plausible. The study is therefore only regarded as additional information.~~

Table B.8.1-45: Overview of the glyphosate soil photolysis studies[1)]

Reference		Guideline	Application rate (µg/g)	Temp (°C)	Duration of test (days)	Soil characteristics			AMPA (% max)	DT_{50} (days)	
						Soil type	pH	OC (%)		Illuminated	Dark
Studies from the 2001 evaluation	McGahen, 1983, BVL no 1052662	US EPA Section 161-3	30	18 – 37 (light)	8 (Californian sunlinght, 37°) eq. to 8.3 d British sunlight (50°N)	Loamy Sand	5.4[2)]	1.5	24% (light)	5.5[4)] (50°N)	-
	Shepler & McGovern, 1989, BVL no 1932096	US EPA Section 161-3	4.5	22.6 (light) 21.9 (dark)	31 (ca. 12 h light/dark cycle)	Sandy loam	7.6[2)]	0.9	13.0 (light) 9.6 (dark)	90	96
	Van Dijk & Burri, 1993, BVL no 1932050	US EPA Section 161-3	3.6	22	30 (12 h light/ dark cycle)	Silt loam	6.1[2)]	1.4	8.2 (light) 6.1 (dark)	101[4)]	1236[4)]
Studies **not** reviewed in the 2001 evaluation	Esser, 1996	US EPA Section 161-3	11.2	24.9 (light) 24.8 (dark)	30 (natural light/ dark cycle: 38°N, 122°W, October)	Sandy loam	8.3[2)]	0.3	28.4 (light) 28.0 (dark)	6.5[3)]	6.6[3)]

1) Temp = temperature; OC= organic carbon; AMPA= aminomethylphosphonic acid
2) buffer solution unknown
3) linear regression analysis (using natural log of percent dose)
4) second order kinetic model

KIIA 7.1.3/1 (Esser, 1996, BVL no 2716559)

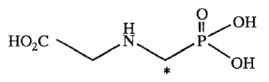
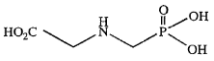
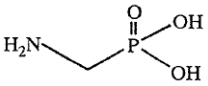
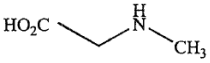
Annex point:	IIA 7.1.3/1
Author(s):	Esser,T.
Title:	(P-Methylene-^{14}C)Glyphosate acid - Photodegradation in/on soil by natural sunlight (WRC-96-066) (WINO19887)
Date:	July 7, 1996
Guideline(s):	EPA Pesticide Assessment Guidelines, Subdivision N. Chemistry: Environmental Fate 161-3, published October 18, 1982
Deviations:	None (Fate, transport and transformation test guidelines OPPTS 835.2410, Photodegradation on soil, October 2008)
GLP:	Yes
Acceptability:	Yes

Material and methods

The photodegradation in/on soil by natural sunlight of glyphosate was studied by Esser (1996). [P-Methylene-14C]glyphosate acid ([14C]-PMG, 97.3% pure by HPLC) was added in an aqueous solution to a thin layer of Visalia sandy loam soil in quartz containers. The application rate was 10.19 ± 0.60 ppm. Dark-control soil samples dosed at the same rate were contained in borosilicate containers wrapped in aluminium foil to prevent irradiation. The moisture of the soil in the containers was adjusted to 75% of water holding capacity at 1/3 bar just after dosing. Containers were sealed with Teflon® septa and placed outdoors in a temperature-controlled water bath for exposure to natural sunlight for up to 30 days. The average temperatures of the light-exposed and dark control soil samples for the study period were 24.91 ± 0.03 and 24.80 ± 0.04 °C.

The test materials are characterised in the following table.

Table B. 8.1-46: Test materials

	Test material (radiolabelled)	**Analytical Reference Standards**		
Name	[P-Methylene-14C]glyphosate acid ([14C]_PMG) (specific activity 42.7 mCi/mmol)	N-(Phosphonomethyl)glycine (PMG)	Aminomethylphosphonic acid (AMPA)	N-Methylglycine (Sarcosine)
Identification	 * Position of radiolabeled carbon atom			
Purity	97.3 %	99.2%	99%	98%
CAS	1071-83-6	1071-83-6	1066-51-9	107-97-1
Stability of test compound	After its use in the study, the stability of the test substance under the conditions of administration was determined by HPLC.	-	-	-

The sandy loam soil Visalia used in the study was collected on May 12, 1994 at ZENECA's Western Regional Technical Center, Visalia, CA. Soil physicochemical properties are given in the following table.

Table B. 8.1-47: Soil physicochemical properties

Soil origin	Soil type (USDA)	pH	CEC (meq/ 100g)	OM (%)	Sand (%)	Silt (%)	Clay (%)	WHC (%) 1/3 bar	Bulk density (g/cc)
Visalia, USA	sandy loam	8.3	8.14	0.80	71.2	20.0	3.8	11.92	1.46

The sandy loam soil used in this study was analyzed for microbial viability prior to the experimental start date. Soil viability was evaluated by enumerating the total colony-forming units (CFU) of aerobic bacteria, actinomycetes and fungi.

Study design

Duplicate light-exposed sample containers were specially designed round quartz chambers (50 mm diameter, 20 mm height), which were sealed with a screw cap fitted with a Teflon® septum. The dark-control containers were identical, but made of borosilicate glass and were covered with aluminium foil to prevent irradiation. The sample containers were submerged in a bath containing deionized water at an approximate 30° angle with respect to the horizon to maximize irradiation during periods of strong sunlight intensity. The water was circulated using a Lauda™ Constant Temperature Circulator (Model RC-20 and RM-20) and maintained at approximately 25.0 ± 1.0 °C. Two small submersible pumps were placed in the bath to prevent local temperature differences. The temperature was acquired each 10 seconds using Type T thermocouples. The mean was calculated and recorded at 20 minute intervals throughout the study. Three thermocouples were used, one was placed in the water bath and one each placed inside and attached to the bottom of the irradiated and dark containers before adding the soil slurry. Volatiles from each individual container were trapped by inserting a needle with tubing attached to a series of traps connected to a water aspirator pump. The traps consisted of an ethylene glycol trap (50 mL) to collect organic volatiles, and two 10% NaOH traps to trap CO_2. From day 20 on, after the regular trapping of headspace, the soil samples were acidified by injecting extraction buffer (5 mL) through the septum, the headspace was purged again through a new series of the same traps. Cloud cover data were compiled using data reported in the San Francisco Chronicle and from observations by PTRL West personnel. The exposure phase was carried out in Richmond, CA at latitude 38° N, longitude 122° W, between October 18 and November 17,1995. Sunlight intensity and cumulative energy (250-700 nm integration range) were measured and recorded at 20 minute intervals throughout the study using an International Light Radiometer (Model 1700) equipped with a Model SE 400 photo detector oriented at a 60° angle with respect to the horizon. The photo detector is calibrated annually by the manufacturer.

Prior to preparation of the soil plates, the moisture content of the sandy loam soil was determined by comparison of the weight of soil subsamples before and after oven drying. The equivalent of 3.1 g of dry soil (3.565 g of moist soil according to the wet/dry ratio) was weighed into each sample container. Deionized water (3 mL) was added to each dish to form slurry, and the slurries were allowed to air dry, forming a thin soil layer corresponding to approximately 3.1 g dry soil on the bottom of the containers. The containers were reweighed after dosing, and the necessary volume of deionized water was added individually to each container to achieve 75% of the water holding capacity at 1/3 bar.

The dosing solution for the study was prepared by adding aqueous [14C]-PMG stock solution (0.238 mL) to 2.562 mL of deionized water. Aliquots (100 µL) of the dosing solution were applied as evenly as possible to each of the previously prepared soil containers. Deionized water (177 µL) was then added to achieve 75% water holding capacity at 1/3 bar. This method resulted in nearly complete coverage of the soil area. Aliquots (5 µL) of the dosing solution taken prior to, during and after the application process were diluted in 4 mL of deionized water, shaken to mix, and aliquots (3 x 200 µL) of each dilution were radio assayed by LSC to determine the applied radiocarbon. The final concentration of test substance in the soil was 10.19 µg/g.

A total of 28 soil containers were prepared for light-exposed and dark-control samples. This allowed for two samples for analysis at time zero, duplicate light-exposed and dark-control samples for five subsequent points (2, 6, 12, 20 and 30 days) and 6 extra soil containers. At 0 time, duplicate dosed soils were extracted immediately, as described below, without headspace trapping. At day 2 and the following sample times, duplicate light-exposed and dark-control samples were removed from the water bath. Volatiles were collected as described previously. Aliquots (0.5 mL) were taken from each trapping solution and radio assayed by LSC. Intermittent trapping of the headspace using the same setup and procedure was performed once a week starting approximately one week after dosing. Samples were weighed following each intermittent trapping to assure that moisture content was maintained at 75% of soil water holding capacity at 1/3 bar. After intermittent trapping the punctured septa were replaced by new ones, and the sealed containers placed back into the water bath. Since some radiocarbon recoveries were low and large amounts of $^{14}CO_2$ were being produced, additional trapping experiments were conducted at day 20 and 30 samplings after purging and trapping the headspace gases. Acidic phosphate buffer (~ pH 2.0) was injected through each septum, the containers were connected to the trapping system, and the mixtures vortexed to release $^{14}CO_2$ adsorbed to the moist soil. At each sampling time the soils were transferred from the containers into pre-weighed Teflon® centrifuge tubes (50 mL), by rinsing the containers with a total of 15 mL of 1 M aqueous KH_2P0_4 adjusted to ~ pH 2.0 with concentrated H_3PO_4. The mixture was shaken for ten minutes with a Wrist Action Shaker. After centrifugation with a Sorvall centrifuge (5,000 rpm, 10 minutes) the supernatant was separated from the residue, and the residue extracted once more with the extraction solvent (total of 15 mL) in the same fashion as the first extraction. The supernatants were combined, the volumes recorded, and aliquots (3 x 1 mL) radio assayed (LSC). For HPLC analyses, subsamples of each replicate sample were filtered using Acrodisc™ CRPTFE filters (Gelman Sciences). Aliquots of the filtrates were co-injected with solutions of mixed analytical reference standard PMG and AMPA.

Following extraction, soil residues were weighed, and aliquots were removed for combustion to determine the levels of un-extracted radiocarbon. Combustions were carried out using a Harvey OX-400 Biological Oxidizer, and the $^{14}CO_2$ generated was trapped with carbon-14 cocktail (RJ. Harvey Instrument Corporation). The radiocarbon content was determined by LSC.

Bound ^{14}C-residues present at ≥ 10% of the applied dose were further characterized. Humic and fulvic acids residue were determined.

The NaOH trapping solutions collected on day 6 were selected for the experiment. Aliquots were taken from each sample for radio assay to determine radiocarbon before precipitation. A

total volume of 3 mL from each NaOH trapping solution was mixed with 1 mL saturated $BaCl_2$ and stored overnight in a refrigerator to facilitate precipitation. The samples were centrifuged for 10 min at 1,500 rpm, and aliquots were taken for radio assay to determine radiocarbon in the supernatant. The difference between the radiocarbon in the NaOH trapping solution before and after precipitation was calculated to determine the radiocarbon in the precipitate.

Test, reference substances and their solutions were stored frozen (< 0 °C) when not in use. Trapping solutions and soil extracts were analyzed for radiocarbon by LSC on the same day as they were collected. Extracts were analyzed by HPLC (Bio-Rad HRLC) within 24 hours of sampling, with the exception of day 2 samples, which were analyzed after three days. All LSC utilized Beckman LS 5000CE or LS 6000IC liquid scintillation spectrometers. Chromatographic methods were validated with reference standards to achieve the necessary resolution and sensitivity. For HPLC radio chromatograms, the limits of detection for individual degradates observed in the HPLC radio chromatograms were determined by the dpm injected, and the liquid scintillation counting detection limit. Peaks containing 2x background dpm are considered to be real.

Results and discussion
Data

Table B. 8.1-48: Accountability of radiocarbon in/on light-exposed soil

Days after application	% applied			
	^{14}C in soil extract	^{14}C non-extracted in residue soil	^{14}C as total volatiles	Total recovery
0 d Rep A	91.6	3.0	n.d.	94.7
0 d Rep B	92.4	3.1	n.d.	95.6
2 d Rep A	70.9	6.3	12.2	89.4
2 d Rep B	65.3	6.2	18.8	90.3
6 d Rep A	49.8	6.4	25.7	81.8
6 d Rep B	48.4	7.2	30.6	86.2
12 d Rep A	40.7	13.0	37.8	91.5
12 d Rep B	40.1	14.8	29.1	84.0
20 d Rep A	36.2	16.2	37.4	89.8
20 d Rep B	41.8	19.0	28.5	89.4
30 d Rep A	25.3	36.1	30.3	91.7
30 d Rep B	26.8	31.0	31.5	89.2

Table B. 8.1-49: Accountability of radiocarbon in/on dark-control soil

Days after application	% applied			
	^{14}C in soil extract	^{14}C non-extracted in residue soil	^{14}C as total volatiles	Total recovery
0 d Rep A	91.6	3.0	n.d.	94.7
0 d Rep B	92.4	3.1	n.d.	95.6
2 d Rep A	70.4	6.1	16.5	93.0
2 d Rep B	73.4	6.4	18.0	97.6
6 d Rep A	51.5	6.2	32.8	90.6
6 d Rep B	43.7	6.6	36.4	86.7
12 d Rep A	37.9	6.1	40.2	84.1
12 d Rep B	40.1	6.7	37.4	84.1
20 d Rep A	36.3	6.1	24.4	66.8

20 d Rep B	36.3	6.5	43.5	86.3
30 d Rep A	28.8	7.4	28/.2	64.4
30 d Rep B	29.2	7.2	43.6	80.0

Table B. 8.1-50: Distribution of [14C]-PMG and its degradates in extracts of light-exposed soil

Days after application	% applied				
	^{14}C in soil extract	PMG	AMPA	Degradate 1	Others
0 d Rep A	91.6	88.69	2.49	0.21	0.21
0 d Rep B	92.4	89.60	1.89	0.17	0.74
2 d Rep A	70.9	57.82	12.58	0.00	0.50
2 d Rep B	65.3	50.99	13.67	0.64	0.00
6 d Rep A	49.8	26.34	22.08	1.38	0.00
6 d Rep B	48.4	26.62	20.69	1.02	0.08
12 d Rep A	40.7	10.85	26.82	3.02	0.00
12 d Rep B	40.1	10.48	27.54	2.07	0.00
20 d Rep A	36.2	6.36	26.60	3.15	0.09
20 d Rep B	41.8	8.05	30.10	3.65	0.00
30 d Rep A	25.3	3.41	18.61	2.57	0.71
30 d Rep B	26.8	2.91	21.06	2.83	0.00

Table B. 8.1-51: Distribution of [14C]-PMG and its degradates in extracts of dark-control soil

Days after application	% applied				
	^{14}C in soil extract	PMG	AMPA	Degradate 1	Others
0 d Rep A	91.6	88.69	2.49	0.21	0.21
0 d Rep B	92.4	89.60	1.89	0.17	0.74
2 d Rep A	70.4	57.17	12.07	0.61	0.56
2 d Rep B	73.2	60.93	11.83	0.34	0.09
6 d Rep A	51.5	30.16	20.85	0.00	0.49
6 d Rep B	43.7	19.51	23.47	0.72	0.00
12 d Rep A	37.9	10.32	26.36	1.22	0.00
12 d Rep B	40.1	13.64	25.46	0.99	0.00
20 d Rep A	36.3	7.57	27.36	1.38	0.00
20 d Rep B	36.3	5.92	28.70	1.60	0.09
30 d Rep A	28.8	2.78	25.00	1.02	0.00
30 d Rep B	29.3	4.36	23.61	1.24	0.00

Mass balance

The material balance of [^{14}C]-PMG applied to the soil, expressed as percent of applied radiocarbon, was determined as the sum of radiocarbon in the soil extracts, volatile traps and bound radiocarbon remaining in the soil. Recoveries averaged 89.5 ± 4.0% (average ± SD, n = 12) and 89.3 ± 5.9% (average ± SD, n = 10) for light-exposed and dark-control samples, respectively. Since the amounts of extracted and bound radiocarbon were usually consistent between replicates, losses of radiocarbon that occurred after day 2 were attributed to the rapid and steady formation of large amounts of $^{14}CO_2$. This caused some leakage from the headspace of the sample containers resulting in lower recoveries in some replicates. Intermittent purging of the headspace at 7 day intervals helped to mitigate the losses, but did not completely solve the problem in the dark-control samples.

Transformation of the parent compound

[^{14}C]-PMG degraded rapidly to the major, terminal metabolite $^{14}CO_2$, which represented a maximum of 32.9 and 36.7% of the applied dose at 12 days in the light-exposed and dark-control soil samples, respectively. Radiocarbon found in the light-exposed and dark-control ethylene glycol traps reached a maximum of 1.9 and 5.8% of the applied dose, respectively, and was not further characterized. Considering unaccounted ^{14}C as CO_2, more CO_2 was formed in the dark-control than in the light-exposed samples. Since the dark-control is essentially a soil metabolism experiment, it is enlightening to note that 64.3% $^{14}CO_2$ was found after 30 days in the soil metabolism study compared to 54% $^{14}CO_2$ (corrected) in the dark-control samples.

[^{14}C]-PMG degraded rapidly in the light-exposed and dark control soil samples and represented only 3.2 and 3.6% of the applied radiocarbon (average of replicates), respectively, after 30 days of exposure. The major degradate detected in the soil extracts was AMPA, which reached a maximum of 28.4 and 28.0% of applied dose (averages of replicates) respectively, in light-exposed and dark-control soil samples at day 20, and represented 19.8 and 24.3% of the applied close (averages of replicates) for light-exposed and dark-control soil extracts, respectively, at day 30. An unidentified degradate, designated Degradate 1, reached 3.4 and 1.5% of applied dose, respectively, in light exposed and dark-control soil samples at 20 days. With the exception of AMPA no other degradates at ~ 10% of the applied dose were detected.

Bound ^{14}C-residues, defined as unextractable with phosphate buffer (~ pH 2.0), reached a maximum of 7.3% of applied dose (average of replicates) at day 30 in the dark-control residue soils; however, in the light-exposed residue soils, the bound ^{14}C-residues reached maximum of 33.6% (average of replicates) at day 30. Light exposed extracted soils from day 30 replicates A and B were therefore selected for additional extraction using 0.l M NaOH for characterization of the bound 14C-residues. Only 3.6 and 6.3% of applied dose (average of replicates) were associated with the humic and fulvic acid fractions, respectively.

The degradation rate of [^{14}C]-PMG in sandy loam soil was determined by linear regression analysis of the natural log of percent dose versus days after treatment. The calculated half-life of [^{14}C]-PMG in light-exposed samples was determined to be 6.5 days (R^2= 0.940). The calculated half-life of [^{14}C]-PMG in dark-control samples was determined to be 6.6 days (R^2 = 0.922).

Conclusion

Radiocarbon recoveries for the study averaged 89.5 ± 4.0% and 89.3 ± 5.9% for the light exposed and dark-control samples, respectively, based on the applied radiocarbon. Small losses of radiocarbon occurred throughout the study, due to the rapid and steady formation of the terminal metabolite, $^{14}CO_2$. Up to 32.9% and 36.7% of the applied dose in the light-exposed and dark-control NaOH traps, respectively, was recovered as $^{14}CO_2$ at day 12. PMG rapidly degraded in both, light-exposed and dark-controls, representing only 3.2% and 3.6 % of the applied dose, respectively, at the end of the study. The half-life of PMG was calculated to be 6.5 days and 6.6 days, respectively, in the light-exposed and dark-control soil test systems.

The major product detected in the light-exposed and dark-control soil extracts was AMPA, which represented 19.8 and 24.3% of the applied dose, respectively, at day 30. A minor non-identified degradate, Degradate 1, represented only 2.7 and 1.1 % of applied dose, in the light-

exposed and dark-·control soil extracts, at the end of the study. No degradates, other than AMP A, were detected at ≥ 10% of the applied dose. A pattern of steady increase of the major terminal metabolite CO_2, and the rise and slight decline of the metabolite AMP A was clearly established.

The only significant difference between light-exposed and dark-control samples was increased post extraction soil residues in the irradiated samples. The unextracted radiocarbon in the dark-control soil reached 7.3% of the applied dose at day 30. The unextracted radiocarbon in the light-exposed soil reached 33.6% of the applied dose at day 30. Additional extractions of the light-exposed residual soil using 0.1 M NaOH were performed on the day 30 post extracted soils. Only 3.6% and 6.3% of the applied dose was found to be associated with the humic and fulvic acid fractions, respectively; the rest of the unextractable ^{14}C remained bound to humin.

Exposure of PMG treated soil to light had no effect on the degradation rate of PMG or extractable residues found. The results of the study indicate that photolysis in/on soil is not likely to be a significant route of dissipation for PMG, compared to rapid microbial degradation in soil.

RMS Comment
The study meets the criteria set in the current guideline on photodegradation on soil (Fate, transport and transformation test guidelines OPPTS 835.2410, Photodegradation on soil, October 2008) and can be accepted.

B.8.1.2 Rate of degradation in soil

B.8.1.2.1 Laboratory studies

During the first EU review of glyphosate (2001), among the submitted studies, only two route of degradation studies (Galicia and Morgenroth, 1993, BVL no 1932059 and one soil of Matla and Vonk, 1993, BVL no 2151389) and two rate studies (Schneider, 1991, BVL no 1062355; Galicia & Flückiger, 1993 BVL no 1932046 together with two additional soils of Matla and Vonk, 1993, BVL no 2151389) conducted with glyphosate in the laboratory under aerobic conditions were assessed as “acceptable” based on the evaluation criteria and guidance in force at that time. However, when re-evaluating the previously submitted studies, it was concluded by the RMS that the route study Kesterson & Atkins (1991, BVL no 1932061)/ Honegger (1992, BVL no 2325652) (previously considerd as not acceptable) is now considered acceptable by RMS according to current guidelines and requirement (for details see B.8.1.1).

Summaries of the studies can be found in the glyphosate Monograph.

Additionally, rate studies with glyphosate-trimesium were submitted during the first EU review of glyphosate (2001). The route studies McBain (1985, BVL no 1052660) and McBain et al. (1985, BVL no 1052661) and the rate studies Lewis & Turnbull (1992, BVL no 1052659) and Runnalls (1991, BVL no 2154349) were considered acceptable on the evaluation criteria and guidance in force at that time. Study summaries of the four studies can be found in the glyphosate-trimesium Monograph.

In order to refine risk assessment endpoints and more importantly in order to comply with current guidelines (OECD Guideline 307, 2002), an additional laboratory aerobic soil rate of

degradation study (Ponte, 2010, BVL no 2310255) was conducted together with the new route study Ponte (2010, BVL no 2310242) summarised under B.8.1.1.1. The GTF concluded that a new rate study was needed since the older studies have deficiencies according to the newer guidance provided by FOCUS (2006, 2011) regarding data quality and data handling that are relevant to the kinetic analysis.

Table B.8.1-52 gives an overview of experimental conditions and soil characteristics of all aerobic soil rate and route of degradation studies with glyphosate evaluated during the first EU glyphosate review, that were considered acceptable in addition to the new route and rate of degradation studies not reviewed in the 2001 evaluation together with the new studies submitted for this review. Since, the study Kesterson & Atkins (1991, BVL no 1932061)/ Honegger (1992, BVL no 2325652) that was previously not considered acceptable by the RMS, is now considered acceptable for the soil dupo, the experimental conditions and soil characteristics are included.

Table B.8.1-53 gives an overview of experimental conditions and soil characteristics of all aerobic soil rate and route of degradation studies with glyphosate-trimesium evaluated during the first EU glyphosate review.

Table B.8.1-52: Overview of the laboratory aerobic rate of degradation studies with glyphosate

Reference	Guideline	Temp (°C)	Duration of test	Soil charactieristics				
				Soil origin	Soil type	pH	OC (%)	Moisture
Galicia & Morgenroth, 1993 BVL no 1932059	US EPA Section 162-1, BBA Part IV, 4-1	20	364 d	Les Evouettes	Silt loam	6.1[2)]	1.4	40 % MWHC
Kesterson & Atkins, 1991, BVL no 1932061 & add. Honegger, 1992, BVL no 2325652	US EPA Section 162-1	25	12 month	Dupo	Silt Loam	7.5	0.6	75 % FC
Matla & Vonk, 1993, BVL no 2151389	Dutch Regulations for Biocides	20	100 d	Droevendaal	Loamy sand	5.2[3)]	2.3	1/3 bar
		20	100 d	Lisse	Sand	7.2[3)]	0.6	1/3 bar
		20	100 d	Maasdijk	Sandy Loam	7.5[3)]	1.1	1/3 bar
Dean, 1995, BVL no 2310244	US EPA Section 162-1, BBA Part IV, 4-1, Japanese MAFF, Draft Guidelines for 91/414	20	180 d	Arrow	Sandy Loam	5.9[4)]	2.2	40 % MWHC
Goodyear, 1996, BVL no 2310246	US EPA Section 162-1, Japanese MAFF	25	121 d	Soil A	Loam	5.9[5)]	6.8	75 % of 1/3 bar
		25	121 d	Soil B	Sandy Loam	6.7[5)]	0.7	75 % of 1/3 bar
Esser, 1996, BVL no 2310248	US EPA Section 162-1	25	31 d	Visalia	Sandy Loam	8.32)	0.3	75 % of 1/3 bar
McLaughlin & Schanné, 1996,	BBA Part IV, 4-1, FAO	20	90 d	Speyer 2.1	Sand	5.9[4)]	0.6	45 % MWHC

Reference	**Guideline**	**Temp (°C)**	**Dura-tion of test**	**Soil charactieristics**				
				Soil origin	**Soil type**	**pH**	**OC (%)**	**Moisture**
BVL no 2310250	guidelines, SETAC (1995)	20	120 d	Speyer 2.2	Loamy Sand	5.6[4)]	2.3	45 % MWHC
		20	60 d	Speyer 2.3	Loamy Sand	6.4[4)]	1.2	45 % MWHC
Galicia & Flückiger, 1993, BVL no 1932046 & add. Mamouni, 2002, BVL no 2437068	BBA Part IV, 4-1	20	105 d	Speyer 2.1	Sand	6.1[2)]	0.70	40 % MWHC
		20	105 d	Speyer 2.2	Sand	6.0[2)]	2.29	40 % MWHC
		20	105 d	Speyer 2.3	Loamy sand	6.9[2)]	1.34	40 % MWHC
Schneider, 1991, BVL no 1062355	BBA Part IV, 4-1	22-26	100 d	F1	Sand	5.7	0.70	40 % MWHC
		22-26	100 d	F2	Silt sand	6.4	1.34	40 % MWHC
		22-26	100 d	2.2	Loamy sand	5.6	2.29	40 % MWHC
		22-26	100 d	Eigen-boden	un-known	un-know n	un-know n	40 % MWHC
Ponte, 2010, BVL no 2310242	US EPA OPPTS 835.4100, OECD 307	20	132 d	Gartenacker	Loam	7.1[5)]	2.0	pF 2.5
Ponte, 2010, BVL no 2310255	US EPA OPPTS 835.4100, OECD 307	20	70 d	Drusenheim	Loam	7.4[5)]	1.7	pF 2.5
		20	120 d	Pappelacker	Sandy Loam	7.0[5)]	1.9	pF 2.5
		20	120 d	18-Acres	Clay Loam	5.7[5)]	2.5	pF 2.5

1) Temp = temperature; OC= organic carbon; MWHC= maximum water-holding capacity; FC=field capacity; AMPA= aminomethylphosphonic acid
2) buffer solution unknown
3) buffer solution = KCl
4) buffer solution = $CaCl_2$
5) buffer solution = H_2O
6) mean of two sampling dates

Table B.8.1-53: Overview of the laboratory aerobic rate of degradation studies with glyphosate-trimesium[1)]

Reference	Guideline	Temp (°C)	Duration of test	Soil charactieristics				
				Soil origin	Soil type	pH	OC (%)	Moisture
McBain, 1985, BVL no 1052660	US EPA Subdivision N, Section 162-1 (1982)	18 – 26.7	376	Sorrento	Loam	6.8	1.8	Field capacity
McBain, 1985, BVL no 1052661		18 – 26.7	211	Sorrento	loam	6.8	1.8	Field capacity
Runnalls, 1991, BVL no 2154349	BBA Part IV, 4-1	20	108	Speyer 2.1	Loamy sand	6.6[5)]	0.5	40 % MWHC
		20	108	Speyer 2.2	Loamy sand	6.0[5)]	2.4	40 % MWHC
		20	108	East Jubilee	Sandy loam	5.7[5)]	3.0	40 % MWHC
		20	108	18 Acres	Sandy clay loam	6.2[5)]	5.2	40 % MWHC
Lewis & Turnbull, 1992, BVL no 1052659	Unknown (test conditions according to BBA Part IV, 4-1)	20	104	Speyer 2.1	Sand	6.6[2, 6)]	0.5	40 % MWHC
		20	104	Speyer 2.1	Sand	6.6[2, 6)]	0.5	20 % MWHC
		8	104	Speyer 2.1	Sand	6.6[2, 6)]	0.5	40 % MWHC
		20	104	Speyer 2.1	Sand	6.6[2, 6)]	0.5	40 % MWHC
		20	104	Beedor Manor	Clay loam	7.8[2)]	2.1	40 % MWHC

1) Temp = temperature; OC= organic carbon; MWHC= maximum water-holding capacity; FC=field capacity; AMPA= aminomethylphosphonic acid
2) buffer solution unknown
3) buffer solution = KCl
4) buffer solution = $CaCl_2$
5) buffer solution = H_2O
6) mean of two sampling dates

On re-evaluation of the rate studies with glyphosate and with glyphosate-trimesium submitted for the first EU review of glyphosate (2001), defiencies were noted for some of the studies.

These deficiencies are summarised in Table B.8.1-54 and Table B.8.1-55 and include the use of not representative soils, not well defined incubation conditions, and low or not reported mass balances and the use of oxygen instead of moistened air to vent the headspace of the test vessels. Due to the described defiencies, the results of soil Visalia of the study Esser (1996, BVL no 2310248), of the soils Droevendaal and Lisse of the study Matla & Vonk (1993, BVL no 2151389), of the soil A of the study Goodyear (1996, BVL no 2310246), of the soil Speyer 2.1 of the study Galicia & Flückiger (1993, 1993 BVL no 1932046)/ Mamouni (2002, BVL no 2437068), of all soils of the study Schneider (1991, BVL no 1062355), of the soil of the studies McBain (1985, BVL no 1052660) and McBain et al. (1985, BVL no 1052661) and of the soils Speyer 2.1, Jubilee and 18-Acres of the study Runnalls (1991, BVL no 2154349) are not considered acceptable by the RMS anymore and will thus not be used for endpoint derivation.

However, when re-evaluating the study Kesterson &Atkins (1991, BVL no 1932061)/ Honegger (1992, BVL no 2325652) it was concluded that the residue data of the soil Dupo can be used for endpoint derivation provided the overall mass balances at the respective sample points are >90 %. It was also concluded by the RMS, that the residue data of the soil Speyer 2.2 (Runnalls, 1991, BVL no 2154349) can still be used, since the average overall mass balance for this soil remained above the 70 % recommended in the OECD guideline 307 (2002) for studies with non-radioactive labelled compounds and the soil does represent soil characteristics of typical soils under agricultural use. Since, the overall mass balance at day 0 of the study Lewis & Tunrbull (1992, BVL no 1052659) were between 90-110 % and the study conditions in the study were well defined, it was also concluded by the RMS that the residue data of both soils can still be included for end point derivation.

Table B.8.1-54: Deficiencies of laboratory aerobic rate of degradation studies with glyphosate

Reference	Guideline	Defiencies	Acceptablilty by RMS
Galicia & Morgenroth, 1993 BVL no 1932059	US EPA Section 162-1, BBA Part IV, 4-1	None	Yes
Kesterson & Atkins, 1991, BVL no 1932061, & add. Honegger, 1992, BVL no 2325652	US EPA Section 162-1	- Total recoveries <90 % for most samples of the soil Kickapoo and some of the duplicate samples and sample points of soil Dupo - Microbial biomass only measured at the beginning of the study	- No for soil Kickapoo - Yes for soil Dupo (exclusion of data points with mass balance <90 %)
Matla & Vonk, 1993, BVL no 2151389	Dutch Regulations for Biocides	- Mass balance missing for the soils Droevendaal and Lisse - Microbial biomass in soil Lisse at day 0 and at the end of study < 1 % of oc-content - Residues of glyphosate in soil Droevendaal indicate an analytical error and don't allow a kinetic evaluation (sudden increase in glyphosate concentration after 35 days, no decline afterwards)	- No for soils Droevendaal and Lisse - Yes for soil Maasdijk
Dean, 1995, BVL no 2310244	US EPA Section 162-1, BBA Part IV, 4-1, Japanese MAFF, Draft Guidelines for 91/414	None	Yes
Goodyear, 1996, BVL no 2310246	US EPA Section 162-1, Japanese MAFF	- oc-content of soil A is > 6.8%, not representative for soil under agricultural use	- No for soil A - Yes for soil B
Esser, 1996, BVL no 2310248	US EPA Section 162-1	- oxygen was used instead of ambient air for airing of the test vessels	No
McLaughlin & Schanné, 1996, BVL no 2310250	BBA Part IV, 4-1, FAO guidelines, SETAC (1995)	None	Yes
Galicia & Flückiger, 1993, BVL no 1932046 &	BBA Part IV, 4-1	- 4 last data points with overal mass balance <90% for soil Speyer 2.1, only three remaining data points	- No for soil Speyer 2.1 - Yes for soils

Reference	Guideline	Defiencies	Acceptablilty by RMS
add. Mamouni, 2002, BVL no 2437068		with mass balance >90% - 1 duplicate sample with overal mass balance <90% for soil Speyer 2.2 - 2 last data data points with overal mass balance <90% for soil Speyer 2.3	Speyer 2.2 & 2.3 (exclusion of data points with mass balance <90%)
Schneider, 1991, BVL no 1062355	BBA Part IV, 4-1	- incubation temperature between 22-26 °C (not clearly defined) - low mass balance at time 0 for all four soils (36 – 84%) - no mass balance values stated for later sample points - no values for each duplicate only mean values stated - low application rate (1ppm)	No
Ponte, 2010, BVL no 2310242	US EPA OPPTS 835.4100, OECD 307	None	Yes
Ponte, 2010, BVL no 2310255	US EPA OPPTS 835.4100, OECD 307	None	Yes

Table B.8.1-55: Defiencies of laboratory aerobic rate of degradation studies with glyphosate-trimesium

Reference	Guideline	Defiencies	Acceptablilty by RMS
McBain, 1985, BVL no 1052660 McBain, 1985, BVL no 1052661	US EPA Subdivision N, Section 162-1 (1982)	- incubation temperature not clearly defined - oxygen was used instead of ambient air for airing of the test vessels	No
Runnalls, 1991, BVL no 2154349	BBA Part IV, 4-1	- non-radioactive labelled substance used - only average mass balances stated: 82 % for soil Speyer 2.1, 80 % for soil Speyer 2.2, 60 % for soil Jubilee and 55 % for soil 18-Acres - oc-content of soil Speyer 2.1 very low (0.46%) and of soil 18-Acres very high (5.22 %)	- No for soil Speyer 2.1, Jubilee and 18-Acres - Yes for soil Speyer 2.2
Lewis & Turnbull, 1992, BVL no 1052659	Unknown (test conditions according to BBA Part IV, 4-1)	- Overall mass balance only reported for day 0 (with > 90- < 110 % AR for all dose groups)	Yes (soil incubations at 20 °C and 40 % MWHC with 4 ppm glyphosate-trimesium used for further evaluation)

The end points reported in the studies evaluated during the first EU review are not appropriate for risk assessment and exposure modelling of the environmental fate of glyphosate in the EU anymore. Thus, the DT_{50} and DT_{90} values derived by study authors are not presented in the here anymore and are not included in the study summaries.

Instead, the aerobic degradation rates of glyphosate and AMPA from the old and new route and rate of degradation studies Ponte (2010, BVL no 2310242 &, BVL no 2310255), Goodyear (1996, BVL no 2310246), Esser (1996, BVL no 2310248), Dean (1995, BVL no 2310244), Galicia & Morgenroth (1993, BVL no 1932059), Galicia &Flückinger 1993, BVL no 1932046 with addendum Mamouni (2002, BVL no 2437068) and Matla & Vonk (1993, BVL no 2151389) were re-calculated in the study Dorn (2012, BVL no 2315991) in accordance with the kinetic approaches recommended in the latest guidance (FOCUS, 2006, 2011). A detailed summary of the updated kinetic evaluation (Dorn, 2012, BVL no 2315991) is provided under B.8.1.2.1. Additionally, aerobic degradation rates of glyphosate and AMPA in the soil Dupo of Kesterson & Atkins (1991, BVL no 1932061)/ Honegger (1992, BVL no 2325652), the soil Speyer 2.2 of Runnalls (1991, BVL no 2154349) and in the soil~~s~~ Speyer 2.1 ~~and Beedon Manor~~ of Lewis & Turnbull (1992, BVL no 1052659) were re-calculated according to FOCUS (2006, 2011) guidance by the RMS. Only the residues with 4 ppm applied glyphosate-trimesium incubated at 20°C and 40 % MWHC of study Lewis & Turnbull (1992, BVL no 1052659) were used for kinetic re-evaluation.

Table B.8.1-112, Table B.8.1-113, Table B.8.1-114 and Table B.8.1-115 list the resulting DT_{50} and DT_{90} values of glyphosate selected by the RMS in accordance with FOCUS (2006, 2011) guidance to be used for persistence calculations and modelling and for evaluation of the P-criterion of PBT substances.

Table B.8.1-116 and Table B.8.1-117 list all DT_{50} and DT_{90} values of AMPA selected by the RMS in accordance with FOCUS (2006, 2011) guidance to be used for persistence calculations and modelling.

Under aerobic conditions, degradation of glyphosate in soil mostly follows biphasic kinetic with a few occasions of SFO kinetic. DT_{50} and DT_{90} values of glyphosate under different soil moisture conditions and temperatures of 20 or 25 °C to be used for persistence calculations range from 1.0 - 67.7 d and from 9.3 - 471.4 d, respectively. At 10 °C, the degradation of glyphosate followed DFOP kinetic with a DT_{50} value of 8.1 d and a DT_{90} value of 50.8 d. Normalised SFO or back-calculated SFO DT_{50} at 20 °C and pF2 derived from best fit kinetics to be used for evaluation of the P-criterion of potential PBT, vPvB or POP substances range from 3.6 d to 133.8 d with a geometric mean of 18.7 d. The geometric mean of normalised SFO or back-calculated SFO DT_{50} of glyphosate at 20 °C and pF2 to be used for modelling is 21.0 d.

The metabolite AMPA was formed during aerobic degradation of glyphosate with formation fractions between 0.1817 - 0.6076 and mean formation fraction of 0.3680. AMPA subsequently degraded following SFO kinetics with DT_{50} and DT_{90} values under different soil moisture conditions and temperatures of 20 or 25 °C to be used for persistence calculations ranging from 39.0 - 300.7 d and 129.5 - 998.9 d, respectively. The geometric mean of normalized SFO DT_{50} of AMPA at 20°C and pF2 is 88.8 d.

KIIA 7.2.1/1 (Ponte, 2010, BVL no 2310255)

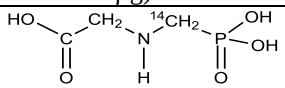
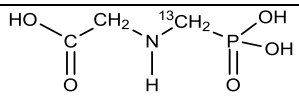
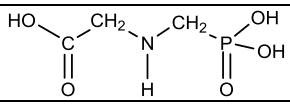
Annex point:	IIA 7.2.1/1
Author(s):	Ponte, M.
Title:	Rate of degradation of [14C]glyphosate in three soils incubated under aerobic conditions
Date:	October 6, 2010
Guideline(s):	U.S. EPA Fate, Transport and Transformation Test Guidelines OPPTS 835.4100, Aerobic, Soil Metabolism (adopted October 2008) and OECD Guideline 307 for Testing of Chemicals: Aerobic and Anaerobic Transformation in Soil (adopted April 2002)
Deviations:	The mass balances for the Drusenheim, Pappelacker soils were not always between 90 % and 110 % as outlined in OECD 307 (labelled chemicals). Therefore, RMS proposes to use only the sampling data for kinetic evaluation where the recoveries ranged from 90 % to 110 %.
GLP:	Yes
Acceptability:	Yes

Material and methods

The rate of degradation of glyphosate (N-(phosphonomethyl)glycine) was studied under aerobic conditions using [^{14}C]-glyphosate in three soils, Drusenheim loam, Pappelacker loamy sand and 18-Acres sandy clay loam soil. The soil samples with moisture contents maintained at 50 ± 10 % water holding capacity (WHC) at pF 2.5, were treated with [^{14}C]glyphosate and incubated in the dark at 20 ºC for up to 120 days. Throughout the incubation period, soil samples were continuously aerated. The [^{14}C]-glyphosate dose rate was 3.80 µg/g of soil, which corresponds to a glyphosate recommended field rate of 2.88 kg/ha, based on a soil density of 1.5 g/cm^3 and a penetration depth of 5 cm.

The test materials are characterised in Table B.8.1-56.

Table B.8.1-56: Test materials

	Test material (radiolabelled)	**Test material (radiolabelled)**	**Test material (non-radiolabelled)**
Name	[^{14}C] N-(phosphonomethyl) glycine (glyphosate, specific activity 47 mCi/mmol or 10.28 MBq/mg or 6.17 x 105 DPM/µg)	[^{13}C] N-(phosphonomethyl) glycine (glyphosate, analytical reference standard))	N-(phosphonomethyl) glycine (glyphosate)
Identification			
Purity	HPLC Radiochemical purity: ≥ 98 %	98.0 %	99.8 %
CAS	-	-	1071-83-6
Stability of test compound	Received as a neat compound and was stored in a freezer (< 0 °C). The stability of the test substance was confirmed under conditions of the dose administration.	Stored in the refrigerator, expiration date: June 30, 2011	Stored at ambient lab temperature; expiration date: January 31, 2011

Three soils were used for the study: a loam soil (Drusenheim), a loamy sand soil (Pappelacker) and a sandy clay loam soil (18-Acres) from Alsace (France), Basel (Switzerland) and Bracknell (United Kingdom), respectively. Soil was collected from a location with restricted access with no pesticides or organic fertiliser treatments for at least five years prior to collection. Soil physicochemical properties are given in Table B.8.1-57.

Table B.8.1-57: Soil physicochemical properties

Soil property	**Soil series name**		
	Drusenheim	**Pappelacker**	**18-Acres**
Sand	47	75	51
Silt	28	20	24
Clay	25	5	25
Classification[1)]	Loam	Loamy sand	Sandy clay loam
Organic carbon (%)	1.7	1.9	2.5
Organic matter (%)	2.9	3.2	4.4
Cation exchange capacity (meq/100g)	23.6	11.7	18.1
pH[2)]	7.4	7.0	5.7
Microbial biomass (µg C/g dry basis)[3)]	255.2 (day 0) 134.8 (day 70)	164.4 (day 0) 256.3 (day 91) 157.3 (day 120)	487.8 (day 0) 615.7 (day 91) 305.2 (day 120)
Maximum water holding capacity (gm/100 gm)	34.3	40.7	51.5
Moisture (%) at 1/3 bar	17.6	12.4	19.7
Bulk density (gm/cc)	1.14	0.98	1.03

1) USDA textual class (hydrometer method)
2) Measured in a 1 : 1 soil : water suspension
3) Based on a Fumigation an Extraction Method by: Vance, E.D. (1987) An Extraction Method for Measuring Soil Microbial Biomass C. Soil Biol. Biochem., Vol. 19, No. 6, pp. 703-707.

Study design

The target dose for all samples was 3.8 ppm, corresponding to a maximum recommended field application rate of 2.88 kg glyphosate/ha, based on a soil bulk density of 1.5 g/cm^3 and a penetration depth of 5 cm.

Portions of sieved Drusenheim soil, Pappelacker soil and 18-Acres soil (50.0 g dry weight equivalent for all soils) were weighed and adjusted to 50 ± 10 % maximum water holding capacity (MWHC) at pF 2.5. Prior to test substance application, samples were placed in a constant temperature chamber, covered with cheesecloth and allowed to equilibrate in the dark at 20 ± 1 °C for at least one week prior to dosing.

The dosing solution was prepared by combining an aliquot (7.76 mg) of [^{12}C]-glyphosate standard with an aliquot (8.83 mg) of [^{13}C]-glyphosate standard and an aliquot of [^{14}C]-glyphosate test substance (4.23 mg, 1.176 mCi) and dissolving in water to a final volume of 54.5 mL. The resulting [^{14}C]-glyphosate treatment solution used for dosing had a specific activity of 1.26 x 10^5 DPM/µg.

A total of 28 samples, each containing 50 g of soil (dry weight) and consisting of 2 samples for time 0 in Teflon centrifuge bottles, 4 samples for biomass in amber bottles and 22 samples in amber jars, were prepared for each soil. The soil samples (except time 0 samples and initial soil samples for biomass determination) were placed in large glass soil containers connected to the trapping systems inside a walk-in constant temperature chamber and maintained at 20 ± 1 °C in the dark. Biomass samples were not dosed but were incubated in the same manner as the dosed samples. Time 0 samples were extracted immediately after dosing.

Immediately following test substance application, all samples were aerated and headspace was trapped in trapping solutions continuously during the incubation period. Approximately once every two weeks the samples were weighed. To maintain moisture levels, deionised water was added as required. Trapping solutions for soil containers were exchanged at each sampling time and when required during longer incubation intervals.

All samples were extracted on the day of collection and initial HPLC analysis was performed within eight days of collection. All samples and standard solutions were stored frozen (<0 °C) when not in use.

For Drusenheim soil, samples were collected immediately after treatment (time 0) and after 1, 3, 8, 14, 27, 48 and 70 days of exposure. For Pappelacker soil, samples were collected at dosing (time 0) and after 1, 3, 8, 14, 27, 48, 70, 91 and 120 days of incubation. Samples were collected at the time of treatment (time 0) and after 8, 14, 21, 41, 63, 91 and 120 days for 18-Acres soil.

The microbial biomass of the Drusenheim soil was determined prior to dosing and at the end of the incubation period. The microbial biomass for the Pappelacker and 18-Acres soils were determined at experimental start, during the incubation period and at the end of the study.

At each sampling time, duplicate samples were removed from their respective container in the constant temperature chamber. Trap solutions for the entire container were exchanged with fresh ones. Total volumes in each trap were measured and aliquots were radioassayed.

After sampling, the soil sample was transferred to a pre-weighed centrifuge bottle and an aliquot (100 mL) of the extraction solvent (0.5 M ammonium hydroxide solution) was added. Then the samples were placed on a Wrist-Action shaker for 1 hour followed by centrifugation for 30 minutes at 4000 rpm. The supernatant (soil extract) was decanted into a graduated cylinder and the extraction procedure was repeated two or three more times with 100 mL of fresh extraction solvent. The soil extracts were combined and a sub-aliquot was transferred to a centrifuge tube. The sub-aliquot was centrifuged for 30 minutes at 10,000 rpm to remove any soil remaining in the combined extracts. Aliquots of the centrifuged combined extracts were taken for radioassay.

Soil extracts were concentrated via roto-vac, Savant Speed-Vac or by rotary evaporation under reduced pressure and cleaned up before HPLC analysis. Dried samples were reconstituted in HPLC water and sonicated to dissolve. The reconstituted supernatants were analysed by LSC to monitor any loss of radioactivity.

All soil extracts and radiochemical purity checks were analysed using a cation-exchange HPLC (Transgenomic Glyphosate Analysis Column, 300 x 4.6 mm; Transgenomic Glyphosate pre column; flow rate 0.5 mL/min; 40 °C; UV detector 215 nm) The mobile phase was an isocratic solvent of 0.005M KH2PO4 in HPLC water with 4 % methanol (pH 1.80).

The quantitative identification of glyphosate and AMPA was conducted by secondary anion exchange HPLC analysis of selected concentrated extracts and by comparison to co-elution of the reference standard. The anion-exchange HPLC column (Allsphere SAX, 250 mm x 4.6 mm x 5 µm) eluted with a solvent gradient of 5 mM to 100 mM KH_2PO_4 in HPLC water at pH 2 with methanol (96/4 v/v) were used to confirm the identity of glyphosate and AMPA. For ^{14}C detection, fractions (0.5 min/fraction for 50 minutes) of eluents were collected and radioassayed on LSC for all HPLC analyses.

Traps for volatiles included an ethylene glycol trap to collect organic volatiles and two 10 % aqueous NaOH traps to collect CO_2. For quantification of volatiles the 10 % NaOH and volatile organic traps were radioassayed directly by Liquid Scintillation Counting (LSC). Selected caustic traps were treated with a saturated $BaCl_2$ solution to confirm the presence of dissolved $^{14}CO_2$. Aliquots were taken of the composite trap solutions for radioassay by LSC. Then, 4 mL aliquots of the composite solutions were combined with 4 mL aliquots of aqueous saturated $BaCl_2$ solution and vortexed for 30 seconds to mix. The samples were then allowed to precipitate at room temperature for 1 hour followed by overnight refrigeration prior to centrifugation for 10 minutes at 2,500 rpm to separate the layers. Aliquots of the supernatants were radioassayed by LSC and compared to the composite aliquots before treatment. The absence of radioactivity in the supernatant after precipitation confirmed the formation of $Ba^{14}CO_3$ from trapped $^{14}CO_2$.

Limits of detection for radioassay were determined by the aliquot size and the liquid scintillation counting detection limit. The limits of detection for combustion samples were determined to be 0.0028 ppm. The limits of detection for individual metabolites observed in the HPLC radio chromatogram were determined to be 0.003 ppm.

Results and discussion

Data

The mass balance and the distribution of glyphosate and its degradation products in the soil Drusenheim, Pappelacker and 18-Acres are given in Table B.8.1-58, Table B.8.1-59 and Table B.8.1-60.

Table B.8.1-58: Degradation product distribution over 70 days aerobic incubation of treated soil [^{14}C]-glyphosate – Drusenheim

Days after application	% of Applied dose					
	Glyphosate	AMPA	Bound residues	Organic volatiles	CO_2	Mass balance
0 Rep A	91.4	0.5	9.1	NA	NA	102.2
0 Rep B	90.5	0.3	9.1	NA	NA	100.9
1 Rep A	64.9	9.6	13.4	0.0	6.7	96.0
1 Rep B	66.2	7.7	12.5	0.0	6.7	94.8
3 Rep A	43.5	15.0	14.1	0.0	16.3	92.0
3 Rep B	44.1	15.1	14.2	0.0	16.3	91.7
8 Rep A	18.3	21.2	13.4	0.0	31.9	87.7
8 Rep B	18.1	21.1	13.5	0.0	31.9	87.2
14 Rep A	10.2	19.7	13.5	0.0	42.1	88.4
14 Rep B	10.8	18.9	13.8	0.0	42.1	90.3
27 Rep A	4.9	17.5	14.3	0.0	51.4	91.2
27 Rep B	3.3	15.9	13.2	0.0	51.4	86.7
48 Rep A	1.6	9.5	11.9	0.0	60.6	85.9
48 Rep B	1.5	9.8	13.1	0.0	59.8	86.7
70 Rep A	1.1	6.2	15.8	0.0	62.1	87.1
70 Rep B	0.9	6.1	14.6	0.0	62.1	86.1
Average total % recovery						90.9
Std. dev. ±						5.2

NA = not applicable

Table B.8.1-59: Degradation product distribution over 120 days aerobic incubation of treated soil [^{14}C]-glyphosate – Pappelacker

Days after application	% of Applied dose					
	Glyphosate	AMPA	Bound residues	Organic volatiles	CO_2	Mass balance
0 Rep A	99.4	0.4	1.8	NA	NA	102.2
0 Rep B	98.0	0.3	1.9	NA	NA	102.0
1 Rep A	77.1	4.2	10.1	0.0	4.8	98.2
1 Rep B	77.2	3.9	9.7	0.0	4.8	96.7
3 Rep A	59.0	7.4	13.9	0.0	12.1	94.9
3 Rep B	58.1	7.9	14.1	0.0	12.1	94.5
8 Rep A	27.4	14.5	14.7	0.0	27.2	86.4
8 Rep B	29.2	13.7	13.6	0.0	27.2	86.3
14 Rep A	19.1	14.2	17.6	0.0	36.3	90.7
14 Rep B	29.6	12.2	18.0	0.0	36.3	100.3
27 Rep A	10.1	13.7	15.0	0.0	46.0	88.9
27 Rep B	18.2	13.2	16.8	0.0	46.0	98.9
48 Rep A	4.5	13.6	15.9	0.0	53.2	90.8
48 Rep B	9.1	15.4	17.3	0.0	45.4	91.1
70 Rep A	2.3	10.4	16.0	0.0	49.7	81.3
70 Rep B	2.9	11.6	18.8	0.0	49.7	86.4
91 Rep A	2.0	10.0	13.7	0.0	52.0	80.3
91 Rep B	1.8	9.5	13.3	0.0	52.0	79.1
120 Rep A	2.0	9.1	18.4	0.0	54.4	87.1
120 Rep B	2.2	9.0	21.9	0.0	54.4	91.0
Average total % recovery						91.4
Std. dev. ±						7.0

NA = not applicable

Table B.8.1-60: Degradation product distribution over 120 days aerobic incubation of treated soil [^{14}C]-glyphosate - 18-Acres

Days after application	% of Applied dose					
	Glyphosate	AMPA	Bound residues	Organic volatiles	CO_2	Mass balance
0 Rep A	95.5	0.6	3.8	NA	NA	101.3
0 Rep B	93.3	1.0	3.8	NA	NA	99.5
8 Rep A	73.9	3.3	13.0	0.0	4.0	95.6
8 Rep B	73.9	3.4	12.8	0.0	4.0	95.3
14 Rep A	69.4	3.9	14.8	0.0	5.9	95.0
14 Rep B	73.1	2.9	16.0	0.0	5.9	98.4
21 Rep A	65.6	6.4	15.4	0.0	7.7	98.2
21 Rep B	65.3	7.2	15.2	0.0	7.7	98.2
41 Rep A	55.9	9.1	18.5	0.0	10.6	97.2
41 Rep B	54.4	8.5	18.2	0.0	10.6	96.6
63 Rep A	47.0	11.7	16.9	0.0	13.7	92.4
63 Rep B	49.3	12.0	17.0	0.0	13.7	95.1
91 Rep A	44.7	13.3	15.6	0.0	15.5	91.0
91 Rep B	46.7	13.2	17.7	0.0	15.5	95.1
120 Rep A	42.1	14.3	22.6	0.0	16.9	98.5
120 Rep B	41.3	12.1	20.6	0.0	16.9	95.8
Average total % recovery						96.5
Std. dev. ±						2.6

NA = not applicable

Mass balance
The material balance was determined as the sum of radiocarbon in soil extracts, volatile traps and residual soil radiocarbon. Radiocarbon mass balance averaged 90.9 ± 5.2 % (from 86.3 % to 101.6 %), 91.4 ± 7.0 % (from 79.7 % to 102.1 %) and 96.5 ± 2.6 % (from 93.1 % to 100.4 %) of applied dose for the Drusenheim, Pappelacker and 18 Acres soils, respectively.

Bound and extractable residues
For the Drusenheim soil, the average extractable radiocarbon yielded 92.5 % AR at time 0 and steadily declined to an average of 9.3 % AR at day 70. Bound residues (unextracted soil residues) increased from 9.1 % AR at time 0 to a maximum average of 15.2 % AR at day 70.

For the Pappelacker soil, the average extractable radiocarbon averaged 100.3 % AR at time 0 and steadily declined to 14.2 % AR at day 91, then slightly increased to 14.5 % AR at day 120. Bound residues (unextracted soil residues) increased from 1.9 % AR at time 0 to a maximum average of 20.2 % AR at day 120.
For the 18-Acres soil, the average extractable radiocarbon yielded 96.6 % AR at time 0 and declined to an average of 58.7 % AR at day 120. Bound residues (unextracted soil residues) increased from 3.8 % AR at time 0 to a maximum average of 21.6 % AR at day 120.

Volatilisation
In all three soils, negligible amounts of organic volatiles were detected during the course of incubation. The major degradate observed in all soil sets was $^{14}CO_2$, which reached maximum averages of 62.1 % AR at day 70, 54.4 % AR at day 120 and 16.9 % AR at day 120 in Drusenheim, Pappelacker and 18-Acres soils, respectively.

Transformation of the parent compound
In all three test soils glyphosate degraded under aerobic conditions. In Drusenheim loam soil glyphosate decreased from an average of 91.0 % AR at time 0 to an average of 43.8 % AR after 3 days of incubation and then declined to an average of 1.0 % AR at termination of incubation at day 70. In the Pappelacker loamy sand soil glyphosate represented an average of 28.3 % AR following 8 days of incubation and then declined to an average of 2.1 % AR at the end of the study (day 120). In the 18-Acres sandy clay loam soil, glyphosate decreased from an average of 94.4 % AR at time 0 to an average of 41.7 % AR at day 120.

In all three soils the main metabolite observed in soil extract was AMPA, which represented a maximum average of 21.2 % AR following 8 days of incubation in Drusenheim soil, a maximum average of 14.5 % AR following 48 days of incubation in Pappelacker soil and a maximum average of 13.3 % AR following 91 days of incubation in 18-Acres soil.

Mineralisation to $^{14}CO_2$ was another main degradation pathway of glyphosate with a maximum average of 62.1 % AR (Drusenheim), 54.4 % AR (Pappelacker soil) and 16.9 % AR (18-Acres soil) at the end of the study.

Conclusion
An aerobic soil degradation study of glyphosate was conducted on three soils using a [^{14}C] glyphosate dose rate of 3.80 ppm. Individual samples with moisture contents maintained at 50 ± 10 % maximum water holding capacity (MWHC) at pF 2.5 were treated and incubated in the dark at 20 ºC for up to 120 days.

The only significant metabolite observed in soil extract was AMPA. For AMPA, a decline in soil concentration was observed during the incubation period.

RMS Comment

The mass balances for the Drusenheim, Pappelacker soils were not always between 90 % and 110 % as outlined in OECD 307 (labelled chemicals). Therefore, RMS proposes to use only the sampling data for kinetic evaluation where the recoveries ranged from 90 % to 110 %.

A new kinetic evaluation (Dorn, 2012, BVL no 2315991) according with the guidance of FOCUS (2006, 2001) of the study results is available which is described under B.8.1.2.1. Thus, the DT_{50} and DT_{90} values of glyphsoate derived by the study author are not presented here anymore.

KIIA 7.2.1/2 (Mamouni, 2002, BVL no 2437068 – addendum to Galicia & Flückiger, 1993, BVL no 1932046)

Annex point:	KIIA 7.2.1/2
Author(s):	Mamouni, A.
Title:	First amendment (addendum) to report - Degradation of ^{14}C-glyphosate in three soils incubated under aerobic conditions
Date:	03/06/2002
Guideline(s):	BBA RL IV, 1-4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Material and methods

The study Galicia & Flückiger (1993, BVL no 1932046) was submitted for the first EU review of glyphosate (2001). The study is summarised in the monograph (Dec 1998) for glyphosate and was considered acceptable by the RMS. However, only results for glyphsate but not for the metabolite AMPA were reported in the original study. However, soil concentrations of AMPA via TLC were determined at all sampling points but were not included in the original study report since the objective of the study was the determination of the degradation rate of of the parent compound only. The concentrations of AMPA are presented in this addendum to allow the derivation of degradation half lifes of the metabolite from the soil study.

Results

The material balance (extraction and recovery of the radioactivity) taken from Galicia & Flückiger (1993, BVL no 1932046), the distribution of glyphosate and the formation and degradation of AMPA soils Speyer 2.1, 2.2 and 2.3 are presented in Table B.8.1-61, Table B.8.1-62 and Table B.8.1-63.

Table B.8.1-61: Material balance (extraction and recovery of radioactivity) from soil Speyer 2.1 and distribution of glyphosate and its degradation products (% of applied dose)

Time (day)	Glyphosate	AMPA	Non-Extractables	Volatiles	CO_2	Mass balance
0	86.7	1.4	0.3	n.d.	n.d.	91.1
7	56.0	21.7	2.3	<0.1	12.3	92.3
14	38.1	41.2	2.5	<0.1	15.1	99.6
28	22.6	32.6	2.0	<0.1	20.5	81.1
56	9.7	40.0	2.5	<0.1	23.7	81.3
84	9.7	38.7	1.6	<0.1	25.2	80.1
105	8.0	23.5	1.6	<0.1	26.1	75.6

n.d. not determined

Table B.8.1-62: Material balance (extraction and recovery of radioactivity) from soil Speyer 2.2 and distribution of glyphosate and its degradation products (% of applied dose)

Time (day)	Glyphosate	AMPA	Non-Extractables	Volatiles	CO_2	Mass balance
0	91.3	0.0	0.8	n.d.	n.d.	97.6
7	41.4	42.4	6.8	<0.1	5.8	96.4
14	48.8	31.4	7.3	<0.1	9.0	99.7
28	33.3	33.1	7.1	<0.1	13.9	98.6
56	31.3	34.6	7.3	<0.1	18.9	99.2
84	19.3	33.9	4.6	<0.1	20.9	88.8
105	13.5	35.4	8.6	<0.1	23.5	94.7

n.d. not determined

Table B.8.1-63: Material balance (extraction and recovery of radioactivity) from soil Speyer 2.3 and distribution of glyphosate and its degradation products (% of applied dose)

Time (day)	Glyphosate	AMPA	Non-Extractables	Volatiles	CO_2	Mass balance
0	90.9	0.0	1.4	n.d.	n.d.	92.3
7	39.4	13.6	7.7	<0.1	31.0	97.7
14	19.7	25.1	7.0	<0.1	39.8	91.6
28	5.5	25.1	7.0	<0.1	50.3	89.9
56	4.3	18.9	8.6	<0.1	56.9	90.1
84	3.0	18.5	6.0	<0.1	58.9	86.4
105	2.5	12.1	5.0	<0.1	61.4	84.7

n.d. not determined

AMPA was formed >10 % in all three soils. Maximum concentrations of 41.2 % AR were formed after day 14 in the soil Speyer 2.1 with a subsequent slow decline to 23.5 % AR at day 105 (end of study). In the soil Speyer 2.2, maximum concentrations of 42.4 % AR were measured after only 7 days with a subsequent decline to 31.4 % AR at day 14. Afterwards, concentrations remained at this level and 35.4 % AR AMPA were still found at the end of the study. In the soil Speyer 2.3, AMPA concentrations reached a maximum of 25.1 % AR after 14 and 28 days and afterwards declined to 12.1 % AR at the end of the study.

RMS Comment

The additional information on the concentrations of AMPA can be used to derive degradation half lifes for AMPA. However, only the concentrations of AMPA and of glyphosate should be used to derive DT_{50} and DT_{90} values where the total recovery is >90 %.

KIIA 7.2.1/3 (Dorn, 2012, BVL no 2315991)

Annex point:	IIA 7.2.1/3
Author(s):	Dorn, S.
Title:	Kinetic modelling analysis of the degradation behaviour of glyphosate and its metabolite AMPA from aerobic laboratory soil degradation studies
Date:	October 6, 2010
Guideline(s):	FOCUS kinetics guidance document (FOCUS, 2006, 2011)
Deviations:	Overall mass balance was used as initial glyphosate concentration, data points after 120 days incubation were excluded from kinetic modelling
GLP:	No (kinetic evaluation: does not contain laboratory work)
Acceptability:	Partly acceptable

Material and methods

Studies on aerobic laboratory soil degradation of glyphosate and its major metabolite AMPA were kinetically evaluated following FOCUS guidance (FOCUS, 2006, 2011a) to derive persistence and modelling endpoints for comparison against trigger values and for use in environmental fate models, respectively.

In total, 9 aerobic soil degradation studies (Ponte, 2010, BVL no 2310242; Dean, 1995, BVL no 2310244; Goodyear, 1996, BVL no 2310246; Galicia & Morgenroth, 1993, BVL no 1932059; Esser, 1996, BVL no 2310248; Matla & Vonk, 1993, BVL no 2151389; Ponte, 2010, BVL no 2310255; Galicia & Flückiger, 1993, BVL no 1932046/Mamouni, 2002, BVL no 2437068; McLaughlin & Schanné, 1996, BVL no 2310250) with glyphosate and its metabolite AMPA considering 15 independent data sets were considered.

The residues of glyphosate and AMPA in Gartenacker soil from the study Ponte, 2010, BVL no 2310242 are presented in Table B.8.1-8. The residues of glyphosate and AMPA in Arrow soil from the study Dean, 1995, BVL no 2310244 are presented in Table B.8.1-26. The residues of glyphosate and AMPA in soil A and B from the study Goodyear, 1996, BVL no 2310246 are presented in Table B.8.1-12 and Table B.8.1-13. The mean of the concentrations measured with HPLC and TLC were used for for kinetic re-evaluation. No kinetic re-evaluation was performed for the soil A of Goodyear, 1996, BVL no 2310246. The residues of glyphosate and AMPA in the soil ‘Les Evouttes’ from the study Galicia & Morgenroth 1993, BVL no 1932059 are presented below in Table B.8.1-64, since these data were not pre-

sented in the study summary of the glyphosate monograph. The residues of glyphosate and AMPA in the soil 'Visalia' from the study Esser, 1996, BVL no 2310248 are presented in Table B.8.1-23. The residues of glyphosate in the soils 'Droevendaal', 'Lisse' and 'Maasdijk' from the study Matla & Vonk, 1993, BVL no 2151389 are presented in Table B.8.1-65, since these data were not shown in the study summary of the glyphosate monograph. No kinetic re-evaluation was performed for the soils 'Doevendaal' and 'Lisse' of Matla & Vonk, 1993, BVL no 2151389. The residues of glyphosate and AMPA in Drusenheim soil, Pappelacker soila and 18-Acres soil from the study Ponte, 2010, BVL no 2310255 are presented in Table B.8.1-58, Table B.8.1-59 and Table B.8.1-60. The residues of glyphosate and AMPA in the soils Speyer 2.1, Speyer 2.2 and Speyer 2.3 from the study Galicia & Flückiger, 1993, BVL no 1932046 together with the addendum Mamouni, 2002, BVL no 2437068 used for kinetic analysis are presented in Table B.8.1-61, Table B.8.1-62 and Table B.8.1-63. The residues of glyphosate and AMPA in the soils Speyer 2.1, Speyer 2.2 and Speyer 2.3 from the study McLaughlin & Schanné, 1996, BVL no 2310250 are presented in Table B.8.1-16, Table B.8.1-17, Table B.8.1-18 and Table B.8.1-19.

Table B.8.1-64: Distribution of glyphosate and its metabolite AMPA in the extracts "Les Evouettes" soil. Values in % of applied radioactivity (Galicia & Morgenroth, 1993 BVL no 1932059)

Time (days)	Glyphosate	AMPA
0	78.3	4
3	65.6	6.2
3	69	6.4
7	49.5	14.9
7	58.6	11.5
14	48.9	12.2
14	38.7	13.5
28	36.7	19.7
28	36.1	21.9
56	24.3	21.1
56	25.4	22.7
84	19.4	28.3
84	19.6	30.4
112	16.3	28.3
112	21.8	26.9
168	9.4	16.6
168	10.8	21.7
252	8.3	17.7
252	8.4	18.8
364	7.4	21.2
364	6	21.4

Table B.8.1-65: Distribution of glyphosate in the soils 'Droevendaal', 'Lisse' and 'Maasdijk' (Matla & Vonk, 1993, BVL no 2151389)

Soil Droevendaal humic sand		Soil Lisse low humic sand		Soil Maasdijk Sandy Loam	
Time (days)	Glyphosate	Time (days)	Glyphosate	Time (days)	Glyphosate
0	73.7	0	98.2	0	89.4
0	74	0	97.9	0	91.3
7	62.7	7	83.3	7	37.2
7	61.1	7	84.5	7	36.7

14	46.4	14	80.3	14	24.9
14	47.8	14	81.1	14	25.2
35	56.6	35	68.5	35	16.1
35	58.3	35	68.7	35	15.5
70	58.5	70	55.7	70	8.7
70	58.5	70	56.4	70	8.2
100	56.7	100	58.2	100	4.2
100	55.7	100	53.6	100	3.9

According to OECD guideline 307 (OECD, 2002), sampling points should not be considered for which the overall recovery is < 90 % AR. Following this guidance principle the following data points were excluded:

(1) Esser, 1996, BVL no 2310248, soil Visalia: day 14 replicate A and B, day 18 replicate A and day 31 replicate B samples.
(2) Ponte, 2010, BVL no 2310255, soil Drusenheim: day 8 replicate A and B, day 14 replicate A, day 27 replicate B, day 48 replicate A and B as well as day 70 replicate A and B.
(3) Ponte, 2010, BVL no 2310255, soil Pappelacker: day 8 replicate A and B, day 27 replicate A, day 70 replicate A and B, day 91 replicate A and B as well as day 120 replicate A.
(4) Galicia & Flückiger, 1993, BVL no 1932046/Mamouni, 2002, BVL no 2437068, soil Speyer 2.1: day 28, day 56, day 84 and day 105. Since after exclusion only 3 data points remained in the data set, the soil Speyer 2.1 was excluded from kinetic analysis.
(5) Galicia & Flückiger, 1993, BVL no 1932046/Mamouni, 2002, BVL no 2437068, soil Speyer 2.3: day 84 and day 105.

For soil Speyer 2.2 (Galicia & Flückiger, 1993, BVL no 1932046/Mamouni, 2002, BVL no 2437068), day 84 (88.8 % AR) was kept in the data set because the recovery rate was only slightly < 90 % and the residue level was well between levels of the previous (day 56) and subsequent (day 105) days.

The exclusion of sampling intervals beyond the typical duration of 120 days is justified when taking into account that viability of soil microbes is known to suffer during prolonged storage of soils under laboratory conditions (SETAC, 1995). The following data points were therefore excluded:

(1) Dean, 1995, BVL no 2310244, soil Arrow: day 180 samples
(2) Galicia & Morgenroth, 1993 (BVL no 1932059): day 168, day 252 and day 364 samples.

For soil Gartenacker (Ponte, 2010, BVL no 2310242) day 132 was kept in the data set since the study duration only slightly exceeded the recommended duration of 120 days and no decline in microbial activity could be observed until the end of the study.

For soil Visalia (Esser, 1996, BVL no 2310248), an experimental artefact concerning extraction of day 8 samples was recognized during the study. Therefore, the extraction of day 8 samples was repeated and the sum of the residues of both extractions was used as input for kinetic evaluations at day 8 for both glyphosate and AMPA.

The following procedures in accordance with FOCUS (2006, 2011) guidance were used by the author for all residues to adjust the experimental data for the kinetic modelling:

The initial pesticide concentration in laboratory samples is well known, as a defined amount of the pesticide is added to the system. According to FOCUS (2006, 2011), the best estimate of the amount of material dosed into the system may be derived from the dose checks and the chemical purity of the test item. Thus, the parent value at time 0 was set to the value of the total mass balance at this time point. The time-zero concentration for metabolites was set to 0% of the total applied radioactivity (AR). The processed residues for glyphosate and AMPA at time 0 are presented in Table B.8.1-66.

Table B.8.1-66: Processed residues of glyphosate and AMPA at time 0 used for kinetic analysis

Study	Soil/ Conditions	Time (days)	Glyphosate	AMPA
Ponte, 2010, BVL no 2310242	Gartenacker (loam, 20 °C)	0, Rep A	100.1	0.0
		0, Rep B	99.2	0.0
Dean, 1995, BVL no 2310244	Arrow (sandy loam, 20 °C)	0, Rep A	97.6	0.0
		0, Rep B	96.6	0.0
Goodyear, 1996, BVL no 2310246	soil B (loam, 25 °C)	0, Rep A	100.3	0.0
		0, Rep B	99.2	0.0
Galicia & Morgenroth, 1993 BVL no 1932059	Les Evouettes (silt loam, 20 °C)	0	98.6	0.0
Esser, 1996, BVL no 2310248	Visalia (sandy loam, 25 °C)	0, Rep A	96.8	0.0
		0, Rep B	96.7	0.0
Matla & Vonk, 1993 BVL no 2151389	Maasdijk (sandy loam, 20 °C)	0, Rep A	92.7	No data
		0, Rep B	95.1	No data
Ponte 2010, BVL no 2310255	Drusenheim (loam, 20 °C)	0, Rep A	102.2	0.0
		0, Rep B	100.9	0.0
	Pappelacker (loamy sand, 20 °C)	0, Rep A	102.2	0.0
		0, Rep B	102.0	0.0
	18-Acres (sandy clay loam, 20 °C)	0, Rep A	101.3	0.0
		0, Rep B	99.5	0.0
Galicia & Flückiger, 1993, BVL no 1932046/ Mamouni, 2002, BVL no 2437068	Speyer 2.2 (sand, 20 °C)	0	97.6	0.0
	Speyer 2.3 (loamy sand, 20 °C)	0	92.3	0.0
McLaughlin & Schanné, 1996, BVL no 2310250	Speyer 2.1 (sand, 20 °C)	0	97.7	0.0
	Speyer 2.2 (loamy sand, 20 °C)	0	103.8	0.0
	Speyer 2.3 (loamy sand, 20 °C)	0	99.1	0.0
	Speyer 2.3 (loamy sand, 10 °C)	0	99.3	0.0

It is recommended that values between LOQ and LOD should be set to the measured value. Values below LOD should be replaced by half the LOD. If the concentrations of the applied substance in soil decline to values below LOD, the curve should be cut off after the first value below LOD, unless detections above LOQ are made later in the experiment. However, based on the values for LOQ and LOD provided in the studies (Ponte, 2010, BVL no 2310242; Dean, 1995, BVL no 2310244; Goodyear, 1996, BVL no 2310246; Galicia & Morgenroth, 1993, BVL no 1932059; Esser, 1996, BVL no 2310248; Matla & Vonk, 1993, BVL no 2151389; Ponte, 2010, BVL no 2310255; Galicia & Flückiger, 1993, BVL no 1932046 /Mamouni, 2002, BVL no 2437068; McLaughlin & Schanné, 1996, BVL no 2310250) corrections of soil residue data as mentioned above were only necessary for glyphosate in soil Spey-

er 2.3 in McLaughlin & Schanné (1996, BVL no 2310250) for the soil Speyer 2.3 incubated at 20°C. Here the value below LOD at day 29 for glyphosate were replaced by 0.05 % AR.

Four kinetic models were used to determine an adequate description of the kinetic behaviour of glyphosate and its metabolite AMPA in soil: the single first-order (SFO), the first-order multi-compartment (FOMC = Gustafson and Holden model), the double first-order in parallel (DFOP) and the hockey-stick (HS) model.

The kinetic analysis of the respective applied compound was conducted using the fitting software KinGUI (version 1.1) in the framework of MatLab 7.0.4.365 (Schäfer & Mikolasch, 2006; MatLab, 2005).

Optimisations were carried out for the initial soil residue (M_0), degradation rate constants (k), (α), (β), (g) or breakpoint (t_b), depending on the kinetic model. The parameters are optimised by minimising the sum of squared differences between measured and calculated data using the Levenberg-Marquardt algorithm. The set of differential equations which define the kinetic model were solved numerically using a Runge-Kutta method.

The initial guess for the parameters was specified manually. A stepwise modification of the fitting routine was performed for data sets including the metabolite AMPA. Data were not weighted and the initial concentration was not constrained in any of the fits.

Results

The results of soil degradation kinetic analysis of glyphosate and AMPA (DT_{50}, DT_{90}) are summarised in the following tables, including the criteria for selection of appropriate kinetic model χ^2-test, t-test probability and the standard error of the parameter estimated as well as an assessment of the visual fit by using the terms “very good”, “good”, “acceptable” and “poor”.

KIIA 7.2.1/4 (Ponte, 2010, BVL no 2310242- Kinetic re-evaluation):

The results of soil degradation kinetic analysis of glyphosate and AMPA (DT_{50}, DT_{90}) in the soil ‘Gartenacker’ of the study Ponte, 2010, BVL no 2310242 are presented in Table B.8.1-67 and Table B.8.1-68.

Table B.8.1-67: Ponte, 2010, BVL no 2310242: Kinetic evaluation for degradation of glyphosate in the Gartenacker soil (parent only)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
SFO	M_0	90.5095	3.2836	-	13.1	11.48	38.13	poor
	k_SFO	0.0604	0.0055	1.0e-009				
FOMC	M_0	98.2709	1.4967	-	4.6	8.28	59.05	good
	α	1.2564	0.1162	2.4e-009				
	β	11.2449	1.6928	2.1e-006				
DFOP	M_0	99.3516	1.1030	-	3.0	7.86	56.29	good
	k1_DFOP	0.2474	0.0304	2.2e-007				
	k2_DFOP	0.0304	0.0022	1.2e-10				
	g	0.4459	0.0355	5.4e-10				
HS[1)]	M0	Not tested						
	k1_HS							
	k2_HS							
	tb							

Table B.8.1-68: Ponte, 2010, BVL no 2310242: Kinetic evaluation for degradation of AMPA in the Gartenacker soil (parent with metabolite)

Kinetic model	Parent or met.	Para-meter	Opti-mised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
FOMC (parent) & SFO (met)	Met.	M_0	0.0000	-	-	8.9	133.696	444.12	acceptable
		k_SFO	0.0052	0.0011	2.3e-005				
		ff	0.1817	0.0123	-				
	Parent	M_0	98.1589	1.1709	-	4.6	8.324	58.98	-[1)]
		α	1.2658	0.0919	5.3e-016				
		β	11.4162	1.3406	2.4e-010				
DFOP (parent) & SFO (met)	Met.	M_0	0.0000	-	-	9.2	120.07	398.87	acceptable
		k_SFO	0.0058	0.0009	1.6e-007				
		ff	0.1825	0.0098	-				
	Parent	M_0	99.1663	0.9548	-	3.0	7.96	55.76	-[1)]
		k1	0.2483	0.0270	4.6e-011				
		k2	0.0310	0.0019	9.0e-018				

[1)] not shown

KIIA 7.2.1/5 (Dean, 1995, BVL no 2310244- Kinetic re-evaluation):

The results of soil degradation kinetic analysis of glyphosate and AMPA (DT_{50}, DT_{90}) in Arrow soil from the study Dean, 1995, BVL no 2310244 are presented in Table B.8.1-69 and Table B.8.1-70.

Table B.8.1-69: Dean, 1995, BVL no 2310244: Kinetic evaluation for degradation of glyphosate in the Arrow soil (parent only)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
SFO	M_0_parent	85.7657	2.7358	-	8.28	59.98	199.23	Poor
	k_ parent	0.0116	0.0011	4.1E-08				
FOMC	M_0_parent	95.7132	1.4875	-	2.15	37.80	>1000	Very good
	α_parent	0.4807	0.0463	5.9e-08				
	β_parent	11.7058	2.5122	2.2e-04				
DFOP	M_0_parent	96.2976	1.5791	-	2.21	43.21	259.8	Very good
	k1_parent	0.1265	0.0285	4.9e-4				
	k2_parent	0.0074	7.5e-4	2.1e-7				
	g_parent	0.3128	0.0365	9.2e-7				
HS	M0_parent	96.3771	1.8445	-	3.06	47.54	235.31	Very good
	k1_parent	0.0388	0.0049	2.3e-6				
	k2_parent	0.0086	6.1e-4	4.3e-9				
	tb_parent	9.4382	1.1475	1.4e-6				

Table B.8.1-70: Dean, 1995, BVL no 2310244: Kinetic evaluation for degradation of AMPA in the Arrow soil (parent with metabolite)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
FOMC (parent) & SFO (met)	M0_met	0.000	-	-	9.43	*>1000*	*>1000*	acceptable
	ff_met	0.4016	0.0344	-				
	k_met	2.3e-014	0.0012	*0.500*				
	M_0_parent	94.8458	1.2784	-	2.24	39.4362	>1000	Not shown
	α_parent	0.5146	0.0.0457	5.2e-012				
	β_parent	13.8604	2.6127	6.7e-006				
DFOP (parent) & SFO (met)	M0_met	0.000			9.03	*>1000*	*>1000*	acceptable
	ff_met	0.3981	0.0357	-				
	k_met	1.1e-008	0.0013	*0.500*				
	M_0_parent	95.1446	1.3312	-	2.43	44.87	255.04	Not shown
	k1_parent	0.1146	0.0229	1.7e-005				
	k2_parent	0.0076	6.9e-004	1.2e-009				
	g_parent	0.2980	0.0350	2.6e-009				

KIIA 7.2.1/6 (Goodyear, 1996, BVL no 2310246- Kinetic re-evaluation):

The results of soil degradation kinetic analysis of glyphosate and AMPA (DT_{50}, DT_{90}) in soil B from the study Goodyear, 1996, BVL no 2310246 are presented in Table B.8.1-71 and Table B.8.1-72.

Table B.8.1-71: Goodyear, 1996, BVL no 2310246: Kinetic evaluation for degradation of glyphosate in soil B (parent only)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
SFO (parent)	M_0_parent	90.3050	5.6479	-	25.59	2.20	7.30	poor
	k_ parent	0.3153	0.0514	7.2e-06				
FOMC (parent)	M_0_parent	99.3637	1.8219	-	6.92	1.2	20.78	Very good
	α_parent	0.6565	0.0524	1.2e-09				
	β_parent	0.6406	0.1189	3.8e-05				
DFOP (parent)	M_0_parent	99.6771	0.3872	2.8e-004	9.01	1.04	16.07	acceptable
	k1_parent	1.7209	0.3872	2.8e-04				
	k2_parent	0.0938	0.0154	1.4e-05				
	g_parent	0.5490	0.0471	6.8e-09				
HS (parent)	M0_parent	Not tested						
	k1_parent							
	k2_parent							
	tb_parent							

Table B.8.1-72: Goodyear, 1996, BVL no 2310246: Kinetic evaluation for degradation of AMPA in soil B (parent with metabolite)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
FOMC (parent) + SFO (met)	M0_met	0.000	-	-	6.98	99.07	329.09	good
	ff_met	0.2646	0.0140	-				
	k_ met	0.0070	0.0011	1.8e-07				
	M_0_parent	99.6952	1.6785	-	10.16	1.16	21.19	not shown
	α_parent	0.6378	0.0456	3.1e-015				
	β_parent	0.5890	0.0991	7.2e-07				

KIIA 7.2.1/7 (Galicia & Morgenroth, 1993, BVL no 1932059- - Kinetic re-evaluation):

The results of soil degradation kinetic analysis of glyphosate and AMPA (DT_{50}, DT_{90}) in the soil Les Evouettes from the study Galicia & Morgenroth, 1993, BVL no 1932059 are presented in Table B.8.1-73 and Table B.8.1-74.

Table B.8.1-73: Galicia & Morgenroth, 1993, BVL no 1932059: Kinetic evaluation for degradation of glyphosate in soil 'Les Evouettes' (parent only)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
SFO (parent)	M_0_parent	70.2655	5.2755	-	22.57	36.83	122.36	poor
	k_ parent	0.0188	0.0038	1.2e-04				
FOMC (parent)	M_0_parent	97.9982	3.4837	-	3.00	9.57	601.6	Very good
	α_parent	0.4083	0.0388	1.0e-07				
	β_parent	2.1460	0.6123	0.0022				
DFOP (parent)	M_0_parent	97.6802	3.8200	-	3.97	8.76	159.38	Very good
	k1_parent	0.2717	0.0567	2.8e-04				
	k2_parent	0.0100	0.0015	2.1e-05				
	g_parent	0.5052	0.0404	3.8e-08				
HS (parent)	M0_parent	93.8596	4.2263	-	6.92	7.96	162.29	Very good
	k1_parent	0.0871	0.0119	7.7e-06				
	k2_parent	0.0102	0.0018	6.5e-05				
	tb_parent	8.5103	0.9425	1.0e-06				

Table B.8.1-74: Galicia & Morgenroth, 1993, BVL no 1932059: Kinetic evaluation for degradation of AMPA in soil 'Les Evouettes' (parent with metabolite)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
DFOP (parent) + SFO (met)	M0_met	0.000	-	-	11.70	*>1000*	*>1000*	good
	ff_met	0.3353	0.0389	-				
	k_ met	1.6e-09	*0.0016*	*0.500*				
	M_0_parent	96.6597	3.2879	-	4.60	10.09	154.76	Not presented
	k1_parent	0.2564	0.0477	8.0e-06				
	k2_parent	0.0106	0.0014	3.7e-08				
	g_parent	0.4841	0.0379	1.7e-012				

KIIA 7.2.1/8 (Esser, 1996, BVL no 2310248 - Kinetic re-evaluation):

The results of soil degradation kinetic analysis of glyphosate and AMPA (DT_{50}, DT_{90}) in the soil Visalia from the study Esser, 1996, BVL no 2310248 are presented in Table B.8.1-75 and Table B.8.1-76.

Table B.8.1-75: Esser, 1996, BVL no 2310248: Kinetic evaluation for degradation of glyphosate in soil 'Visalia' (parent only)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
SFO (parent)	M_0_parent	94.5112	3.4543	-	14.11	1.23	4.09	poor
	k_ parent	0.5628	0.0399	9.5e-011				
FOMC (parent)	M_0_parent	96.6259	1.7883	-	6.26	0.9901	6.1447	Very good
	α_parent	1.4977	0.2292	4.7e-006				
	β_parent	1.6824	0.3974	3.6e-004				
DFOP (parent)	M_0_parent	96.8532	1.4789	-	4.90	1.02	7.16	Very good
	k1_parent	0.8819	0.0757	6.9e-009				
	k2_parent	0.0891	0.0237	0.0011				
	g_parent	0.8135	0.0378	2.0e-012				
HS (parent)	M0_parent	Not tested						
	k1_parent							
	k2_parent							
	tb_parent							

Table B.8.1-76: Esser, 1996, BVL no 2310248: Kinetic evaluation for degradation of AMPA in the soil 'Visalia' (parent with metabolite)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
FOMC (parent) & SFO (met)	M0_met	0.000	-	-	5.30	*68.57*	227.79	Very good
	ff_met	0.2793	0.0113	-				
	k_met	0.0101	0.0029	7.2e-004				
	M_0_parent	96.8804	1.3729	-	6.17	0.97	6.30	Not shown
	α_parent	1.4137	0.1550	1.4e-010				
	β_parent	1.5370	0.2651	1.1e-006				
DFOP (parent) & SFO (met)	M0_met	0.000	-	-	4.68	70.73	234.95	Very good
	ff_met	0.2799	0.0096	-				
	k_met	0.0098	0.0025	2.1e-004				
	M_0_parent	96.7151	1.1533	-	4.94	1.04	7.00	Not shown
	k1_parent	0.8613	0.0550	2.8e-016				
	k2_parent	0.0860	0.0185	3.0e-005				
	g_parent	0.8210	0.0288	1.4e-23				

KIIA 7.2.1/9 (Matla & Vonk, 1993, BVL no 2151389 - Kinetic re-evaluation):

The results of soil degradation kinetic analysis of glyphosate and AMPA (DT_{50}, DT_{90}) in the soil Maasdijk from the study Matla & Vonk, 1993, BVL no 2151389 are presented in Table B.8.1-75.

Table B.8.1-77: Matla & Vonk, 1993, BVL no 2151389: Kinetic evaluation for degradation of glyphosate in soil 'Maasdijk' (parent only)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
SFO (parent)	M_0_parent	91.5769	5.7340	-	19.44	6.67	22.17	poor
	k_ parent	0.1039	0.0148	1.8e-005				
FOMC (parent)	M_0_parent	93.86	1.1675	-	3.79	4.42	56.62	Very good
	α_parent	0.7508	0.0612	3.2e-007				
	β_parent	2.9124	0.5206	1.7e-004				
DFOP (parent)	M_0_parent	93.8998	0.5122	-	0.84	4.61	62.00	Very good
	k1_parent	0.2638	0.0146	4.6e-008				
	k2_parent	0.0192	0.000	4.1e-008				
	g_parent	0.6715	0.0138	1.8e-011				
HS (parent)	M0_parent	Not tested						
	k1_parent							
	k2_parent							
	tb_parent							

KIIA 7.2.1/10 (Ponte, 2010, BVL no 2310255 - Kinetic re-evaluation):

The results of soil degradation kinetic analysis of glyphosate and AMPA (DT_{50}, DT_{90}) in the soil 'Drusenheim' of the study Ponte, 2010, BVL no 2310255 are presented in Table B.8.1-78 and Table B.8.1-79.
The results of soil degradation kinetic analysis of glyphosate and AMPA (DT_{50}, DT_{90}) in the soil 'Pappelacker' of the study Ponte, 2010, BVL no 2310255 are presented in Table B.8.1-80 and Table B.8.1-81.
The results of soil degradation kinetic analysis of glyphosate and AMPA (DT_{50}, DT_{90}) in the soil '18_acres' of the study Ponte, 2010, BVL no 2310255 are presented in Table B.8.1-82and Table B.8.1-83.

Table B.8.1-78: Ponte, 2010, BVL no 2310255: Kinetic evaluation for degradation of glyphosate in the Drusenheim soil (parent only)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
SFO	M_0	97.6963	4.3394	-	9.0	2.35	7.81	poor
	k_SFO	0.2949	0.0401	1.6e-004				
FOMC	M_0	100.8839	1.9286	-	3.5	2.057	17.78	good
	α	1.0359	0.1884	0.0014				
	β	2.1592	0.6025	0.0079				
DFOP	M_0	101.5418	1.0173	-	2.4	2.06	15.38	good
	k1_DFOP	1.2566	0.1794	0.0011				
	k2_DFOP	0.1161	0.0107	2.1e-004				
	g	0.4038	0.0336	1.4e-004				
HS[1)]	M0	-	-	-	-	-	-	-
	k1_HS	-	-	-				
	k2_HS	-	-	-				
	tb	-	-	-				

[1)] not tested

Table B.8.1-79: Ponte, 2010, BVL no 2310255: Kinetic evaluation for degradation of AMPA in the Drusenheim soil (parent with metabolite)

Kinetic model	Parent or met.	Para-meter	Opti-mised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
FOMC (parent) & SFO (met)	Met.	M_0	0.0000	-	-	2.1	47.04	156.27	very good
		k_SFO	0.0147	0.0065	0.0222				
		ff	0.2578	0.0223	-				
	Parent	M_0	100.9119	1.3320	-	3.5	2.05	17.83	-[1)]
		α	1.0319	0.1280	3.0e-006				
		β	2.1445	0.4074	1.3e-004				
DFOP (parent) & SFO (met)	Met.	M_0	0.0000	-	-	3.3	38.98	129.48	very good
		k_SFO	0.0178	0.0038	4.6e-004				
		ff	0.2602	0.0127	-				
	Parent	M_0	101.4991	0.7541	-	2.4	2.05	15.44	-[1)]
		k1	1.2202	0.1242	9.3e-007				
		k2	0.1150	0.0078	2.2e-008				

[1)] not shown

Table B.8.1-80: Ponte, 2010, BVL no 2310255: Kinetic evaluation for degradation of glyphosate in the Pappelacker soil (parent only)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
SFO	M_0	92.1699	4.5647	-	13.4	6.49	21.559	poor
	k_SFO	0.1068	0.0188	1.0e-004				
FOMC	M_0	100.9888	2.4555	-	4.1	3.94	43.45	good
	α	0.8550	0.1251	3.8e-005				
	β	3.1539	0.8615	0.0026				
DFOP	M_0	100.6804	2.5380	-	4.1	3.78	39.36	good
	k1_DFOP	0.3923	0.0915	0.0013				
	k2_DFOP	0.0367	0.0103	0.0036				
	g	0.5756	0.0911	1.1e-004				
HS[1)]	M0	Not tested						
	k1_HS							
	k2_HS							
	tb							

[1)] not tested

Table B.8.1-81: Ponte, 2010, BVL no 2310255: Kinetic evaluation for degradation of AMPA in the Pappelacker soil (parent with metabolite)

Kinetic model	Parent or met.	Para-meter	Opti-mised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
FOMC (parent) & SFO (met)	Met.	M_0	0.0000	-	-	6.2	126.57	420.46	good
		k_SFO	0.0055	0.0028	0.0305				
		ff	0.1835	0.0207	-				
	Parent	M_0	100.9163	1.7311	-	4.1	3.95	43.42	-[1)]
		α	0.8574	0.0882	4.2e-009				
		β	3.1770	0.6064	2.3e-005				

1) not shown

Table B.8.1-82: Ponte, 2010, BVL no 2310255: Kinetic evaluation for degradation of glyphosate in the 18-Acres soil (parent only)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
SFO	M_0	84.1367	3.5853	-	9.9	90.9309	302.0660	poor
	k_SFO	0.0076	0.0011	3.0e-006				
FOMC	M_0	100.0879	1.3671	-	2.0	60.223	>1000	good
	α	0.2605	0.0179	9.8e-010				
	β	4.5232	0.8779	9.3e-005				
DFOP	M_0	99.7577	1.7741	-	2.9	67.72	471.37	good
	k1_DFOP	0.1129	0.0228	1.7e-004				
	k2_DFOP	0.0040	6.4e-004	2.3e-005				
	g	0.3453	0.0339	1.5e-007				
HS[1)]	M0	100.3791	2.2165	-	3.7	70.23	373.27	good
	k1_HS	0.0382	0.0047	1.5e-006				
	k2_HS	0.0053	4.9e-004	7.6e-008				
	tb	9.7436	1.1317	8.8e-007				

1) not tested

Table B.8.1-83: Ponte, 2010, BVL no 2310255: Kinetic evaluation for degradation of AMPA in the 18-Acres soil (parent with metabolite)

Kinetic model	Parent or met.	Para-meter	Opti-mised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
FOMC (parent) & SFO (met)	Met.	M_0	0.0000	-	-	*16.3*	>1000	>1000	acceptable
		k_SFO	2.3e-014	*0.0017*	*0.5000*				
		ff	0.2144	0.0242	-				
	Parent	M_0	99.6068	1.2619	-	2.0	61.13	>1000	-[1)]
		α	0.2696	0.0173	2.5e-015				
		β	5.0594	0.8959	2.7e-006				
DFOP (parent) & SFO (met)	Met.	M_0	0.0000	-	-	*16.1*	>1000	>1000	acceptable
		k_SFO	6.5e-010	*0.0021*	*0.5000*				
		ff	0.2169	0.0292	-				
	Parent	M_0	99.0833	1.4677	-	2.9	68.05	478.55	-[1)]
		k1	0.1022	0.0169	1.1e-006				
		k2	0.0039	0.0006	1.1e-007				

[1)] not shown

KIIA 7.2.1/11 (Galicia & Flückiger, 1993, BVL no 1932046/ Mamouni, 2002, BVL no 2437068- Kinetic re-evaluation):

The results of soil degradation kinetic analysis of glyphosate and AMPA (DT_{50}, DT_{90}) in the Speyer 2.2 soil of the study Galicia & Flückiger, 1993, BVL no 1932046/ Mamouni, 2002, BVL no 2437068 are presented in Table B.8.1-84 and Table B.8.1-85.

The results of soil degradation kinetic analysis of glyphosate and AMPA (DT_{50}, DT_{90}) in the Speyer 2.3 soil of the study Galicia & Flückiger, 1993, BVL no 1932046/ Mamouni, 2002, BVL no 2437068 are presented in Table B.8.1-86 and Table B.8.1-87.

Table B.8.1-84: Galicia & Flückiger, 1993, BVL no 1932046/ Mamouni, 2002, BVL no 2437068: Kinetic evaluation for degradation of glyphosate in the Speyer 2.2 soil (parent only)

Kinetic mod-el	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
SFO (parent)	M_0_parent	74.1562	12.0156	-	*26.67*	32.35	107.45	poor
	k_ parent	0.0214	0.0085	0.0269				
FOMC (par-ent)	M_0_parent	97.373	7.6894	-	12.26	7.09	>1000	good
	α_parent	0.3330	0.1264	0.0289				
	β_parent	1.0102	1.3098	*0.2418*				
DFOP (par-ent)	M_0_parent	97.5342	5.6135	-	8.54	1.59	150.62	acceptable
	k1_parent	3.8122	*>1000*	*0.500*				
	k2_parent	0.0108	0.0035	0.0276				
	g_parent	0.4925	0.1029	0.0087				
HS (parent)	M0_parent	97.6067	5.6133	-	8.54	6.08	150.61	acceptable
	k1_parent	0.1139	*50.8230*	*0.4492*				
	k2_parent	0.0108	0.0029	0.0176				
	tb_parent	6.5842	*>1000*	*0.4990*				

Table B.8.1-85: Galicia & Flückiger, 1993, BVL no 1932046/ Mamouni, 2002, BVL no 2437068: Kinetic evaluation for degradation of AMPA in the Speyer 2.2 soil (parent with metabolite)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
DFOP (parent) + SFO (met)	M0_met	0.000	-	-	10.34	156.43	360.18	poor
	ff_met	0.6936	1.6822	-				
	k_ met	0.0064	0.0137	*0.3267*				
	M_0_parent	97.8763	4.8491	-	8.61	1.37	108.43	Not presented
	k1_parent	2.8170	*>1000*	*0.4997*				
	k2_parent	0.0102	0.0022	8.5e-04				
	g_parent	0.5037	0.0491	3.5e-06				

Table B.8.1-86: Galicia & Flückiger, 1993, BVL no 1932046/ Mamouni, 2002, BVL no 2437068: Kinetic evaluation for degradation of glyphosate in the Speyer 2.3 soil (parent only)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
SFO (parent)	M_0_parent	91.7974	2.8632	-	5.58	6.06	20.14	acceptable
	k_ parent	0.1144	0.0079	3.6e-04				
FOMC (parent)	M_0_parent	92.3702	2.1375	-	3.84	5.48	23.83	Very good
	α_parent	3.2209	1.5897	0.0900				
	β_parent	22.8266	13.8501	*0.1205*				
DFOP (parent)	M_0_parent	92.1728	1.5156	-	2.41	5.78	21.99	Very good
	k1_parent	0.1277	0.0123	0.0305				
	k2_parent	2.3e-014	*0.0252*	*0.500*				
	g_parent	0.9578	0.0515	0.0171				
HS (parent)	M0_parent	Not tested						
	k1_parent							
	k2_parent							
	tb_parent							

Table B.8.1-87: Galicia & Flückiger, 1993, BVL no 1932046/ Mamouni, 2002, BVL no 2437068: Kinetic evaluation for degradation of AMPA in the Speyer 2.3 soil (parent with metabolite)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
FOMC (parent) + SFO (met)	M0_met	0.000	-	-	11.41	70.92	235.59	good
	ff_met	0.3435	0.0377	-				
	k_ met	0.0098	0.0040	0.0300				
	M_0_parent	85.6885	2.4873	-	3.98	5.65	23.31	Not presented
	α_parent	3.9509	2.662	0.0990				
	β_parent	29.4703	*23.4313*	*0.1322*				
DFOP (parent) + SFO (met)	M0_met	0.000	-	-	10.18	77.50	257.43	good
	ff_met	0.3401	0.0329	9.7e-005				
	k_ met	0.0089	0.0036	0.0329				
	M_0_parent	91.8965	2.1971	-	2.56	5.86	22.02	Not presented
	k1_parent	0.1254	0.0167	8.4e-040				
	k2_parent	0.1254	0.0386	*0.500*				
	g_parent	0.9608	0.0732	9.7e-05				

KIIA 7.2.1/12 (McLaughlin & Schanné, 1996, BVL no 2310250 - Kinetic re-evaluation):

The results of soil degradation kinetic analysis of glyphosate and AMPA (DT_{50}, DT_{90}) in the Speyer 2.1 soil at 20 °C of the study McLaughlin & Schanné, 1996, BVL no 2310250 are presented in Table B.8.1-88 and
Table B.8.1-89.
The results of soil degradation kinetic analysis of glyphosate and AMPA (DT_{50}, DT_{90}) in the Speyer 2.2 at 20 °C of the study McLaughlin & Schanné, 1996, BVL no 2310250 are presented in Table B.8.1-90 and Table B.8.1-91.
The results of soil degradation kinetic analysis of glyphosate and AMPA (DT_{50}, DT_{90}) in the Speyer 2.3 at 20 °C of the study McLaughlin & Schanné, 1996, BVL no 2310250 are presented in Table B.8.1-92Table B.8.1-93.
The results of soil degradation kinetic analysis of glyphosate and AMPA (DT_{50}, DT_{90}) in the Speyer 2.3 at 10 °C of the study McLaughlin & Schanné, 1996, BVL no 2310250 are presented in Table B.8.1-94 and Table B.8.1-95.

Table B.8.1-88: McLaughlin & Schanné, 1996, BVL no 2310250: Kinetic evaluation for degradation of glyphosate in the Speyer 2.1 at 20 °C soil (parent only)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
SFO (parent)	M_0_parent	87.9758	4.0619	-	9.53%	11.54	38.35	poor
	k_ parent	0.0600	0.0091	1.5e-04				
FOMC (parent)	M_0_parent	94.89	3.331	-	5.72%	8.24	64.69	Very good
	α_parent	1.1337	0.3102	0.0053				
	β_parent	9.7701	4.3539	0.0330				
DFOP (parent)	M_0_parent	98.2487	1.7152	-	2.45%	8.31	51.27	Very good
	k1_parent	0.4736	0.1045	0.0031				
	k2_parent	0.0372	0.0033	4.5e-05				
	g_parent	0.3278	0.0384	1.8e-04				

HS (parent)	M0_parent	Not tested
	k1_parent	
	k2_parent	
	tb_parent	

Table B.8.1-89: McLaughlin & Schanné, 1996, BVL no 2310250: Kinetic evaluation for degradation of AMPA in the Speyer 2.1 at 20 °C soil (parent with metabolite)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
FOMC (parent) + SFO (met)	M0_met	0.000	-	-	13.14	*>1000*	*>1000*	good
	ff_met	0.5202	0.0639	-				
	k_ met	2.3e-14	*0.0020*	*0.500*				
	M_0_parent	97.5697	3.7221	-	6.24	7.17	76.71	not shown
	α_parent	0.8737	0.1937	2.9e-04				
	β_parent	5.9234	2.3359	0.0124				
DFOP (parent) + SFO (met)	M0_met	0.000	-	-	9.41	*>1000*	*>1000*	good
	ff_met	0.5060	0.0420	-				
	k_ met	4.5e-04	0.0014	*0.3735*				
	M_0_parent	100.2543	2.6428	-	3.19	7.57	53.60	not shown
	k1_parent	0.5298	0.1403	0.0013				
	k2_parent	0.0347	0.0045	2.5e-06				
	g_parent	0.3583	0.0492	4.8e-06				

Table B.8.1-90: McLaughlin & Schanné, 1996, BVL no 2310250: Kinetic evaluation for degradation of glyphosate in the Speyer 2.2 at 20 °C soil (parent only)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
SFO (parent)	M_0_parent	90.2877	4.5356	-	11.0	34.53	114.69	poor
	k_ parent	0.0201	0.0035	2.4e-04				
FOMC (parent)	M_0_parent	101.2190	2.5523	-	4.04	18.74	428.05	very good
	α_parent	0.5770	0.0508	4.6e-06				
	β_parent	8.0642	1.8407	0.0016				
DFOP (parent)	M_0_parent	100.3681	3.3544	-	4.97	19.32	178.89	very good
	k1_parent	0.1279	0.0509	0.0229				
	k2_parent	0.0096	0.0027	0.0058				
	g_parent	0.4432	0.0963	0.0018				
HS (parent)	M0_parent	98.6536	3.5938	-	6.29	22.78	162.48	very good
	k1_parent	0.0493	0.0079	3.9e-04				
	k2_parent	0.0155	0.0022	9.8e-04				
	tb_parent	11.3980	*NaN*	*NaN*				

Table B.8.1-91: McLaughlin & Schanné, 1996, BVL no 2310250: Kinetic evaluation for degradation of AMPA in the Speyer 2.2 at 20 °C soil (parent with metabolite)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment

FOMC (parent) + SFO (met)	M0_met	0.000	-	-	4.05	636.15	>1000	good
	ff_met	0.570	0.0541	-				
	k_ met	0.0011	*0.0013*	*0.1990*				
	M_0_parent	100.8498	2.1584	-	8.27	19.14	412.58	Not shown
	α_parent	0.5916	0.0730	3.6e-07				
	β_parent	0.570	0.0541	8.1e-07				
DFOP (parent) + SFO (met)	M0_met	0.000	-	-	5.05	382.85	>1000	good
	ff_met	0.6076	0.0692	-				
	k_ met	0.0018	*0.0016*	*0.1430*				
	M_0_parent	99.1344	2.3957	-	9.21	19.74	193.22	Not shown
	k1_parent	0.0986	0.0262	0.0010				
	k2_parent	0.0084	0.0024	0.0020				
	g_parent	0.4929	0.0893	3.8e-05				

Table B.8.1-92: McLaughlin & Schanné, 1996, BVL no 2310250: Kinetic evaluation for degradation of glyphosate in the Speyer 2.3 at 20 °C soil (parent only)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
SFO (parent)	M_0_parent	98.3115	4.1211	-	8.78	2.93	9.74	acceptable
	k_ parent	0.2365	0.0243	3.4e-05				
FOMC (parent)	M_0_parent	100.2861	4.1469	-	7.67	2.71	12.48	good
	α_parent	2.6790	1.6892	0.0868				
	β_parent	9.1595	7.2065	*0.1298*				
DFOP (parent)	M_0_parent	100.0885	4.0870	-	7.45	2.70	13.03	Very good
	k1_parent	0.3162	0.098	0.0160				
	k2_parent	0.0494	0.0677	*0.2530*				
	g_parent	0.8355	0.2145	0.0088				
HS (parent)	M0_parent	Not tested						
	k1_parent							
	k2_parent							
	tb_parent							

Table B.8.1-93: McLaughlin & Schanné, 1996, BVL no 2310250: Kinetic evaluation for degradation of AMPA in the Speyer 2.3 at 20 °C soil (parent with metabolite)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
FOMC (parent) + SFO (met)	M0_met	0.000	-	-	16.48	42.14	139.99	good
	ff_met	0.4283	0.0532	-				
	k_ met	0.0164	0.0054	0.0055				
	M_0_parent	102.0027	4.4178	-	8.15	2.5	13.49	Not presented
	α_parent	1.8755	0.8283	0.0224				
	β_parent	5.5886	3.3197	*0.0602*				
DFOP (parent) + SFO (met)	M0_met	0.000	-	-	16.23	41.87	139.10	good
	ff_met	0.4312	0.0537	-				
	k_ met	0.0166	0.0055	0.0064				
	M_0_parent	101.7403	0.0055	0.0064	8.11	2.49	15.36	Not presented
	k1_parent	0.3899	0.1327	0.0074				
	k2_parent	0.0629	0.0561	*0.1441*				

	g_parent	0.7461	0.2163	0.0031				

Table B.8.1-94: McLaughlin & Schanné, 1996, BVL no 2310250: Kinetic evaluation for degradation of glyphosate in the Speyer 2.3 at 10 °C soil (parent only)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
SFO (parent)	M_0_parent	91.8815	3.9195	-	7.72	10.82	35.93	poor
	k_ parent	0.0641	0.0089	1.8e-04				
FOMC (parent)	M_0_parent	98.2720	2.2675	-	3.25	8.13	64.81	good
	α_parent	1.1171	0.2260	0.0022				
	β_parent	9.4539	3.0416	0.0133				
DFOP (parent)	M_0_parent	99.4484	1.8142	-	2.31	8.07	50.79	Very good
	k1_parent	0.300	0.0790	0.0096				
	k2_parent	0.0361	0.0050	0.0010				
	g_parent	0.3756	0.0686	0.0027				
HS (parent)	M0_parent	Not tested						
	k1_parent							
	k2_parent							
	tb_parent							

Table B.8.1-95: McLaughlin & Schanné, 1996, BVL no 2310250: Kinetic evaluation for degradation of AMPA in the Speyer 2.3 at 10 °C soil (parent with metabolite)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
FOMC (parent) + SFO (met)	M0_met	0.000	-	-	11.59	200.69	666.69	acceptable
	ff_met	0.4389	0.0450	-				
	k_ met	0.0035	0.0030	*0.1368*				
	M_0_parent	99.6843	2.4287	-	3.49	7.60	72.25	Not presented
	α_parent	0.9556	0.1708	8.1e-05				
	β_parent	7.1320	2.1336	0.0033				
DFOP (parent) + SFO (met)	M0_met	0.000	-	-	8.96	134.79	447.75	good
	ff_met	0.4490	0.0360	-				
	k_ met	0.0051	0.0024	0.0305				
	M_0_parent	100.5782	1.9668	-	2.55	7.81	51.28	Not presented
	k1_parent	0.3497	0.0857	0.0011				
	k2_parent	0.0359	0.0048	1.1e-05				
	g_parent	0.3702	0.0606	5.7e-05				

Conclusion

The DT_{50} and DT_{90} values chosen by the study author as persistence and as modelling endpoints for glyphosate are presented in Table B.8.1-96.

The DT_{50} and DT_{90} values chosen by the study author as persistence endpoints for the metabolite AMPA are presented in Table B.8.1-97.

Table B.8.1-96: Dorn, 2012, BVL no 2315991: Persistence and modelling end points of glyphosate chosen by the study author

Study	Soil	Temp. (°C)	Persistence endpoints			Modelling endpoints		
			Model	$DegT_{50}$ (d)	$DegT_{90}$ (d)	Model	Non-referenced $DegT_{50}$ (d)	Normal-ised $DegT_{50}$ (d) (20°C, pF2)
Glyphosate								
Ponte (2010), BVL no 2310242)	Gartenacker	20	DFOP	7.9	56.3	FOMC	17.8 [1)]	9.4 [1)]
Dean (1995), BVL no 2310244	Arrow	20	FOMC	37.8	> 1000 [5)]	DFOP	93.7 [2)]	63.1 [2)]
Goodyear (1996), BVL no 2310246	Soil B	25	FOMC	1.2	20.7	FOMC	6.2 [1)]	6.9 [1)]
Galicia & Morgenroth (1993) BVL no 1932059	Les Evouettes	20	DFOP	8.8	159.4	DFOP	69.3 [2)]	42.2 [2)]
Esser (1996), BVL no 2310248	Visalia	25	DFOP	1.0	7.2	FOMC	1.9 [1)]	2.1 [1)]
Matla & Vonk (1993) BVL no 2151389	Maasdijk	20	DFOP	4.6	62.0	FOMC	18.0 [1)]	15.2 [1)]
Ponte (2010), BVL no 2310255	Drusenheim	20	DFOP	2.1	15.4	FOMC	5.4 [1)]	2.9 [1)]
	Pappelacker	20	FOMC	3.9	43.4	FOMC	13.1 [1)]	5.9 [1)]
	18-Acres	20	FOMC	60.2	> 1000 [5)]	DFOP	173.3 [2)]	92.7 [2)]
Galicia & Flückiger (1993), BVL no 1932046 Mamouni (2002), BVL no 2437068	Speyer 2.2	20	DFOP	1.6	150.6	- [3)]	- [3)]	- [3)]
	Speyer 2.3	20	DFOP	5.8	22.0	FOMC	7.2 [1)]	5.5 [1)]
McLaughlin & Schanné (1996), BVL no 2310250	Speyer 2.1	20	DFOP	8.3	51.3	FOMC	19.5 [1)]	18.1 [1)]
	Speyer 2.2	20	FOMC	18.7	428.0 [6)]	DFOP	72.2 [2)]	60.2 [2)]
	Speyer 2.3	20	DFOP	2.7	13.0	FOMC	3.8 [1)]	4.7 [1), 4)]
	Speyer 2.3	10	DFOP	8.1	50.8	FOMC	19.5 [1)]	

1) Back-calculated from FOMC DegT90/3.32
2) Calculated from slower k-rate of DFOP model
3) No acceptable fit for the derivation of modelling end points could be obtained
4) Arithmetic mean of normalised $DegT_{50}$ (20 °C, pF2) of 10 °C and 20 °C soil
5) Value could not be extrapolated
6) Compared with the results from the other data sets, the DT_{90} estimated with the FOMC model and extrapolated beyond the study duration seems to be unrealistically long (FOCUS, 2006, 2011). The value was therefore excluded from statistics.

Table B.8.1-97: Dorn, 2012, BVL no 2315991: Persistence and modelling end points of AMPA chosen by the study author

Study	Soil	Temp. (°C)	Persistence endpoints			Modelling endpoints		
			Model	$DegT_{50}$ (d)	$DegT_{90}$ (d)	Model	Non-referenced $DegT_{50}$ (d)	Normalised $DegT_{50}$ (d) (20°C, pF2)
Metabolite AMPA								
Ponte (2010) BVL no 2310242	Gartenacker	20	SFO	120.1	398.9	SFO	133.7	70.9
Dean (1995), BVL no 2310244	Arrow	20	- 1)	- 1)	- 1)	- 1)	- 1)	- 1)
Goodyear (1996), BVL no 2310246	Soil B	25	SFO	99.1	329.1	SFO	99.1	110.3
Galicia & Morgenroth (1993) BVL no 1932059	Les Evouettes	20	- 1)	- 1)	- 1)	- 1)	- 1)	- 1)
Esser (1996), BVL no 2310248	Visalia	25	SFO	70.7	234.9	SFO	68.6	76.3
Matla & Vonk (1993) BVL no 2151389	Maasdijk	20	- 2)	- 2)	- 2)	- 2)	- 2)	- 2)
Ponte (2010), BVL no 2310255	Drusenheim	20	SFO	39.0	129.5	SFO	47.0	25.6
	Pappelacker	20	SFO	126.6	420.5	SFO	126.6	57.2
	18-Acres	20	- 1)	- 1)	- 1)	- 1)	- 1)	- 1)
Galicia & Flückiger (1993), BVL no 1932046 Mamouni (2002), BVL no 2437068	Speyer 2.2	20	- 1)	- 1)	- 1)	- 1)	- 1)	- 1)
	Speyer 2.3	20	SFO	77.5	257.4	SFO	70.9	54.5
McLaughlin & Schanné (1996), BVL no 2310250	Speyer 2.1	20	- 1)	- 1)	- 1)	- 1)	- 1)	- 1)
	Speyer 2.2	20	- 1)	- 1)	- 1)	- 1)	- 1)	- 1)
	Speyer 2.3	20	SFO	41.9	139.1	SFO	42.1	35.1
	Speyer 2.3	10	SFO	134.8	447.7	- 3)	- 3)	- 3)

1) No decline of AMPA can be observed in parent study; no acceptable fit for AMPA could be obtained
2) Metabolite AMPA was not detected in study
3) T-test for k-rate not significant; $DegT_{50}$ was excluded from obtained modelling endpoints
4) Maximum at 10 °C and 20 °C

RMS Comment and re-calculations

Review of the included soil studies and the data processing of the soil residues of the soil residue data

The study Esser (1996, BVL no 2310248) was considered not acceptable by the RMS since oxygen was used instead of CO_2-free air for airing the headspace of the test vessels thus creating a best case regarding the supply with oxygen for the soil microbes and possibly accelerating glyphosate degradation. Thus the derived DT_{50} and DT_{90} values of glyphosate and AMPA from this study willl not be used for modelling or persistence calculations.

The exclusion of data points with a overall recovery of < 90 % AR before performing kinetic calculations as it has been done for the study results of Ponte (2010, BVL no 2310255), Galicia & Flückiger, (1993, BVL no 1932046)/ Mamouni (2002, BVL no 2437068) is considered an acceptable approach by the RMS to allow the derivation of endpoints even if not all data points achieve a sufficient overall recory.

The approach to set the initial metabolite concentration of AMPA to 0 is according to FOCUS (2006, 2011) and is considered acceptable by the RMS.

~~The approach of the study author, to use the overall mass balance as initial glyphosate concentration is not exactly according to FOCUS (2006, 2011) where it is only suggested to convert the initial metabolite concentration to mass parent and to add it to the value measured for the parent. However, the differences in the resulting DT_{50} and DT_{90} values between the two approaches are expected to be small by the RMS. Thus, the approach is considered acceptable by the RMS.~~

However, the general exclusion of data points beyond study duration of 120 days as has been done for the study residues of Dean, 1995, BVL no 2310244 and Galicia & Morgenroth, 1993, BVL no 1932059 is not considered acceptable by the RMS. In both studies, the microbial biomass was monitored at day 0 and day 364 in the study Galicia & Morgenroth, 1993, BVL no 1932059 and at day 0, 120 and 217 in the study Dean, 1995, BVL no 2310244. The microbial biomass in the study Galicia & Morgenroth, 1993, BVL no 1932059 declined from 58.5 to 22 mgC/100g at the end of the study. However, with a soil organic carbon-content of 1.4 %, more than 1 % of the oc-content (1.6 %) still consisted of microbial biomass at day 364 as is recommended for soils in the OECD guideline 307 (2002). The microbial biomass in the study Dean, 1995, BVL no 2310244 even remained constant at 337 µgC/g from day 0 to day 120 and declined only afterwards to 256 µg C/g. The latter is equal to 1.2 % of the organic carbon-content of the soil. Besides, a decline of glyphosate could still be observed at the data points beyond 120 days in both investigated soils suggesting that degradation of the active substance still occured. Thus, the kinetics calculations by Dorn, 2012, BVL no 2315991 for soil Arrow (Dean, 1995, BVL no 2310244) and soil 'Les Evouttes' (Galicia & Morgenroth, 1993, BVL no 1932059) are not considered acceptable by the RMS and recalculations for these soils were performed using all data points by the RMS. However, since an increase of AMPA at day 364 was observed after the previous decline after day 84, the last data points at day 364 for the metabolite were excluded as outlier.

Finally, no kinetic-re-evalution were performed for the soil Dupo of Kesterson & Atkins (1991, BVL no 1932061)/ Honegger (1992, BVL no 2325652), ~~and~~for the soil Speyer 2.2 of Runnalls (1991, BVL no 2154349) and in the soil Speyer 2.1 of Lewis & Turnbull (1992,

BVL no 1052659). Thus, data processing and kinetic re-calculations according to FOCUS guideline (2006, 2011) for these ~~two~~ soils were performed by the RMS.

The kinetic modelling programme KinGuiII with NLLS (non-linear least-square estimation) as statistical optimizer was used by the RMS for the kinetic re-calculations.

KIIA 7.2.1/13 (Dean, 1995, BVL no 2310244- Kinetic re-evaluation by RMS):

The results of soil degradation kinetic re-analysis by the RMS of glyphosate and AMPA (DT_{50}, DT_{90}) in Arrow soil from the study Dean, 1995, BVL no 2310244 are presented in Figure B. 8.1-11, Figure B. 8.1-12, Figure B. 8.1-13; Table B.8.1-98 and Table B.8.1-99.

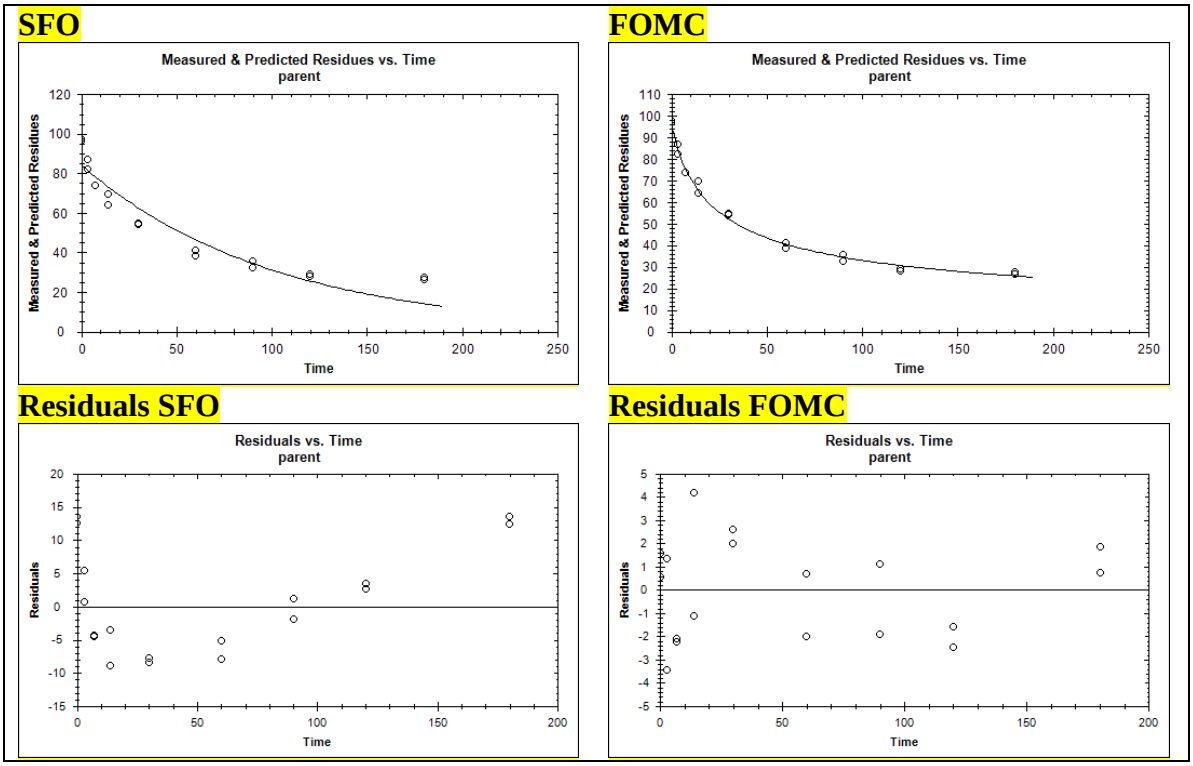
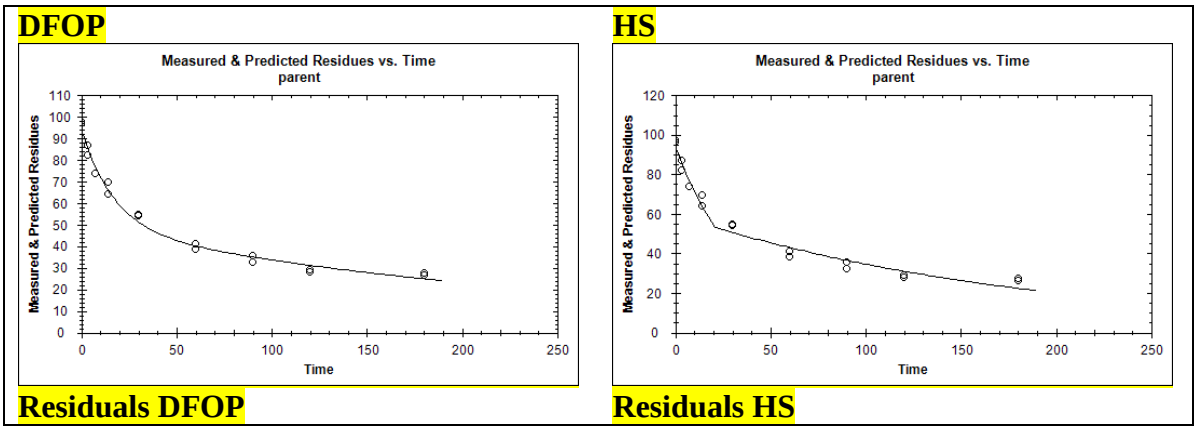


Figure B. 8.1-11: Optimised SFO and FOMC kinetic models for degradation of glyphosate in the Arrow soil (parent only)



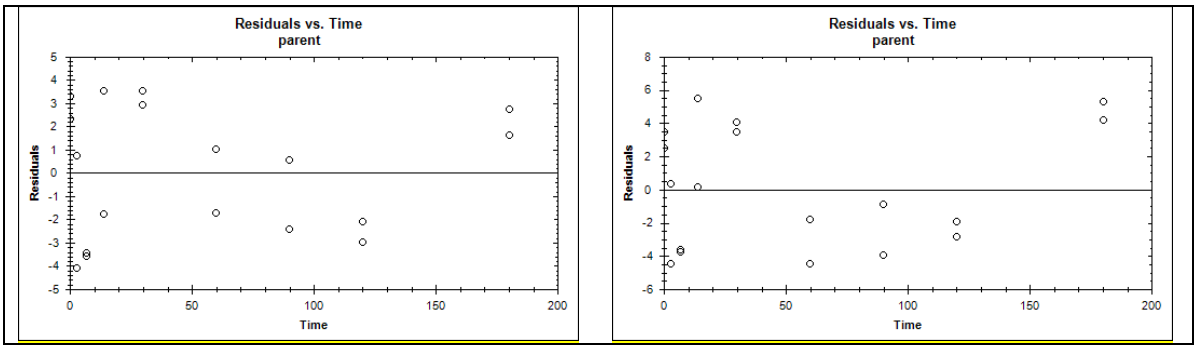


Figure B. 8.1-12: Optimised DFOP and HS kinetic models for degradation of glyphosate in the Arrow soil (parent only)

Table B.8.1-98: Dean, 1995, BVL no 2310244: Kinetic re-evaluation by the RMS for degradation of glyphosate in the Arrow soil (parent only)

~~Kinetic model~~	~~Parameter~~	~~Optimised value~~	~~Std. error~~	~~p-value (t-test)~~	~~% error χ^2 test~~	~~DT_{50} (d)~~	~~DT_{90} (d)~~	~~Visual assessment~~
~~SFO (parent)~~	~~M_0_parent~~	~~80.446451~~	~~2.956416~~	~~1.75e-14~~	~~9.83~~	~~75.67~~	~~251.03~~	~~poor~~
	~~k_parent~~	~~0.009173~~	~~0.001033~~	~~1.16e-07~~				
~~FOMC (parent)~~	~~M_0_parent~~	~~91.48393~~	~~1.61158~~	~~<2e-16~~	~~2.10~~	~~44.97~~	~~1368~~	~~Very good~~
	~~α_parent~~	~~0.51502~~	~~0.04702~~	~~1.49e-08~~				
	~~β_parent~~	~~15.82632~~	~~3.36220~~	~~0.000168~~				
~~DFOP (parent)~~	~~M_0_parent~~	~~89.18~~	~~1.619~~	~~<2e-16~~	~~2.47~~	~~44.76~~	~~300.73~~	~~Very good~~
	~~k1_parent~~	~~2.305e-03~~	~~1.154e-03~~	~~0.033554~~				
	~~k2_parent~~	~~3.825e-02~~	~~7.817e-03~~	~~0.00417~~				
	~~g_parent~~	~~0.4429~~	~~0.06926~~	~~1.18e-05~~				
~~HS (parent)~~	~~M0_parent~~	~~91.199860~~	~~6.869604~~	~~3.08e-09~~	~~9.27~~	~~61.97~~	~~247.36~~	~~poor~~
	~~k1_parent~~	~~0.172886~~	~~0.092233~~	~~0.04176~~				
	~~k2_parent~~	~~0.008681~~	~~0.001018~~	~~5.51e-07~~				
	~~tb_parent~~	~~0.944952~~	~~0.333247~~	~~0.00702~~				

Kinetic model	**Parameter**	**Optimised value**	**Std. error**	**p-value (t-test)**	**% error χ^2 test**	**DT50 (d)**	**DT90 (d)**	**Visual assessment**
SFO (parent)	M0_parent	83.981720	3.291018	1.09e-14	11.24	69.77	231.77	poor
	k_ parent	0.009935	0.001217	2.12e-07				
FOMC (parent)	M0_parent	96.02318	1.41719	< 2e-16	2.305	37.75	1661.5	Very good
	α_parent	0.45389	0.03436	5.75e-10				
	β_parent	10.47275	1.88856	2.80e-05				
DFOP (parent)	M0_parent	94.293705	2.025502	< 2e-16	3.521	37.44	186.64	Very good
	k1_parent	0.059441	0.016962	0.00175				
	k2_parent	0.003714	0.001234	0.00469				
	g_parent	0.485715	0.071654	4.45e-06				
HS (parent)	M0_parent	94.4	2.247	< 2e-16	4.981	43.079	337.98	acceptable
	k1_parent	0.02750	0.003643	1.34e-06				
	k2_parent	0.005458	0.0007745	2.9e-06				
	tb_parent	20.78	3.582	2.3e-05				

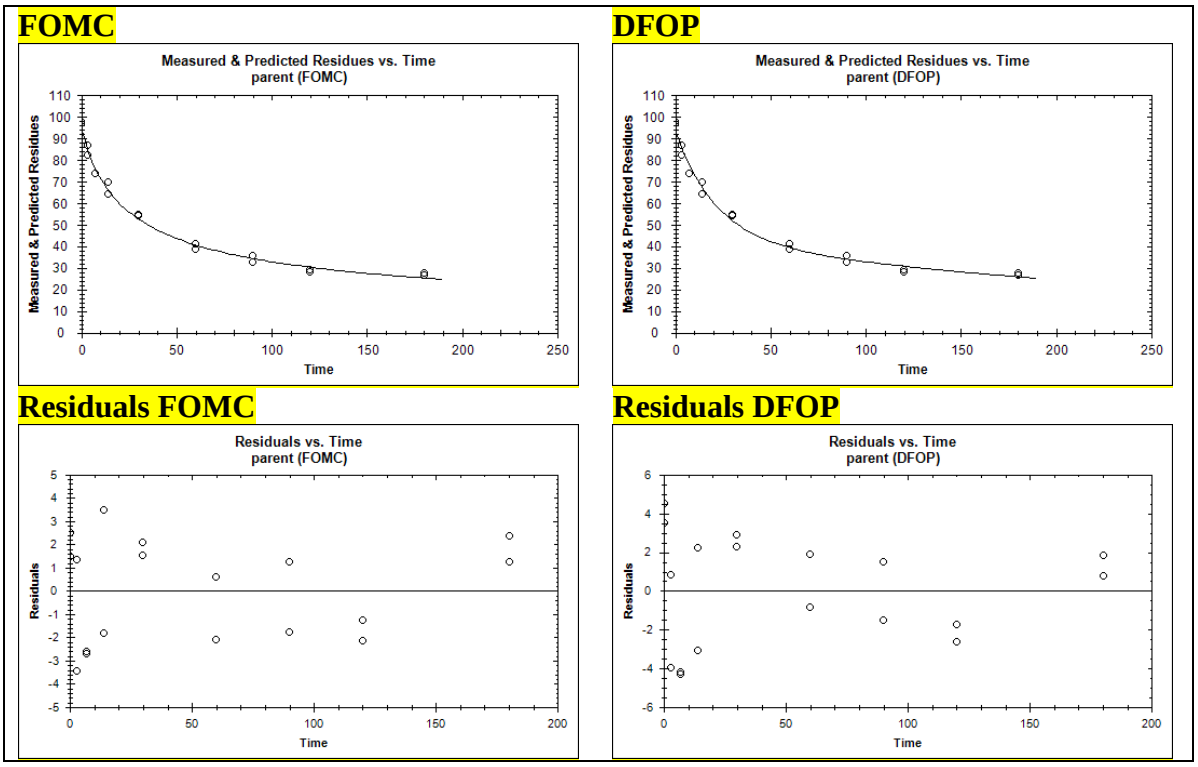


Figure B. 8.1-13: Optimised FOMC and DFOP kinetic models for degradation of AMPA in the Arrow soil (parent with metabolite)

Table B.8.1-99: Dean, 1995, BVL no 2310244: Kinetic re-evaluation by the RMS for degradation of AMPA in the Arrow soil (parent with metabolite)

~~Kinetic model~~	~~Parameter~~	~~Optimised value~~	~~Std. error~~	~~p-value (t-test)~~	~~% error χ^2 test~~	~~DT_{50} (d)~~	~~DT_{90} (d)~~	~~Visual assessment~~
~~FOMC (parent) + SFO (met)~~	~~M0_met~~	~~0.000~~	~~-~~	~~-~~	~~7.40~~	~~1115~~	~~3703~~	~~good~~
	~~ff_met~~	~~0.4496~~	~~0.03228~~	~~-~~				
	~~k_.met~~	~~0.0006217~~	~~0.000657~~	~~*0.176*~~				
	~~M_0_parent~~	~~90.61394~~	~~1.38987~~	~~<2e-16~~	~~2.25~~	~~46.88~~	~~1213~~	~~Very good~~
	~~α_parent~~	~~0.548067~~	~~0.043195~~	~~5.97e-13~~				
	~~β_parent~~	~~18.43663~~	~~3.39389~~	~~3.82e-06~~				
~~DFOP (parent) + SFO (met)~~	~~M0_met~~	~~0.000~~	~~-~~	~~-~~	~~6.41~~	~~1153~~	~~3830~~	~~good~~
	~~ff_met~~	~~0.4698~~	~~0.0349792~~	~~-~~				
	~~k_.met~~	~~0.0006011~~	~~0.0007561~~	~~*0.217*~~				
	~~M_0_parent~~	~~88.4427987~~	~~1.2303103~~	~~<2e-16~~	~~2.63~~	~~46.09~~	~~451.33~~	~~Very good~~
	~~k1_parent~~	~~0.0329918~~	~~0.0056223~~	~~1.31e-06~~				
	~~k2_parent~~	~~0.0015358~~	~~0.0011823~~	~~*0.102*~~				
	~~g_parent~~	~~0.6053567~~	~~0.0697525~~	~~*9.97e-10*~~				

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
FOMC (parent) + SFO (met)	M_0_parent	95.11	283.4	0.487	2.475	39.592	1432.9	Very good
	α_parent	0.4841	61.42	0.497				
	β_parent	12.43	433.6	0.499				
	ff_met	0.3925	71.2		9.118	1.02e+10	3.38e+10	acceptable
	k_ met	6.812e-11	2.477	0.500				
DFOP (parent)	M_0_parent	93.09	2555	0.486	3.765	3.882	243.57	Very good
	k1_parent	0.04690	12.36	0.498				

+ SFO (met)	k2_parent	0.002846	2.078	0.499				
	g_parent	0.5386	119.7	0.498				
	ff_met	0.41	61.66		8.584	12049	40027	acceptable
	k_ met	0.00005753	2.089	0.500				

KIIA 7.2.1/14 (Galicia & Morgenroth, 1993, BVL no 1932059- - Kinetic re-evaluation by RMS):

The results of soil degradation kinetic re-analysis by the RMS of glyphosate and AMPA in the soil Les Evouettes from the study Galicia & Morgenroth, 1993, BVL no 1932059 are presented in Figure B. 8.1-14, Figure B. 8.1-15, Figure B. 8.1-16, Table B.8.1-100 and Table B.8.1-101.

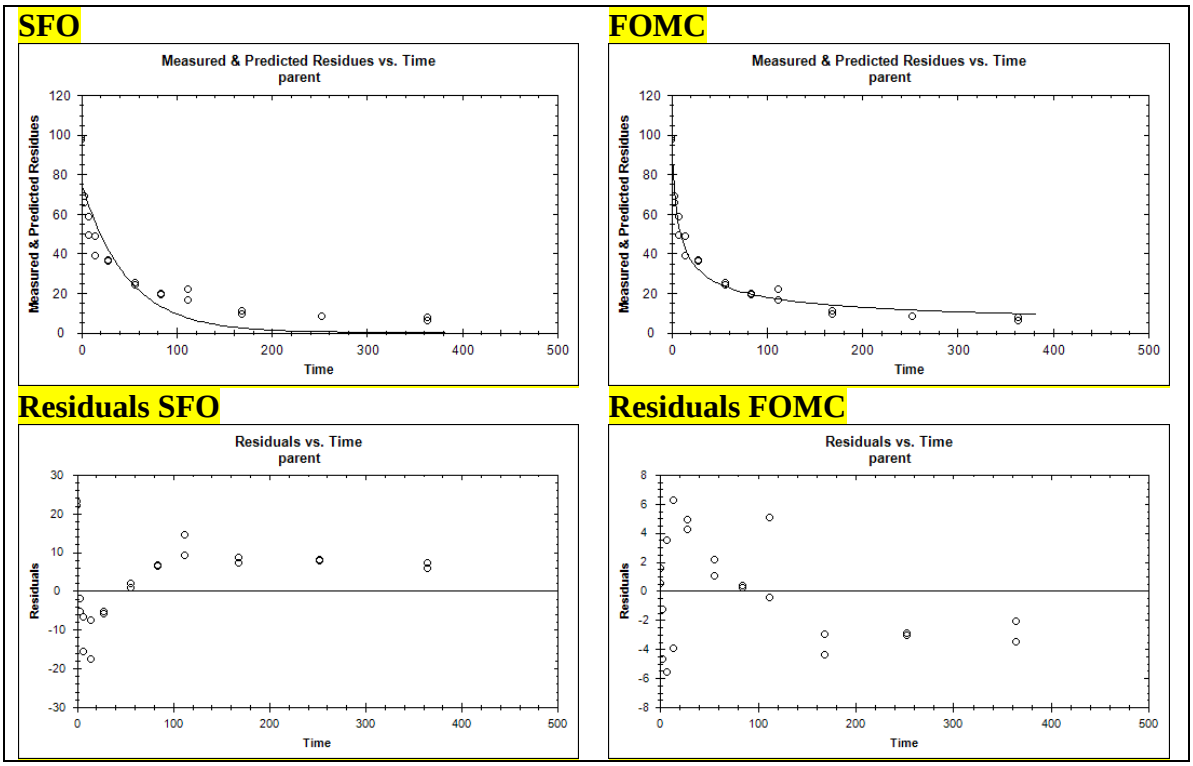
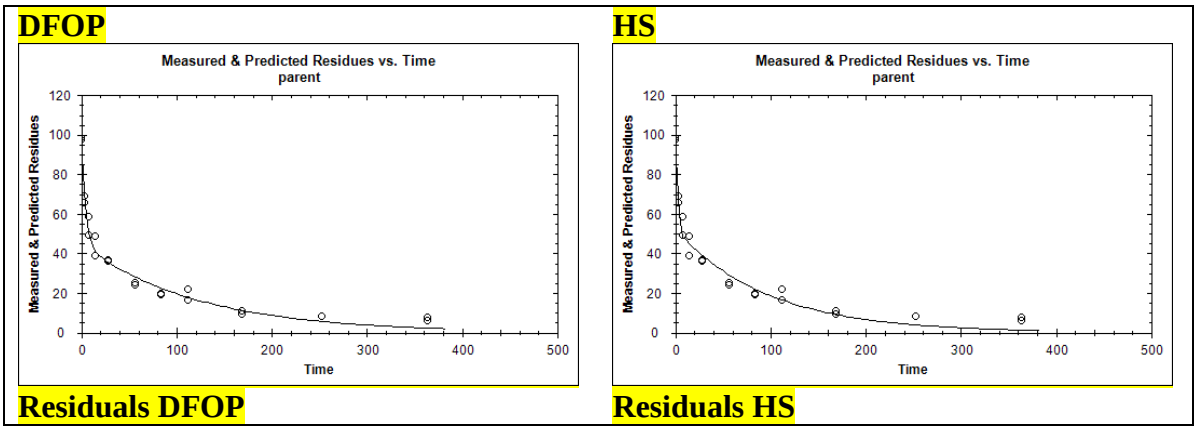


Figure B. 8.1-14: Optimised SFO and FOMC kinetic models for degradation of glyphosate in soil 'Les Evouettes' (parent only)



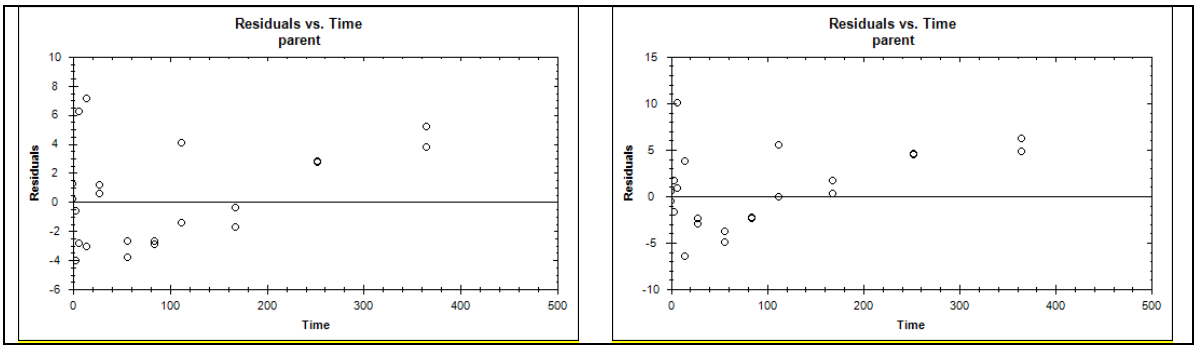
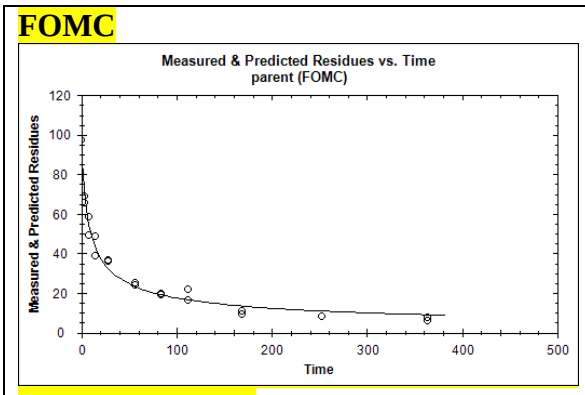


Figure B. 8.1-15: Optimised DFOP and HS kinetic models for degradation of glyphosate in soil 'Les Evouettes' (parent only)

Table B.8.1-100: Galicia & Morgenroth, 1993, BVL no 1932059: Kinetic re-evaluation by the RMS for degradation of glyphosate in soil 'Les Evouettes' (parent only)

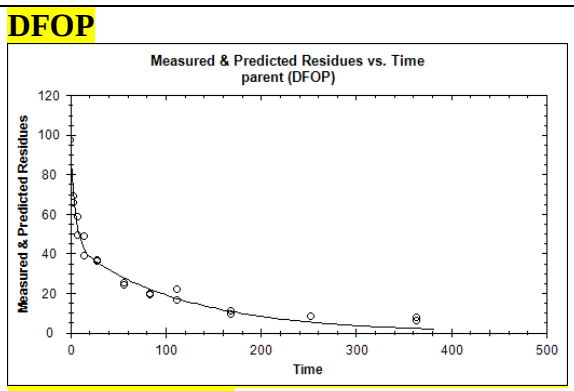
Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
SFO (parent)	M_0_parent	75.520930	5.617182	8.9e-12	*24.66*	33.13	110.04	poor
	k_ parent	0.020925	0.005412	0.00048				
FOMC (parent)	M_0_parent	97.06926	3.16768	< 2e-16	6.17	10.00	347.00	acceptable
	α_parent	0.49124	0.03334	3.76e-12				
	β_parent	3.22603	0.70985	0.000111				
DFOP (parent)	M_0_parent	97.366601	2.831998	<2e-16	5.93	8.55	83.92	good
	k1_parent	0.234970	0.046727	4.36e-05				
	k2_parent	0.008260	0.001173	7.14e-07				
	g_parent	0.541289	0.036307	7.14e-12				
HS (parent)	M0_parent	98.100398	3.213175	< 2e-16	8.26	6.08	160.90	acceptable
	k1_parent	0.125607	0.019145	1.82e-06				
	k2_parent	0.01395	0.001349	2.08e-07				
	tb_parent	5.467707	0.906982	5.32e-06				

FOMC



Residuals FOMC

DFOP



Residuals DFOP

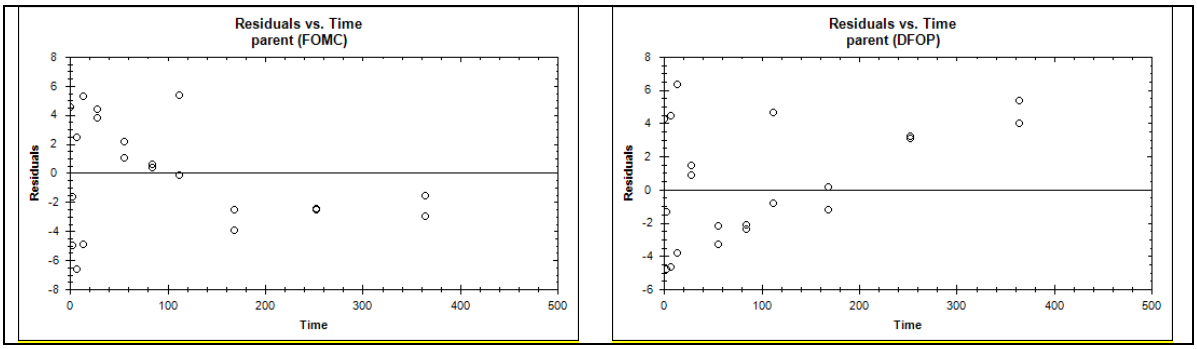


Figure B. 8.1-16: Optimised FOMC and DFOP kinetic models for degradation of AMPA in soil 'Les Evouettes' (parent with metabolite)

Table B.8.1-101: Galicia & Morgenroth, 1993, BVL no 1932059: Kinetic re-evaluation for degradation of AMPA in soil 'Les Evouettes' (parent with metabolite)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
FOMC (parent) + SFO (met)	M0_met	0.000	-	-	14.00	337.34	1255.9	acceptable
	ff_met	0.3618	0.03572	-				
	k_ met	1.833e-03	6.664e-04	0.004668				
	M_0_parent	93.05	3.607	<2e-16	7.17	11.87	378.07	acceptable
	α_parent	0.5283	0.04518	6.07e-14				
	β_parent	4.372	1.092	0.000154				
DFOP (parent) + SFO (met)	M0_met	0.000	-	-	16.06	300.71	998.93	acceptable
	ff_met	0.3721	0.04214	-				
	k_ met	2.305e-03	7.735e-04	1.38e-13				
	M_0_parent	93.33	3.828	<2e-16	7.15	10.59	81.49	good
	k1_parent	0.1974	0.04903	0.00015				
	k2_parent	8.506e-03	1.455e-03	6.87e-07				
	g_parent	0.5238	0.04546	1.38e-13				

KIIA 7.2.1/15 (Kesterson & Atkins (1991, BVL no 1932061)/ Honegger (1992, BVL no 2325652) - Kinetic re-evaluation by RMS):

The residues of glyphosate and AMPA in the soil Dupo together with the total recovery of the applied radioactivity are presented in Table B.8.1-102.

Table B.8.1-102: Kesterson & Atkins (1991, BVL no 1932061)/ Honegger (1992, BVL no 2325652): residues of glyphosate and AMPA and total radioactive recovery in the soil Dupo

Sample description	Time	Glyphosate	AMPA	Total recovery
		% of applied		
Rep A	0 days	64.3	14.3	84
Rep B	0 days	82.2	18.7	103.2
Rep A	1 days	49.7	22	99.5
Rep B	1 days	61.5	19.1	87.1
Rep A	3 days	27.3	26.3	104.4
Rep B	3 days	23.9	21.7	84.8
Rep A	7 days	13.9	28	87.4
Rep B	7 days	11.7	23.4	81.4
Rep A	14 days	7	34.6	94.1
Rep B	14 days	5.6	22.7	93.6
Rep A	1 month	2.7	22.3	92.6
Rep B	1 month	3.3	26.3	88.4
Rep A	2 month	1.5	14.7	93.5
Rep B	2 month	1.5	14.9	87.2
Rep A	3 month	1.7	10	89.3
Rep B	3 month	1.4	11.4	93.6
Rep A	4 month	1.1	5.1	89.8
Rep B	4 month	1.3	8.8	92.8
Rep A	6 month	0.7	2.8	91.7
Rep B	6 month	0.7	4.3	92.9
Rep A	9 month	0.8	2.1	92.6
Rep B	9 month	0.6	2.2	94.1
Rep A	12 month	0.4	1.6	86.1
Rep B	12 month	0.7	1.5	88.3

For kinetic calculation, the sample points with total recovery <90 % were excluded, the monthly sampling points were converted into days assuming 30.4 days per month (= 365/12). Additionally, the AMPA concentration measured at day 0 of the experiment was converted to mass parent and added to the value measured for the parent as rcommended by FOCUS (2006, 2011).

The processed residue data of glyphosate and AMPA used for kinetic evaluation are given in Table B.8.1-103.

Table B.8.1-103: Kesterson & Atkins (1991, BVL no 1932061)/ Honegger (1992, BVL no 2325652): processed residue data of glyphosate and AMPA used for kinetic evaluation

Time (days)	Glyphosate	AMPA
	% of applied	
0	94.48	0
1	49.7	22
3	27.3	26.3
14	7	34.6
14	5.6	22.7
30	2.7	22.3
61	1.5	14.7
91	1.4	11.4
122	1.1	5.1
122	1.3	8.8
183	0.7	2.8
183	0.7	4.3
274	0.8	2.1
274	0.6	2.2

The results of soil degradation kinetic re-analysis by the RMS of glyphosate and AMPA in the soil Dupo are presented in Figure B. 8.1-17; Figure B. 8.1-18, Figure B. 8.1-19, Table B.8.1-104 and Table B.8.1-105.

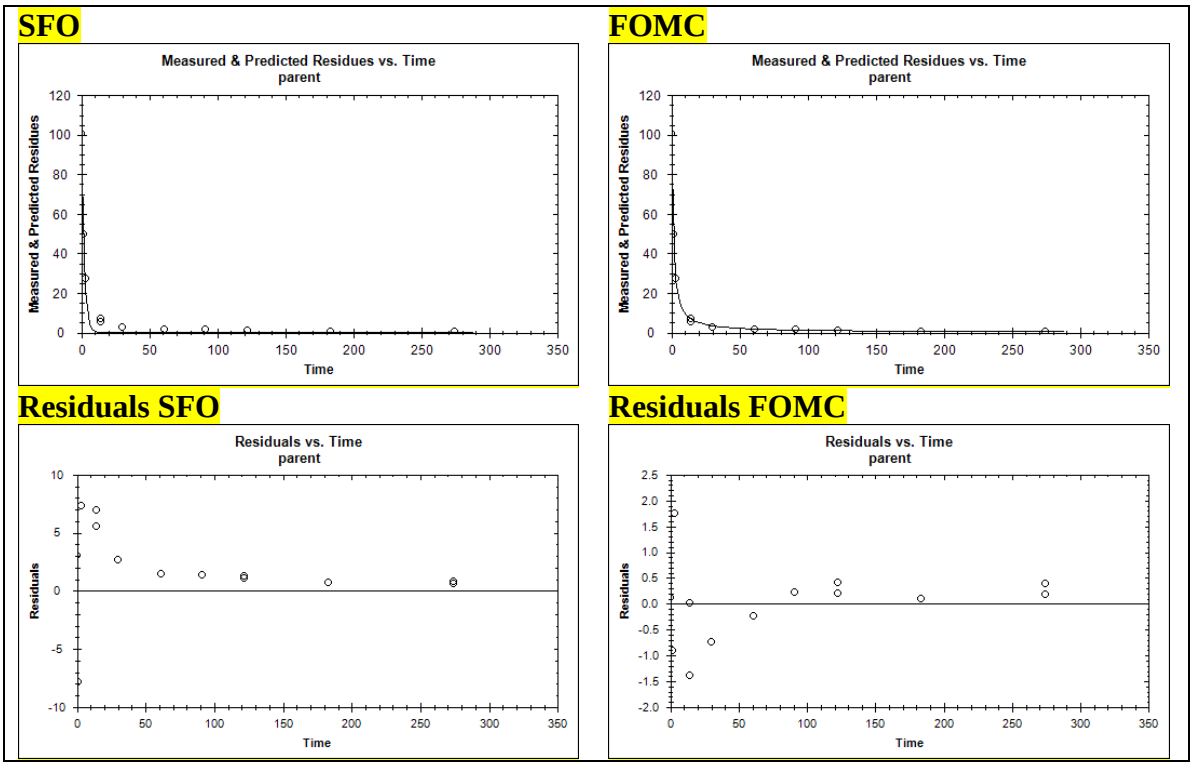


Figure B. 8.1-17: Optimised SFO and FOMC kinetic models for degradation of glyphosate in soil 'Dupo' (parent only)

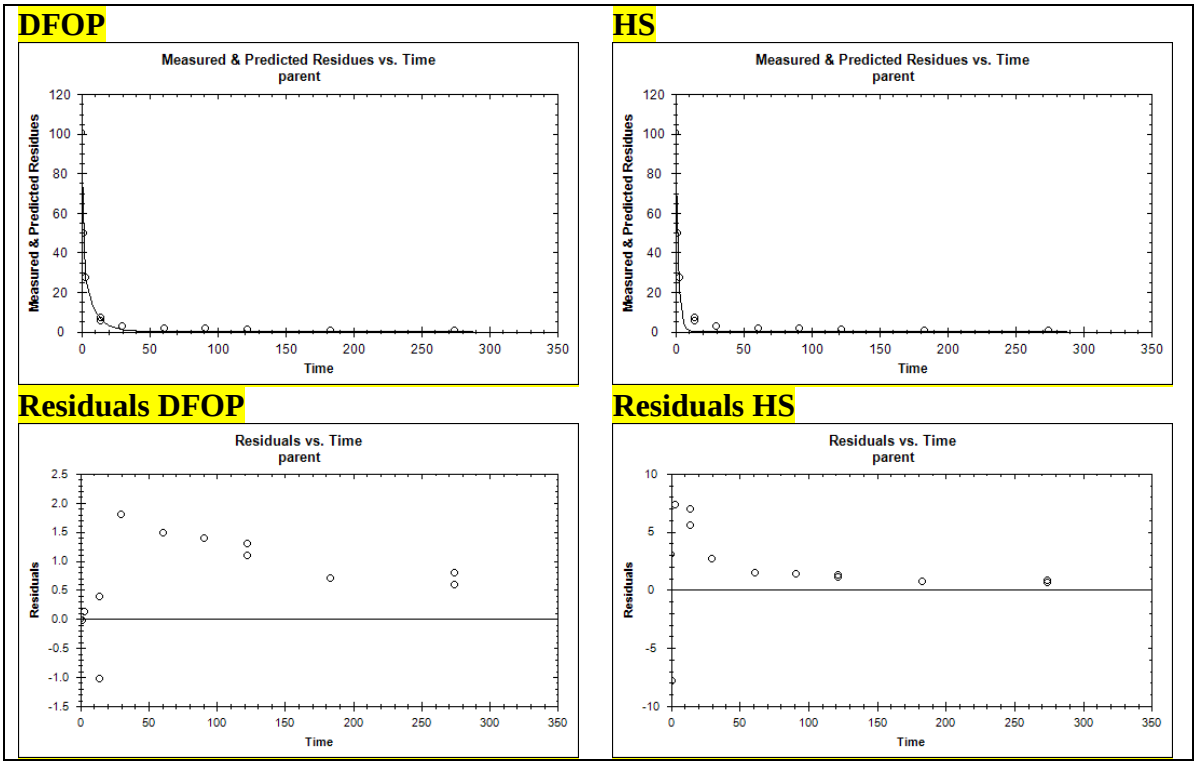
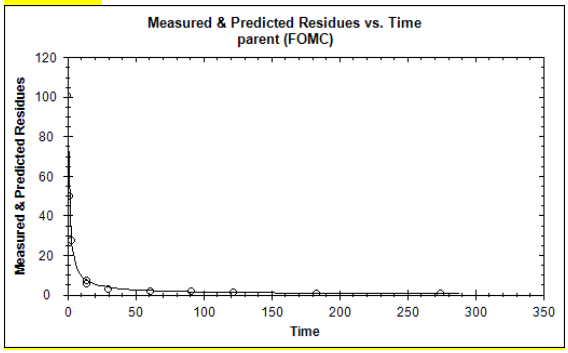


Figure B. 8.1-18: Optimised DFOP and HS kinetic models for degradation of glyphosate in soil 'Dupo' (parent only)

Table B.8.1-104: Kesterson & Atkins (1991, BVL no 1932061)/ Honegger (1992, BVL no 2325652): Kinetic re-evaluation by the RMS for degradation of glyphosate in soil 'Dupo' (parent only)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
SFO (parent)	M_0_parent	97.79708	3.99075	6.43e-12	*22.49*	1.34	4.34	poor
	k_ parent	0.53039	005925	5.84e-07				
FOMC (parent)	M_0_parent	100.78	0.82691	<2e-16	3.80	1.01	9.31	Very good
	α_parent	0.97868	0.04549	1.22e-10				
	β_parent	0.97899	0.08415	7.99e-08				
DFOP (parent)	M_0_parent	100.89570	1.13792	4.07e-16	5.74	0.97	5.56	acceptable
	k1_parent	1.35513	0.14257	1.26e-06				
	k2_parent	0.12469	0.01323	1.37e-06				
	g_parent	0.62404	0.03328	2.01e-09				
HS (parent)	M0_parent	97.80	*1428*	*0.473*	*24.1*	*24.1*	*<0.0001*	poor
	k1_parent	0.002596	*58.25*	*0.500*				
	k2_parent	0.5304	*38.26*	*0.495*				
	tb_parent	7.220e-08	*38.50*	*0.500*				

FOMC



Residuals FOMC

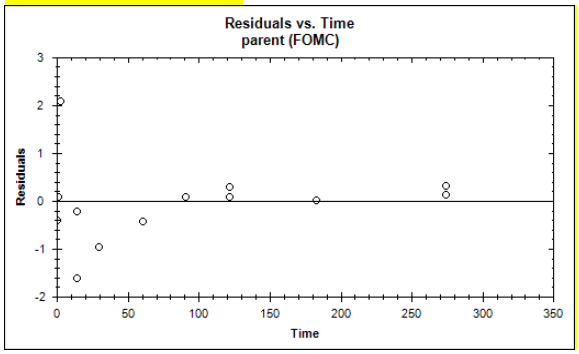


Figure B. 8.1-19: Optimised FOMC kinetic model for degradation of AMPA in soil 'Dupo' (parent with metabolite)

Table B.8.1-105: Kesterson & Atkins (1991, BVL no 1932061)/ Honegger (1992, BVL no 2325652): Kinetic re-evaluation by the RMS for degradation of AMPA in soil 'Dupo' (parent with metabolite)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
FOMC (parent) + SFO (met)	M_0_parent	101.3	2.147	<2e-16	4.45%	0.96	9.45	Very good
	α_parent	0.9284	0.1109	9.69e-09				
	β_parent	0.8640	0.1931	8.61e-05				
	ff_met	0.3637	0.01720	-	7.57%	48.32	160.51	good
	k_ met	0.01435	0.001352	1.24e-10				

KIIA 7.2.1/16 (Runnalls (1991, BVL no 2154349) - Kinetic re-evaluation by RMS):

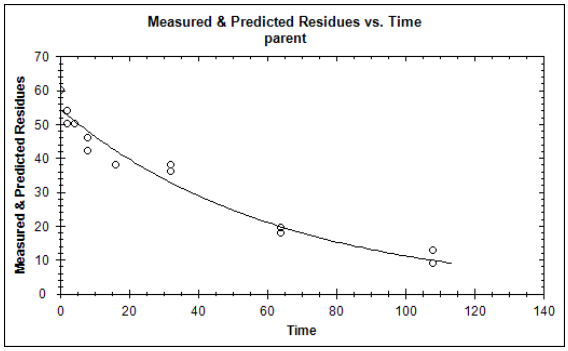
The residues of PMG in the soil Speyer 2.2 used for kinetic evaluation are presented in Table B.8.1-106.

Table B.8.1-106: Runnnalls (1991, BVL no 2154349): residues of glyphosate in the soil Speyer 2.2

Time (days)	PMG (% of applied)
0	60.0
0	60.0
2	50.0
2	54.0
4	50.0
4	50.0
8	42.0
8	46.0
16	38.0
16	38.0
32	36.0
32	38.0
64	19.4
64	18.0
108	12.8
108	8.8

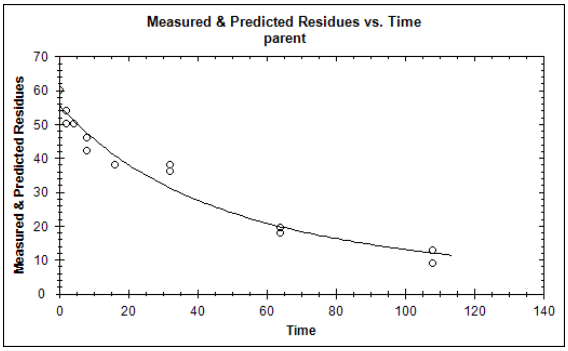
The results of soil degradation kinetic re-analysis by the RMS of PMG (DT_{50}, DT_{90}) in the soil Speyer 2.2 are presented in Figure B. 8.1-20 and Table B.8.1-107.

SFO



Residuals SFO

FOMC



Residuals FOMC

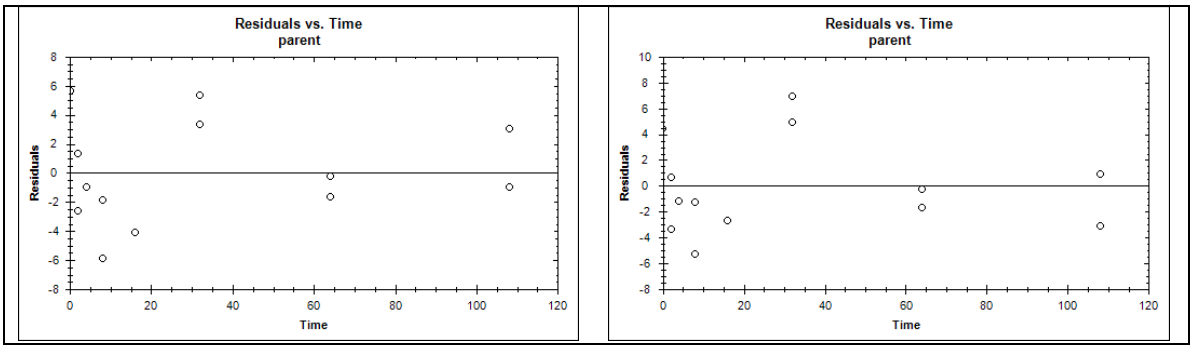


Figure B. 8.1-20: Optimised SFO and FOMC kinetic models for degradation of PMG in soil 'Speyer 2.2'

Since SFO gave a good fit for glyhosate-trimesium, DFOP and HS kinetics were not applied.

Table B.8.1-107: Runnnalls (1991, BVL no 2154349): Kinetic re-evaluation by the RMS for degradation of PMG in soil 'Speyer 2.2'

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
SFO (parent)	M_0_parent	54.34	1.45	9.8e-16	6.95	43.53	144.61	good
	k_ parent	0.015923	0.001526	2.75e-08				
FOMC (parent)	M_0_parent	55.58	2.042	3.78e-13	6.83	39.12	198.77	good
	α_parent	2.115	*2.039*	*0.159*				
	β_parent	100.872	*125.261*	*0.218*				

KIIA 7.2.1/17 (Lewis & Turnbull (1992, BVL no 1052659 - Kinetic re-evaluation by RMS):

The residues of PMG and AMPA in the soil Speyer 2.1 incubated at 20 °C and 40 % MWHC following an application of 4 ppm glyphosate-trimesium labelled in the phosphonomethylglycine anion (PMG) is presented in Table B.8.1-108.

Table B.8.1-108: Lewis & Turnbull, 1992, BVL no 1052659: residues of PMG and AMPA in the soil Speyer 2.1 (following an application of 4 ppm glyphosate-trimesium and an incubation at 20 °C and 40 % MWHC)

Time	PMG	AMPA
(days)	(% of applied)	
0	74.96	7.90
0	74.80	4.24
2	53.68	7.09
2	64.98	9.41
4	54.58	15.31
4	54.95	15.34
8	46.86	18.62
8	41.84	17.40
16	34.95	25.45
16	32.03	24.18
33	21.03	29.23
33	21.20	29.81
64	13.09	33.92
64	13.31	29.68
104	7.46	29.93

For kinetic calculation, the AMPA concentration measured at day 0 in the soil Speyer 2.1 was converted to mass parent and added to the value measured for the parent as recommended by FOCUS (2006, 2011). The thus processed data of PMG and AMPA in the soil Speyer 2.1 incubated at 20 °C and 40 % MWHC following an application of 4 ppm glyphosate-trimesium are presented in Table B.8.1-109. ~~The residue data of PMG in the soils Speyer 2.1 and Beedon Manor can be used without further processing.~~

Table B.8.1-109: Lewis & Turnbull, 1992, BVL no 1052659: processed residue data for kinetic re-evaluation of PMG and AMPA in the soil Speyer 2.1 (following an application of 4 ppm glyphosate-trimesium and an incubation at 20 °C and 40 % MWHC)

Time	PMG	AMPA
(days)	(% of applied)	
0	80.14	0
0	74.80	0
2	53.68	7.09
2	64.98	9.41
4	54.58	15.31
4	54.95	15.34
8	46.86	18.62
8	41.84	17.40
16	34.95	25.45
16	32.03	24.18
33	21.03	29.23
33	21.20	29.81
64	13.09	33.92
64	13.31	29.68
104	7.46	29.93

The results of soil degradation kinetic re-analysis by the RMS of PMG and AMPA (DT_{50}, DT_{90}) in the soil Speyer 2.~~2~~1 are presented in Figure B. 8.1-21, Figure B. 8.1-22, Figure B. 8.1-23, Table B.8.1-110 and Table B.8.1-111.

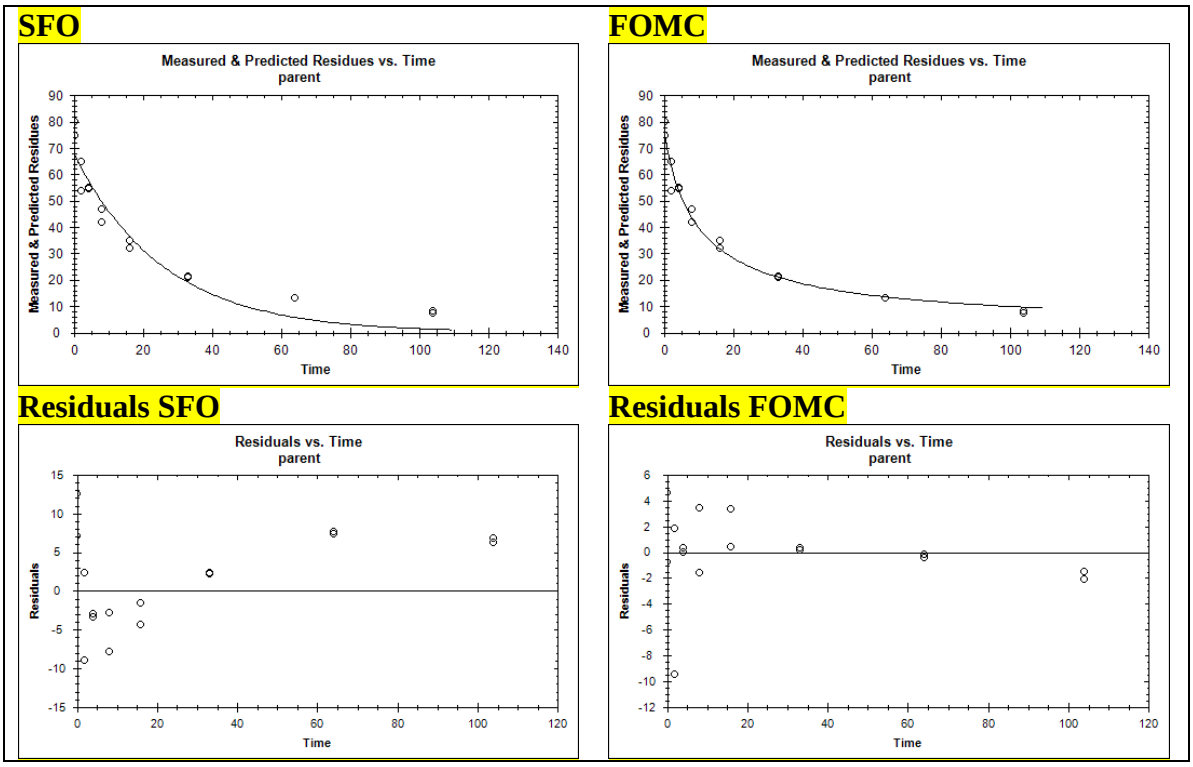


Figure B. 8.1-21: Optimised SFO and FOMC kinetic models for degradation of PMG and AMPA in the soil Speyer 2.1 (following an application of 4 ppm glyphosate-trimesium and an incubation at 20 °C and 40 % MWHC) – parent only

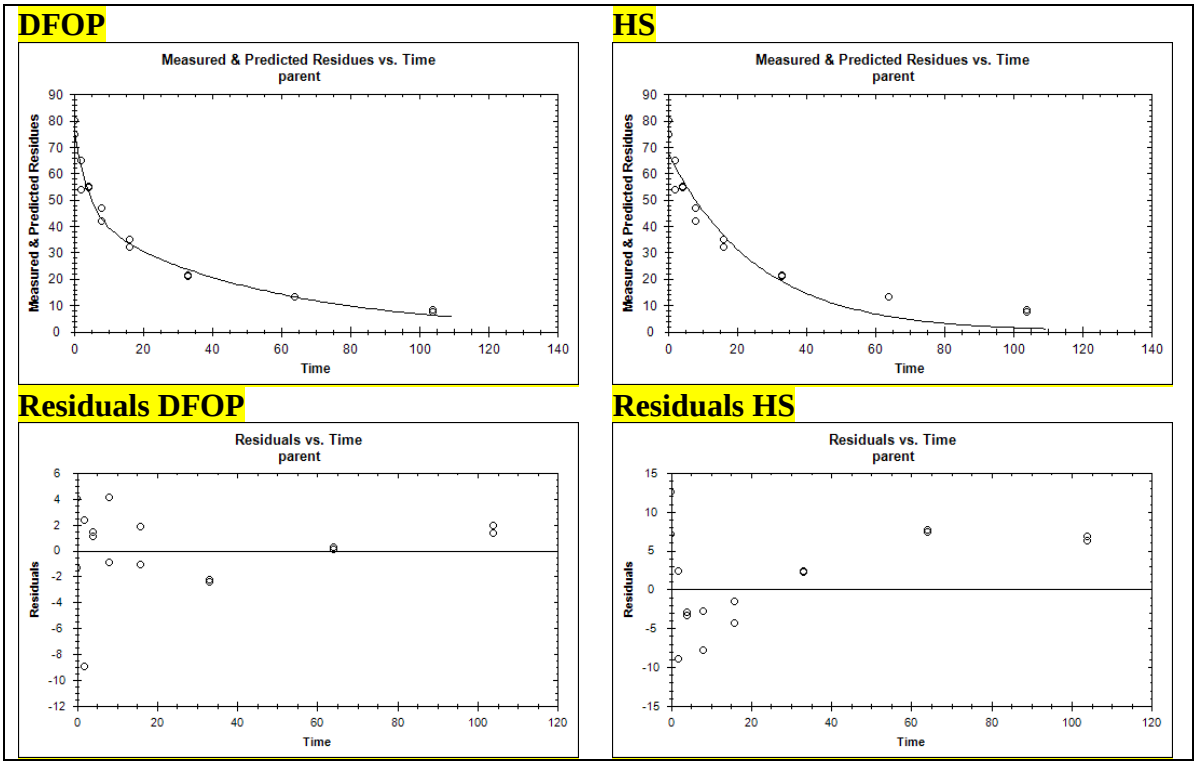
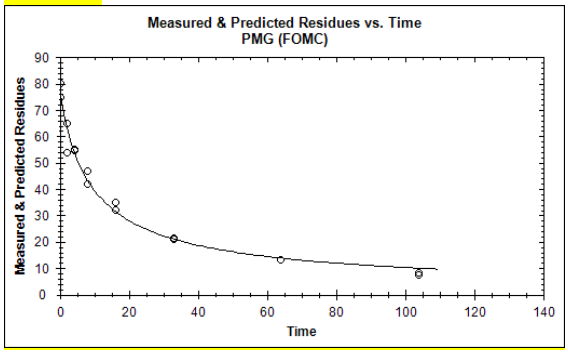


Figure B. 8.1-22: Optimised DFOP and HS kinetic models for degradation of PMG and AMPA in the soil Speyer 2.1 (following an application of 4 ppm glyphosate-trimesium and an incubation at 20 °C and 40 % MWHC) – parent only

Table B.8.1-110: Lewis & Turnbull, 1992, BVL no 1052659: Kinetic re-evaluation by the RMS for degradation of PMG and AMPA in the soil Speyer 2.1 (following an application of 4 ppm glyphosate-trimesium and an incubation at 20 °C and 40 % MWHC) – parent only

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
SFO (parent)	M_0_parent	67.635915	3.210297	2.65e-12	12.0	17.92	59.5~~63~~	poor
	k_ parent	0.038678	0.006697	2.40e-05				
FOMC (parent)	M_0_parent	75.5588	2.3681	4.92e-14	**3.91**	**11.11**	**144.25**	Very good
	α_parent	0.7683	0.1347	3.63e-05				
	β_parent	7.5833	2.6494	0.00667				
DFOP (parent)	M_0_parent	76.150180	2.496916	4.84e-13	3.95	11.02	36.70	Very good
	k1_parent	0.228215	0.0096585	0.017933				
	k2_parent	0.018885	0.004297	0.000437				
	g_parent	0.426854	0.087240	0.000185				
HS (parent)	M0_parent	67.64	*865.8*	*0.470*	12.73	17.92	59.53	poor
	k1_parent	0.03155	*74.57*	*0.500*				
	k2_parent	0.03868	*0.6286*	*0.476*				
	tb_parent	3.106e-06	*155.9*	*0.500*				

FOMC



Residuals FOMC

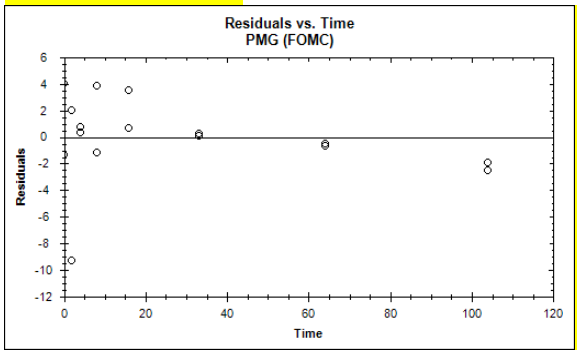


Figure B. 8.1-23: Optimised FOMC kinetic model for degradation of PMG and AMPA in the soil Speyer 2.1 (following an application of 4 ppm glyphosate-trimesium and an incubation at 20 °C and 40 % MWHC) –parent with metabolite

Table B.8.1-111: Lewis & Turnbull, 1992, BVL no 1052659: Kinetic re-evaluation by the RMS for degradation of PMG and AMPA in the soil Speyer 2.1 (following an application of 4 ppm glyphosate-trimesium and an incubation at 20 °C and 40 % MWHC) –parent with metabolite

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)	Visual assessment
FOMC (parent) + SFO (met)	M_0_parent	76.13	1.671	< 2e-16	4.09	10.71	153.14	Very good
	α_parent	0.7259	0.08016	5.71e-10				
	β_parent	6.699	1.472	5.09e-05				
	ff_met	0.5851	3.278e-02	-	4.29	230.7	766.33	Very good
	k_met	3.005e-03	9.848e-04	0.00253				

Summary of persistence and modelling end points of glyphosate and AMPA selected by the RMS

Review of the selected end points for persistence and modelling

The goodness of the fits presented in Dorn (2012, BVL no 2315991) and the goodness of fits recalculated by the RMS were assessed by the RMS to derive reliable endpoints for persistence and modelling according to FOCUS Kinetics guidance (2006). First, a visual inspection of diagrams of estimated and measured values vs. time and of the diagrams of residuals versus time was assessed. Generally, the error at which χ^2-test is passed of less than 15 % as well as a t-test probability of 0.05 was considered to indicate a good fit for soil degradation (FOCUS 2006, 2011). Additionally, it was checked that the standard errors of the parameters estimated were sufficiently low (at least < 100 %) to assure that these are reliable. However, on a case-by-case basis, the soil degradation end points were still be considered acceptable even though one or more of the indices are not met, as long as the end point value can be considered conservative, or can be justified based on weight of evidence from other studies.

Endpoints with regard to the P criterion of potential POP, vPvB or PBT substances

Persistence end points were derived by the Notifier selecting best fit kinetics. Apart from the fact that the RMS has corrected some endpoints (see above), the RMS recommends using the endpoints from FOMC, HS or DFOP kinetics only to determine whether various aquatic or terrestrial ecotoxicology studies are triggered, but not to decide whether the P criterion is fulfilled. To maintain a conservative approach with regard to the P criterion, the RMS proposes to use recalculated SFO DT_{50} values in cases when biphasic kinetics gave the best fit. Thereby, SFO DT_{50} values should be recalculated from the overall DT_{90} of best-fit biphasic kinetics (DT_{90}/3.32) according to EFSA DG SANCO working document on evidence needed to identify POP, PBT and vPvB properties for pesticides from 25.09.2012- rev.3.

All DT_{50} values of glyphosate in soil selected by the RMS for persistence and modelling and for evaluating of the P-criterion are reported in Table B.8.1-112 to Table B.8.1-115.

All DT_{50} values of AMPA in soil selected by the RMS for persistence and modelling are reported in Table B.8.1-116 and Table B.8.1-117.

Table B.8.1-112: Persistence degradation endpoints of glyphosate (at 20 and 25 °C) selected by the RMS

Study	Soil	pH H_2O	Persistence endpoints				
			DT_{50} (d)	DT_{90} (d)	Model	χ2 error (%)	Parameter
Glyphosate							
Ponte (2010), BVL no 2310242	Gartenacker, loam	7.1	7.86	56.29	DFOP	3.0	k_1: 0.2474 k_2: 0.0304 g: 0.4459
Dean (1995), BVL no 2310244	Arrow, sandy loam	6.5*	~~44.76~~ 37.75	~~300.73~~ 1661	~~DFOP~~ FOMC	2.47 2.31	~~k_1: 0.03825~~ ~~k_2: 0.002305~~ ~~g: 0.4429~~ α: 0.45389 β: 10.47275
Goodyear (1996), BVL no 2310246	Soil B, sandy loam	6.7	1.2	20.8	FOMC	6.92	α: 0.6565 β: 0.6406
Galicia & Morgenroth (1993) BVL no 1932059	Les Evouettes, silt loam	6.1	8.55	83.92	DFOP	5.93	k_1: 0.23497 k_2: 0.00826 g: 0.541289
Matla & Vonk (1993) BVL no 2151389	Maasdjik, sandy loam	7.5*	4.61	62.00	DFOP	0.84	k_1: 0.2638 k_2: 0.0192 g: 0.6715
Ponte (2010), BVL no 2310255	Drusenheim, loam	7.4	2.06	15.38	DFOP	2.4	k_1: 1.2566 k_2: 0.1161 g: 0.4038
	Pappelacker, loamy sand	7.0	3.94	43.45	FOMC	4.1	α: 0.8550 β: 3.1539
	18-Acres, clay loam	5.7	67.72	471.37	DFOP	2.9	k_1: 0.1129 k_2: 0.0040 g: 0.3453
Galicia & Flückiger (1993),	~~Speyer 2.2, Sand~~	~~6.0~~	~~8.54~~	~~150.62~~	~~DFOP~~	~~1.59~~	~~k_1: 3.8122~~ ~~k_2:~~

Study	Soil	pH H_2O	Persistence endpoints				
			DT_{50} (d)	DT_{90} (d)	Model	χ2 error (%)	Parameter
Glyphosate							
BVL no 1932046 / Mamouni, 2002 BVL no 2437068							~~0.01808~~ ~~g: 0.4925~~
	Speyer 2.3, Lomay Sand	6.9	5.78	21.99	DFOP	2.41	k_1: 0.1277 k_2: 2.3e-014 g: 0.9578
McLaughlin & Schanné (1996), BVL no 2310250	Speyer 2.1, sand	6.5*	8.3	51.3	DFOP	2.45	k_1: 0.4736 k_2: 0.0372 g: 0.3278
	Speyer 2.2, loamy sand	6.2*	18.7	428	FOMC	4.04	α: 0.5770 β: 8.0642
	Speyer 2.3, loamy sand	6.9*	2.70	13.03	DFOP	7.45	k_1: 0.3162 k_2: 0.0494 g: 0.8355
Kesterson & Atkins, 1991, BVL no 1932061 & add. Honegger, 1992, BVL no 2325652	Dupo, silt loam	7.3§	1.01	9.31	FOMC	3.80	α: 1.01 β: 9.31
Runnalls, 1991, BVL no 2154349	Speyer 2.2, loamy sand	6.0	43.53	144.61	SFO	6.95	k: 0.015923
Lewis & Turnbull 1992, BVL no 1052659	Speyer 2.1, sand	6.9§	11.11$	144.25$	FOMC$	3.91	α: 0.7683 β: 7.5833
	~~Beedon Manor, clay loam~~	~~7.8$~~	~~1.32~~	~~62.99~~	~~FOMC~~	~~3.33~~	~~α: 0.44208~~ ~~β: 0.34642~~
Maximum (n = ~~17~~ 15)**			~~67.72~~ **37.75**	~~471.4~~ **1661**	~~DFOP~~ ~~18 Acres~~ **Arrow FOMC**	~~k_1: 0.1129~~ ~~k_2: 0.0040~~ ~~g: 0.3453~~ **α: 0.45389** **β: 10.47275**	

1) Acceptable visual fit for formation phase of AMPA, however no statistically acceptable fit c for AMPA ould be obtained in this pathway
2) no statistically reliable fit could be obtained (high chi^2 error and/ or t-test not passed at 0.05)
3) not statistically not appropriate, according to FOCUS (2006, 2011) the geometric mean should be used for averaging degradation rates and half-lives while the arithmetic mean should be used for formation fractions
* converted from given pH value in $CaCl_2$ and KCl in order to allow pH dependency tests of the degradation
§ buffer solution unknown
$ labelled in the phosphonomethyl-glycine anion of glyphosate-trimesium
** maximum, which would result to the highest PECsoil

Table B.8.1-113: Persistence degradation endpoints of glyphosate (at 10 °C) selected by the RMS

Study	Soil	pH H_2O	Persistence endpoints				
			DT_{50} (d)	DT_{90} (d)	Model	χ2 error (%)	Parameter

Glyphosate							
McLaughlin & Schanné (1996), BVL no 2310250	Speyer 2.3, loamy sand	6.9*	8.07	50.79	DFOP	2.31	k_1: 0.300 k_2: 0.0361 g: 0.3756

* converted from given pH value in $CaCl_2$ and KCl in order to allow pH dependency tests of the degradation

Table B.8.1-114: Degradation endpoints of glyphosate for the P-criterion selected by the RMS

Study	**Soil**	**pH H_2O**	**Endpoints for the P-criterion**			
			recalculated SFO DT_{50} actual (d)	**Normalised SFO DT_{50} (days), 20 °C, pF2**	**χ2 error (%)**	**Model**
Glyphosate						
Ponte (2010), BVL no 2310242	Gartenacker, loam	7.1	16.95	15.2	3.0	DFOP, DT_{90}/3.32
Dean (1995), BVL no 2310244	Arrow, sandy loam	6.5*	~~90.6~~ 500.3	~~77.5~~ 427.8	~~2.46~~ 2.31	~~DFOP, DT_{90}/3.32~~ FOMC DT_{90}/3.32
Goodyear (1996), BVL no 2310246	Soil B, sandy loam	6.7	6.27	6.7	6.92	FOMC DT_{90}/3.32
Galicia & Morgenroth (1993) BVL no 1932059	Les Evouettes, silt loam	6.1	25.28	22.6	5.93	DFOP, DT_{90}/3.32
Matla & Vonk (1993) BVL no 2151389	Maasdjik, sandy loam	7.5*	18.7	14.1	0.84	DFOP, DT_{90}/3.32
Ponte (2010), BVL no 2310255	Drusenheim, loam	7.4	4.63	3.6	2.4	DFOP, DT_{90}/3.32
	Pappelacker, loamy sand	7.0	13.09	12.0	4.1	FOMC DT_{90}/3.32
	18-Acres, clay loam	5.7	141.9	133.8	2.9	DFOP, DT_{90}/3.32
Galicia & Flückiger (1993), BVL no 1932046 / Mamouni, 2002, BVL no 2437068	~~Speyer 2.2, Sand~~	~~6.0~~	~~45.4~~	~~45.4~~	~~1.59~~	~~FOMC DT_{90}/3.32~~
	Speyer 2.3, Lomay Sand	6.9	6.6	6.6	2.41	DFOP, DT_{90}/3.32
McLaughlin & Schanné (1996), BVL no 2310250	Speyer 2.1, sand	6.5*	15.45	15.45	2.45	DFOP, DT_{90}/3.32
	Speyer 2.2, loamy sand	6.2*	129	129	4.04	FOMC DT_{90}/3.32
	Speyer 2.3, loamy sand	6.9*	3.93	3.93	7.45	DFOP, DT_{90}/3.32
Kesterson & Atkins, 1991, BVL no 1932061 & add. Honegger, 1992, BVL no 2325652	Dupo, silt loam	7.3§	2.80	3.70	3.80	FOMC DT_{90}/3.32
Runnalls, 1991, BVL no 2154349	Speyer 2.2, loamy sand	6.0	43.53	40.6	6.95	SFO

Lewis & Turnbull 1992, BVL no 1052659	Speyer 2.1, sand	6.9[§]	43.06[$]	43.06	3.91	FOMC DT_{90}/3.32 [$]
	~~Beedon Manor, clay loam~~	~~7.8[§]~~	~~18.97~~	~~18.97~~	~~3.33~~	~~FOMC DT_{90}/3.32~~
Maximum (n = ~~17~~ 15)				**~~133.8~~ 427.8**	according to EFSA DG SANCO working document on evidence needed to identify POP, PBT and vPvB properties for pesticides from 25.09.2012- rev.3 -	
Geometric mean (n = ~~17~~ 15)				**~~18.70~~ 19.74**		

1) Acceptable visual fit for formation phase of AMPA, however no statistically acceptable fit c for AMPA ould be obtained in this pathway
2) no statistically reliable fit could be obtained (high chi^2 error and/ or t-test not passed at 0.05)
3) not statistically not appropriate, according to FOCUS (2006, 2011) the geometric mean should be used for averaging degradation rates and half-lives while the arithmetic mean should be used for formation fractions
* converted from given pH value in $CaCl_2$ and KCl in order to allow pH dependency tests of the degradation
§ buffer solution unknown
$ labelled in the phosphonomethyl-glycine anion of glyphosate-trimesium

Table B.8.1-115: Modelling degradation endpoints of glyphosate and formation fractions of AMPA selected by the RMS

Study	Soil	pH H_2O	Modelling endpoints				
			recalculated SFO DT_{50} actual (d)	Normalised SFO DT_{50} (d) 20 °C, pF2	f.f. AMPA	$\chi 2$ error (%)	Model
Glyphosate							
Ponte (2010), BVL no 2310242	Gartenacker, loam	7.1	17.79	16.0	0.1817	4.6	DT_{90} FOMC/ 3.32
Dean (1995), BVL no 2310244	Arrow, sandy loam	6.5*	~~300.7~~ 186.6	~~257.1~~ 159.6	~~0.4698[1)]~~	~~2.47~~ 3.52	DFOP slow phase
Goodyear (1996), BVL no 2310246	Soil B, sandy loam	6.7	6.2	6.6	0.2646	6.92	DT_{90} FOMC/ 3.32
Galicia & Morgenroth (1993) BVL no 1932059	Les Evouettes, silt loam	6.1[§]	~~48.46~~ 104.5	~~43.3~~ 93.3	0.3618	6.17	DT_{90} FOMC/ 3.32
Matla & Vonk (1993) BVL no 2151389	Maasdjik, sandy loam	7.5*	17.05	15.2	n.m.	3.79	DT_{90} FOMC/ 3.32
Ponte (2010), BVL no 2310255	Drusenheim, loam	7.4	5.35	4.2	0.2578	3.5	DT_{90} FOMC/ 3.32
	Pappelacker, loamy sand	7.0	13.09	12.0	0.1835	4.1	DT_{90} FOMC/ 3.32
	18-Acres, clay loam	5.7	173.2	160.5	0.2169[1)]	2.9	DFOP slow phase
Galicia & Flückiger (1993),	~~Speyer 2.2, Sand~~	~~6.0~~	~~45.4~~	~~45.4~~	~~2)~~	~~1.59~~	~~DT_{90} FOMC/~~

Study	Soil	pH H_2O	Modelling endpoints				
			recalculated SFO DT_{50} actual (d)	Normalised SFO DT_{50} (d) 20 °C, pF2	f.f. AMPA	χ2 error (%)	Model
Glyphosate							
BVL no 1932046 / Mamouni, 2002, BVL no 2437068							~~3.32~~
	Speyer 2.3, Lomay Sand	6.9	7.2	7.2	0.3435	3.84	DT_{90} FOMC/ 3.32
McLaughlin & Schanné (1996), BVL no 2310250	Speyer 2.1, sand	6.5*	19.5	19.5	0.520[1)]	5.72	DT_{90} FOMC/ 3.32
	Speyer 2.2, loamy sand	6.2*	72.2	72.2	0.6076[1)]	4.97	DFOP slow phase
	Speyer 2.3, loamy sand	6.9*	3.76	3.76	0.4283	7.67	DT_{90} FOMC/ 3.32
Kesterson & Atkins, 1991, BVL no 1932061 & add. Honegger, 1992, BVL no 2325652	Dupo, silt loam	7.3[§]	2.80	3.70	0.3637	3.80	DT_{90} FOMC/ 3.32
Runnalls, 1991, BVL no 2154349	Speyer 2.2, loamy sand	6.0	43.53	40.6	n.m.	6.95	SFO
Lewis & Turnbull 1992, BVL no 1052659	Speyer 2.1 sand	6.9[§]	43.06[$]	43.06	0.5851[$]	3.91[$]	DT_{90} FOMC/ 3.32
	~~Beedon Manor clay loam~~	~~7.8[§]~~	~~18.97~~	~~18.97~~	~~n.m.~~		~~DT_{90} FOMC/ 3.32~~
Geometric mean (n = ~~17~~ 15)				**~~21.03~~ 20.51**	-[3)]		
pH dependency				**No**	**-**		
Arithmetic mean (n = ~~13~~ 12)				-[3)]	**~~0.3680~~ 0.3595**		

1) Acceptable visual fit for formation phase of AMPA, however no statistically acceptable fit for the transformation rate of AMPA could be obtained in this pathway
2) no statistically reliable fit could be obtained (high chi^2 error and/ or t-test not passed at 0.05)
3) statistically not appropriate, according to FOCUS (2006, 2011) the geometric mean should be used for averaging degradation rates and half-lives while the arithmetic mean should be used for formation fractions

n.m. not measured
* converted from given pH value in $CaCl_2$ and KCl in order to allow pH dependency tests of the degradation
§ buffer solution unknown
$ labelled in the phosphonomethyl-glycine anion of glyphosate-trimesium

Table B.8.1-116: Persistence degradation endpoints of AMPA selected by the RMS

Study	Soil	pH H_2O	Persistence endpoints			
			DT_{50} (days)	DT_{90} (days)	χ2 error (%)	Model Parent_Met.

Metabolite AMPA						
Ponte (2010), BVL no 2310242	Gartenacker, loam	7.1	120.07	398.9	9.2	DFOP_SFO
Dean (1995), BVL no 2310244	Arrow, sandy loam	6.5*	-[1)]	-[1)]	-[1)]	FOMC_SFO
Goodyear (1996), BVL no 2310246	Soil B, sandy loam	6.7	99.1	329	6.98	FOMC_SFO
Galicia & Morgenroth (1993) BVL no 1932059	Les Evouettes, silt loam	6.1[§]	300.71	998.9	16.06	DFOP_SFO
Matla & Vonk (1993), BVL no 2151389	Maasdjik, sandy loam	7.5*	n.m.	n.m.	n.m.	n.m.
Ponte (2010), BVL no 2310255	Drusenheim, loam	7.4	38.98	129.5	3.3	DFOP_SFO
	Pappelacker, loamy sand	7.0	126.57	420.5	6.2	FOMC_SFO
	18-Acres, clay loam	5.7	-[1)]	-[1)]	-[1)]	FOMC_SFO
Galicia & Flückiger (1993), BVL no 1932046 / Mamouni, 2002, BVL no 2437068	Speyer 2.2, Sand	6.0	-[2)]	-[2)]	-[2)]	-[2)]
	Speyer 2.3, Lomay Sand	6.9	77.50	257.43	10.18	DFOP_SFO
McLaughlin & Schanné (1996), BVL no 2310250	Speyer 2.1, sand	6.5*	-[1)]	-[1)]	-[1)]	DFOP_SFO
	Speyer 2.2, loamy sand	6.2*	-[1)]	-[1)]	-[1)]	FOMC_SFO
	Speyer 2.3, loamy sand	6.9*	41.87	139.10	16.23	DFOP_SFO
Kesterson & Atkins, 1991, BVL no 1932061 & add. Honegger, 1992, BVL no 2325652	Dupo, silt loam	7.3[§]	48.32	160.5	7.57	FOMC_SFO
Runnalls, 1991, BVL no 2154349	Speyer 2.2, loamy sand	6.0	n.m.	n.m.	n.m.	n.m.
Lewis & Turnbull 1992, BVL no 1052659	Speyer 2.1, sand	6.9[§]	230.7[$]	766[$]	4.29	FOMC_SFO
	~~Beedon Manor clay loam~~	~~7.8[§]~~	~~n.m.~~	~~n.m.~~	~~n.m.~~	~~n.m.~~
Minimum (n = 9)			**38.98**	**129.5**	**SFO**	
Maximum (n = 9)			**300.71**	**998.9**	**SFO**	

[1)] No decline of AMPA was observed in parent study; thus, no acceptable fit for AMPA could be obtained
[2)] no statistically reliable fit could be obtained (high chi^2 error and/ or t-test not passed at 0.05) for parent and metabolite
n.m. not measured
* converted from given pH value in $CaCl_2$ and KCl in order to allow pH dependency tests of the degradation
§ buffer solution unknown
$ labelled in the phosphonomethyl-glycine anion of glyphosate-trimesium

Table B.8.1-117: Modelling degradation endpoints of AMPA selected by the RMS

Study	Soil	pH H_2O	Modelling endpoints			
			recalculated SFO DT_{50} actual (d)	Normalised SFO DT_{50} (days), 20 °C pF2	χ2 error (%)	Model Parent_Met.
Metabolite AMPA						
Ponte (2010), BVL no 2310242	Gartenacker, loam	7.1	133.69	119.9	8.9	FOMC_SFO
~~Dean (1995), BVL no 2310244~~	~~Arrow, sandy loam~~	~~6.5*~~	~~-[1)]~~	~~-[1)]~~	~~-[1)]~~	~~DFOP_SFO~~
Goodyear (1996), BVL no 2310246	Soil B, sandy loam	6.7	99.1	106.2	6.98	FOMC_SFO
Galicia & Morgenroth (1993) BVL no 1932059	Les Evouettes, silt loam	6.1	337	300.9	14.00	FOMC_SFO
Matla & Vonk (1993), BVL no 2151389	Maasdjik, sandy loam	7.5*	n.m.	n.m.	n.m.	n.m.
Ponte (2010), BVL no 2310255	Drusenheim, loam	7.4	47.04	36.8	2.1	FOMC_SFO
	Pappelacker, loamy sand	7.0	126.5	116.3	6.2	FOMC_SFO
	18-Acres, clay loam	5.7	-[1)]	-[1)]	-[1)]	DFOP_SFO
Galicia & Flückiger (1993), BVL no 1932046 / Mamouni (2002), BVL no 2437068	Speyer 2.2, sand	6.0	-[2)]	-[2)]	-[2)]	-[2)]
	Speyer 2.3, loamy sand	6.9	70.92	70.92	11.41	FOMC-SFO
McLaughlin & Schanné (1996), BVL no 2310250	Speyer 2.1, sand	6.5*	-[1)]	-[1)]	-[1)]	FOMC_SFO
	Speyer 2.2, loamy sand	6.2*	-[1)]	-[1)]	-[1)]	DFOP_SFO
	Speyer 2.3, loamy sand	6.9*	42.14	42.14	~~9.41~~ 16.48	~~DFOP_SFO~~ FOMC_SFO
Kesterson & Atkins, 1991, BVL no 1932061 & add. Honegger, 1992, BVL no 2325652	Dupo, silt loam	7.3[§]	48.32	30.5	7.57	FOMC_SFO
Runnalls, 1991, BVL no 2154349	Speyer 2.2, loamy sand	6.0	n.m.	n.m.	n.m.	n.m.
Lewis & Turnbull, 1992, BVL no 1052659	Speyer 2.1, sand	6.9	230.7[$]	230.7[$]	4.29[$]	FOMC_SFO
	~~Beedon Manor clay loam~~	~~7.8[§]~~	~~n.m.~~	~~n.m.~~	~~n.m.~~	~~n.m.~~
Minimum				**30.5**		
Maximum				**300.9**		
pH dependency				**No**		
Geometric mean (n = 9)				**88.84**		

1) No decline of AMPA was observed in parent study; thus, no acceptable fit for AMPA could be obtained
2) no statistically reliable fit could be obtained (high chi^2 error and/ or t-test not passed at 0.05) for parent and metabolite
n.m. not measured
* converted from given pH value in $CaCl_2$ and KCl in order to allow pH dependency tests of the degradation

§ buffer solution unknown
$ labelled in the phosphonomethyl-glycine anion of glyphosate-trimesium

B.8.1.2.2 Field studies

Several investigations of the field soil dissipation of glyphosate and glyphosate-trimesium in different areas of Central Europe -multiple field locations in Germany and Switzerland- (Hill, 1992, BVL no 2154352; Schulz, 1992 (a-d), BVL no 1932128, 1932131, 1932073 and 1932133) and in areas of the USA (Tennessee, California, Georgia) and Canada (Iwata (1983a-c), Roper & Hoag (1994), Runnals (1992), Danhaus (1983 & 1982), Oppenhuizen (1993a & b) have been conducted were evaluated during the 2001 EU evaluation of glyphosate. The results of these studies were discussed in detail in the glyphosate and the glyphosate-trimesium Monographs. The studies were considered acceptable based on the evaluation criteria and guidance in force at that time.

No new field dissipation studies were conducted for the renewal dossier but the a new kinetic evaluation used to derive dissipation half-lives were submitted by the GTF in accordance with the latest guidance (FOCUS, 2006, 2011) in order to refine risk assessment endpoints. Details of the updated kinetic evaluation are provided in Annex Point IIA 7.3.1 (Kreschnak, 2012, BVL no 2315993).

KIIA 7.3.1/1 (Kreschnak, 2012, BVL no 2315993)

Annex point:	IIA 7.2.1/1
Author(s):	Kreschnak, C.
Title:	Kinetic modelling analysis of the degradation behaviour of glyphosate and its metabolite AMPA in field soil dissipation studies
Date:	August 27, 2012
Guideline(s):	Kinetic evaluation according to: FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the Work Group on Degradation Kinetics of FOCUS. EC Document Reference SANCO/10058/2005 version 2.0, June 2006. FOCUS (2011a): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.0. KINGUI v1.1 in the framework of MatLab 7.0.4.365 was used as fitting software. Microsoft Excel™ 2003 was used for statistical evaluation of the optimised parameters. Persistence endpoints were derived for glyphosate and AMPA: Deviations: none
Deviations:	None
GLP:	No (kinetic evaluation: does not contain laboratory work)
Acceptability:	Yes

Material and methods

The kinetic evaluation of both AMPA and glyphosate was based on soil residue data from five field soil dissipation studies (10 trials) with different soil types and application rates where glyphosate was applied as the test substance. In addition to the four studies of Schulz

(1992 (a-d), BVL no 1932128, 1932131, 1932073 and 1932133), where glyphosate formulated as isopropylamine salt was applied as the test substance, the RMS accept the inclusion of the field dissipation study of Hill (1992, BVL no 2154352) conducted with glyphosate-trimesium as test substance. Glyphosate is a strong acid, therefore, in spray solutions and in soil, glyphosate acid and the corresponding counter ions are rapidly ionized resulting in the formation of the freely dissociated anion (glyphosate) and the corresponding cation *(e.g.,* isopropylamine and trimesium cations). Regardless of the glyphosate formulation used in the soil dissipation studies, the exact ionic form of glyphosate in the environment will be determined mainly by the pH and the presence of other naturally occurring counter ions in soil and spray solutions. Thus, soil samples from studies conducted with both salts and both formulations of glyphosate were analysed for glyphosate and its metabolite AMPA in accordance with the degradation scheme obtained from the lab studies.

Table B.8.1-118 provides information on the site locations and characteristics of soils accepted by RMS for the derivation of reliable endpoints for persistence and as trigger for higher tier experiments according to FOCUS Kinetics guidance (2006). The study of Danhaus (1983) with the addendum of Mueth (1988) and the studies of Oppenhuizen et al. (1993a-d) are excluded and not provided in the summary table because the study by Danhaus (1983) was not considered acceptable for the updated kinetic analyses due to missing soil characterisation (e.g. cation exchange capacity, particle size distribution, water holding capacity and microbial biomass) and the illegible pesticide history of the field plots. Although the studies of Oppenhuizen at al. (1993) conducted in USA (Tennessee, California, Georgia) and Canada, were included in the assessment of persistence endpoints by the Notifier, in the opinion of RMS the climatic conditions in the mentioned regions are not representative for Europe. The Notifier has not provided additional information on the comparability of weather conditions during the duration of field dissipation studies in USA and Canada and the studies conducted in Europe. Apart from that there are enough half live values from European locations to derive reliable persistence endpoints for glyphosate and its metabolite AMPA.

Table B.8.1-118: Site characteristics from field soil dissipation studies with glyphosate formulations

References	Site, country	Guideline	Application rate (kg a.s./ha)	Duration (days)	Characteristics upper soil layer								AMPA (% max)**
					Soil type	Sand (%)	Silt (%)	Clay (%)	OM (%)	pH	ρbulk (g/cm³)	CEC (meq/100g)	
Schulz, 1992a, BVL no 1932128	Diegten, Switzerland	BBA Part IV, 4-1	3.53	282	Sandy clay	47.6	13.3	39.2	2.8	7.1	1.5	31.0	24.78
Schulz, 1992b, BVL no 1932131,	Egerkingen, Switzerland	BBA Part IV, 4-1	3.87	202	Clay loam	34.2	28.8	37.1	2.7	7.3	1.5	31.3	19.93
Schulz, 1992c, BVL no 1932073	Bad Krozingen, Germany	BBA Part IV, 4-1	3.67	61	Sandy loam	55.0	27.1	17.9	0.6	6.0	1.5	8.9	26.48
Schulz, 1992d, BVL no 1932133	Menslage, Germany	BBA Part IV, 4-1	3.67	315	Sandy loam	90.6	2.1	7.2	0.4	4.7	1.5	4.9	53.76
Hill, 1992, BVL no 2154352	Buchen, Germany	unknown	5.20*	475	Loamy sand	80.0	14.0	6.0	2.8	6.4	1.4	6.5	32.52
	Kleinzecher, Germany	unknown	5.7*	567	Sandy loam	66.0	21.0	13.0	1.9	7.0	1.6	7.7	28.40
	Unzhurst, Germany	unknown	4.8*	418	Loam	48.0	39.0	13.0	1.8	6.7	1.4	6.6	28.30
	Rohrbach, Germany	unknown	5.0*	582	Silt loam	12.0	77.0	11.0	1.8	8.5	1.3	12.7	28.93
	Herrngiersdorf, Germany	unknown	4.6*	541	Clay loam	23.0	47.0	30.0	2.8	8.0	1.5	14.4	19.65
	Wang-Inzkofen, Germany	unknown	4.8*	549	Silt loam	25.0	51.0	24.0	2.1	7.2	1.6	14.0	36.85

* glyphosate-trimesium as test substance
** calculated as glyphosate equivalent (molar based)

The details of the field dissipation studies relevant for Europe were provided in the monographs of glyphosate (2001) and glyphosate-trimesium (2001) excepting the tables with residue data. To ensure the conformability of the further modelling analysis the processed residue data of the trials in Schulz (1992 a-d, BVL no 1932128, 1932131, 1932073 and 1932133) and Hill (1992, BVL no 2154352) are presented in Table B.8.1-119 to Table B.8.1-120.

Table B.8.1-119: Processed residue data (kg/ha and % of DAT0) of glyphosate and AMPA from the field soil dissipation studies of Schulz (1992 a-d, BVL no 1932128, 1932131, 1932073 and 1932133)

Time (days)	**Sum of horizon 1-3 (0-30 cm)**			
	Glyphosate (kg/ha)	**AMPA (kg/ha)**	**Glyphosate (% of DAT0)**	**AMPA (% of DAT0)[1]**
Diegten/ Switzerland (Sandy clay)				
0	3.64	0.00	100.00	0.00
7	1.66	0.56	45.57	23.32
15	0.88	0.33	24.21	13.85
30	0.45	0.29	12.27	11.97
62	0.49	0.54	13.50	22.75
194	0.36	0.57	9.96	24.01
282	0.13	0.39	3.54	16.17
Egerkingen/ Switzerland (Clay loam)				
0	2.21	0.00	100.00	0.00
7	0.97	0.19	43.92	12.92
15	0.97	0.37	43.92	25.32
30	0.72	0.47	32.72	32.25
62	0.68	0.51	30.55	34.93
202	0.15	0.34	6.86	23.46
Bad Krozingen/ Germany (Sandy loam)				
0	4.26	0.00	100.00	0.00
7	1.34	0.35	31.43	12.49
15	1.22	0.40	28.58	14.25
30	0.65	0.45	15.35	16.08
61	0.59	0.64	13.73	22.78
Menslag/ Germany (Sandy loam)				
0	4.22	0.00	100.00	0.00
7	1.99	0.35	47.26	12.67
15	0.89	0.48	20.98	17.43
30	1.03	0.58	24.47	20.79
60	0.77	0.79	18.35	28.43
192	0.36	0.64	8.43	23.07
271	0.44	1.29	10.35	46.73
315	0.20	0.64	4.69	23.12

1) expressed as glyphosate equivalent = percentage of glyphosate amount at DATO corrected for molar mass difference between parent and metabolite (169 g/mol /111 g/mol)

Table B.8.1-120: Processed residue data (kg/ha and % of DATO) of glyphosate and AMPA from the field soil dissipation study of Hill (1992, BVL no 2154352)

Time (days)	Sum of horizon 1-2 (0-20 cm)			
	Glyph. (kg/ha)	AMPA (kg/ha)	Glyph. (% of DAT0)	AMPA (% of DAT0)[1]
Buchen/ Germany (Loamy sand)				
0	3.25	0.00	100.00	0.00
7	3.15	0.23	96.94	10.61
14	2.76	0.33	84.73	15.51
28	2.17	0.37	66.71	17.37
61	1.12	0.48	34.42	22.26
91	0.89	0.77	27.45	35.83
121	0.39	0.32	12.12	14.93
182	0.44	0.60	13.59	28.24
240	0.31	0.49	9.39	23.09
322	0.26	0.32	8.04	14.76
475	0.26	0.51	7.89	23.81
Kleinzecher/ Germany (Sandy loam)				
0	2.93	0.00	100.00	0.00
7	2.94	0.43	100.10	22.16
14	2.22	0.48	75.69	25.03
28	1.55	0.47	52.74	24.64
61	1.30	0.67	44.16	34.94
91	0.74	0.53	25.28	27.42
119	0.92	0.59	31.26	30.83
201	0.75	0.73	25.55	38.06
244	0.65	0.70	22.15	36.15
298	0.30	0.58	10.36	29.89
479	0.18	0.68	6.09	35.51
567	0.04	0.52	1.42	26.80
Unzhurst/ Germany (Loam)				
0	3.47	0.00	100.00	0.00
7	2.59	0.24	74.61	10.63
13	2.47	0.31	71.12	13.72
27	1.92	0.27	55.36	11.91
57	0.71	0.55	20.52	23.92
90	0.53	0.61	15.22	26.91
117	0.35	0.54	9.98	23.63
187	0.25	0.53	7.22	23.04
251	0.24	0.62	7.05	26.98
314	0.22	0.55	6.30	24.21
418	0.15	0.43	4.26	18.74

Time (days)	**Sum of horizon 1-2 (0-20 cm)**			
	Glyph. (kg/ha)	**AMPA (kg/ha)**	**Glyph. (% of DAT0)**	**AMPA (% of DAT0)[1]**
Rohrbach/ Germany (Silt loam)				
0	2.28	0.00	100.00	0.00
7	2.68	0.34	117.47	22.36
14	2.05	0.46	89.68	30.53
28	1.36	0.44	59.67	29.28
56	0.44	0.66	19.20	43.65
85	0.20	0.63	8.66	41.86
231	0.04	0.56	1.53	37.58
282	-	0.52	-	34.41
418	-	0.28	-	18.60
582	-	0.23	-	15.61
Herrngiersdorf/ Germany (Clay loam)				
0	2.05	0.00	100.00	0.00
6	1.96	0.36	95.89	26.44
13	1.48	0.29	72.53	21.64
28	1.46	0.41	71.60	30.46
58	0.47	0.41	23.08	30.44
90	0.30	0.41	14.61	30.47
125	0.18	0.38	8.81	27.93
168	0.04	0.27	1.96	20.11
330	-	0.28	-	20.91
464	-	0.13	-	9.64
541	-	-	-	-
Wanz-Inzkofen/ Germany (silt loam)				
0	2.97	0.00	100.00	0.00
7	2.11	0.57	71.03	29.03
15	1.44	0.66	48.50	33.71
29	1.37	0.80	46.22	41.12
58	0.69	0.64	23.10	32.67
94	0.40	0.65	13.60	33.39
114	0.36	0.66	12.24	33.96
275	0.24	0.59	7.93	30.06
414	0.14	0.46	4.88	23.55
549	0.04	0.35	1.32	17.82

1) It is stated In the study that samples were taken up to 30 cm but no results are reported for that depth and no reason is given for this.
2) expressed as glyphosate equivalent =percentage of glyphosate amount at DATO corrected for molar mass difference between parent and metabolite (169 g/mol /111 g/mol)

The standard procedures recommended by FOCUS (2006, 2011) were followed in this report for all residues to adjust the experimental data for the kinetic modelling.

The time-zero concentration for metabolites was set to zero and the initial metabolite amount was added to the parent substance considering the molar weight difference between the com-

pounds. Values between LOO and LOO were set to the measured value. Values below LOO were replaced by half the LOO. If the concentrations of the applied substance in soil declined to values below LOO, the curve was cut off after the first value below LOO, unless detections above LOO were made later in the experiment. These corrections were performed along the time course as well as along the horizons.

The measured residues (mg/kg) of the different soil layers were converted into residues expressed in kg/ha (considering the layer depth and bulk density) and then summed up. To gain a better overview they were expressed as percentage values of the residue at DAT0 (so the time zero value is 100 %). Thus, if the maximum concentration occurs after DAT0, the respective maximum percentage values will be higher than 100 %.

The input values of AMPA were expressed as percentage values of the parent residue at DAT0 as well (correcting for the molar weight difference).

According to FOCUS (2006, 2011) true replicates and not mean concentration values at each sampling point should be used for the kinetic evaluation if available. For the trials of Schulz (1992 a-d, BVL no 1932128, 1932131, 1932073 and 1932133) and Hill (1992, BVL no 2154352) no replicates are available.

The updated kinetic modelling analysis for glyphosate and its major metabolite AMPA for field soil dissipation studies was conducted in order to derive dissipation endpoints using best-fit kinetics ("persistence" end points) for use in PEC_{Soil} calculations and as trigger endpoints for higher-tier environmental fate studies. The decision flow sheet for deriving persistence endpoints, as recommended by FOCUS kinetics guidelines (FOCUS, 2006, 2011) was considered. The dissipation rate of glyphosate was evaluated using KINGUI v.1.1 (Schäfer & Mikolasch, 2006; MatLab, 2005).

Persistence endpoints for glyphosate and AMPA were derived following the guidance of FOCUS (2006, 2011a). Additionally, formation fractions for AMPA were determined.

The initial hypothesis for each dataset was that the glyphosate residue data were best described by single first-order (SFO) kinetics. As a first step, first-order kinetics were fitted to the glyphosate residue data. The fit of the SFO model was compared to the fit of the First-Order Multi Compartment (FOMC) kinetic model to test this hypothesis. Where the hypothesis was rejected the bi-exponential or Double First-Order in Parallel (DFOP) bi-phasic model was compared to the FOMC model to assess which was the most appropriate bi-phasic model for describing the dissipation kinetics of glyphosate in soil. In each case the data were fitted un-weighted with the complete data set. The goodness of fit of the kinetic models was assessed both statistically and visually in accordance with the guidance provided by FOCUS (2006, 2011). The Chi-square test (χ^2-test) was employed as a statistical measure of the goodness of fit. For fits conducted with the SFO and DFOP models, parameter confidence was additionally assessed using the t-test function. For fits conducted with the FOMC model, however, the t-test is not appropriate as a measure of confidence (FOCUS, 2006, 2011). Therefore 95 % confidence intervals were assessed for every parameter estimated using the SFO, FOMC and DFOP models, and a fit was considered acceptable if the confidence intervals of all estimated parameters did not include zero.

As suggested by FOCUS guidance (2006, 2011), the metabolite AMPA was fitted, applying the SFO model for the metabolite, in a "pathway fit" simulating the simultaneous formation of AMPA from glyphosate and its subsequent degradation in soil. If the pathway fit did not provide visual and statistically acceptable results, the fitting procedure was repeated using the decline phase of AMPA from the point of maximum concentration observed.

Results

The optimisation of the kinetic analysis was conducted following decision schemes as defined in the FOCUS kinetics guidance document (FOCUS, 2006, 2011).

For trigger endpoints of the parent compound the SFO model was compared to the FOMC model. In cases, where SFO was not appropriate as best-fit model, a modified fitting procedure was applied by constraining M0 if necessary.

In some trials initial increases in glyphosate concentration were observed resulting in maximum concentrations after DAT 0. Depending on how much time after DATO the decline phase started, kinetic fitting could be difficult. In those cases, only the decline phase was used for the fitting procedure. If FOMC still has a better fit than the SFO model, DFOP was tested as a further bi-phasic model. The modified fitting was also applied to the biphasic models if no acceptable results could be achieved otherwise. In a second step, the metabolite AMPA was fitted, applying the SFO model for the metabolite, in a pathway fit together with the respective model selected for the trigger endpoint determination of the parent substance. In the initial pathway fit the parameters of the parent, derived from the best-fit parent-only model, were fixed. If the initial fit provided acceptable results, the fitting procedure was repeated using the derived best-fit parameters for the parent (from the parent-only fit) and for the metabolite (from the initial pathway fit) as initial values. If the initial pathway fit did not provide acceptable results, the fitting procedure was repeated using the decline phase of the metabolite only. In that case, the same decision scheme as for the parent (see description above) was followed.

Regarding the parent compound glyphosate, robust kinetic fits could be derived for 7 soils of the 11 sites with sufficient data to describe the decline of glyphosate. DT_{50} values for glyphosate dissipation in field ranged from 5.7 to 38.3 days. The degradation patterns were biphasic in 7 cases with four soils exhibiting DFOP, one soil exhibits FOMC kinetic and for the other two soils the SFO model provided the best fit. The corresponding DT_{90} values ranged from 66.9 to 386.6 days.

Due to pronounced and continuous non-linear transformation of glyphosate to AMPA in soil, only 5 out of 11 soil dissipation locations contain sufficient data to adequately describe the pattern of decline of AMPA in these studies. Pathway fits were acceptable in three soils resulting in formation fractions between 0.33 and 0.55. For the other two soils, only the decline phase of AMPA was used for the kinetic fitting. Persistence DT_{50} values of AMPA ranged from 283.6 to 633.1 days, but were typically longer than one year in all trials. All pathway fits were conducted using the SFO kinetic model for AMPA.
The detailed results of the best fit kinetic modelling of each trial for glyphosate and its metabolite AMPA are provided in the Table B.8.1-121 to Table B.8.1-137.

KIIA 7.3.1/2 Field dissipation study Schulz (1992a, BVL no 1932128)

The kinetic evaluation results of field dissipation study of the study Schulz (1992a, BVL no 1932128) in Diegten/Germany are provided in Table B.8.1-121 and Table B.8.1-122. Visual fits are provided in Figure B. 8.1-24 to Figure B. 8.1-27.

Table B.8.1-121: Kinetic evaluation results of field dissipation study of the study Schulz (1992a, BVL no 1932128), Diegten/Germany, sandy clay - persistence and trigger end points of glyphosate

Glyphosate							
Kinetic model	**DT_{50} (days)**	**DT_{90} (days)**	**χ2 error (%)**	**Parameter**	**Prob>t**	**Std**	**Visual fit**
SFO	7.23	24.02	19	k 0.0958	0.0016	0.0181	not acceptable
FOMC	4.88	67.12	10.1	alpha 0.742	0.012	0.21	acceptable
				beta 3.157	0.091	1.958	
DFOP	6.1	116.1	4.96	k1 0.1437	9.60E-4	0.0139	acceptable
				k2 0.0033	0.059	0.0015	
				g 0.854	3.4E-5	0.0268	
DFOP	**6.1**	**116.1**	**4.96**	**k1 0.1437**	**Best fit kinetic (Diegten/Germany)**		
				k2 0.0033			
				g 0.854			

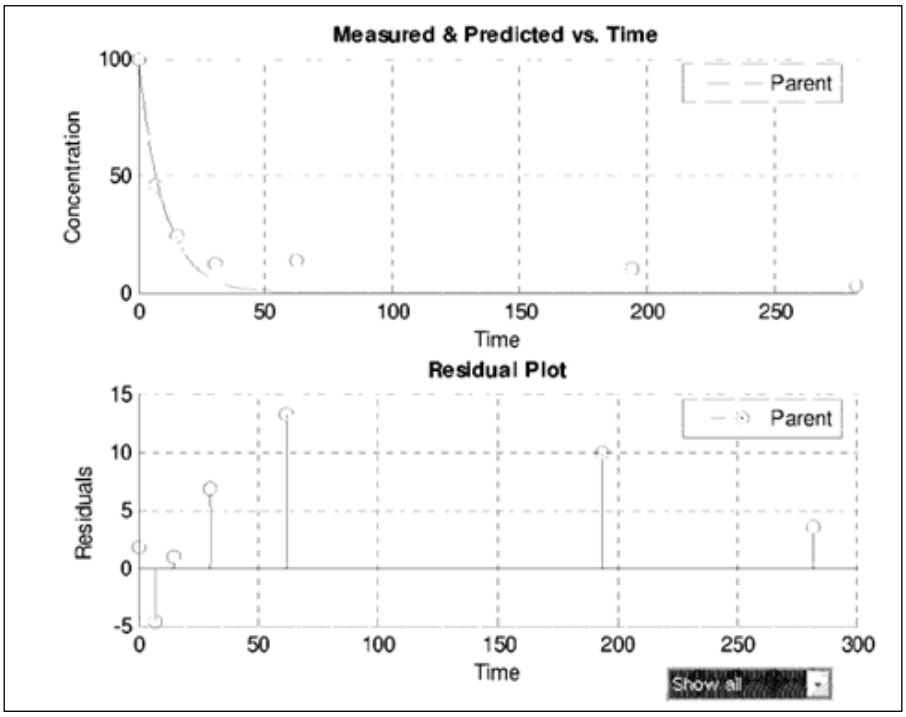


Figure B. 8.1-24: Schulz, 1992a: Optimised SFO kinetic model for degradation of glyphosate in the Diegten soil (parent only)

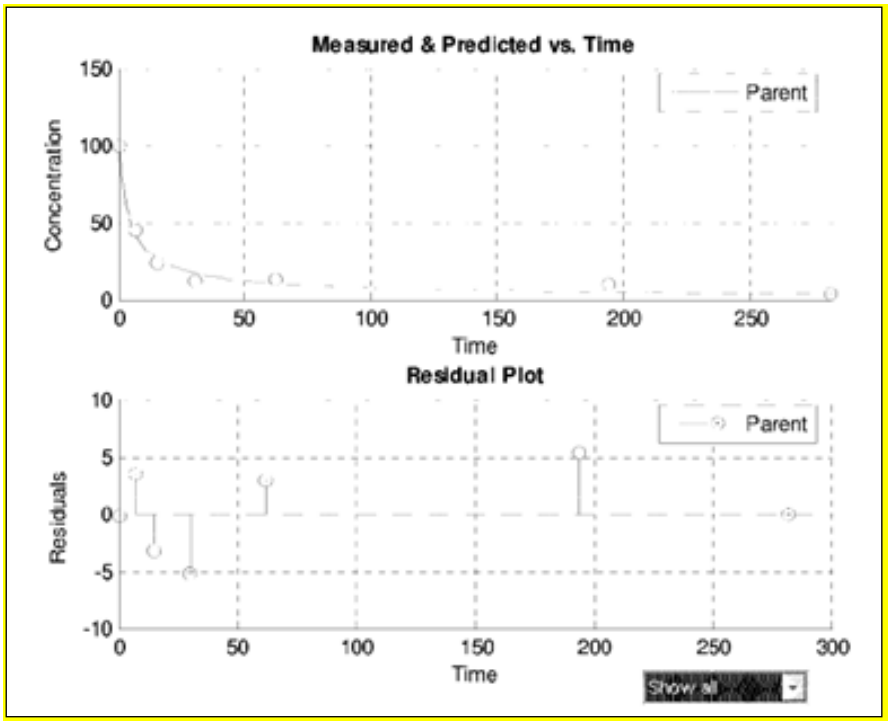


Figure B. 8.1-25: Schulz, 1992a: Optimised FOMC kinetic model for degradation of glyphosate in the Diegten soil (parent only)

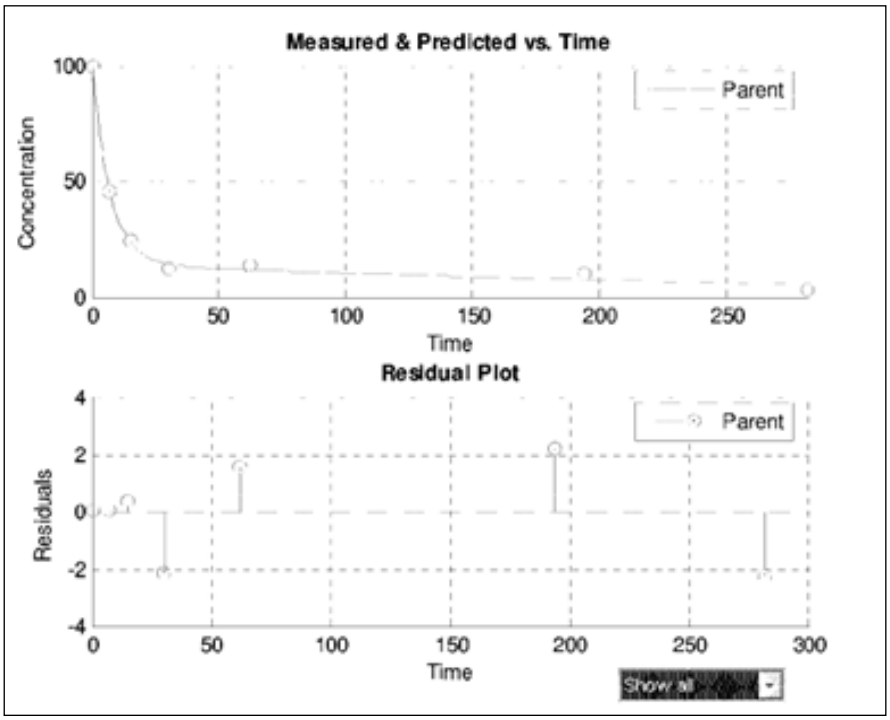


Figure B. 8.1-26: Schulz, 1992a: Optimised DFOP kinetic model for degradation of glyphosate in the Diegten soil (parent only)

Using the SFO model for the parent substance glyphosate, the visual fit was considered not acceptable because the optimisation resulted in a systematic underestimation of the data points from day 15 onward with a χ^2-error of 19.0 %. Using the FOMC model the visual assessment revealed an acceptable fit with a χ^2-error of 10.1 %. Therefore the SFO model was rejected and DFOP was tested additionally. The DFOP model provided a good visual fit with a χ^2-error of 5.0 %. Due to the better χ^2-error, the DFOP model was chosen for trigger endpoints.

Table B.8.1-122: Kinetic evaluation results of field dissipation study of the study Schulz (1992a, BVL no 1932128), Diegten/Germany, sandy clay - persistence and trigger end points of AMPA

AMPA								
Kinetic model pathway fit	**DT_{50} (days)**	**DT_{90} (days)**	**$\chi 2$ error (%)**	**ff**	**Parameter**	**Prob>t**	**Std**	**Visual fit**
DFOP- SFO	>1000	>1000	30.4	0.233	k 5.2E-4	0.3092	0.0010	not acceptable

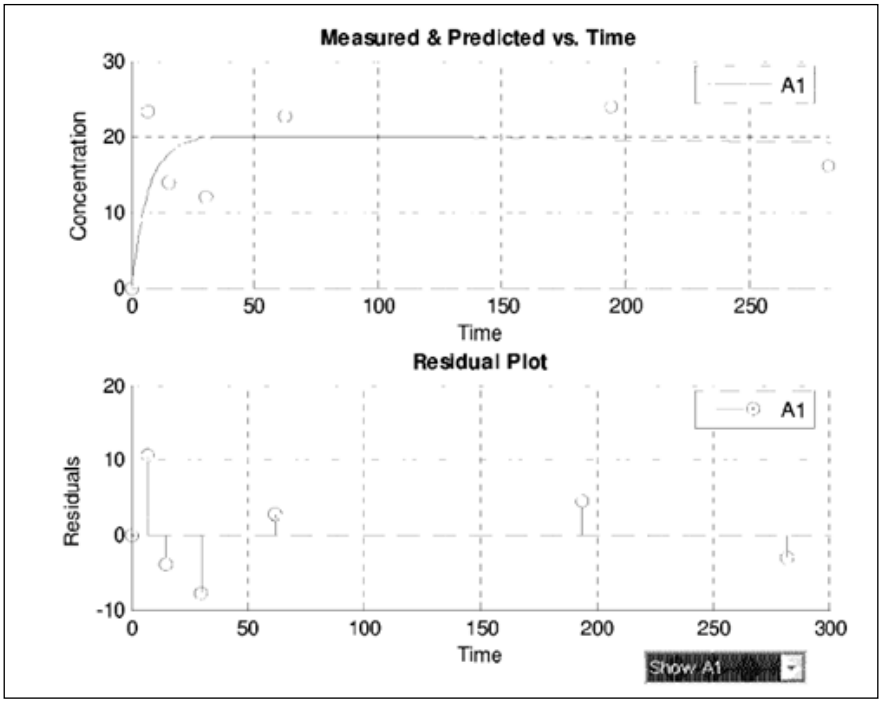


Figure B. 8.1-27: Schulz, 1992a: Optimised SFO kinetic model for degradation of AMPA in the Diegten soil (pathway fit- initial)

The pathway fit for the metabolite AMPA using the DFOP model for the parent and the SFO model for AMPA was visually not acceptable due to a poor fit of the decline phase which is represented by the last two data points only. Subsequently, kinetic fitting using the decline phase only was not possible either. In conclusion, no acceptable kinetic fit could be obtained for the degradation behaviour of AMPA.

KIIA 7.3.1/3 Field dissipation study Schulz (1992b, BVL no 1932131)

The kinetic evaluation results of field dissipation study of the study Schulz (1992b, BVL no 1932131) in Ekergingen/Switzerland are provided in Table B.8.1-123. Visual fits are provided in Figure B. 8.1-28 to Figure B. 8.1-30.

Table B.8.1-123: Kinetic evaluation results of field dissipation study of the study Schulz (1992b, BVL no 1932131), Ekergingen/Switzerland, clay loam - persistence and trigger end points of glyphosate

Glyphosate							
Kinetic model	**DT_{50} (days)**	**DT_{90} (days)**	**χ2 error (%)**	**Parameter**	**Prob>t**	**Std**	**Visual fit**
SFO	21.58	71.7	26.8	k 0.0321	0.0498	0.015	not acceptable
SFO M0 fixed	13.41	44.56	29.7	k 0.0517	0.0121	0.0162	not acceptable
FOMC	6.78	812.77	11.31	alpha 0.3466	0.0452	0.1406	acceptable
				beta 1.061	0.2516	1.3984	
DFOP	1.458	178.659	5.24	k1 1.918	0.4999	>1000	very good
				k2 0.0087	0.0294	0.0022	
				g 0.526	0.0042	0.0488	

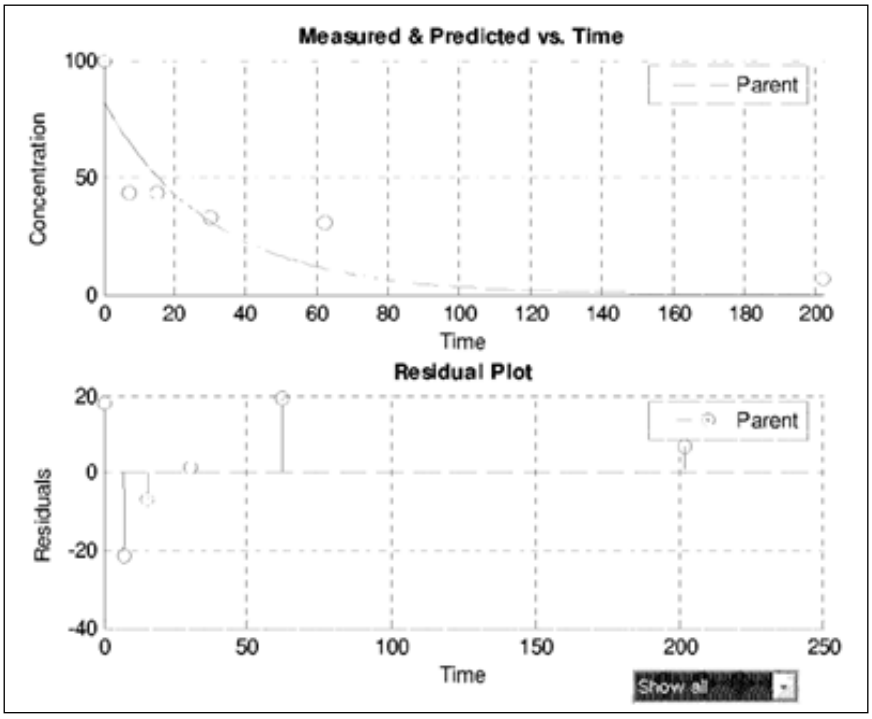


Figure B. 8.1-28: Schulz, 1992b: Optimised SFO kinetic model for degradation of glyphosate in the Egerkingen soil (parent only)

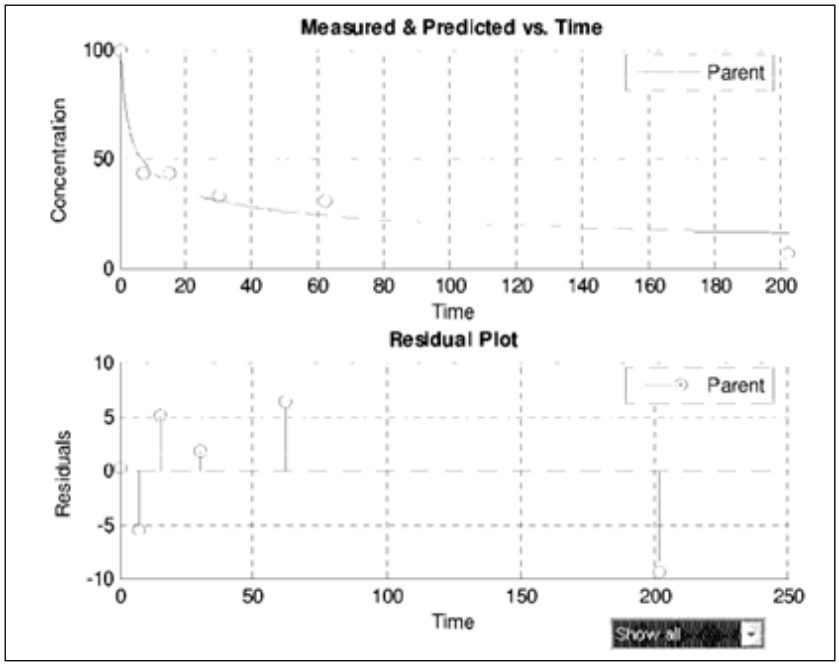


Figure B. 8.1-29: Schulz, 1992b: Optimised FOMC kinetic model for degradation of glyphosate in the Egerkingen soil (parent only)

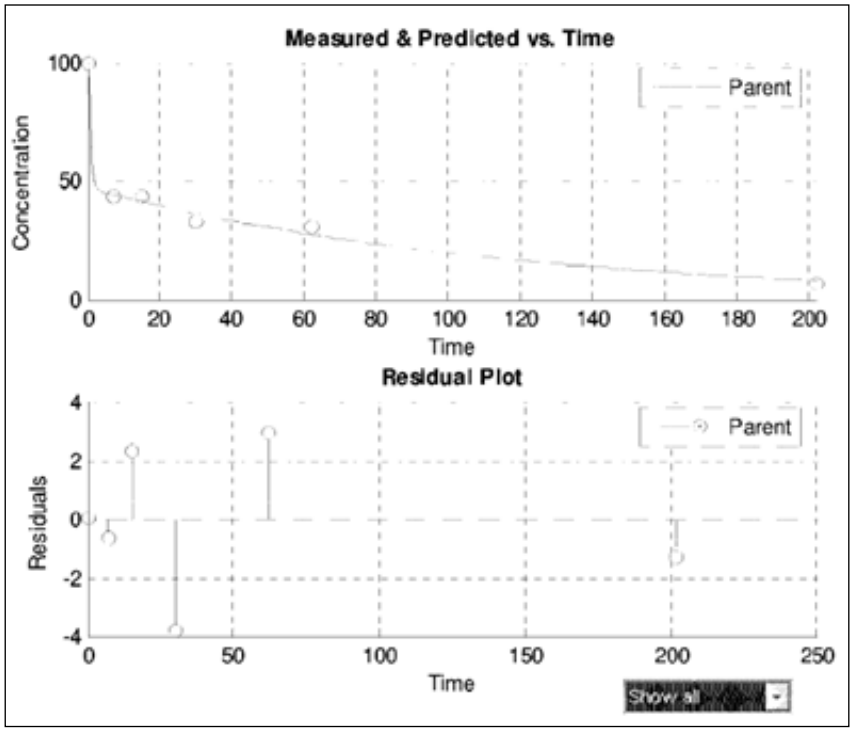


Figure B. 8.1-30: Schulz, 1992b: Optimised DFOP kinetic model for degradation of glyphosate in the Egerkingen soil (parent only)

Using the SFO model for the parent substance glyphosate, the visual fit was considered not acceptable because M0 and the last data points from day 30 onward were underestimated. The FOMC model also revealed a non-acceptable visual fit with an overestimation of the last data point. Following the decision scheme of FOCUS degradation kinetics (2006, 2011) the SFO model fit was repeated with a fixed Mo. The modified SFO model fit still underestimated the last data points (from day 30 onward) and was therefore considered visually non-acceptable. Therefore the SFO and the FOMC model were rejected and DFOP was tested additionally. The DFOP model fit resulted in a visually acceptable fit with a χ^2-error of 5.2 %. However, due the large confidence interval of k1' the DFOP model was rejected as well. In conclusion, no acceptable kinetic fit could be obtained for the degradation behaviour of glyphosate.

No pathway fit could be conducted for the metabolite AMPA because no reliable kinetic model could be obtained for the parent compound. Furthermore, AMPA reaches its maximum not until the second to last sampling point at day 62, which made it impossible to conduct an optimisation for the metabolite's decline phase only (see Table B.8.1-119). In conclusion, no acceptable kinetic fit could be obtained for the degradation behaviour of AMPA.

KIIA 7.3.1/4 Field dissipation study Schulz (1992c, BVL no 1932073)

The kinetic evaluation results of field dissipation study of the study Schulz (1992, BVL no 1932073) in Bad Krozingen/Germany are provided in Table B.8.1-124. Visual fits are provided in Figure B. 8.1-31 to Figure B. 8.1-33.

Table B.8.1-124: Kinetic evaluation results of field dissipation study of the study Schulz (1992c, BVL no 1932073), Bad Krozingen/Germany, sandy loam - persistence and trigger end points of glyphosate

Glyphosate							
Kinetic model	**DT_{50} (days)**	**DT_{90} (days)**	**$\chi 2$ error (%)**	**Parameter**	**Prob>t**	**Std**	**Visual fit**
SFO	6.33	21.02	23.4	0.1096	0.0286	0.0364	not acceptable
FOMC	2.28	127.16	5.84	alpha 0.422	0.044	0.135	good
				beta 0.547	0.231	0.606	
DFOP	2.3	66.84	7.67	k1 0.5905	0.369	1.349	good
				k2 0.0186	0.179	0.0118	
				g 0.653	0.0571	0.1185	

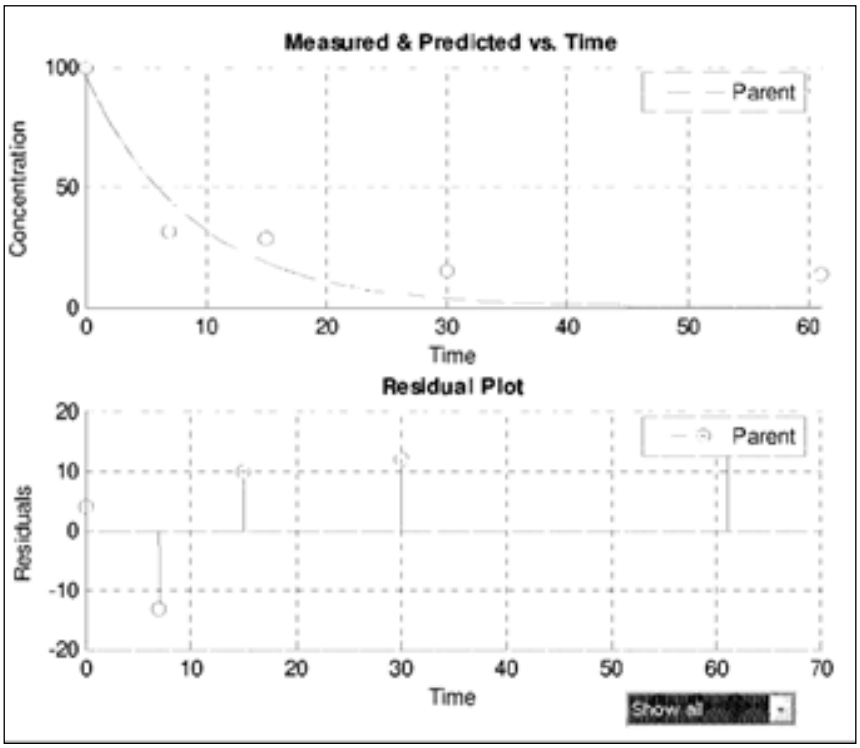


Figure B. 8.1-31: Schulz, 1992c: Optimised SFO kinetic model for degradation of glyphosate in the Bad Krozingen soil (parent only)

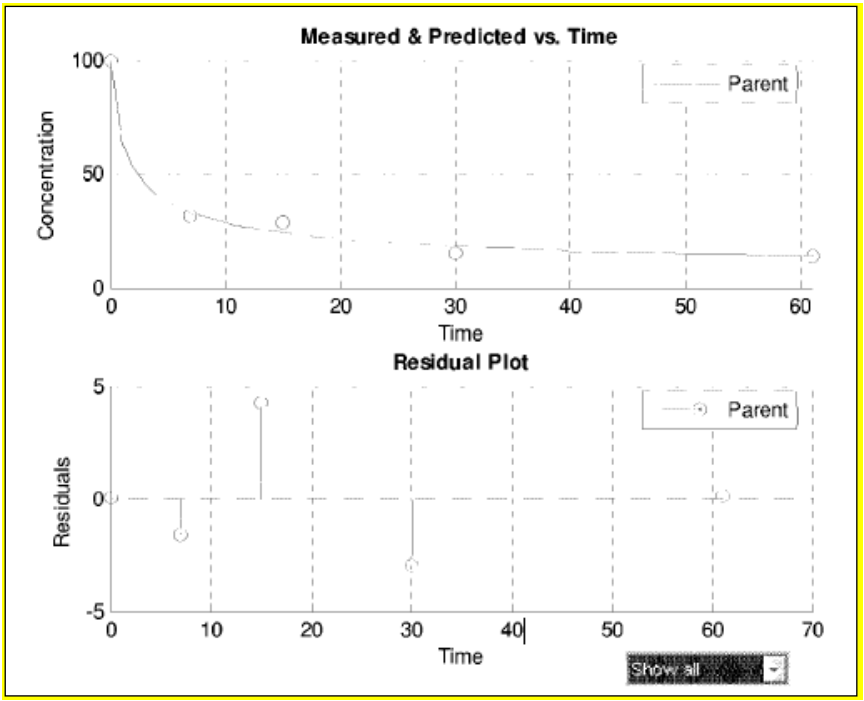


Figure B. 8.1-32: Schulz, 1992c: Optimised FOMC kinetic model for degradation of glyphosate in the Bad Krozingen soil (parent only)

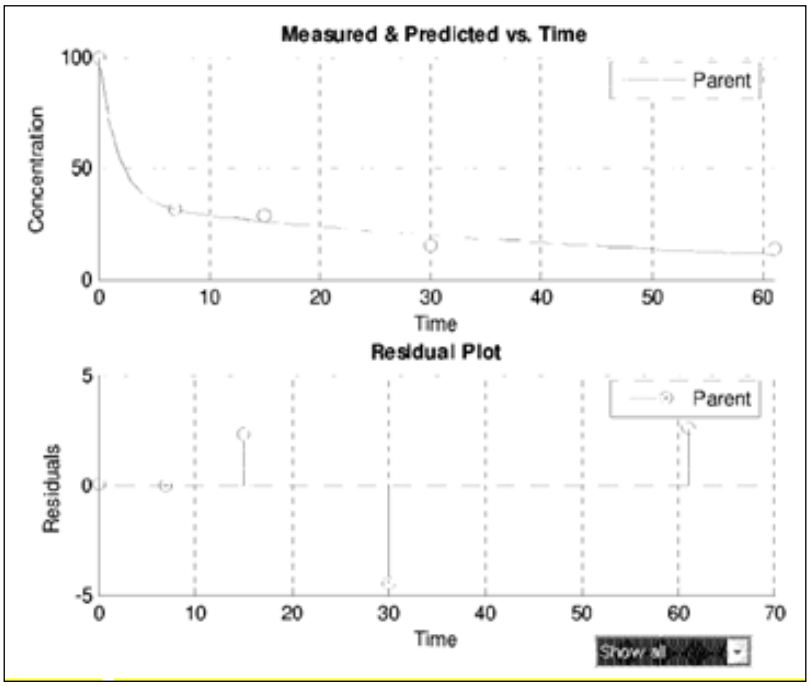


Figure B. 8.1-33: Schulz, 1992c: Optimised DFOP kinetic model for degradation of glyphosate in the Bad Krozingen soil (parent only)

Using the SFO model for the parent substance glyphosate, the visual fit was considered not acceptable because the last data points from day 15 onward were underestimated. The FOMC model revealed an acceptable visual fit with a χ^2-error of 5.8 %. However, the confidence intervals of the parameters were quite large and included zero. Therefore the SFO and the FOMC model were rejected and DFOP was tested additionally. The DFOP model resulted in a visually acceptable fit with χ^2-error of 7.7 %. However, due the large confidence interval of k1' the DFOP model was rejected as well. In conclusion, no acceptable kinetic fit could be obtained for the degradation behaviour of glyphosate.

No pathway fit could be conducted for the metabolite AMPA because no reliable kinetic model could be obtained for the parent compound. Furthermore, the maximum value of AMPA occurs at the last sampling point at day 61, which made it impossible to conduct an optimisation for the metabolite's decline phase only (see Table B.8.1-119). In conclusion, no acceptable kinetic fit could be obtained for the degradation behaviour of AMPA.

KIIA 7.3.1/5 Field dissipation study Schulz (1992d, BVL no 1932133)

The kinetic evaluation results of field dissipation study of the study Schulz (1992d, BVL no 1932133) in Menslage/Germany are provided in Table B.8.1-125. Visual fits are provided in Figure B. 8.1-34 to Figure B. 8.1-37.

Table B.8.1-125: Kinetic evaluation results of field dissipation study of the study Schulz (1992d, BVL no 1932133), Menslage/Germany, sandy loam - persistence and trigger end points of glyphosate

Glyphosate							
Kinetic model	**DT_{50} (days)**	**DT_{90} (days)**	**χ2 error (%)**	**Parameter**	**Prob>t**	**Std**	**Visual fit**
SFO	8.05	26.73	28.6	0.0861	0.0053	0.0235	not acceptable
FOMC	4.64	147.51	12.1	alpha 0.5066	0.0047	0.1235	good
				beta 1.583	0.1073	1.114	
DFOP	5.7	200.8	9.4	k1 0.1786	0.0036	0.0353	good
				k2 0.0041	0.0314	0.0016	
				g 0.771	3.1E-5	0.0440	
DFOP	**5.7**	**200.8**	**9.4**	**k1 0.1786**	**Best fit kinetic (Menslage/Germany)**		
				k2 0.0041			
				g 0.771			

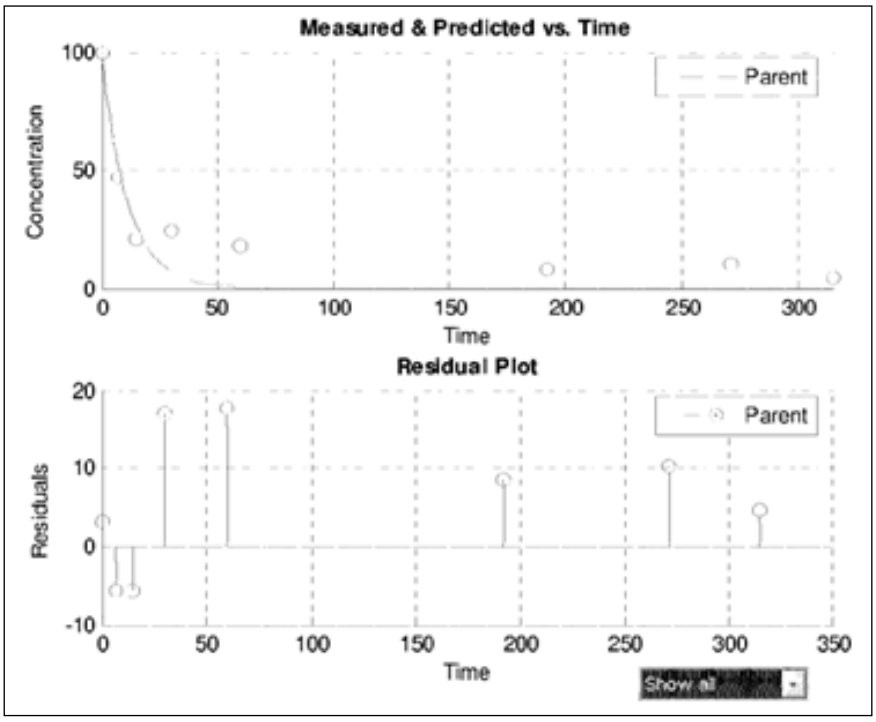


Figure B. 8.1-34: Schulz, 1992d: Optimised SFO kinetic model for degradation of glyphosate in the Menslage soil (parent only)

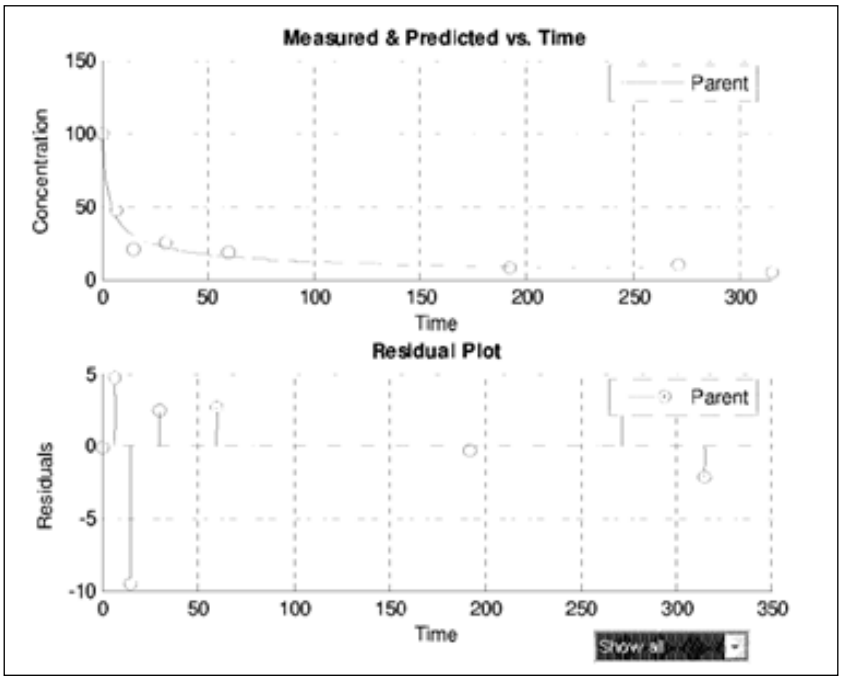


Figure B. 8.1-35: Schulz, 1992d: Optimised FOMC kinetic model for degradation of glyphosate in the Menslage soil (parent only)

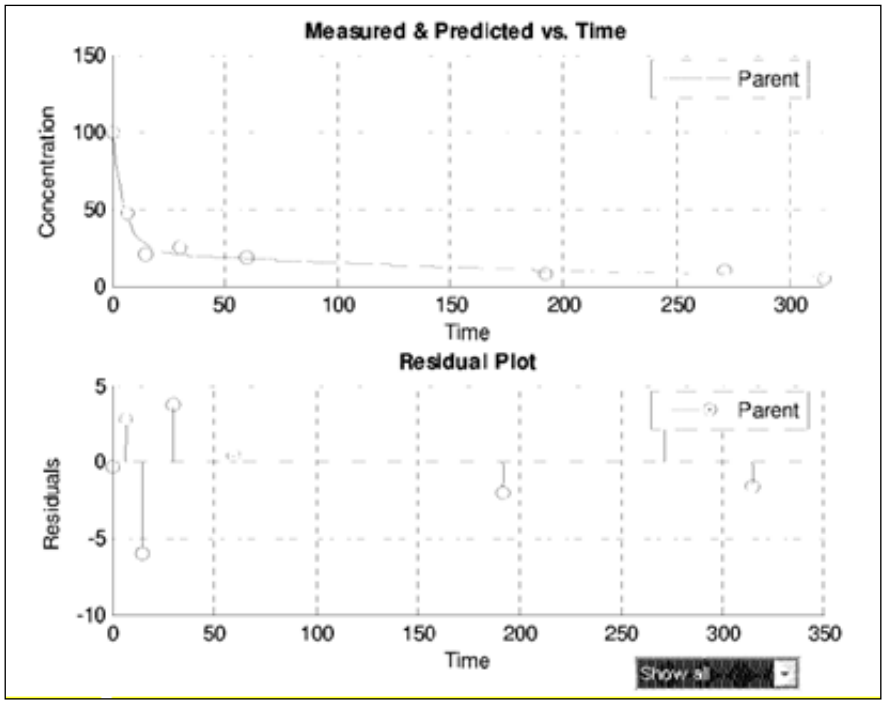


Figure B. 8.1-36: Schulz, 1992d: Optimised DFOP kinetic model for degradation of glyphosate in the Menslage soil (parent only)

Using the SFO model for the parent substance glyphosate, the visual fit was considered not acceptable because the optimisation resulted in a systematic underestimation of the data points from day 30 onward with a χ^2-error of 28.6 %. Using the FOMC model the visual assessment revealed an acceptable fit with a χ^2-error of 12.1 %. Therefore, the SFO model was

rejected and DFOP was tested additionally. The DFOP model provided a good visual fit with a χ^2-error of 9.4 %. Due to the better l-error, the DFOP model was chosen for trigger end points.

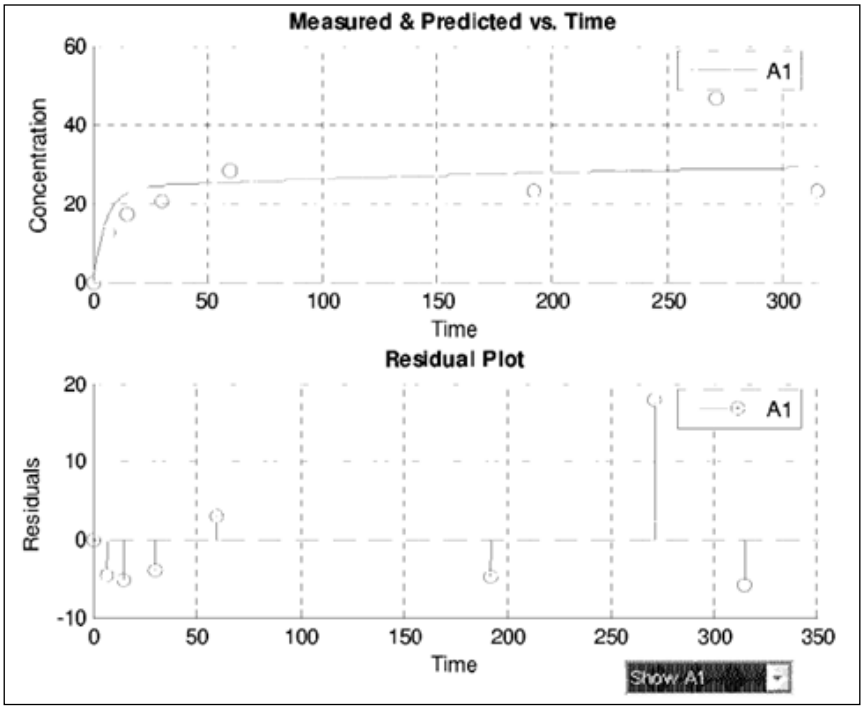


Figure B. 8.1-37: Schulz, 1992d: Optimised DFOP kinetic model for degradation of AMPA in the Menslage soil (pathway fit- decline)

The pathway fit for the metabolite AMPA using the DFOP model for the parent and the SFO model for AMPA was visually not acceptable because the maximum value was not represented. Kinetic fitting using the decline phase only was not possible either because the maximum is not reached until the second to last sampling point (see Table B.8.1-119). In conclusion, no acceptable kinetic fit could be obtained for the degradation behaviour of AMPA.

KIIA 7.3.1/6 Field dissipation study Hill (1992, BVL no 2154352)

The kinetic evaluation results of field dissipation study of the study Hill (1992, BVL no 2154352) in Buchen/Germany are provided for glyphosate in Table B.8.1-126 and for AMPA in Table B.8.1-127. Visual fits of degradation kinetics for glyphosate are provided in Figure B. 8.1-38 to Figure B. 8.1-40. Visual fits of degradation kinetics for AMPA are provided in Figure B. 8.1-41 to Figure B. 8.1-43.

Table B.8.1-126: Kinetic evaluation results of field dissipation study of the study Hill (1992, BVL no 2154352), Buchen/Germany, loamy sand – persistence and trigger end points of glyphosate

Glyphosate							
Kinetic model	**DT_{50} (days)**	**DT_{90} (days)**	**χ2 error (%)**	**Parameter**	**Prob>t**	**Std**	**Visual fit**
SFO	45.3	150.3	10.04	0.0153	1.30E-06	0.0015	not acceptable
FOMC	40.13	188.82	8.8	alpha 2.536	0.0482	1.347	not acceptable
				beta 127.680	0.0956	89.421	
DFOP	40.9	187.3	6.6	k1 0.019	1.40E-04	0.0028	good
				k2 2.3E-14	0.5	0.0026	
				g 0.927	1.10E-06	0.0656	
DFOP	**40.9**	**187.3**	**6.6**	**k1 0.019**	**Best fit kinetic (Buchen/Germany)**		
				k2 2.3E-14			
				g 0.927			

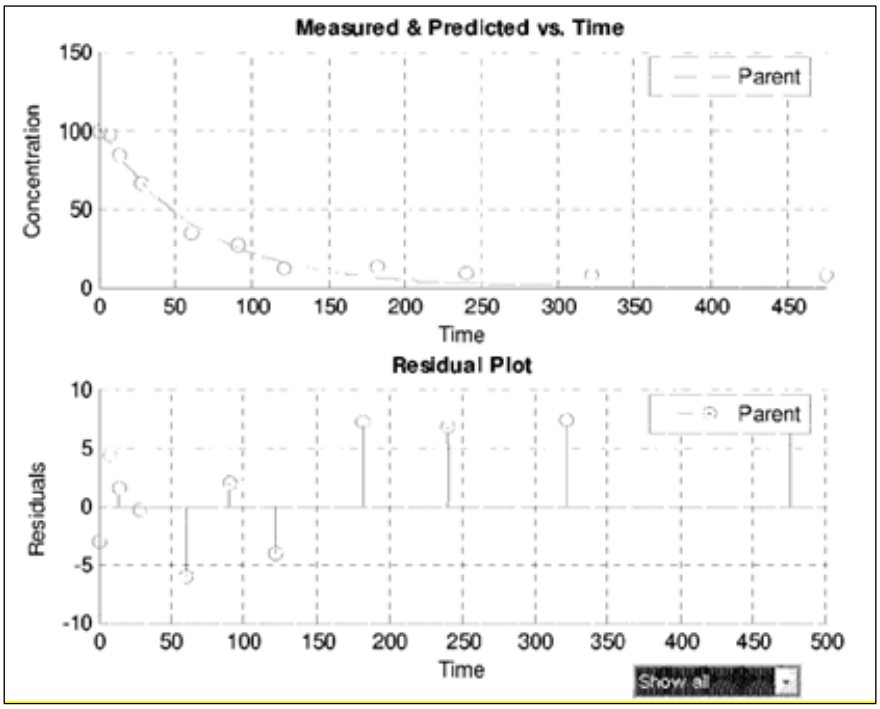


Figure B. 8.1-38: Hill, 1992: Optimised SFO kinetic model for degradation of glyphosate in the Buchen soil (parent only)

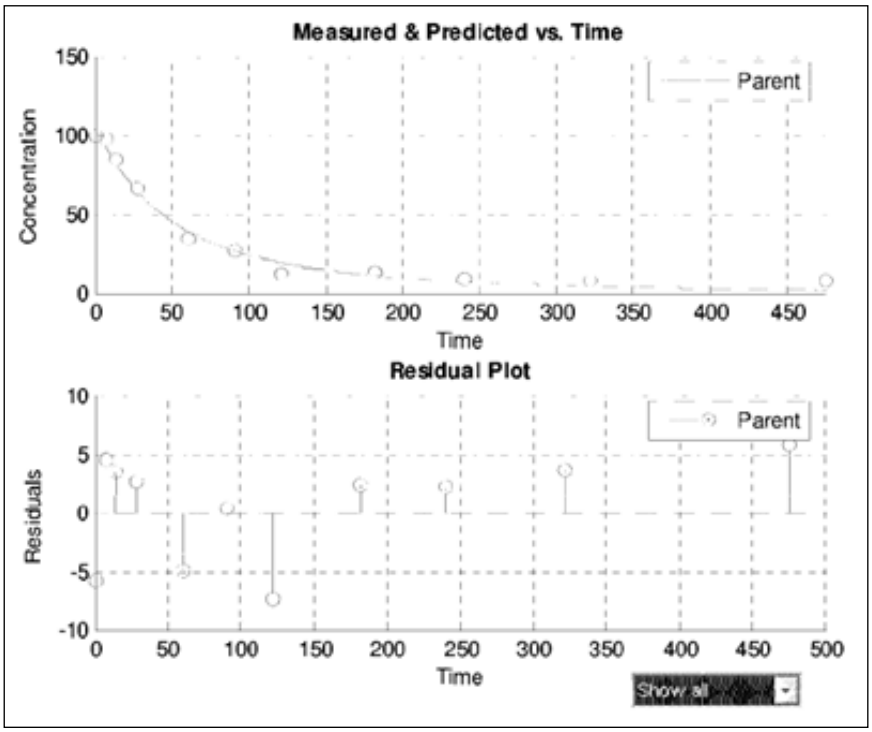


Figure B. 8.1-39: Hill, 1992: Optimised FOMC kinetic model for degradation of glyphosate in the Buchen soil (parent only)

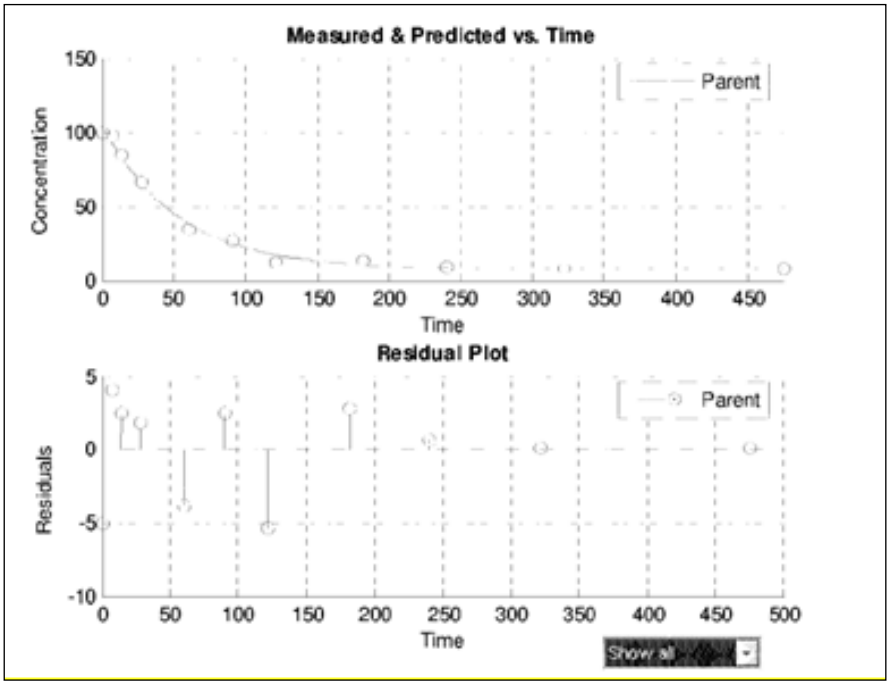


Figure B. 8.1-40: Hill, 1992: Optimised DFOP kinetic model for degradation of glyphosate in the Buchen soil (parent only)

Using the SFO model for the parent substance glyphosate, the visual fit was considered not acceptable due to a systematic underestimation of the data points from day 182 onward with a χ^2-error of 10.0 %. Using the FOMC model the visual assessment also revealed an unacceptable fit due to a systematic underestimation of the data points from day 182 onward with a

slightly lower χ^2-error of 8.8 %. Therefore, the SFO and the FOMC model were both rejected and DFOP was tested additionally. The DFOP model provided a good visual fit with a good estimation of the last data points and a χ^2-error of 6.6 %. Consequently, the DFOP model was chosen for trigger end points.

Table B.8.1-127: Kinetic evaluation results of field dissipation study of the study Hill (1992, BVL no 2154352), Buchen/Germany, loamy sand - persistence and trigger end points of AMPA

AMPA								
Kinetic model	**DT_{50} (days)**	**DT_{90} (days)**	**$\chi 2$ error (%)**	**ff**	**Parameter**	**Prob>t**	**Std**	**Visual fit**
DFOP- SFO	357.8	>1000	29	0.369	0.0019	0.0119	7.90E-04	not acceptable
SFO Top down	805.7	>1000	23.6	nd	8.60E-04	0.263	0.0012	not acceptable
SFO Top down M0 fixed	283.7	942.6	29.9	nd	0.0024	0.042	0.0011	not acceptable

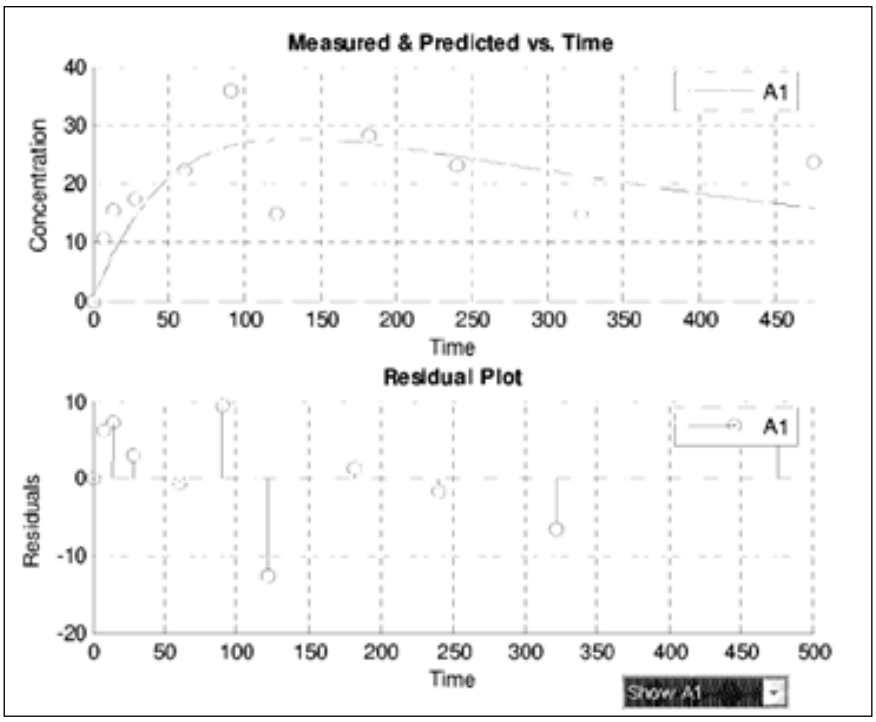


Figure B. 8.1-41: Hill, 1992: Optimised DFOP kinetic model for degradation of AMPA in the Buchen soil (pathway fit - initial)

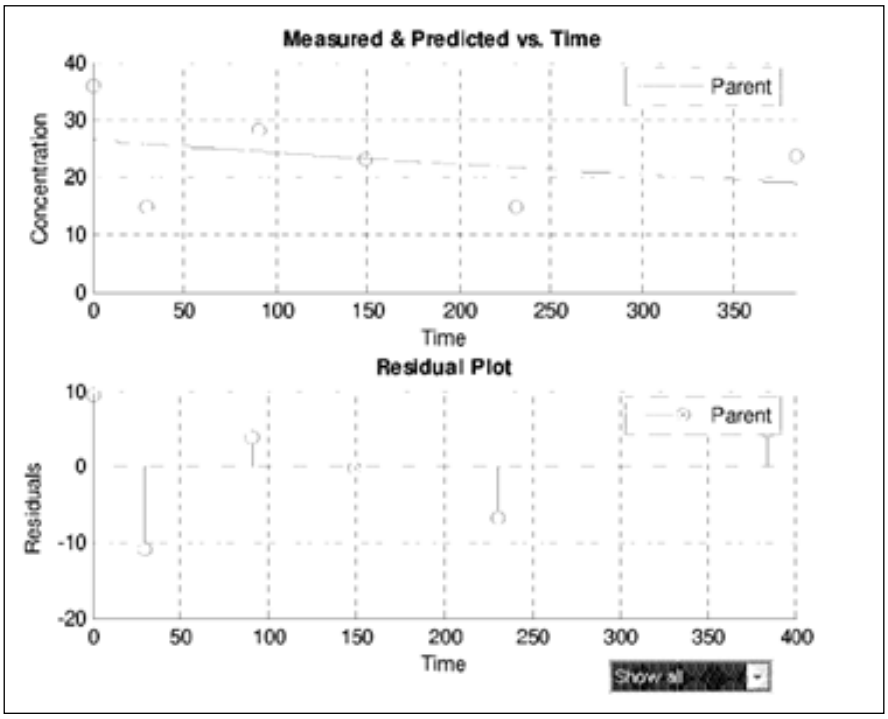


Figure B. 8.1-42: Optimised DFOP kinetic model for degradation of AMPA in the Buchen soil (decline fit)

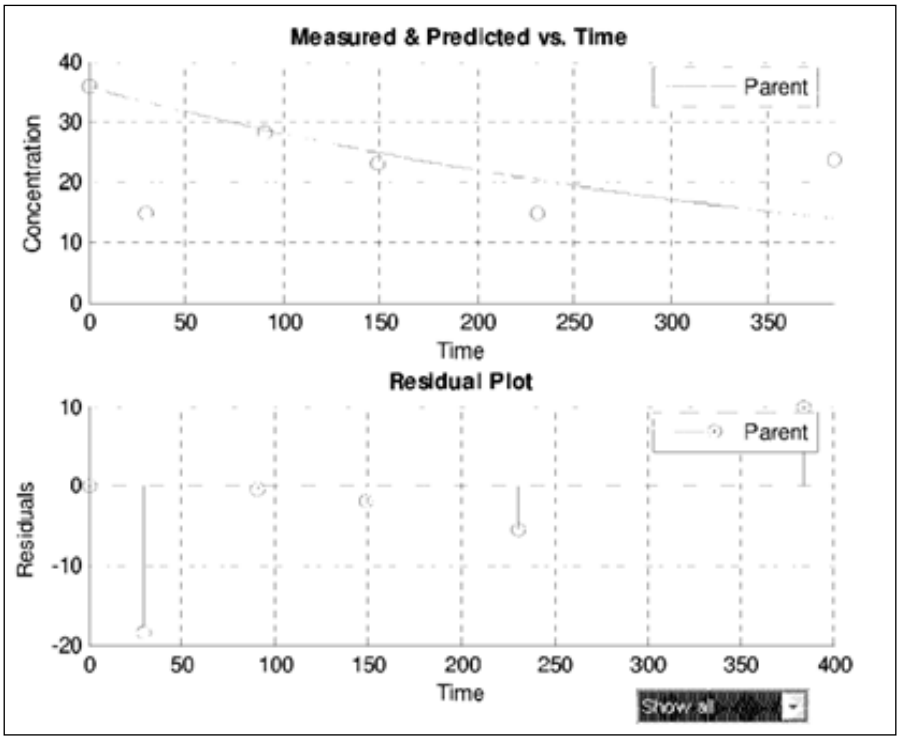


Figure B. 8.1-43: Hill, 1992: Optimised DFOP kinetic model for degradation of AMPA in the Buchen soil (decline fit- M0 fixed)

The pathway fit for the metabolite AMPA using the DFOP model for the parent and the SFO model for AMPA was visually not acceptable due to a poor estimate of the maximum and an underestimation of the last data point. Therefore, the SFO model was tested using the decline phase only, but no acceptable visual fit could be obtained with and without fixed Mo due to strong data scattering in the decline phase. No biphasic degradation pattern is visible within the data points and, thus, no improvement could be expected from testing the bi-phasic models. Furthermore, only a small decrease in AMPA concentration could be observed during the study with more than 50 % of the maximum still being detected at the end of the study. In conclusion, no acceptable kinetic fit could be obtained for the degradation behaviour of AMPA.

The kinetic evaluation results of field dissipation study of Study Hill (1992, BVL no 2154352) in Kleinzecher/ Germany are provided for glyphosate in Table B.8.1-128 and for AMPA Table B.8.1-129. Visual fits of degradation kinetics for glyphosate are provided in Figure B. 8.1-44 to Figure B. 8.1-46. Visual fit of degradation kinetics for AMPA is provided in Figure B. 8.1-47.

Table B.8.1-128: Kinetic evaluation results of field dissipation study of the study Hill (1992, BVL no 2154352), Kleinzecher/Germany, sandy loam - persistence and trigger end points of glyphosate

Glyphosate							
Kinetic model	**DT_{50} (days)**	**DT_{90} (days)**	**χ2 error (%)**	**Parameter**	**Prob>t**	**Std**	**Visual fit**
SFO	66.8	221.7	19.5	0.0104	1.60E-04	0.0019	not acceptable
FOMC	39.5	398.3	12.6	alpha 0.9124	4.50E-03	0.2576	not acceptable
				beta 34.714	6.24E-02	20.508	
DFOP	38.3	386.6	11.7	k1 0.0384	2.40E-02	0.0165	good
				k2 0.0037	2.11E-02	0.0015	
				g 0.575	0.0011	0.129	
DFOP	**38.3**	**386.6**	**11.7**	**k1 0.0384**	**Best fit kinetic (Kleinzecher/Germany)**		
				k2 0.0037			
				g 0.575			

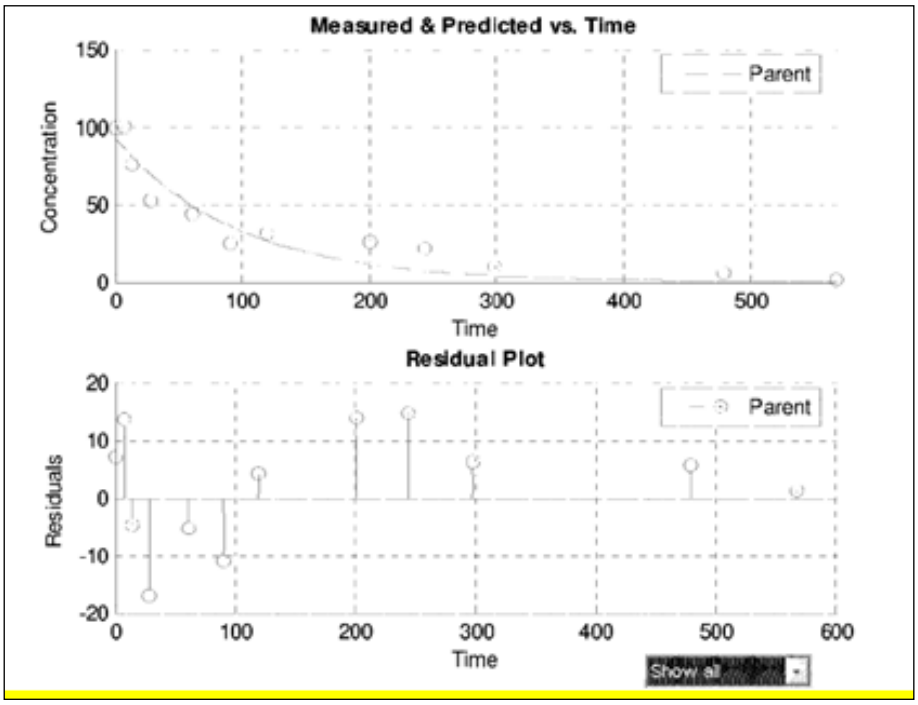


Figure B. 8.1-44: Hill, 1992: Optimised SFO kinetic model for degradation of glyphosate in the Kleinzecher soil (parent only)

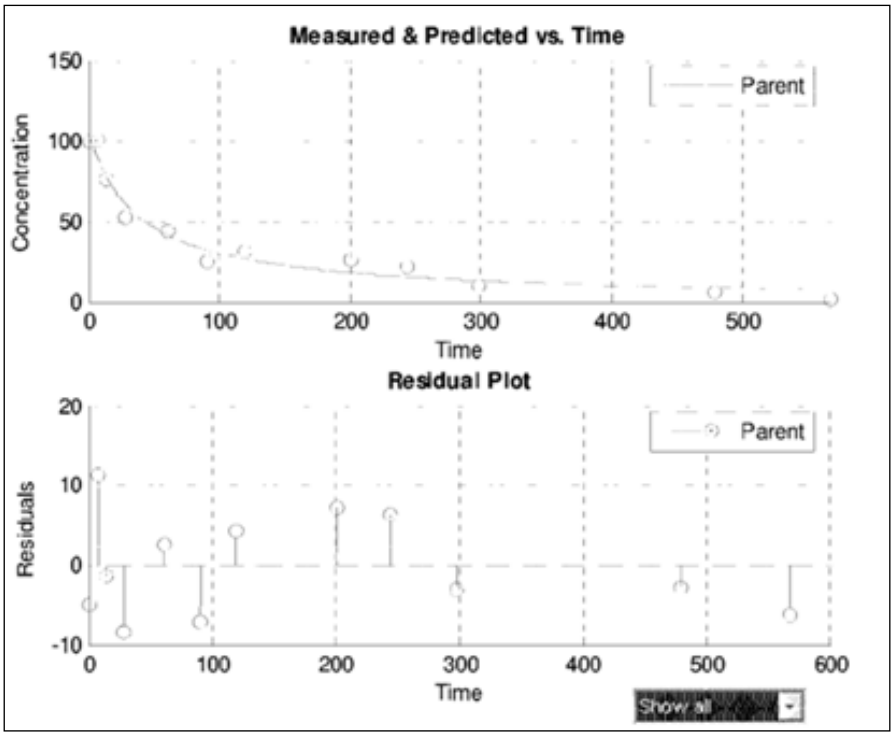


Figure B. 8.1-45: Hill, 1992: Optimised FOMC kinetic model for degradation of glyphosate in the Kleinzecher soil (parent only)

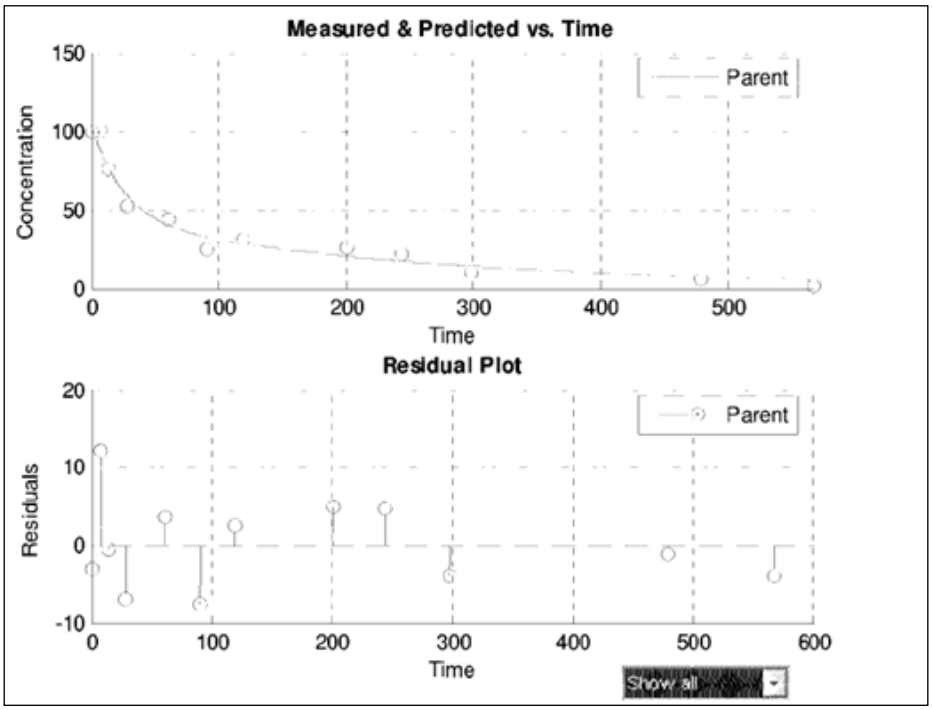


Figure B. 8.1-46: Hill, 1992: Optimised DFOP kinetic model for degradation of glyphosate in the Kleinzecher soil (parent only)

Using the SFO model for the parent substance glyphosate, the visual fit was considered not acceptable due to a systematic underestimation of the data points from day 119 onward with a χ^2-error of 19.5 %. Using the FOMC model the visual assessment revealed an acceptable fit with a slight overestimation of the last data points and a χ^2-error of 12.6 %. Therefore, the SFO model was rejected and DFOP was tested additionally. The DFOP model provided a good visual fit with a better estimation of the last data points (lower residuals than for FOMC) and a χ^2-error of 11.7 %. Consequently, the DFOP model was chosen for trigger end points.

Table B.8.1-129: Kinetic evaluation results of field dissipation study of the study Hill (1992, BVL no 2154352), Kleinzecher/Germany, sandy loam - persistence and trigger end points of AMPA

AMPA								
Kinetic model pathway fit	**DT_{50} (days)**	**DT_{90} (days)**	**$\chi2$ error (%)**	**ff**	**Parameter**	**Prob>t**	**Std**	**Visual fit**
DFOP- SFO	514.9	>1000	15.9	0.5082	0.0013	0.0102	5.30E-04	good
DFOP- SFO	**514.9**	**>1000**	**15.9**	**0.5082**	**Best fit kinetic (Kleinzecher/Germany)**			

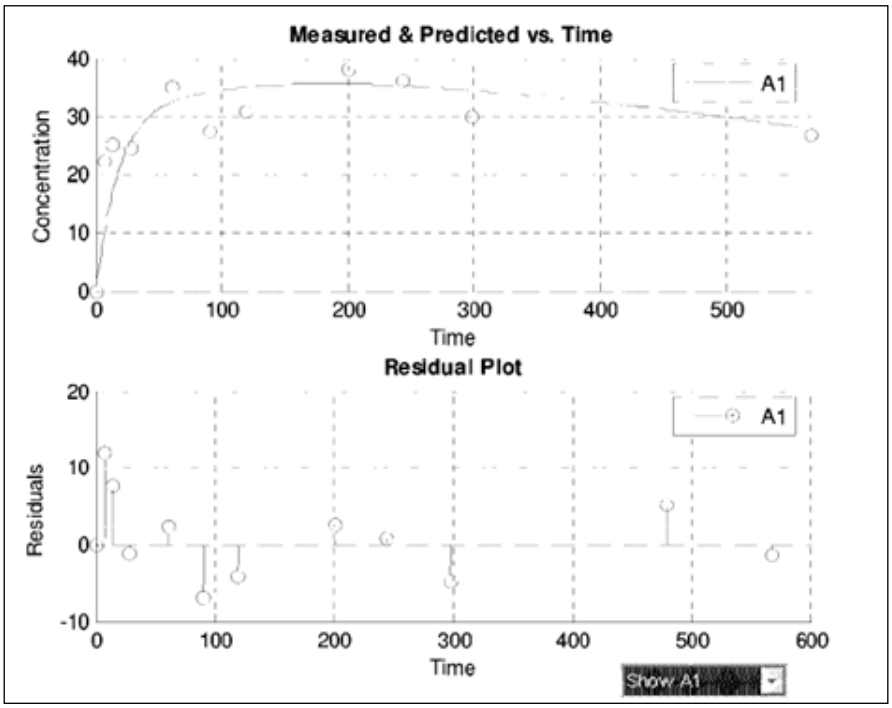


Figure B. 8.1-47: Hill, 1992: Optimised SFO kinetic model for degradation of AMPA in the Kleinzecher soil (pathway fit- simultaneous)

The pathway fit for the metabolite AMPA using the DFOP model for the parent and the SFO model for AMPA was considered visually acceptable due to a good estimation of the formation as well as the decline phase. The χ^2-error is 15.9 % which is a reasonable value for the kinetic fitting of metabolites. Therefore, the SFO model considering a pathway fit using DFOP for the parent was chosen for trigger endpoints. The formation fraction of AMPA was estimated to 0.5082.

The kinetic evaluation results of field dissipation study of the study Hill (1992, BVL no 2154352) in Unzhurst/Germany for glyphosate and AMPA are provided in Table B.8.1-130 and Table B.8.1-131, respectively. Visual fits of degradation kinetics for glyphosate are provided in Figure B. 8.1-48 to Figure B. 8.1-50. Visual fit of degradation kinetics for AMPA is provided in Figure B. 8.1-51.

Table B.8.1-130: Kinetic evaluation results of field dissipation study of the study Hill (1992, BVL no 2154352), Unzhurst/Germany, loam - persistence and trigger end points of glyphosate

Glyphosate							
Kinetic model	**DT_{50} (days)**	**DT_{90} (days)**	**$\chi 2$ error (%)**	**Parameter**	**Prob>t**	**Std**	**Visual fit**
SFO	30.8	102.3	11.8	0.0225	3.60E-06	0.0025	not acceptable
FOMC	26.12	135.29	9.8	alpha 2.0319	0.0189	0.8176	not acceptable
				beta 64.252	0.0578	36.41	

DFOP	27.7	122.3	8.4	k1 0.0280	2.70E-04	0.0047	good
				k2 8.9E-4	0.3981	0.0033	
				g 0.922	1.7E-6	0.0701	
DFOP	**27.7**	**122.3**	**8.4**	**k1 0.0280**	**Best fit kinetic (Unzhurst/Germany)**		
				k2 8.9E-4			
				g 0.922			

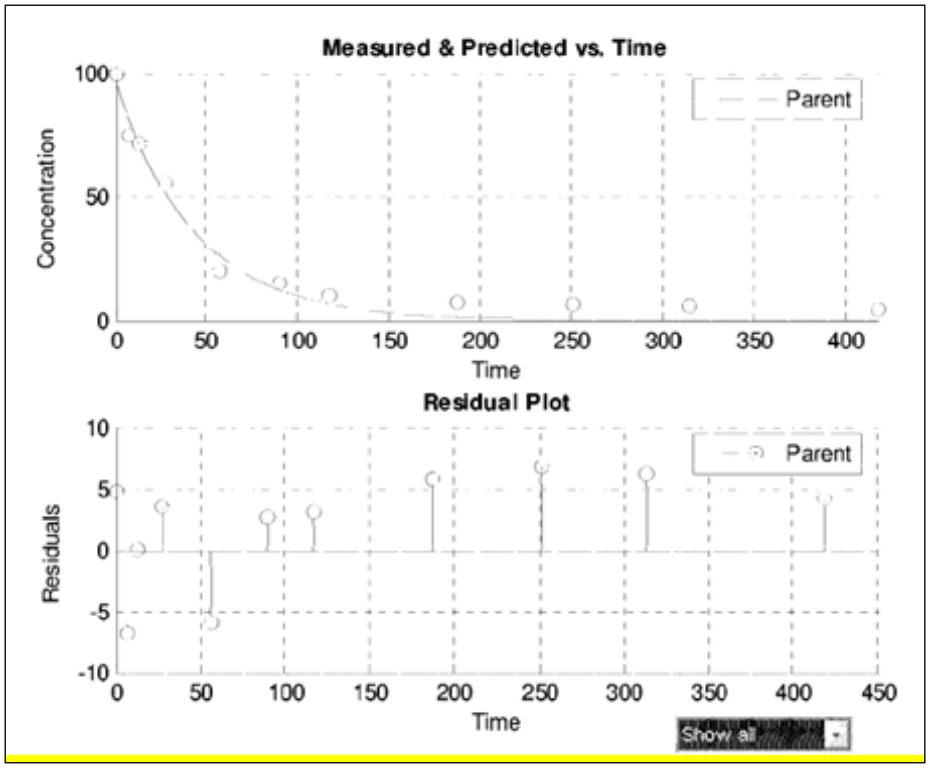


Figure B. 8.1-48: Hill, 1992: Optimised SFO kinetic model for degradation of glyphosate in the Unzhurst soil (parent only)

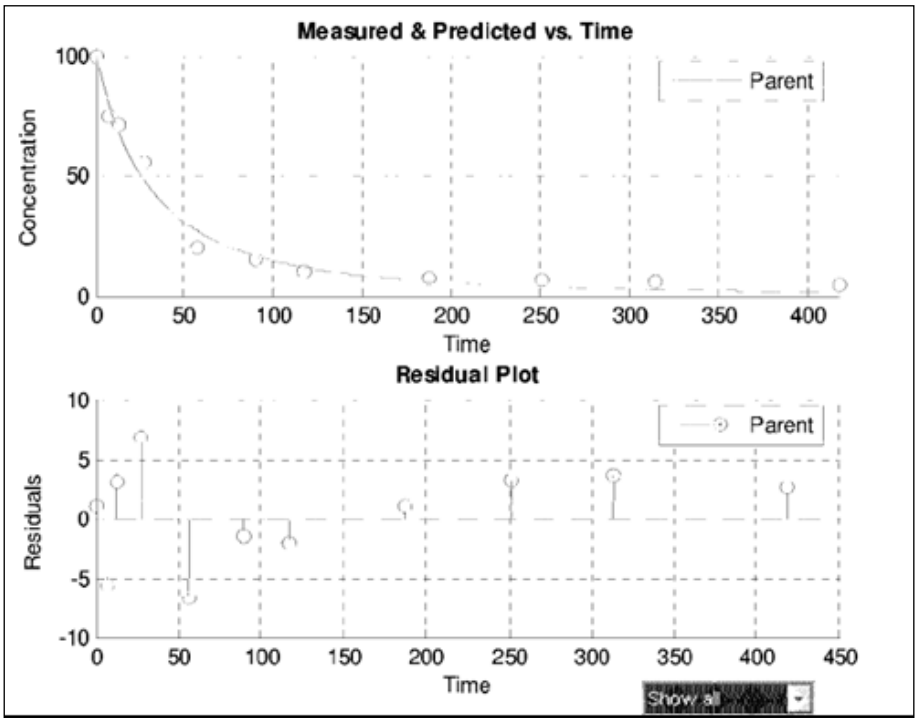


Figure B. 8.1-49: Hill, 1992: Optimised FOMC kinetic model for degradation of glyphosate in the Unzhurst soil (parent only)

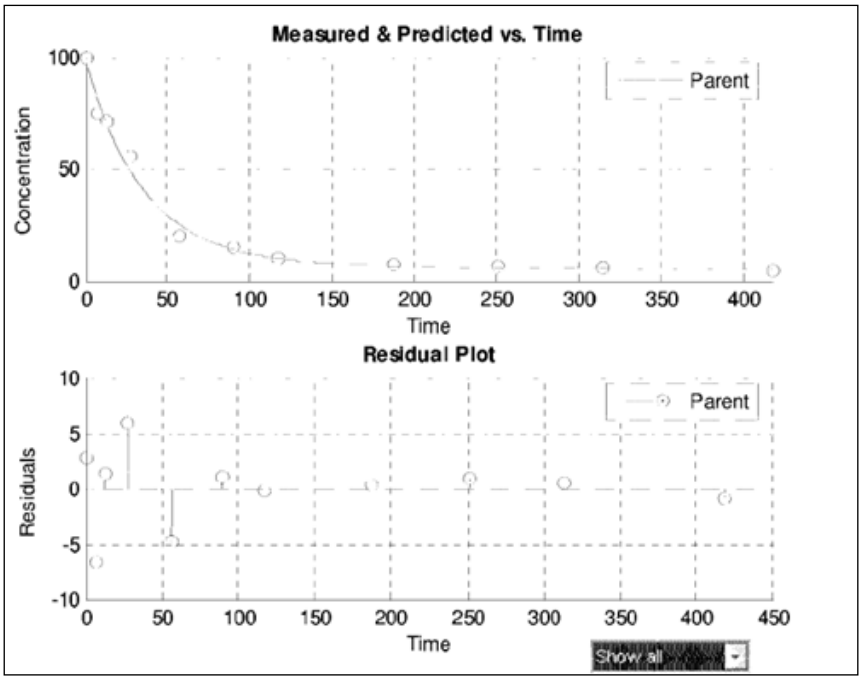


Figure B. 8.1-50: Hill, 1992: Optimised DFOP kinetic model for degradation of glyphosate in the Unzhurst soil (parent only)

Using the SFO model for the parent substance glyphosate, the visual fit was considered not acceptable due to a systematic underestimation of the data points from day 90 onward with a χ^2-error of 11.8 %. Using the FOMC model the visual assessment revealed an acceptable fit

with a slight underestimation of the last data points and a χ^2-error of 9.8 %. Therefore, the SFO model was rejected and DFOP was tested additionally. The DFOP model provided a good visual fit with a better estimation of the last data points (lower residuals than for FOMC) and a slightly lower χ^2-error of 8.4 %. Consequently, the DFOP model was chosen for trigger end points.

Table B.8.1-131: Kinetic evaluation results of field dissipation study of the study Hill (1992, BVL no 2154352), Unzhurst/Germany, loam - persistence and trigger end points of AMPA

AMPA								
Kinetic model pathway fit	**DT_{50} (days)**	**DT_{90} (days)**	**$\chi2$ error (%)**	**ff**	**Parameter**	**Prob>t**	**Std**	**Visual fit**
DFOP- SFO	633.1	>1000	13.13	0.332	0.0011	2.63E-02	5.20E-04	acceptable
DFOP- SFO	**633.1**	**>1000**	**13.13**	**0.332**	**Best fit kinetic (Unzhurst/Germany)**			

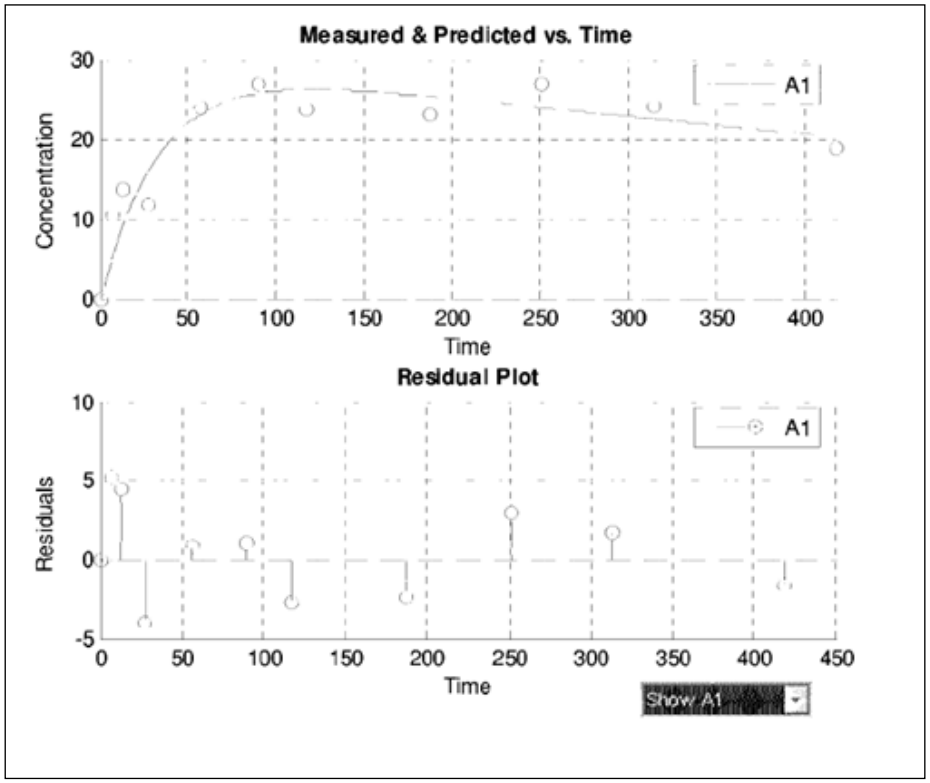


Figure B. 8.1-51: Hill, 1992: Optimised SFO kinetic model for degradation of AMPA in the Unzhurst soil (pathway fit- simultaneous)

The pathway fit for the metabolite AMPA using the DFOP model for the parent and the SFO model for AMPA was considered visually acceptable due to a good estimation of the formation as well as the decline phase. The χ^2-error was 13.1 %. However, since only little degradation is occurring at only 2 data points after the maximum value and, furthermore, the estimated DT_{50} is higher than the study duration, the fit was rejected. A decline fit was not pos-

sible because of the small decline phase. In conclusion, no acceptable kinetic fit could be obtained for the degradation behaviour of AMPA.

RMS comment:
RMS doesn`t follow the argumentation of the Notifier and considers the half life of AMPA resulting from pathway fit acceptable. Even though there are only a few data points of the decline phase, it is not appropriate to reject the fit. The residual plot of the pathway fit shows acceptable deviations of data points from the fitting line in both directions and no systematic underestimation of the half life is considered. The half life of 633 days should be included in the further risk assessment.

The kinetic evaluation results of field dissipation study of the study Hill (1992, BVL no 2154352) in Rohrbach/Germany for glyphosate and AMPA are provided in Table B.8.1-132 and Table B.8.1-133, respectively. Visual fits of degradation kinetics for glyphosate are provided in Figure B. 8.1-52 to Figure B. 8.1-55. Visual fits of degradation kinetics for AMPA are provided in Figure B. 8.1-56 and Figure B. 8.1-57.

Table B.8.1-132: Kinetic evaluation results of field dissipation study of the study Hill (1992, BVL no 2154352), Rohrbach/Germany, silt loam - persistence and trigger end points of glyphosate

Glyphosate							
Kinetic model	**DT_{50} (days)**	**DT_{90} (days)**	**χ2 error (%)**	**Parameter**	**Prob>t**	**Std**	**Visual fit**
SFO	20.1	66.9	18.5	0.025	2.10E-04	0.0043	good
FOMC	27.7	92.23	19.5	alpha 879.16	0.4992	>1000	good
				beta >1000	0.4992	>1000	
SFO top down	20.14	66.92	3.8	0.0344	5.90E-09	0.0011	good
FOMC Top down	20.14	66.95	4.05	alpha >1000	0.4959	>1000	good
				beta >1000	0.4959	>1000	
SFO top down	**20.14**	**66.92**	**3.8**	**0.0344**	**Best fit kinetic (Rohrbach/Germany)**		

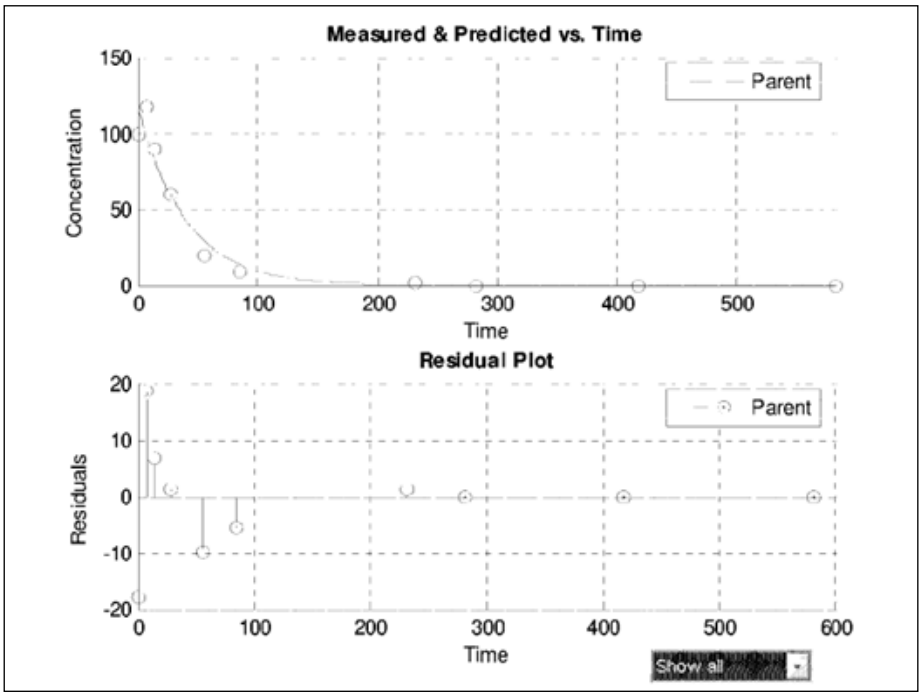


Figure B. 8.1-52: Hill, 1992: Optimised SFO kinetic model for degradation of glyphosate in the Rohrbach soil (parent only)

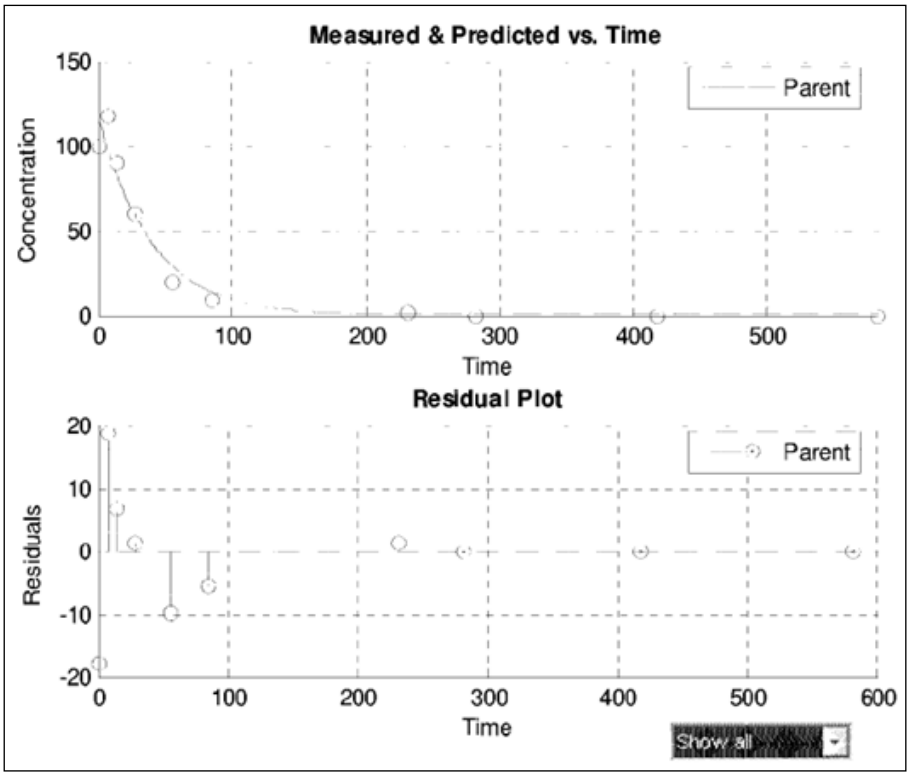


Figure B. 8.1-53: Hill, 1992: Optimised FOMC kinetic model for degradation of glyphosate in the Rohrbach soil (parent only)

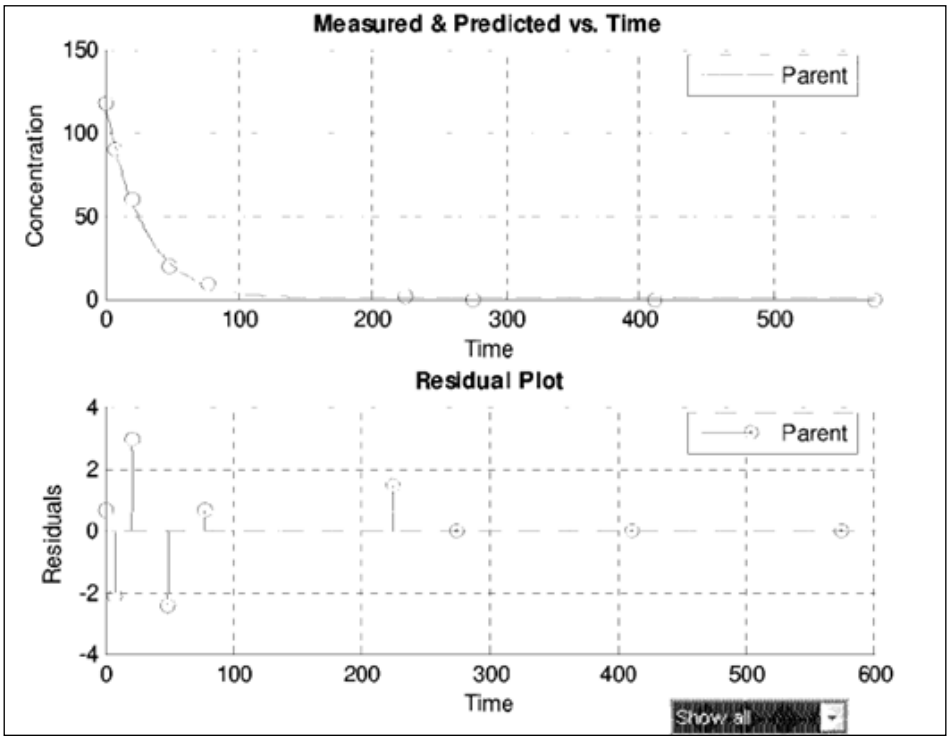


Figure B. 8.1-54: Hill, 1992: Optimised SFO kinetic model for degradation of glyphosate in the Rohrbach soil (parent only)- decline fit

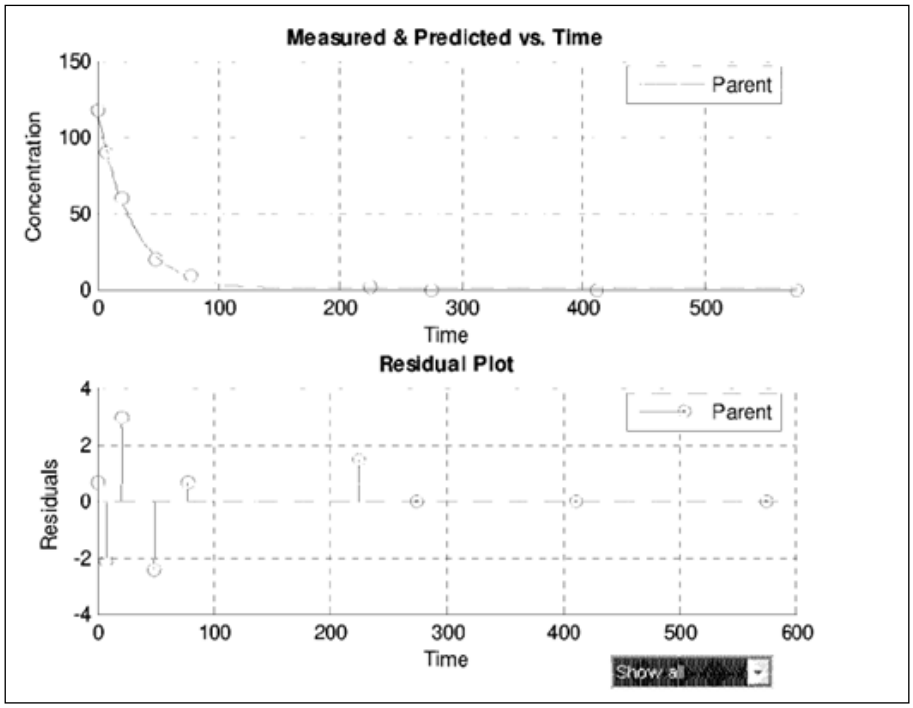


Figure B. 8.1-55: Hill, 1992: Optimised FOMC kinetic model for degradation of glyphosate in the Rohrbach soil (parent only)- decline fit

Using the SFO model for the parent substance glyphosate, the visual assessment was considered acceptable with a χ^2-error of 18.5 %. The FOMC model gave similar results with a

slightly higher χ^2-error of 19.5 %. High residuals occurred at day 0 and 7 for the SFO and FOMC model, because the maximum value of the trial was observed at day 7 with 117.5 % (compared to the amount at day 0). Therefore, the kinetic fitting was repeated considering the decline phase from day 7 only. Both models were visually acceptable which was also reflected in the χ^2-error values of 3.8 % (SFO) and 4.0 % (FOMC). Consequently, the SFO model considering the decline phase only was chosen for trigger end points.

Table B.8.1-133: Kinetic evaluation results of field dissipation study of the study Hill (1992, BVL no 2154352), Rohrbach/ Germany, silt loam - persistence and trigger endpoints of AMPA

AMPA								
Kinetic model pathway fit	**DT_{50} (days)**	**DT_{90} (days)**	**χ2 error (%)**	**ff**	**Parameter**	**Prob>t**	**Std**	**Visual fit**
SFO Top down	374.9	>1000	8.6	-	0.0018	0.0033	3.60E-04	acceptable
FOMC Top down	374.9	>1000	9.4	-	alpha 115.35	>1000	0.4979	acceptable
					beta >1000	>1000	0.4979	
SFO Top down	**374.9**	**>1000**	**8.6**	**-**	**Best fit kinetic (Rohrbach/ Germany)**			

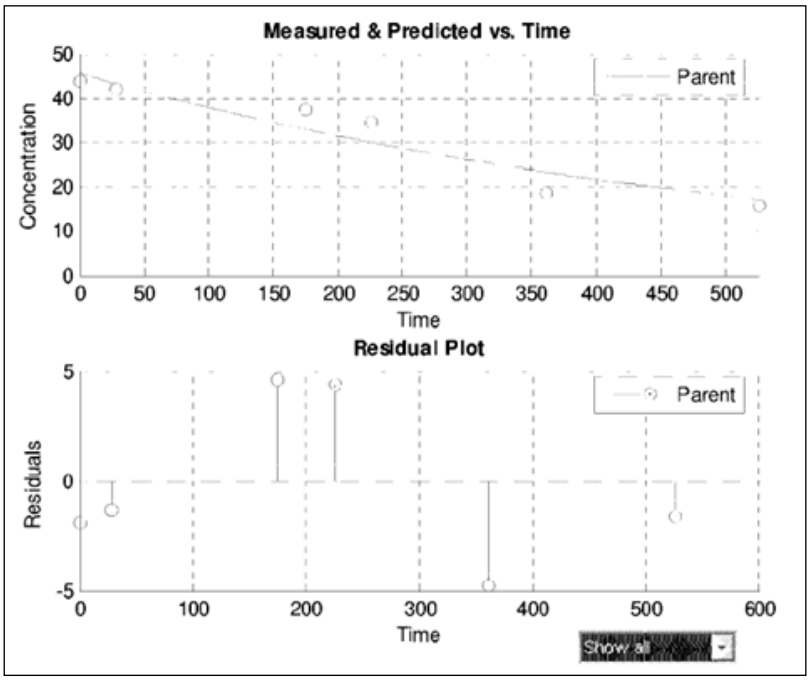


Figure B. 8.1-56: Hill, 1992: Optimised SFO kinetic model for degradation of AMPA in the Rohrbach soil (decline fit)

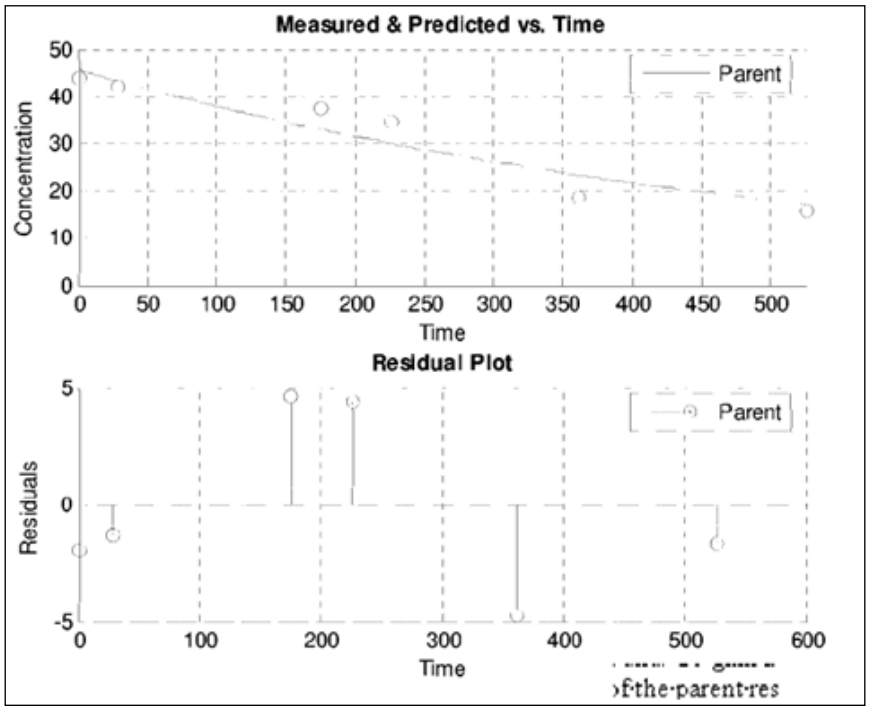


Figure B. 8.1-57: Hill, 1992: Optimised FOMC kinetic model for degradation of AMPA in the Rohrbach soil (decline fit)

No pathway fit could be conducted for the metabolite AMPA because the reliable kinetic model for the parent compound was obtained by considering the decline phase of the parent only. Therefore, the SFO and the FOMC model were tested using the decline phase only. Both fits were visually acceptable with a χ^2-error of 8.6 % for SFO and 9.4 % for FOMC. Since no bi-phasic degradation pattern is visible within the data points and the χ^2-error of the SFO fit is slightly better, the SFO model considering the decline phase only was chosen for trigger end points.

The kinetic evaluation results of field dissipation study of the study Hill (1992, BVL no 2154352) in Herrngiersdorf/Germany are provided for glyphosate and AMPA in Table B.8.1-134 and Table B.8.1-135, respectively. Visual fits of degradation kinetics for glyphosate are provided in Figure B. 8.1-58 and Figure B. 8.1-59. Visual fits of degradation kinetics for AMPA are provided inFigure B. 8.1-60 to Figure B. 8.1-62.

Table B.8.1-134: Kinetic evaluation results of field dissipation study of the study Hill (1992, BVL no 2154352), Herrngiersdorf/Germany, clay loam - persistence and trigger end points of glyphosate

Glyphosate							
Kinetic model	**DT_{50} (days)**	**DT_{90} (days)**	**$\chi 2$ error (%)**	**Parameter**	**Prob>t**	**Std**	**Visual fit**
SFO	33.7	111.9	10.6	0.0206	1.40E-04	0.0027	good
FOMC	33.7	112.05	11.3	alpha 346.4	4.97E-01	>1000	good

				beta >1000	4.97E-01	>1000	
SFO	**33.7**	**111.9**	**10.6**	**0.0206**	**Best fit kinetic (Herrngiersdorf/Germany)**		

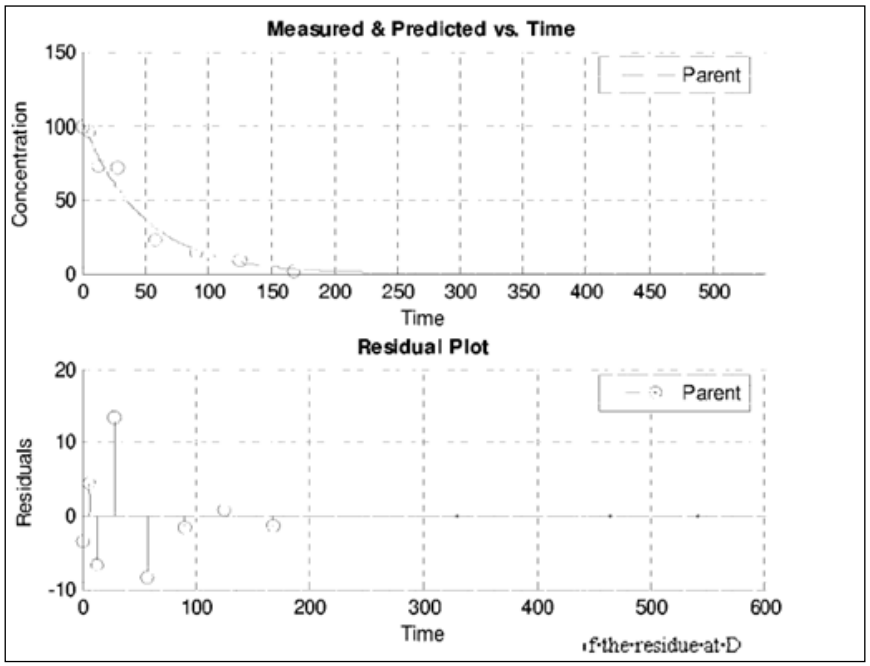


Figure B. 8.1-58: Hill, 1992: Optimised SFO kinetic model for degradation of glyphosate in the Herrngiersdorf soil (parent only)

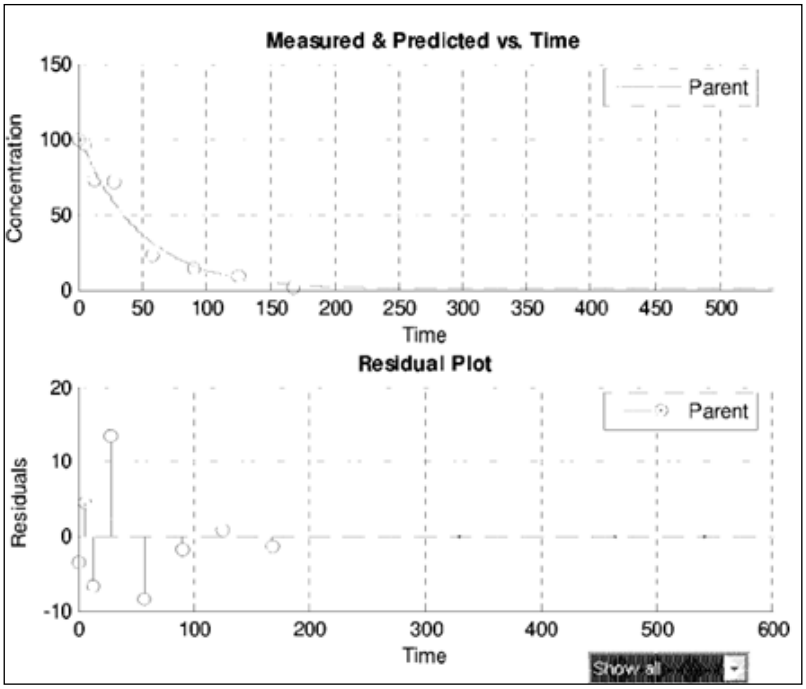


Figure B. 8.1-59: Hill, 1992: Optimised FOMC kinetic model for degradation of glyphosate in the Herrngiersdorf soil (parent only)

Using the SFO model for the parent substance glyphosate, the visual assessment was considered acceptable with a χ^2-error of 10.6 %. The FOMC model was visually acceptable as well with a slightly higher χ^2-error of 11.3 %. Consequently, the SFO model was chosen for trigger end points.

Table B.8.1-135: Kinetic evaluation results of field dissipation study of the study Hill (1992, BVL no 2154352), Herrngiersdorf/Germany, clay loam - persistence and trigger end points of AMPA

AMPA								
Kinetic model pathway fit	**DT_{50} (days)**	**DT_{90} (days)**	**$\chi 2$ error (%)**	**ff**	**Parameter**	**Prob> t**	**Std**	**Visual fit**
SFO-SFO	117.99	391.9	32.4	0.5364	0.0059	0.0156	0.0025	not acceptable
SFO Top down	288.4	958.1	10.98	-	0.0024	0.0251	7.60E-04	acceptable
FOMC Top down	264.3	>1000	12.8	-	alpha 0.711	0.3461	1.554	acceptable
					beta 160.21	0.4013	5.62E+02	
SFO Top down	**288.4**	**958.1**	**10.98**	**-**	**Best fit kinetic (Herrngiersdorf/ Germany)**			

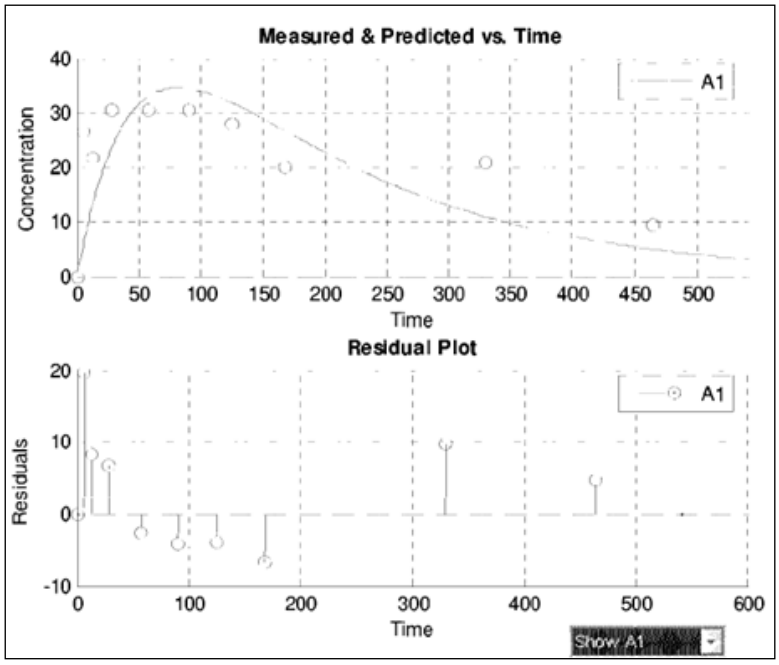


Figure B. 8.1-60: Hill, 1992: Optimised SFO kinetic model for degradation of AMPA in the Herrngiersdorf soil (pathway fit- initial)

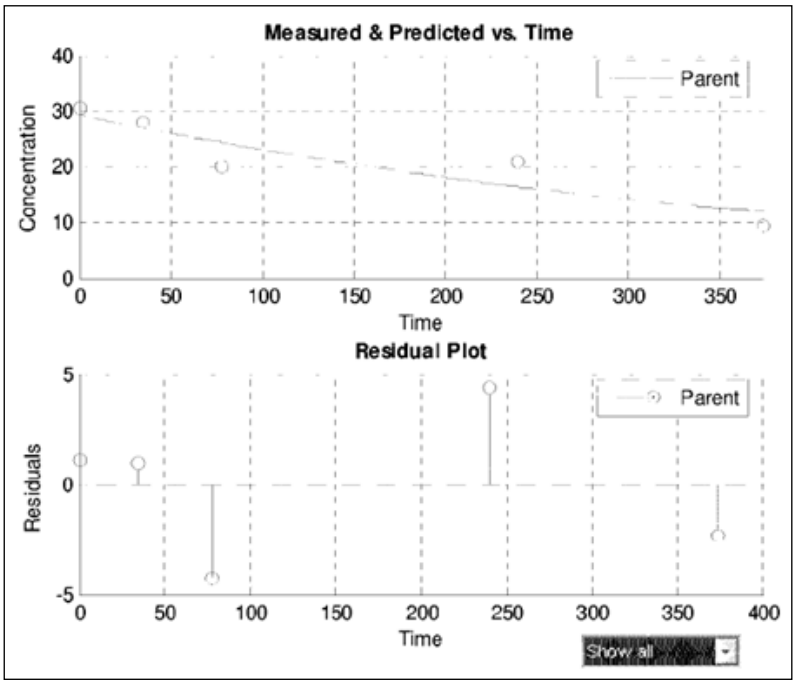


Figure B. 8.1-61: Hill, 1992: Optimised SFO kinetic model for degradation of AMPA in the Herrngiersdorf soil (decline fit)

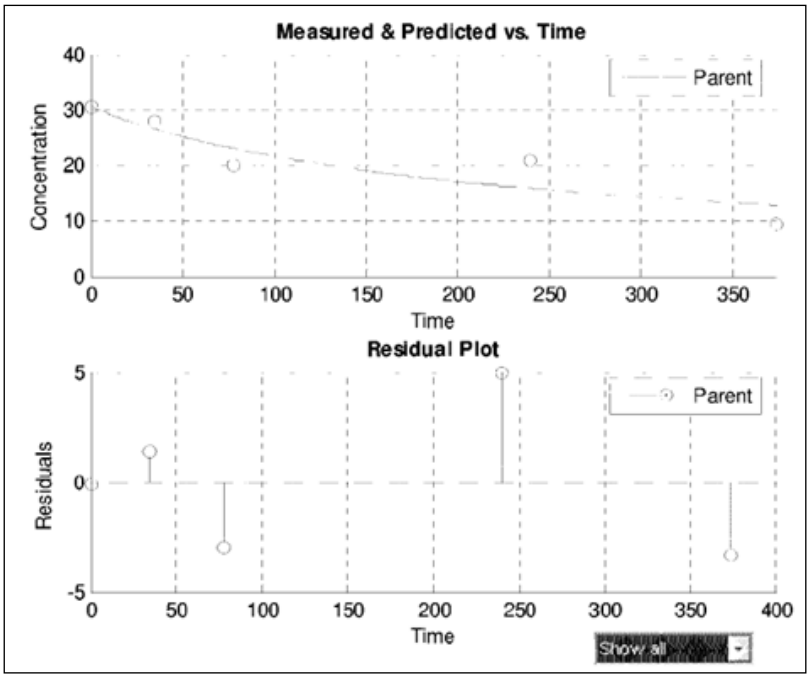


Figure B. 8.1-62: Hill, 1992: Optimised FOMC kinetic model for degradation of AMPA in the Herrngiersdorf soil (decline fit)

The pathway fit for the metabolite AMPA using the SFO model for the parent and for AMPA was considered visually unacceptable due to a poor fit of the decline phase. The χ^2-error was 32.4 %. Therefore, the SFO and the FOMC model were tested using the decline phase only, resulting in acceptable visual fits for both models.

The χ^2-error values were 11.0 % for SFO and 12.8 % for FOMC. Due to the slightly lower χ^2-error, the SFO model considering the decline phase only was chosen for trigger endpoints.

The kinetic evaluation results of field dissipation study of the study Hill (1992, BVL no 2154352) in Wang-Inzkofen/Germany are provided for glyphosate and AMPA in Table B.8.1-136 and Table B.8.1-137, respectively. Visual fits of degradation kinetics for glyphosate are provided in Figure B. 8.1-63 to Figure B. 8.1-66. Visual fit of degradation kinetics for AMPA is provided in Figure B. 8.1-67.

Table B.8.1-136: Kinetic evaluation results of field dissipation study of the study Hill (1992, BVL no 2154352), Wang-Inzkofen/Germany, silt loam - persistence and trigger endpoints of glyphosate

Glyphosate							
Kinetic model	**DT_{50} (days)**	**DT_{90} (days)**	**$\chi2$ error (%)**	**Parameter**	**Prob>t**	**Std**	**Visual fit**
SFO	27.6	91.6	16.8	0.0251	1.50E-04	0.0041	not acceptable
FOMC	17.8	165.5	8.7	alpha 0.975	8.30E-04	0.1969	good
				beta 17.207	1.51E-02	6.3533	
SFO M0 fixed	22.7	75.3	19.1	0.0306	1.90E-05	0.0041	not acceptable
DFOP	18.7	190.3	11.1	k1 0.0578	0.0065	0.0166	good
				k2 0.0055	0.0739	0.0033	
				g 0.714	4.30E-04	0.1167	
FOMC	**17.8**	**165.5**	**8.7**	**alpha 0.975**	**Best fit kinetic (Wang-Inzkofen/ Germany)**		
				beta 17.207			

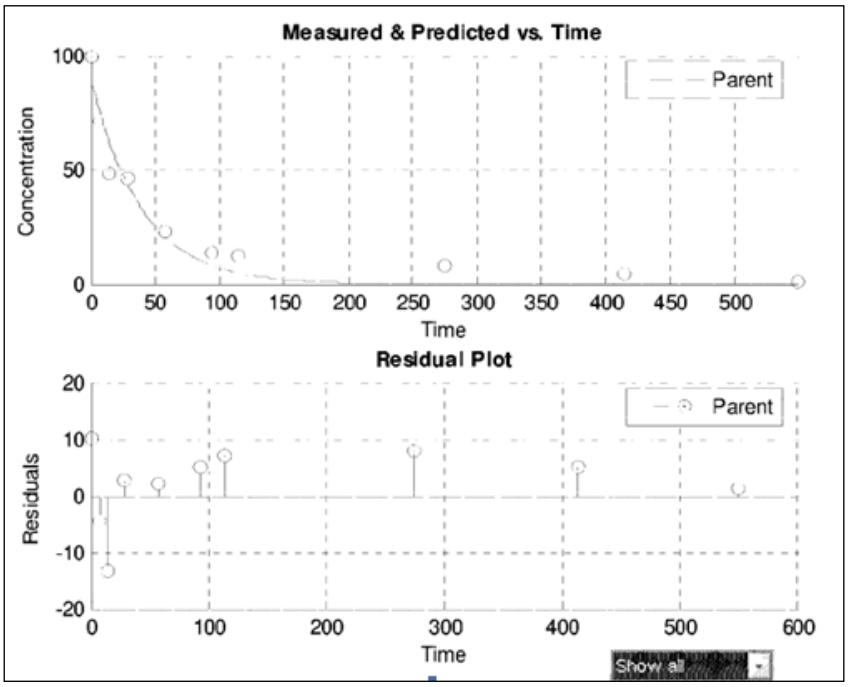


Figure B. 8.1-63: Hill, 1992: Optimised SFO kinetic model for degradation of glyphosate in the Wang-Inzkofen soil (parent only)

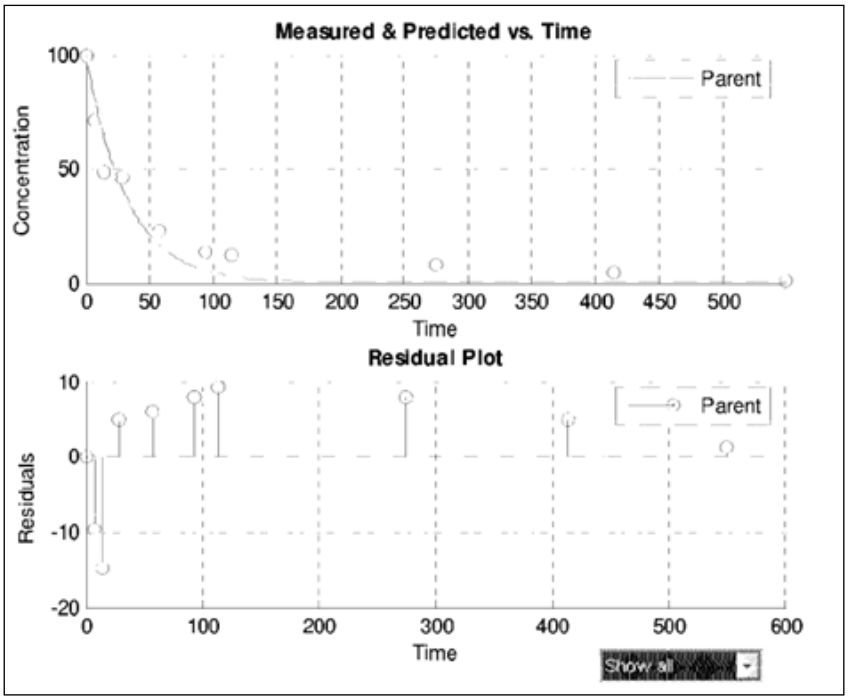


Figure B. 8.1-64: Hill, 1992: Optimised SFO kinetic model for degradation of glyphosate in the Wang-Inzkofen soil (parent only – M0 fixed)

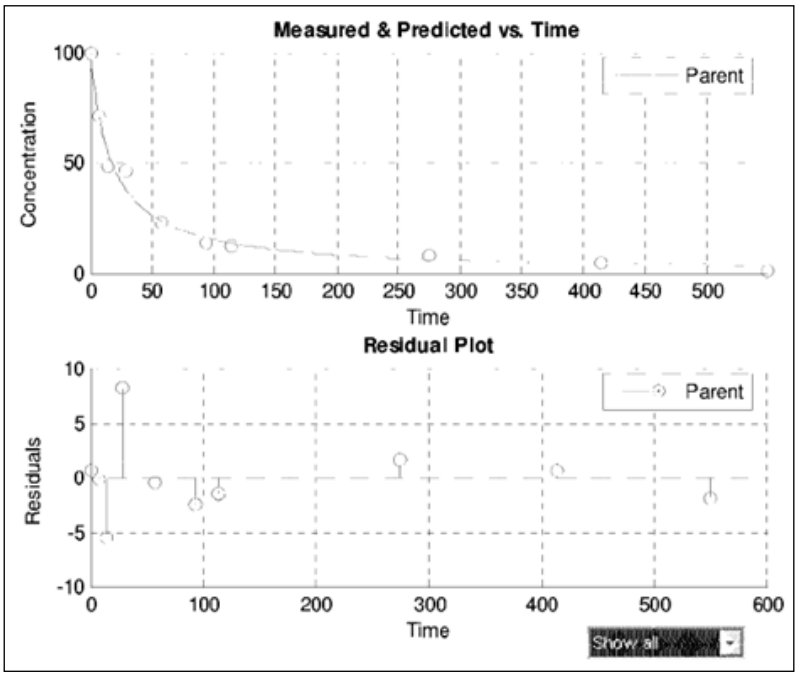


Figure B. 8.1-65: Hill, 1992: Optimised FOMC kinetic model for degradation of glyphosate in the Wang-Inzkofen soil (parent only)

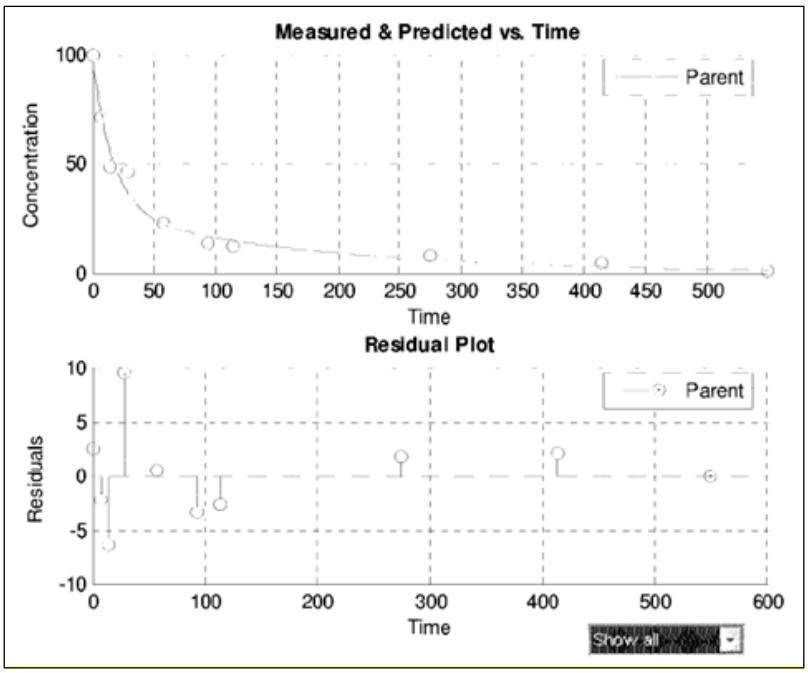


Figure B. 8.1-66: Hill, 1992: Optimised DFOP kinetic model for degradation of glyphosate in the Wang-Inzkofen soil (parent only)

Using the SFO model for the parent substance glyphosate, the visual fit was considered not acceptable due to an underestimation of Mo and a systematic underestimation of the last data points from day 29 onward. The FOMC model revealed an acceptable visual fit with a χ^2-error of 8.7 %. Following the decision scheme of FOCUS (2006, 2011) the SFO model fit was

repeated with a fixed Mo. The modified SFO model fit was still visually not acceptable with a systematic underestimation from day 29 onward. Therefore, the SFO model was rejected and DFOP was tested additionally. The visual assessment of the DFOP model revealed an acceptable visual fit with a χ^2-error of 11.1 %. Due to the slightly better χ^2-error, the FOMC model was chosen for trigger end points.

Table B.8.1-137: Kinetic evaluation results of field dissipation study of the study Hill (1992, BVL no 2154352), Wang-Inzkofen/Germany, silt loam - persistence and trigger endpoints of AMPA

AMPA								
Kinetic model pathway fit	**DT_{50} (days)**	**DT_{90} (days)**	**$\chi2$ error (%)**	**ff**	**Parameter**	**Prob>t**	**Std**	**Visual fit**
FOMC - SFO	283.6	942.3	15.6	0.5466	0.0024	2.00E-04	5.40E-04	good
FOMC - SFO	**283.6**	**942.3**	**15.6**	**0.5466**	**Best fit kinetic (Wang-Inzkofen/ Germany)**			

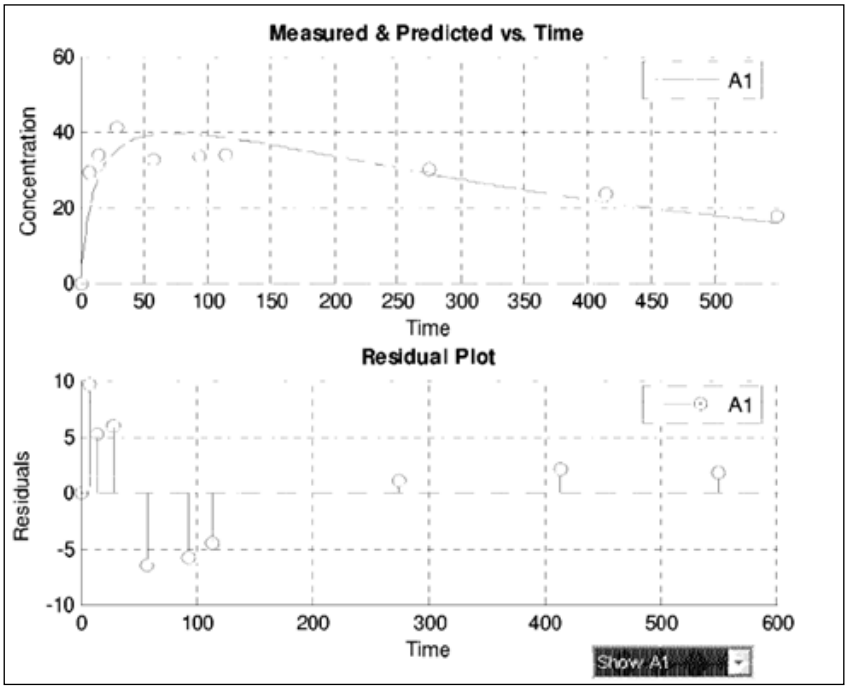


Figure B. 8.1-67: Hill, 1992: Optimised DFOP kinetic model for degradation of AMPA in the Wang-Inzkofen soil (pathway fit- simultaneous)

The pathway fit for the metabolite AMPA using the FOMC model for the parent and the SFO model for AMPA was visually acceptable because the general degradation pattern was displayed well. The χ^2-error was 15.6 %. Therefore, the SFO model considering a pathway fit and using FOMC for the parent was chosen for trigger end points. The formation fraction of AMPA was estimated to 0.5466.

Summary of persistence and trigger end points from field dissipation studies

An overview of the kinetic evaluation results of all field soil dissipation studies for glyphosate and its metabolite AMPA in order to derive DT_{50} and DT_{90} values to be used as trigger end-point and as input parameter in PEC_{soil} calculations is provided in Table B.8.1-138 and Table B.8.1-139.

Table B.8.1-138: Overview of the kinetic evaluation results of field soil dissipation studies for glyphosate (persistence end point and trigger for higher tier studies)

References		Site, country	Characteristics upper soil layer			Persistence endpoint (re-calculated based on FOCUS 2006, 2011)							
			Soil type (USDA)	OM (%)	pH	Glyphosate							
						Kinetic model	DT_{50} (days)	DT_{90} (days)	$\chi 2$ error (%)	Parameter	Prob>t	Std	Visual fit
Studies from the 2001 evaluation	Schulz, 1992a, BVL no 1932128	Diegten, Switzerland	Sandy clay	2.8	7.1	DFOP	6.1	116.1	4.96	k1 0.1437	9.60E-4	0.0139	acceptable
										k2 0.0033	0.059	0.0015	
										g 0.854	3.4E-5	0.0268	
	Schulz, 1992d, BVL no 1932133	Menslage, Germany	Sandy loam	0.4	4.7	DFOP	5.7	200.8	9.4	k1 0.1786	0.0036	0.0353	good
										k2 0.0041	0.0314	0.0016	
										g 0.771	3.1E-5	0.0440	
	Hill, 1992, BVL no 2154352	Buchen, Germany	loamy sand	2.8	6.4	DFOP	40.9	187.3	6.6	k1 0.019	1.40E-04	0.0028	
										k2 2.3E-14	0.5	0.0026	
										g 0.927	1.10E-06	0.0656	
		Kleinzecher, Germany	Sandy loam	1.9	7.0	DFOP	38.3	386.6	11.7	k1 0.0384	2.40E-02	0.0165	good
										k2 0.0037	2.11E-02	0.0015	
										g 0.575	0.0011	0.129	
		Unzhurst, Germany	Loam	1.8	6.7	DFOP	27.7	122.3	8.4	k1 0.0280	2.70E-04	0.0047	good
										k2 8.9E-4	0.3981	0.0033	
										g 0.922	1.7E-6	0.0701	
		Rohrbach,	Silt	1.8	8.5	SFO	20.1	66.9	3.8	k 0.0344	5.90E-09	0.0011	good

		Germany	loam			Top down							
		Herrngiers-dorf, Germa-ny	Clay loam	2.8	8.0	SFO	33.7	111.9	10.6	k 0.0206	1.40E-04	0.0027	good
		Wang-Inzkofen, Germany	Silt loam	2.1	7.2	FOMC	17.8	165.5	8.7	alpha 0.975	8.30E-04	0.1969	good
										beta 17.207	1.51E-02	6.3533	
Worst case kinetic for PEC_{Soil} and as trigger for higher tier studies (n = 8)							**38.3**	**386.6**	**11.7**	**k1 0.0384**	**DFOP Kleinzecher, Germany**		
										k2 0.0037			
										g 0.575			
Maximum with regard to P-criterion (n = 8)							**116.4**	**386.6**			**maximum overall DT90/ 3.32* (DFOP, Kleinzecher)**		
Geomean with regard to P-criterion (n = 8)							**45.2**	**149.96**			**based on overall DT90/3.32***		

* according to EFSA DG SANCO working document on evidence needed to identify POP, PBT and vPvB properties for pesticides from 25.09.2012- rev.3

Table B.8.1-139: Overview of the kinetic evaluation results of field soil dissipation studies for AMPA (persistence end point and trigger for higher tier studies)

References		**Site, country**	**Characteristics upper soil layer**			**Persistence end point (re-calculated based on FOCUS 2006, 2011)**								
			Soil type (USDA)	**OM (%)**	**pH**	**AMPA**								
						Kinetic model	**DT_{50} (days)**	**DT_{90} (days)**	**ff**	**χ2 error (%)**	**Parameter (k)**	**Prob>t**	**Std**	**Visual fit**
	Hill, 1992, BVL no 2154352	Kleinzecher, Germany	Sandy loam	1.9	7.0	DFOP-SFO	514.9	>1000	0.508	15.9	0.0013	0.0102	5.30E-04	good
		Unzhurst, Germany	Loam	1.8	6.7	DFOP-SFO	633.1	>1000	0.332	13.3	0.0011	2.63E-02	5.20E-04	acceptable
		Rohrbach,	Silt	1.8	8.5	SFO	374.9	>1000	n.d.	8.6	0.0018	0.0033	3.60E-04	acceptable

		Germany	loam			Top down								
		Herrngiers-dorf, Germa-ny	Clay loam	2.8	8.0	SFO Top down	288.4	958.1	n.d.	10.9	0.0024	0.0251	7.60E-04	acceptable
		Wang-Inzkofen, Germany	Silt loam	2.1	7.2	FOMC-SFO	283.6	942.3	0.547	15.6	0.0024	2.00E-04	5.40E-04	good
Maximum (n = 5) **for PEC_{Soil}, as trigger for higher tier studies and with regard to P-criterion**							**633.1**	**>1000**		**SFO** **Unzhorst, Germany**				
Arithmetic mean (n = 3)									**0.462**					

Review of the selected end points for persistence and as trigger for higher tier studies

The goodness of the fits presented in Kreschnak (2012, BVL no 2315993) was assessed by the RMS to derive reliable persistence endpoints according to the recommendations of FOCUS degradation kinetics guidance (2006). First, the visual fits of diagrams of estimated and measured values vs. time and of the diagrams of residuals versus time were assessed. Generally, the error at which χ^2-test is passed of less than 15 % as well as a t-test probability of 0.05 was considered to indicate a good fit for soil degradation (FOCUS Kinetics guidance 2006). Additionally, it was checked that the standard errors of the parameters estimated were sufficiently low (at least < 100 %) to assure that these are reliable. However, on a case-by-case basis, the soil degradation endpoints were still be considered acceptable even though one or more of the indices are not met, as long as the endpoint value can be considered conservative, or can be justified based on weight of evidence from other studies.

Persistence end points of glyphosate and AMPA selected by the RMS

The DT_{50} and DT_{90} values of glyphosate derived from field dissipation studies by best fit-kinetics and used as persistence endpoints and triggers for higher-tier experiments range between 6.1 and 40.9 days and between 66.9 and 386.6 days, respectively. The appropriate DT_{50} and DT_{90} values of the metabolite AMPA range between 283.6 and 633.1 days and between 942.39 and >1000 days, respectively.

For the use in PEC_{Soil} calculations the maximum half life value of unnormalised field dissipation studies should be used. In the case of glyphosate in six of eight cases the degradation doesn´t follow first order kinetics, but biphasic models (DFOP and FOMC) were considered best fit kinetics. To select the worst case soil, it is not possible to compare the DT_{50}/DT_{90} values of all soils, but the PEC_{Soil} values of each respective model itself with its kinetic parameters have to be compared afterwards.

As input parameters for PEC_{Soil} calculation of glyphosate the kinetic parameters of the DFOP kinetic (k_1, k_2, g) of the trial Kleinzecher, Germany, should be used in the program ESCAPE 2. The overall DT_{50} and DT_{90} values for this trial are 38.3 and 386.6 days for glyphosate.

The RMS can follow the argumentation of the Notifier concerning the use of default DT_{50} value for soils, for which no reliable degradation rate was able to calculate. If there was no acceptable pathway fit or a clear decline phase for the metabolite AMPA during the study duration, it seems not appropriate to use a default DT_{50} value of 1000 days, because it could demonstrated in other field trials, that a slow degradation of AMPA takes place under field conditions and that reliable half lives could provided in more than four cases. In soils without a decline phase it is only shown, that the formation of the metabolite is higher than its degradation during the duration time of the study, but no information can provided on the rate of degradation of AMPA in these soils. Therefore in the opinion of RMS it is more proper to exclude soils without a clear decline phase from further assessment and to provide the persistence end point for AMPA based on the five soils with reliable half lives according to FOCUS 2006, 2011).

In the peer review process (evaluation table, experts consultation 4.1) RMS was asked to present the kinetic fitting of glyphosate and AMPA to support the expert discussion on including of default DT_{50} of 1000 days in soils without clear decline phase (Schulz 1992 a-d). The statement of GTF, that the apparent persistence could also be due to the continuous replen-

ishment of AMPA from the degradation of glyphosate so that the decline phase of AMPA was not reached during the study period, can be supported by RMS. If the rate of formation of AMPA from glyphosate degradation were to be equal to the rate of degradation of AMPA in soil, this will result in an apparent steady state AMPA concentration in soil but it does not mean that AMPA is persistent and is not degrading. The observed residues of AMPA will be the result of simultaneous formation and degradation of AMPA during the study duration. It was shown in the study of Hill 1992 with longer study duration that AMPA has been degraded with half lives between 284 and 633 d. The study period in Schulz 1992 a-d is too short to derive reliable half lives compared with the study of Hill, in which a similar residue situation at corresponding sample points like in Schulz 1992 was observed. Only if glyphosate was degraded to less than 10 % during study duration, henceforward the clear decline fit of AMPA could shown because of more than 2 available sampling points. The default values of 1000 d would lead to an overestimation of the persistence of AMPA in the field. This approach is supported by results of laboratory studies, which show clear biological degradation with maximum half life of 301 d at 20 °C for AMPA. According to experience studies with the metabolite as parent result in significant lower half lives compared with half lives of the metabolite from degradation studies with parent. Therefore the approach of RMS in the RAR should be assessed as conservative.

As input parameters for PEC_{Soil} calculation of AMPA the kinetic parameters of the SFO kinetic of the trial Unzhorst, Germany, should be used in the program ESCAPE 2. The DT_{50} value for this trial is 633 days for AMPA.

Endpoints with regard to the P criterion of potential POP, vPvB or PBT substances
Persistence end points were derived by the Notifier selecting best fit kinetics. Apart from the fact that the RMS has corrected some endpoints (see above), the RMS recommends using the endpoints from FOMC, HS or DFOP kinetics only to determine whether various aquatic ecotoxicology studies are triggered, but not to decide whether the P criterion is fulfilled. To maintain a conservative approach with regard to the P criterion, the RMS proposes to use recalculated SFO DT_{50} values in cases when biphasic kinetics gave the best fit. Thereby, SFO DT_{50} values should be recalculated from the overall DT_{90} of biphasic kinetics (DT_{90}/3.32) according to EFSA DG SANCO working document on evidence needed to identify POP, PBT and vPvB properties for pesticides from 25.09.2012- rev.3.

Based on the maximum overall DT_{90}/3.32 the DT_{50} value with regard to the P criterion is 116.4 days modelled for the trial Kleinzecher. Based on the geometric mean of all overall DT_{90} /3.32 values the DT_{50} value with regard to the P criterion is 45.2 days.

B.8.1.2.3 Soil residue study

During the 2001 EU review of glyphosate it was concluded that:

> "Soil residues studies for glyphosate are not required because lab DT_{50} of glyphosate is less than one-third of the period between the application and harvest in most cases and absorption by the succeeding crop is not possible."

This conclusion can be confirmed in the renewal process based upon the updated lab DT_{50} values presented in Section 0.

B.8.1.2.4 Soil accumulation studies

During the 2001 EU review of glyphosate it was concluded that:

> "soil accumulation studies are not required for glyphosate because the DT_{90} values for glyphosate in field dissipation studies are in most cases less than 1 year. The differing results for the degradation rates of the relevant metabolite AMPA cause further consideration of its accumulation potential for a confirmatory evaluation."

This conclusion cannot be confirmed in the renewal process based upon the up-dated persistence endpoints derived from field dissipation studies according to FOCUS degradation kinetics (2006, 2011) by Kreschnak (2012, BVL no 2315993) summarised in Table B.8.1-138. The worst case best fit kinetic for glyphosate dissipation in field was calculated from trial Kleinzecher/DE following the DFOP kinetic. This maximum DT_{90} value of 386 days exceeds the trigger of 365 days and justifies the consideration of an accumulation potential of glyphosate in soil. The long-term plateau concentrations in soil (PEC_{Soil}) for the worst-case application scenario of glyphosate in annual and permanent crops were predicted by using the program ESCAPE 2.0 in the section B.8.3. Results are provided in Table B.8.3-8 and Table B.8.3-9.

DT_{90} values of AMPA derived from the glyphosate soil dissipation studies according to FOCUS degradation kinetics (2006, 2011) were generally higher than one year (see Table B.8.1-139. Long-term plateau concentrations in soil (PEC_{Soil}) were predicted for AMPA based on its maximal occurrence in soil degradation studies and the maximum DT_{50}/DT_{90} values of 633/ >1000 days, respectively, for annual and permanent crops considering the worst case application scenario of glyphosate. Results of the calculation with the program ESCAPE 2.0 are provided in Table B.8.3-23 and Table B.8.3-24.

B.8.1.3 Other studies on route and rate of degradation in soil (Review of open literature)

B.8.1.3.1 Route of degradation (open literature)

Soil photolysis (open literature)
Three articles are available on the behaviour of glyphosate when subjected to irradiation in the presence various catalysts in or on a solid phase (solid TiO_2, thermally activated peroxydisulfate, $Fe_3O_4/SiO_2/TiO_2$). Test results are of minor relevance as they are not obtained by a standard test procedure on photodegradation in soil. They can be assessed as additional information only.

To sum up, Echavia et al. (2009) observed a complete (100 %) decomposition of glyphosate within 60 min of irradiation when irradiated in the presence of TiO_2. From photochemical activation of peroxydisulfate in flash-photolysis experiments, the bimolecular rate constants for the reaction of sulfate radical with glyphosate ($1.6 \cdot 10^8\ M^{-1}\ s^{-1}$) was obtained by Kirmser et al. (2010). Photocatalytic degradation of glyphosate in soil by the photocatalyst $Fe_3O_4/SiO_2/TiO_2$ under solar irradiation was investigated by Xu et al. (2011). Results show that 0.5% $Fe_3O_4/SiO_2/TiO_2$ has the best photocatalytic activity. Degradation efficiency of glyphosate reaches 89 % in 2 h when the dosage of photocatalyst is 0.4 g/100 g (soil), and it increased slowly when more photocatalyst was used.

For a more detailed descriptions and the evaluation of the open literature studies, please refer to B.8.10. The respective references are listed there as well.

B.8.1.3.2 Rate of degradation (open literature)

Laboratory studies (open literature)
The degradation of glyphosate in soil under aerobic conditions has been investigated by several authors. It should be noted that most of these publications do not provide sufficient experimental details to evaluate the data quality and validity in these manuscripts. Those publications that do provide details clearly show deficiencies according to the guidance provided by FOCUS (2006, 2011) regarding data quality and data handling that are relevant to the kinetic analysis. These deficiencies include: low or no reported mass balance, lack of duplicate sampling and insufficient valid sampling times. In addition, the majority of these publications do not provide much information in regard to the procedures used for deriving degradation rates that have been reported. Consequently, the results of open literature were not used to derive endpoints or to calculate PEC's.

However, the review of open literature (see B.8.10) shows that glyphosate is degraded through soil micro-organisms. Main metabolic pathway involves the formation of CO_2 and aminomethyl-phosphonic acid (AMPA) which is further degraded to CO_2. AMPA typically represents 15-25 % of the applied glyphosate. Glyphosate mineralization varies extensively, depending on the soil type and biological activity. Glyphosate half-lives (DT_{50}) in laboratory experiments were investigated by several authors. When only those studies are considered studies, which were conducted in line with reliable standard GLP-studies, the DT_{50} values ranged from 9 to 115 days. AMPA is also biodegraded in soil. The DT_{50} values range from 25-75 days under laboratory conditions. In detail, glyphosate degradation in soil is a microbiological process and often shows a high degree of mineralisation. The differences in glyphosate mineralization rate can be attributed to differences in the biological activity of the soils. Gimsing et al. (2004) observed a good correlation between the mineralisation of glyphosate and the abundance of Pseudomonas spp bacteria in soil; although a poor correlation was observed with the overall soil respiration activity. Several authors also reported an inverse correlation with the strength of soil adsorption hypothesising that if glyphosate is strongly adsorbed to soil, it is not bioavailable for degradation. Several authors have also investigated the mineralization of glyphosate in deeper soil layers. Jacobsen et al. (2003) observed that the soil below the ploughing layer had a low potential to mineralise glyphosate (< 10 % CO_2).

For a more detailed descriptions and the evaluation of the open literature studies, please refer to B.8.10. The respective references are listed there as well.

Field studies (open literature)
The dissipation of glyphosate under field conditions has been investigated in several studies. However none of these studies fulfilled the quality criteria of FOCUS degradation kinetics or even the EFSA guidance on soil persistence. Therefore, the results of open literature were not used to derive endpoints or to calculate PEC's.

In detail, the studies performed under outdoor conditions confirm that glyphosate is degraded through the action of soil microorganisms. Main metabolic pathway involves the formation of CO_2 and AMPA, which is further degraded to CO_2. The DT_{50} under typical field conditions ranged from 3-30 days. However, Laitinen et al. (2006) studied the fate of glyphosate in two

Finnish sugar beet cultivated fields for 26 months. The results from this field study demonstrate overwinter persistence of glyphosate residues from an autumn treatment.

For a more detailed descriptions and the evaluation of the open literature studies, please refer to B.8.10. The respective references are listed there as well.

B.8.2 Adsorption, desorption and mobility in soil (Annex IIA 7.4; Annex IIIA 9.3)

B.8.2.1 Adsorption and desorption of the active substance

The adsorption and desorption behaviour of glyphosate in soil was evaluated during the 2001 EU evaluation of glyphosate. One study (Livingston et al., 1986, BVL no 2325589) was evaluated as acceptable based on the evaluation criteria and guidance in force at that time. The adsorption/desorption characteristics of glyphosate resulting from this study have been reported in the Glyphosate Monograph and ranged from 3800 to 60000 mL/g (K_{foc} values). The study of Waring (1992, BVL no 1932008) was also evaluated as acceptable. The K_{doc} values reported in the Glyphosate Monograph ranged from 884 to 50660 mL/g.

There are three additional studies of the adsorption and desorption behaviour of glyphosate in soil available from GTF members but not considered during the 2001 evaluation (Thomas & Lane, 1996, BVL no 2310260; van Noorloos & Slangen, 2001, BVL no 2310257; van der Kolk, 1996, BVL no 2310258), one of which has been evaluated at Member State level already (Thomas and Lane, 1996, BVL no 2310260). Furthermore, there is one more study available (Schneider, 1993, BVL no 1027844) submitted during national authorisation in Germany. Details of these four studies are provided below.

Table B.8.2-1 summarises the results of all compliant adsorption/desorption studies of glyphosate in (including already EU evaluated and additional studies). The K_{foc}/K_{doc} values for glyphosate range from 884 to 60000 mL/g (arithmetic mean: 15844 mL/g). The RMS proposes to use 1/n default values of 0.9 in cases where no reliable 1/n value could be derived in the study and 1.0 in cases where no investigations of the relationship between soil solution concentration and adsorption behaviour were conducted in the study.

Table B.8.2-1: Overview of the glyphosate adsorption/desorption studies

Reference		Guidelines	Soil	Soil type	OC [%]	Clay [%]	Silt [%]	Sand [%]	CEC [mVal/100g]	pH [-]	K_f [mL/g]	K_{foc}/K_{doc} [mL/g]	1/n [-]
Studies from the 2001 evaluation	Living-ston et al. 1986, BVL no 2325589	OECD Test Guideline 106, 1981	Drummer	Silty clay loam	1.45	27.6	56.4	16.0	20.2	6.5 [2)]	324.0	22300	0.92
			Dupo	Silt loam	0.87	14.0	68.0	18.0	8.7	7.4 [2)]	33.0	3800	0.80
			Spinks	Loamy sand	1.10	3.6	22.4	74.0	5.8	5.2 [2)]	660.0	60000	1.16
	Waring, 1992, BVL no 1932008	US EPA Section 163-1	Greenan sand	Sand	0.80	3.0	2.0	95.0	5.0	5.7 [2)]	-	32838 [6)]	1.00 [7)]
			Auchincruive	Sand loam	1.60	13.0	12.0	75.0	12.0	7.1 [2)]	-	50660 [6)]	1.00 [7)]
			Headley Hall	Sandy clay loam	1.40	32.0	21.0	47.0	13.0	7.8 [2)]	-	3598 [6)]	1.00 [7)]
			Californian sandy soil	Loamy sand	0.60	6.0	11.0	83.0	7.0	8.3[2)]	-	884 [6)]	1.00 [7)]
			Les Evouettes II	Silt loam	1.40	11.3	50.7	38.0	15.5	6.1 [2)]	-	3404 [6)]	1.00 [7)]
			Darnconner sediment	Loam	3.00	21.0	40.0	39.0	17.0	7.1 [2)]	-	17010 [6)]	1.00 [7)]
Studies not reviewed in the 2001 evalua-tion or new studies	Thomas & Lane 1996, BVL no 2310260	OECD Test Guideline 106, 1981, US EPA Section 163-1	Lilly Field	Sand	0.29	4.0	4.0	92.0	1.8	5.7 [2)]	64.0	22000	0.75
			Visalia	Sandy loam	0.58	13.0	18.0	69.0	7.3	8.4 [2)]	9.4	1600	0.72
			Wisborough Green	Silty clay loam	2.26	32.0	60.0	8.0	11.9	5.7 [2)]	470.0	21000	0.93
			Champaign	Silty clay loam	2.15	36.0	52.0	12.0	28.3	6.2 [2)]	700.0	33000	0.94
			18 Acres	Sandy loam	1.80	19.0	23.0	58.0	14.4	7.4 [2)]	90.0	5000	0.76
	van Noorloos & Slangen, 2001, BVL no 2310257 [9)]	Commission Directive 95/36/EC Section 7.1.2, US-EPA Section 163-1, OECD Test Guideline 106, 2000	~~Speyer 2.1~~	~~Sand~~	~~0.56~~	~~1.7~~	~~8.8~~	~~90.2~~	~~4.0~~	~~6.0~~ [3)]	~~57.2~~ [4)]	~~10000~~	~~0.90~~ [8)]
			~~Cranfield 115~~	~~Clay loam~~	~~1.70~~	~~32.8~~	~~23.5~~	~~43.7~~	~~19.6~~	~~7.9~~ [2)]	~~216.0~~ [4)]	~~12500~~	~~0.90~~ [8)]
			~~Cranfield 164~~	~~Silt loam~~	~~3.00~~	~~11.1~~	~~72.9~~	~~16.0~~	~~18.1~~	~~7.1~~ [2)]	~~897.0~~ [4)]	~~30000~~	~~0.73~~
			~~Cranfield 243~~	~~Sandy loam~~	~~1.10~~	~~12.1~~	~~16.0~~	~~71.9~~	~~3.3~~	~~5.4~~ [2)]	~~222.5~~ [4)]	~~20000~~	~~0.9~~ [8)]

Table B.8.2-1: Overview of the glyphosate adsorption desorption studies (continued)

Reference		Guidelines	Soil	Soil type	OC [%]	Clay [%]	Silt [%]	Sand [%]	CEC [mVal/100g]	pH [-]	K_f [mL/g]	K_{foc}/K_{doc} [mL/g]	1/n [-]
Studies not reviewed in the 2001 evaluation or new studies	van der Kolk, 1996, BVL no 2310258	OECD Test Guideline 106, 1981	Speyer 2.1 [1)]	sand	0.62	1.9	9.8	88.4	5.0	5.9 [3)]	29.5 [5)]	4762	0.84
			Speyer 2.2 [1)]	loamy sand	2.32	5.5	13.4	81.2	10.9	5.6 [3)]	71.7 [5)]	3091	0.84
			Speyer 2.3 [1)]	loamy sand	1.22	9.5	29.6	60.9	10.2	6.4 [3)]	37.7 [5)]	3092	0.84
	Schneider, 1993, BVL no 1027844	OECD Test Guideline 106, 1981	Soil 2.1	-	0.70	3.5	4.4	92.1	4.9	5.9	-	9486 [6)]	1.00 [7)]
			Soil 2.3	-	1.34	8.3	11.2	80.5	9.5	6.3	-	5709 [6)]	1.00 [7)]
			Soil F3	-	1.20	15.2	14.7	70.1	13.0	7.3	-	4533 [6)]	1.00 [7)]
Arithmetic mean (n = ~~24~~ 20)												**~~15844~~ 15388**	**~~0.914~~ 0.93**
Min (n = ~~24~~ 20)												**884**	**0.72**
Max (n = ~~24~~ 20)												**60000**	**1.16**
pH dependency												**No**	**-**

1) BBA Soil Texture Parametrisation
2) Buffer Solution = H_2O
3) Buffer Solution = $CaCl_2$
4) For this study, the units of the K_f and K_{foc} values were converted from [102 cm^3/g] and [103 cm^3/g] to [mL/g].
5) For this study, the K_{foc} values were not rounded.
6) K_{doc} values determined for a single concentration.
7) default value, due to the fact that no investigations of the relationship between soil solution concentration and adsorption behaviour were conducted in the study
~~8) default value, since no reliable 1/n value could be derived in the original study~~
8) the Pesticides Peer Review Meeting 126 considered that the results of the Van Noorloos & Slangen experiments (2001) should be excluded from the dataset

During the peer review the applicant was asked to provide information on pH dependency of adsorption of glyphosate and AMPA; see reporting table 4(3) from 02.05.2014.

pH Dependency of adsorption coefficient (Koc)
(GTF): Glyphosate comprises of one basic amino function and three ionizable acidic sites. It has a number of pK_as (2.34, 5.73 and 10.2) and therefore exists as multiple species at most pHs, although the dianion predominates at physiologically typical pH 5-9.

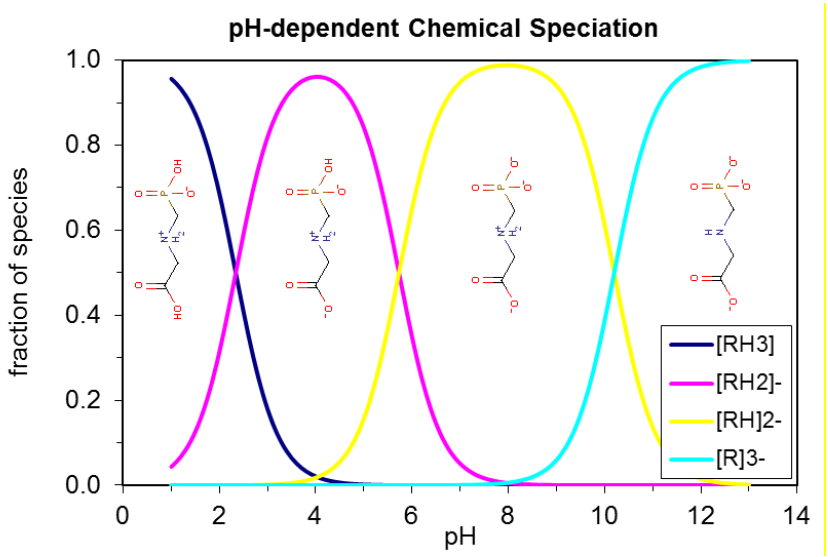


Figure B. 8.2-1: pH dependent chemical specification of glyphosate

A statistical evaluation with simple tools, that evaluate the sigmoid correlation between pH and K_{oc} of glyphosate. is only appropriate for compounds that exist as two distinct species over the pH ranged tested (*e.g.* for simple carboxylic acids). In the opinion of the GTF, standard correlation analysis of glyphosate adsorption with major soil physicochemical properties, including pH, indicates that sorption is not influenced by a single or a dominant soil property (*NB*. correlation of *K*, rather K_{oc}, with pH is more appropriate where sorption is not dominated by organic carbon).

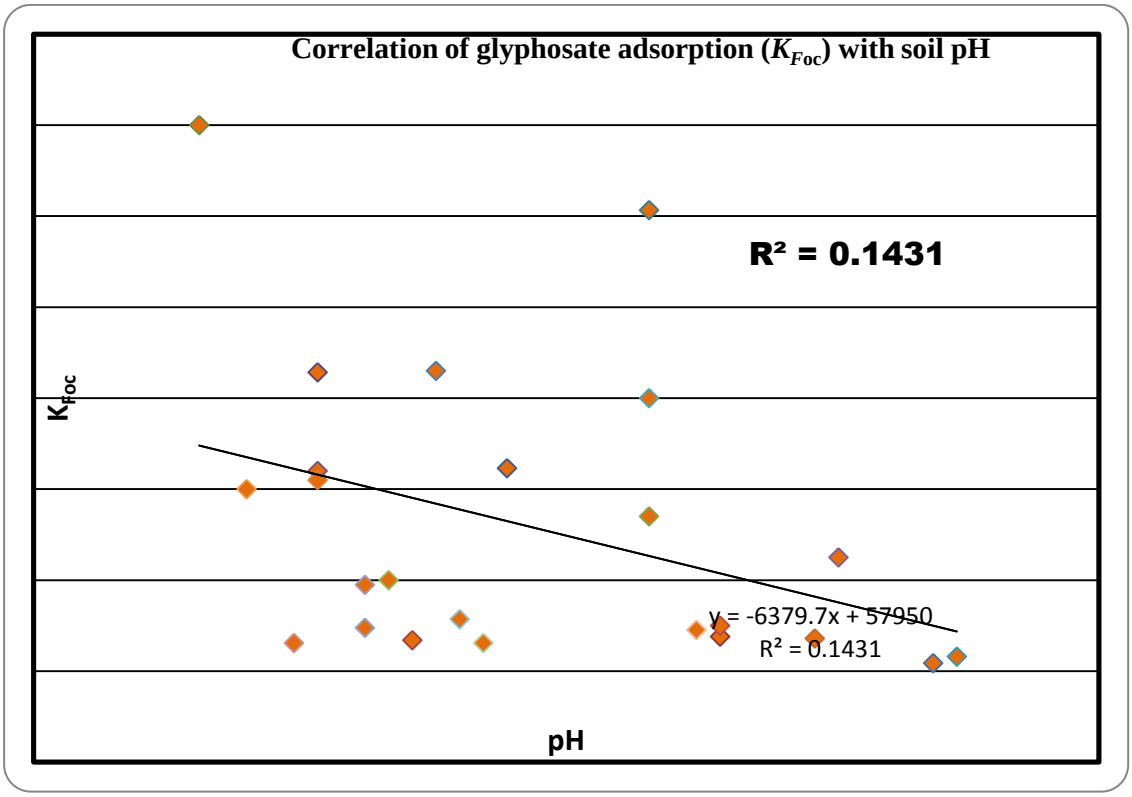


Figure B. 8.2-2. Correlation of glyphosate adsorption (K_{oc} values) with soil pH (H_20)

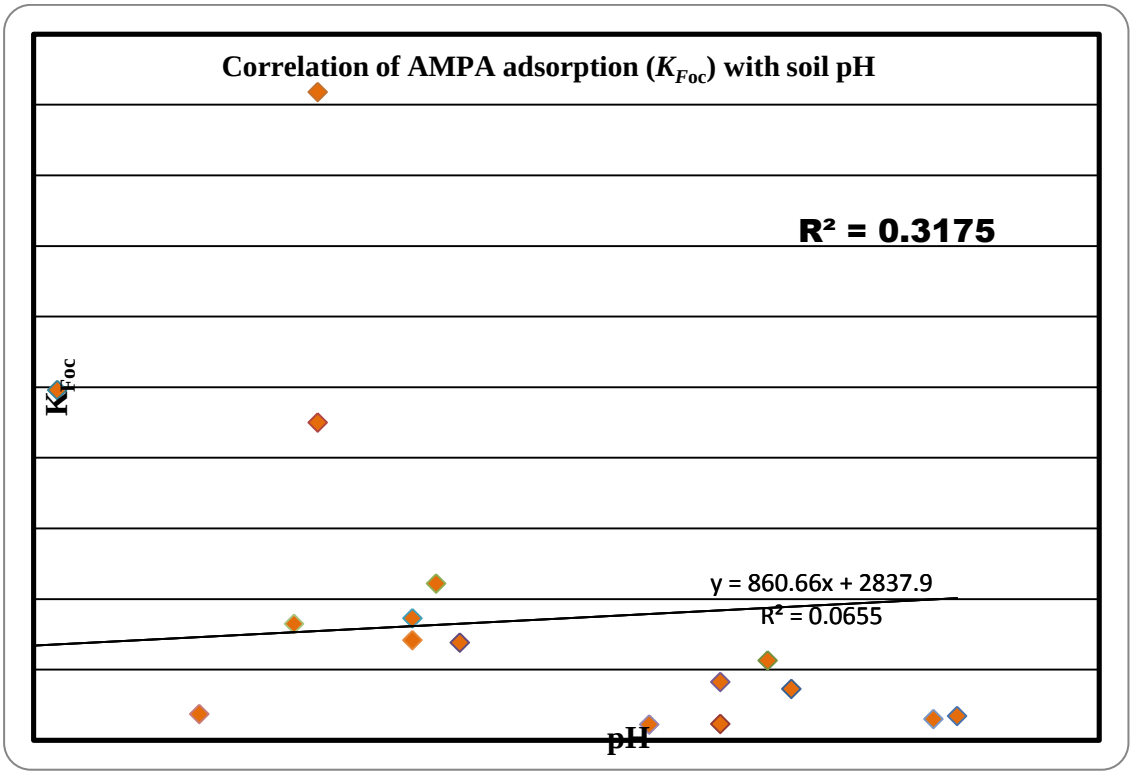


Figure B. 8.2-3: Correlation of AMPA adsorption (K_{oc} values) with soil pH (H_20)

The possibility of pH-dependency of K_{oc} values for glyphosate and AMPA was investigated using Microsoft 'Excel'. The glyphosate and AMPA K_{oc} values were taken from Table B.8.2-1 and Table B.8.2-17 of RAR, respectively. Figure B. 8.2-2 and Figure B. 8.2-3 clearly show lack of correlation between glyphosate and AMPA adsorption coefficients with soil pH values, indicating that sorption is not influenced by soil pH. Lack of pH dependency of glyphosate and AMPA adsorption coefficients is consistent with the scientific weight of evidence and inherent physico chemical properties of these two compounds. Additional support for a lack of pH-dependency on adsorption coefficients is found in the literature review section of RAR (Vol 3 B.8.2.4.1 and Vol 3 B.8.11.3.1) concludes, sorption of glyphosate and AMPA in soil is not influenced by a single or dominating soil physico-chemical property. Although several authors suggested that glyphosate sorption can inversely correlate with the pH of the soil, this is not always the case since increasing pH by liming has been reported[1] to have the opposite effect, i.e. increased glyphosate sorption at increasing pH. Glyphosate and AMPA are unique in that their high affinity for adsorption to soils are influenced by several mechanisms controlled by different soil physico-chemical properties, therefore, no single soil property could predict their adsorption behaviour to soils.

RMS Comment:
The argumentation of GTF regarding the pH dependency of adsorption coefficient of glyphosate and AMPA is considered acceptable by the RMS and will not be considered in further risk assessment.

[1] De Jonge, H., L.W. De Jonge, O.H. Jacobsen, T. Yamaguchi and P. Moldrup. 2001. Glyphosate sorption in soils of different pH and phosphorus content. Soil Science 166: 230-238.

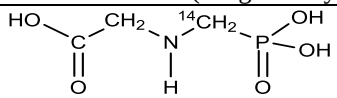
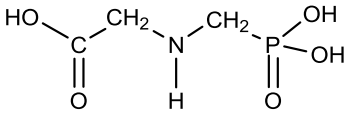
KIIA 7.4.1/1 (van Noorloos and Slangen, 2001, BVL no 2310257)

Annex point:	KIIA 7.4.1/1
Author(s):	van Noorloos, B., Slangen, P.J.
Title:	Adsorption/desorption of glyphosate on soil
Date:	December 10, 2001
Guideline(s):	Commission Directive 95/36/EC amending Council Directive 91/414/EEC. Annex I, Section 7.1.2. Adsorption and desorption, Official Journal of the European Communities no L172 (1995), US-EPA Pesticide Assessment Guidelines, Subdivision N. Chemistry: Environmental fate. Section 163-1. Leaching and adsorption/desorption studies (1982), OECD Guideline no. 106
Deviations:	A preliminary test was conducted with only one soil/solution ratio. No mass balance was carried out during the preliminary test. For Soil II (Cranfield 115), the desorption mass balances of replicates A and B were 78.3 % and 84.6 %, respectively. Nevertheless, the indirect method was used in the main study.
GLP:	Yes
Acceptability:	Yes

Material and methods

The radiolabelled and non-radiolabelled test material is characterised in Table B.8.2-2.

Table B.8.2-2: Test material

	Test material (radiolabelled)	**Test material (non-radiolabelled)**
Common name	N-(phosphonomethyl) glycine (glyphosate, specific activity 1.89 GBq/mmol, 51.20 mCi/mmol (weight assay)	N-(phosphonomethyl) glycine (glyphosate)
Structural formula		
Purity	radiochemical purity 99%; chemical purity 99 %	97.8 %
CAS		1071-83-6
Stability of test compound	Stored in freezer in the dark (expired 1 year after receipt of the test substance)	The substance is stable at room temperature, therefore it was stored at room temperature in the dark.

The adsorption/desorption characteristics of glyphosate was determined in four soil types which represent major agricultural areas – one German origin and three UK origin. The soils used were collected from the top 0 – 22 cm layer in fields that had not been treated with pesticides for at least 18 months before soil were collected for the experiment. Soil physicochemical properties are presented in Table B.8.2-3.

Table B.8.2-3: Soil physicochemical properties

	Soil series name			
Soil property	**Soil I: Speyer 2.1 Speyer standard soil type 2.1**	**Soil II: Cranfield 115 SSLRC Standard soil No 115**	**Soil III: Cranfield 164 SSLRC Standard soil No 164**	**Soil IV: Cranfield 243 SSLRC Standard soil No 243**
Origin	Rheinland-Pfalz, Germany	Worcester, UK	Derbyshire, UK	Warwickshire, UK
Horizon (cm)	20	0-10	15-22	5-15
Soil type (USDA)	Sand	Clay loam	Silt loam	Sandy loam
Clay	1.7	32.76	11.14	12.10
Silt	8.2	23.50	72.91	15.97
Sand	90.2	43.74	15.95	71.93
Organic matter (%)	0.97	2.9	5.2	1.9
Organic carbon (%)	0.56	1.7	3.0	1.1
CEC (meq/100g)	4	19.6	18.1	3.3
pH	6.0 (in aqu. $CaCl_2$)	7.9 (in H_2O); 7.4 (in aqu. KCl)	7.1 (in H_2O); 6.5 (in aqu. KCl)	5.4 (in H_2O); 4.3 (in aqu. KCl)
MWHC (%)	29	55.3	72.8	51.1
Moisture (%) at 1/3 bar	NA	30.4	41.2	22.7

Study design

Experimental conditions

A stock solution containing 10.11 MBq/mL, equivalent to 904 µg/mL ^{14}C-labelled glyphosate in 0.01 M aqueous $CaCl_2$ solution was prepared. In addition, a stock solution containing 2.94 mg/mL of unlabelled glyphosate in 0.01 M aqueous $CaCl_2$ solution was prepared.

For the kinetics experiment a treatment solution was prepared from both stock solutions to afford a final concentration of 10.0 µg/mL of glyphosate. For the isotherms experiment, portions of both stock solutions were mixed to give treatment solutions with concentration ranges of 0.808, 4.04, 20.1, 39.8, and 100 µg/mL of glyphosate. All treatment solutions were used on the day of preparation.

a) Adsorption-desorption kinetics

A preliminary adsorption test was performed with soil I and soil II at an initial substance concentration of 1.0 µg/mL and a soil/solution ratio of 1:2. On the basis of results (97 - 99% adsorption percentage) of preliminary experiments, an optimised soil/solution ratio of 1/50 (0.5 g soil : 25 mL solution) was used in the adsorption-desorption kinetics experiment. Portions of treatment solution were added to two replicates of approximately 0.5 g of each soil sample, resulting in a concentration of approximately 1 µg glyphosate/mL. The mixture was sealed and placed on a shaker at 20 °C ± 2 °C in the dark. Prior to adsorption sampling time (3, 6.75, 24 and 48 hours), soil slurries were centrifuged at 3100 g for 5 minutes at 20 °C and a 100 µL aliquot of supernatant was taken for determination of radioactivity. At the end of the adsorption phase, the remaining supernatant was removed and replaced by an approximately equal, known weight of fresh 0.01 M aqueous $CaCl_2$ solution. The vials were sealed and placed on the shaker in the climate room. At desorption sampling time (3.75, 6.5, 25.25 and

51.75 hours) soil slurries were centrifuged at 3100 g for 5 minutes at 20 °C, and a 100 µL aliquot of supernatant was taken for determination of radioactivity. Appropriate control experiments were also performed to assess potential adsorption to glass test vessels.

b) Adsorption-desorption isotherms
In view of the high grade adsorption observed during kinetics experiments, a soil/solution ratio of 1:100 was chosen in the adsorption/desorption isotherms experiments. The adsorption isotherms experiment was initiated by adding a known volume of treatment solutions to ten pre-equilibrated soil slurries of each soil (two replicates per concentration) in order to provide a test substance concentrations of approximately 0.04, 0.2, 1, 2 and 5 mg/L of glyphosate. The soil slurries were placed on a shaker at 20 ± 2 °C in the dark. After approximately 24 hours of adsorption equilibrium, the soil slurries were centrifuged for 5 minutes at 3100 g at 20 °C, 100 µL of supernatant per replicate was taken for the determination of activity and the remaining supernatant was decanted into glass vials. The decanted supernatant was replaced by an approximately equal volume of fresh 0.01 M aqueous $CaCl_2$ solution. The soil slurries were mixed well and placed on a shaker at 20 ± 2 °C until desorption equilibrium was reached (approximately 24 hours). At desorption equilibrium, soil slurries were centrifuged for 5 minutes at 3100 g at 20 °C. Then, the activity in 100 µL of supernatant was determined.

Analytical procedures
The radiochemical purity of the stock solution and of the isotherms treatment solutions was determined by thin layer chromatography [TLC plate: RP-18; mobile phase: acetonitrile : buffer pH 3 20/80 (v/v)] and HPLC (Partisil SAX column: 250 x 4.6 mm, 10 pm, Waters; UV detector: 195 nm and radio detector; mobile phase: 96 % buffer pH 2.2 (0.859 g KH_2PO_4 in 960 mL water, pH adjusted to 2.2 with phosphoric acid) and 4 % methanol; flow rate: 1.5 mL/min). The concentration of treatment solutions was determined by liquid scintillation counting (LSC) prior to use. The activity of the supernatants of test samples in adsorption/desorption kinetics and isotherms experiments was determined by LSC. Radioactivity in residual soil samples was determined by combustion followed by LSC.

Results and discussion

Mass balance
Mass balances were determined in duplicate for the four soils tested after the desorption stage of the experiment. After the adsorption phase, no mass balances were determined since the soil samples were further used for the desorption stage. However, an acceptable mass balance after the desorption phase indicates an acceptable mass balance after the adsorption phase as well. The mass balances after the desorption stage of the kinetics experiment were in the range of 78 - 94 %.

Transformation of the parent compound
The radiochemical purity of the test substance in the supernatants was determined by HPLC after the adsorption and the desorption step of the isotherms experiment. After adsorption it was in the range 80-97 % (Soils Cranfield 115 and Cranfield 164 < 95 %). After desorption, the radiochemical purity of the test substance in the supernatants of all four soils tested was < 95 %, being as low as 16 % for Soil Cranfield 115. The radiochemical purities of the stock solution (StA) and of the control sample after adsorption kinetics were 95 % and 98 %, respectively.

From these results it appears that glyphosate was not stable during the test and degraded in the presence of soil, primarily during the desorption phase of the isotherms experiment. However, the soils were air-dried and at least nine months old, and had been stored devoid of fresh air. Moreover, the test was performed in aqueous solution (i.e. under anaerobic conditions). Therefore, the soils can be considered to have been biologically inactive. The observation of an apparent degree of degradation of glyphosate during the adsorption phase and a large degree of degradation of glyphosate during the desorption phase suggests an alternative explanation. As at the start of the experiment the radiochemical purity of glyphosate in the treatment solutions was 95 %, 5 % impurity was present which is thought to show adsorption and desorption behaviour differing from that of glyphosate itself. The impurity appears to have adsorbed less strongly than glyphosate and to have desorbed to a much larger extent than glyphosate, causing relatively high concentrations of the impurity in the supernatants and thus apparent lower radiochemical purity for glyphosate in the supernatants.

Findings

a) Adsorption-desorption kinetics
At adsorption equilibrium, K_d, K_{om} and K_{oc} were calculated and at desorption equilibrium, K_{des} was calculated. The amount of material adsorbed to soil at equilibrium ranged from approximately 70 % (Soil I) to 99 % (Soil III). The amount of material desorbed from soil was in the range of 0.5 - 13 %.

Table B.8.2-4: Adsorption/desorption parameters for glyphosate in four soils (soil: solution ratio 1:50)

Soil origin	Soil type (USDA)	pH [2)]	OM (%)	OC (%)	Adsorption			
					K_d (mL/g)[1)]	K_{om} (mL/g)[1)]	K_{oc} (mL/g)[1)]	K_{des} (mL/g)[1)]
Soil I Speyer 2.1	Sand	6.0	0.97	0.56	142.0	15000	25000	331.0
Soil II Cranfield 115	Clay loam	7.9	2.9	1.7	417.0	14000	25000	874.0
Soil III Cranfield 164	Silt loam	7.1	5.2	3.0	4100.0	79000	140000	9150.0
Soil IV Cranfield 243	Sandy loam	5.4	1.9	1.1	1190.0	63000	110000	2210.0

[1)] Mean, rounded values. Calculations were performed with non-rounded values. The units of the K_f and K_{foc} values were converted from [102 cm^3/g] and [103 cm^3/g] to [mL/g] in the tables
[2)] pH in H_2O or $CaCl_2$

b) Adsorption-desorption isotherms
Adsorption isotherms were calculated by linear regression analysis of adsorption or desorption data according to the Freundlich equation.

The Freundlich adsorption coefficients (K_f values) for glyphosate ranged from 56.9–900 mL/g with corresponding K_{foc} values of 10000 – 30000 mL/g for the test soils (Table B.8.2-5). Desorption parameters were determined to be in the range of 139– 1460 mL/g for K_f and of 21000 – 51000 mL/g for K_{foc} respectively (Table B.8.2-6).

Table B.8.2-5: Freundlich adsorption isotherm parameters for glyphosate in four soils

Soil origin	Soil type (USDA)	pH[1)]	OM (%)	OC (%)	Adsorption				
					K_f (mL/g)[2)]	K_{fom} (mL/g)[2]	K_{foc} (mL/g)[2)]	1/n [2)] (-)	r^2 [2)] (-)
Soil I Speyer 2.1	Sand	6.0	0.97	0.56	57.4 56.9	5900.0 5900.0	10000 10000	0.60* 0.60*	0.9879 0.9840
Soil II Cranfield 115	Clay loam	7.9	2.9	1.7	224.0 208.0	7700.0 7200.0	13000 12000	0.67* 0.64*	0.9898 0.9925
Soil III Cranfield 164	Silt loam	7.1	5.2	3.0	894.0 900.0	17000.0 17000.0	30000 30000	0.72 0.73	0.9925 0.9952
Soil IV Cranfield 243	Sandy loam	5.4	1.9	1.1	222.0 223.0	12000.0 12000.0	20000 20000	0.59* 0.59*	0.9886 0.9895

1) pH in H_2O or $CaCl_2$
2) Rounded values. Calculations were performed with non-rounded values. The units of the K_f and K_{foc} values were converted from [102 cm^3/g] and [103 cm^3/g] to [mL/g]
* low Freundlich slope values (< 0.7)

Table B.8.2-6: Freundlich desorption isotherm parameters for glyphosate in four soils

Soil origin	Soil type (USDA)	pH[1)]	OM (%)	OC (%)	Desorption				
					K_f (mL/g)[2)]	K_{fom} (mL/g)[2)]	K_{foc} (mL/g)[2)]	1/n [2)] (-)	r^2 [2)] (-)
Soil I Speyer 2.1	Sand	6.0	0.97	0.56	139.0 148.0	14000 15000	25000 26000	0.71 0.72	0.9967 0.9974
Soil II Cranfield 115	Clay loam	7.9	2.9	1.7	408.0 352.0	14000 12000	24000 21000	0.70 0.67*	0.9897 0.9893
Soil III Cranfield 164	Silt loam	7.1	5.2	3.0	1530.0 1460.0	30000 28000	51000 48000	0.72 0.71	0.9936 0.9953
Soil IV Cranfield 243	Sandy loam	5.4	1.9	1.1	366.0 362.0	19000 19000	33000 33000	0.62* 0.62*	0.9934 0.9937

1) pH in H_2O or $CaCl_2$
2) Rounded values. Calculations were performed with non-rounded values. The units of the K_f and K_{foc} values were converted from [102 cm^3/g] and [103 cm^3/g] to [mL/g]
* low Freundlich slope values (< 0.7)

Conclusions

Based on the adsorption/desorption parameters glyphosate can be considered as immobile in test soils according to the classification scheme of Mensink (1995).

RMS Comment

Deviating from OECD 106, a preliminary test was conducted with only one soil/solution ratio. No mass balance was carried out during the preliminary test.

As described in the study, complete mass balances were obtained in duplicate for all four soils after the desorption kinetics experiment. The mass balances were in the range 78-94 %. With the exception of Soil II (Cranfield 115), for each soil the mass balance of at least one replicate was > 90 %, as specified by the OECD guideline. For Soil II (Cranfield 115), the desorption mass balances of replicates A and B were 78.3 % and 84.6 %, respectively. In the study it is argued that the K_d and K_{oc} for this soil are in the same range as those for the other soils tested. Therefore, it is assumed that (at least) the qualitative results for Soil II are reliable.

Furthermore, glyphosate was not stable during the test and degraded in the presence of soil, primarily during the desorption phase of the isotherms experiment. Especially, for Soil II (Cranfield 115) the radiochemical purity of the supernatants after adsorption/desorption was 80 % and 16 %, respectively.

According to OECD 106 the direct method has to be used in order to quantify adsorption and desorption, if it turns out that the test substance is considered to be unstable in the time and the scale of the test. This aspect was not addressed for the Soil II (Cranfield 115). However, as K_f and K_{foc} for this soil are in the same order of magnitude as those for the other soils tested, RMS proposes to use these values for further risk assessment.

The Freundlich slope values should be assessed according to the ´Clarification note from EFSA concerning data requirements, study guideline, guidance and consensus of member state fate and behaviour experts on soil adsorption measurements and selection of the slope (1/n value) that describe the Freundlich coefficient (2010)´. Values for 1/n are typically in the range from 0.7 to 1.1, though in some valid experiments values outside this range do sometimes occur. FOCUS guidance indicates that an expected average 1/n when looking at a database of a large number of different compounds is 0.9. The RMS considers that the recommendation of the FOCUS guidance to use the often referred to 'FOCUS default' Freundlich slope of 0.9 (average value) is applicable when the experiments attempted to investigate the effect of concentration on adsorption, but a reliable 1/n value could not be derived, as is the case here.

Finally, the RMS proposes to use the K_f/K_{foc} values determined in the study in combination with the FOCUS default' Freundlich slope of 0.9 instead of measured values for the soils I (Speyer 2.1), II (Cranfield 115) and IV (Cranfield 243) for further risk assessment. For the soil III (Cranfield 164) the 1/n value determined in the study can be used.

Outcome of the discussions in the Pesticides Peer Review Meeting 126 (February 2015):

It was also noted that desorped glyphosate was degrading in soil solution within the equilibrium time of batch experiments, though it was noted that the Van Noorloos & Slangen experiment the equilibrium time was longer and more degradation of glyphosate was apparent. On balance the experts considered that the results of the Van Noorloos & Slangen experiments should be excluded from the dataset as the longer batch equilibrium time (compared to other investigations or investigations where soils were sterilised) meant that degradation of glyphosate that occurred during the study resulted in lower confidence in these data.

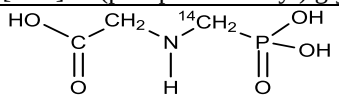
KIIA 7.4.1/2 (Thomas and Lane, 1996, BVL no 2310260)

Annex point:	KIIA 7.4.1/2
Author(s):	Thomas, P.K., Lane, M.C.G.
Title:	Glyphosate Acid: Adsorption and Desorption Properties in 5 Soils
Date:	September 12, 1996
Guideline(s):	OECD Guideline no. 106, US-EPA Pesticide Assessment Guidelines, Subdivision N. Chemistry: Environmental fate. Series 163-1. Leaching and adsorption/desorption studies (1982)
Deviations:	Only one soil ('Visalia') was used for the pre-liminary study.
GLP:	Yes
Acceptability:	Yes

Material and methods

The radiolabelled test material is characterised in Table B.8.2-7.

Table B.8.2-7: Test material

	Test material (radiolabelled)
Common name	[14C] N-(phosphonomethyl) glycine (glyphosate specific activity 1.67 GBq $mmol^{-1}$)
Structural formula	
Purity	95% (by TLC)
Stability of test compound	Assumed to be stable, as shown by subsequent analysis of the various extracts after equilibrium

The adsorption/desorption characteristics of glyphosate were determined in five soil types – three UK origin and two USA origin – using the batch equilibrium method. The soils were air dried and passed through a 2 mm sieve. Each soil sample was gamma irradiated (25-40 kGy) to eliminate any living organisms within the soil. Soil physicochemical properties are presented in Table B.8.2-8.

Table B.8.2-8: Soil physicochemical properties

	Soil series name				
Soil property	**'Lilly Field'**	**'Visalia'**	**'Wisborough Green'**	**'Champaign'**	**'18 Acres'**
Origin	Wishanger Farm, Churt, Surrey, UK	WRTC, 498 N. Mariposa Avenue, CA 93292, USA	Naldretts Court, Wisborough Green Sussex, UK	NRTC, 495 County Road 1300N, IL 61821, USA	Jealotts Hill Farm, Warfield, Berkshire, UK
Soil type (USDA)	Sand	Sandy loam	Silty clay loam	Silty clay loam	Sandy loam
Sand (%)	92	69	8	12	58
Silt (%)	4	18	60	52	23
Clay (%)	4	13	32	36	19
Organic matter (%)	0.5	1.0	3.9	3.7	3.1
CEC (meq/100g)	1.8	7.3	11.9	28.3	14.4
pH in H_2O	5.7	8.4	5.7	6.2	7.4
Moisture	3.1	10.4	30.9	22.7	17.1

Holding Capacity 1/3 bar (%)					
Moisture Holding Capacity 15 bar (%)	1.1	4.8	19.8	13.5	10.4

Study design

Experimental conditions

All solutions of glyphosate free acid were prepared and stored in plastic vials, due to adsorption affinities of glyphosate to glass. A stock solution was prepared in water. The exact radiochemical content was detected using liquid scintillation counting (LSC).

All aqueous $CaCl_2$ solutions used in this study were sterilised by autoclaving prior to use. All soils were sterilised by gamma irradiation prior to use. Soil slurries were prepared with soil:water (0.01M $CaCl_2$) ratios of 1:20.

In a preliminary experiment with 'Visalia' soil, the time taken for ^{14}C-glyphosate (0.2 µg mL^{-1}) to equilibrate between soil and solution phases was studied over a period of 48 hours. After 4 hours the approximate equilibrium plateau of the ^{14}C-glyphosate distribution between soil and aqueous phases was reached. Furthermore, it was shown that even though the aqueous and soil phases had been sterilised the parent was degrading. Thus, it was considered to use an equilibrium time of 4 hours (as short as possible) to limit the degradation of glyphosate. The desorption step was carried out overnight.

a) Adsorption experiment
The adsorption studies were carried out with air dried soils samples (equivalent to 1.0 g oven dry weight, four replicates) weighed directly into Teflon centrifuge tubes with self-sealing caps.

Prior to ^{14}C-glyphosate treatment, each soil sample was equilibrated overnight in 0.01 M $CaCl_2$ (approximately 19 mL) by continuous mixing on an end-over-end shaker at approximately 1200 revolutions per hour (rph) at 20 ± 2 °C.

An appropriate volume of the stock solution was added to a 0.01 M $CaCl_2$ solution in a way that when aliquots (1 mL) of the prepared treatment solutions were added to the soil slurries, the required concentration of glyphosate in the aqueous phase would be achieved (0.05, 0.1, 0.2, 1.0, 2.0 µg mL^{-1}). The final soil:solution ratio was 1:20. The tubes were placed on an end-over-end shaker and continuously mixed at 1100 rph for 4 h at 20 ± 2 °C.

Additional samples were generated as treatment controls (no soil) and soil blanks (no glyphosate).

After 4 h of shaking the slurries were centrifuged at 3500 rpm (1625 g) for 15 minutes. Aliquots of the supernatants (17 mL) were decanted and, if necessary, filtered. Sub-samples were taken and analysed for radiochemical content by LSC. The remainder of the supernatant was stored at -20 ± 5 °C until further analysis.

b) Soil extraction
Glyphosate was extracted from the remaining soil after the removal of the adsorption supernatant (for 2 replicates). To each tube a phosphate buffer solution (pH 2.1, 1 mol L^{-1}, 3 x 10 mL) was added. Between each addition, the soil slurry was agitated on a reciprocating shaker at 120 rpm for 10 minutes and then centrifuged at 3500 rpm for 15 minutes. Subsequently the supernatant buffer solution was decanted. The residual soil was washed with acetone (2 x 2.5 mL), using a similar technique. The buffer extracts and acetone washes were pooled in polythene bottles. These combined extract solutions were then made up to volume with distilled water (50 mL). If required, these soil extract solutions were filtered. The extracted residual soil samples were dried in a fume cupboard at room temperature and the radioactivity remaining in the soil was determined by combustion.

c) Desorption experiment
After adsorption, two replicates were used for a single desorption step. After the initial centrifugation (described in a) Adsorption experiment) and decantation, the volumes of the supernatants were replaced with equivalent volumes of fresh sterile 0.01 M $CaCl_2$ solution. The samples were re-equilibrated on an end-over-end shaker (1100 rph) for 21 hours at 20 ± 5 °C. Once again, the same volumes of supernatant were removed and aliquots were taken for LSC analysis. All supernatant phases were frozen at -20 ± 5°C until further analysis. The soil residues were extracted in the same manner as described for the adsorption samples.

Analytical procedures
All analyses were carried out on individual replicate samples of both the adsorption and desorption supernatants and their respective soil extracts.

The radiochemical content was determined using LSC (LKB 1217 spectrophotometer; LKB Instruments Ltd.) with 'Optiphase Safe' (LKB, Sweden) as scintillation cocktail.

Liquid samples were concentrated by evaporation under a stream of air and an aliquot of the concentrate was analysed by thin layer chromatography (Dowex 50W X8/Cellulose 6/4 plates developed in a 0.1 M HCl solvent system). Autoradiographic images of the developed chromatograms were made using a Fuji bas2000 bio-imaging system.

All extracted residual soil samples were dried in a fume cupboard at room temperature and the radioactivity remaining in the soil determined by combustion (Harvey OX300 Biological Oxidiser). The $^{14}CO_2$ evolved was trapped in 2-methoxyethylamine (part of 'Optiphase Safe'/ 2-methoxyethylamine/distilled water, 50/25/2, v/v/v) and radioassayed by LSC. The recovery of the applied radiochemical was greater than 90 % on all occasions and was used as a correction factor for the combusted samples.

Results and discussion

Mass balance
Recoveries of radioactivity from the various samples assessed ranged from 84-105 % and averaged 96 % of that nominally applied.

Transformation of the parent compound
Results of analysis of aqueous supernatants and soil extracts from adsorption and desorption experiments demonstrated that there had been some degradation of the glyphosate acid during the equilibration process, even though the soil and the aqueous phases had been sterilised pri-

or to use. The single major metabolite was shown to be aminomethylphosphonic acid (AMPA) via co-chromatographic TLC analysis. Aliquots from the aqueous supernatants and the soil phases showed overall similar amounts of AMPA (4.0-33 %). The chromatography of the soil phase was difficult and led to an average of 6 % of applied radioactivity being entrained as baseline of the plates. It is possible that much of this material was either glyphosate or AMPA. In the calculation of the adsorption coefficients it was assumed that all radioactivity present was glyphosate. This assumption might tend to slightly overestimate (< 10 %) the actual partition coefficients for glyphosate.

Findings

a) Adsorption experiment

At adsorption equilibrium, the K_d and K_{oc} values and the percentages of applied radioactivity adsorbed were calculated for each single concentration. Average K_d values in the soils ranged from 18 in the low organic matter and clay content soil 'Visalia' to 1000 in the higher organic matter and clay content soil 'Champaign'. The corresponding K_{oc} values ranged from 3100 in 'Visalia' to 58000 in 'Lilly Field.

Adsorption isotherms were calculated by linear regression analysis of adsorption data according to the Freundlich equation. Freundlich adsorption coefficients (K_f) ranged from 9.4 to 700 and the organic carbon normalised Freundlich coefficients (K_{foc}) from 1600 to 33000. The Freundlich equation showed a good fit for all soils tested, with r^2 values equal to or greater than 0.98. 1/n values ranged from 0.72 to 0.94, demonstrating a decrease in adsorption with increasing rate of application. No saturation of adsorption sites was found at the highest rate of application. These data are consistent with a decrease in K_d values with increasing rate of application.

In the majority of soils, more than 85 % of the applied chemical was adsorbed with the exception of soil 'Visalia' in which adsorption was still strong with an average of 44 %.

Table B.8.2-9: Adsorption parameters for glyphosate in five soils (soil:solution 1:20)

Soil origin	pH[1)]	OM (%)	Adsorption						
			K_d [2)]	K_{oc} [2)]	K_f	K_{foc}	1/n	r^2	% AR adsorbed[2)]
'Lilly Field'	5.7	0.5	170	58000	64	22000	0.75	0.99	87
'Visalia'	8.4	1.0	18	3100	9.4	1600	0.72	0.99	44
'Wisborough Green'	5.7	3.9	680	30000	470	21000	0.93	1.00	97
'Champaign'	6.2	3.7	1000	47000	700	33000	0.94	0.98	98
'18 Acres'	7.4	3.1	230	13000	90	5000	0.76	1.00	91

1) pH in H2O
2) Mean, rounded values. Calculations were performed with non-rounded values.

a) Desorption experiment

Average K_d values after the desorption step ranged from 32 in the low clay and organic matter content soil 'Visalia' to 1600 in the higher organic matter and clay content soil 'Champaign'. The K_d, K_{oc} and K_{foc} values after desorption were all higher than those after the adsorption step, with a large increase (i.e. a 95 % average increase across all soils and rates of application).

Table B.8.2-10: Desorption parameters for glyphosate in five soils (soil:solution 1:20)

Soil origin	pH[1)]	OM (%)	Desorption			
			K_d [2)]	K_{oc} [2)]	K_{foc}	% increase in K_d (adsorption to desorption)[2)]
'Lilly Field'	5.7	0.5	460	160000	50000	170
'Visalia'	8.4	1.0	32	5600	3000	96
'Wisborough Green'	5.7	3.9	1300	55000	21000	80
'Champaign'	6.2	3.7	1600	74000	56000	60
'18 Acres'	7.4	3.1	400	22000	6600	68

1) pH in H2O
2) Mean, rounded values. Calculations were performed with non-rounded values.

Conclusions

Using the McCall Classification scale to assess a chemical´s potential mobility in soil, glyphosate can be classified as having low mobility or being immobile, based on the adsorption results obtained. However, high K_{foc} desorption values led to the conclusion that adsorption of glyphosate was generally not very reversible, resulting in further reduction in the potential mobility of the compound. Thus, the potential mobility of glyphosate has been further reduced from having a 'slight' potential mobility and being immobile.

RMS Comment

Deviating from the OECD 106 specifications, only one soil ('Visalia') was used for the preliminary study.

The stability of glyphosate free acid was checked via LSC and TLC during this phase and the compound was found to degrade during the equilibration process. The compound degraded primarily into AMPA with up to 15 % of the aqueous and soil phases shown to be AMPA after 48 hours of equilibration. As outlined in OECD 106 the study was continued, taking into account the instability of the test compound by analysing both phases (aqueous phase and soil phase) in the main study. Thus, it was decided that in order to minimise the influence of the degradation on adsorption coefficients an equilibration time of 4 hours for the main study was the best compromise.

Furthermore, the chromatography of the soil phase was difficult and led to an average of 6 % of applied radioactivity being entrained as baseline of the plates. It is possible that much of this material was either glyphosate or AMPA. In the calculation of the adsorption coefficients it was assumed that the radioactivity present was glyphosate. This assumption might tend to slightly overestimate (< 10 %) the actual partition coefficients for glyphosate. In the study, it is argued that the adsorption coefficients are so high that this variation will make a negligible difference to the potential mobility or bioavailability of the compound in the environment.

The RMS also considers that adsorption may have been overestimated due to the analytical difficulties in the soil phase. On the other hand a short an equilibration time of 4 hours is very conservative and could led to an underestimation of the adsorption. However, it is likely that both variations will make negligible difference to the potential mobility of glyphosate. Since the resulting K_f/K_{foc} values of the study are in same range as those from the other valid studies regarding the adsorption /desorption behaviour of glyphosate in soil, the RMS suggests using the results of the present study for further risk assessment.

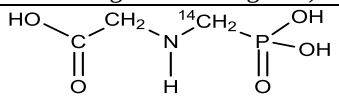
KIIA 7.4.1/3 (van der Kolk, 1996, BVL no 2310258)

Annex point:	KIIA 7.4.1/3
Author(s):	van der Kolk, J.
Title:	Glyphosate: determination of adsorption and desorption properties based on the OECD method 106
Date:	April 26, 1996
Guideline(s):	OECD Guideline no. 106
Deviations:	Even though a preliminary test was performed and glyphosate was found to be stable in all soils within the first 5 hours, HPLC analysis of the supernatants showed that in the Speyer 2.3 soil, degradates were present after 5 hours of the advanced isotherm test (adsorption). Furthermore, HPLC analysis of supernatant showed that after the first desorption step and even more after the second desorption step, most of the radioactivity present in the Speyer 2.3 sample consisted of degradates (mainly AMPA). At the first desorption step 65.9 % of the radioactivity found by HPLC in the Speyer 2.3 soil supernatant were degradates. At the second desorption step it was 71.8 %. With knowledge concerning the premilinary test, the indirect method was used in the main test.
GLP:	Yes
Acceptability:	Yes

Material and methods

The radiolabelled test material is characterised in Table B.8.2-11.

Table B.8.2-11: Test material

	Test material (radiolabelled)
Common name	[^{14}C] N-(phosphonomethyl) glycine (glyphosate, specific activity 316 µCi/mg at a molar weight of 171 mg/mol)
Structural formula	
Purity	99.6% (radiochemical purity)
Stability of test compound	The stability was proven by HPLC.

The soil physicochemical properties are summarised in Table B.8.2-12.

Table B.8.2-12: Soil physicochemical properties

Soil type	**Common name**	**Sand (%)**	**Silt (%)**	**Clay (%)**	**pH**	**CEC (meq/ 100g)**	**OC (%)**	**MWC (g/100 g dry soil)**
Sand	Speyer 2.1	88.4	9.8	1.9	5.9	5.0	0.62	31
Loamy sand	Speyer 2.2	81.2	13.4	5.5	5.6	10.9	2.32	48
Loamy sand	Speyer 2.3	60.9	29.6	9.5	6.4	10.2	1.22	39

Study design

Experimental conditions

a) Adsorption study

Twelve samples of each soil type were prepared by adding 25 ml aliquots of the appropriate stock solution of ^{14}C-glyphosate treatment solution in $CaCl_2$ (0.01 mol/L) to 5 g of pre-conditioned soil (dry weight) in tare tubes to obtain concentrations of 0.04, 0.19, 0.98 and 4.66 mg/L. In addition, three further samples were prepared as control samples for each soil as indicated above, but using blank 0.01 M $CaCl_2$.

For each soil type and concentration of glyphosate, triplicate samples were used except for the highest concentration where two additional samples were set up for each soil to determine the material balance.

The samples were briefly hand-shaken after dosing and then placed on a shaker to ensure that the soil was kept in motion continuously for the duration of the equilibrium period of 5 hours determined in the preliminary test at 20 ± 1 °C. The tubes were weighed and centrifuged at 2000 rpm for 10 minutes. The supernatants were decanted and the volumes determined after which they were transferred to brown crimp cap vials. Two 500 µL samples were taken for LSC analysis. The rest of the supernatant was stored at -20 °C.

The amount of test substance adsorbed onto soil was obtained from the difference between initial and final amount of the substance in aqueous phase. The test solution remaining in soil after a saturation step with $CaCl_2$, was evaluated to determine the adsorbed concentration.

b) Desorption study
As mentioned above, the amount of supernatant after an adsorption step was measured to determine the amount of test solution retained. Although it was expected that the concentration of glyphosate in the interstitial water was very low, it was recorded for further calculations. Thus the initial test substance present before the first desorption step consisted of test substance adsorbed by the soil and the test substance in residual water.

After most of the supernatant was removed, it was replaced with a fresh aliquot of 25 mL of 0.01M $CaCl_2$ solution. The Teflon® centrifuge tubes were hand-shaken vigorously to break up the soil packed at the bottom, and then shaken on a shaker for a pre-determined equilibrium time of 24 hours. Tubes were then removed from the shaker and centrifuged. The supernatants were decanted and the volume was measured. Then the supernatants were transferred to brown crimp vials. Two 500 µL samples were taken for LSC analyses.

Analytical procedures
The two additional samples per soil prepared at the highest concentration were analysed by LSC after the adsorption step. After transferring the supernatant, the wet soil was extracted three times with 12.5 mL, 0.09 M CaCl2/0.35 M H_3PO_4. For the second extraction, after addition of extraction solution samples were stored overnight at -20 °C. Then the extracts were centrifuged for 10 minutes at 2000 rpm and the amount of test substance was quantified by LSC.

To determine if any un-extractable radioactivity remained in the soils, triplicate 0.5 g portions of the soils were transferred into pre-weighed Combusto-Cone® (a pressed, cellulose-based material, Packard Instrument Company). The samples were placed in a Packard Model 306 sample oxidiser and combusted. The resulting $^{14}CO_2$ was trapped as carbonate salt in Carbosorb® and Permafluor V® and the vials were placed in the LS counter and the dpm determined.

Recovery rates of the oxidiser were determined prior to analysing each set of samples by combusting and counting the activity of a standard reference material and comparing the measured values to that of a concurrently fortified standard. To verify whether apart from ^{14}C-glyphosate any degradate was present in the test solutions, HPLC analysis was performed.

Results and discussion

Mass balance
The mean recoveries accounting for glyphosate were 95.3 %, 96.0 % and 95.8 % for Speyer 2.1, Speyer 2.2 and Speyer 2.3 soils, respectively.

Transformation of the parent compound
HPLC analysis of 4.66 mg/L supernatants showed that in the Speyer 2.3 soil, degradates were present after 5 hours. However, no degradates were found for the Speyer 2.1 and Speyer 2.2 soils.

Findings
a) Adsorption
The adsorption/desorption data were fitted to a Freundlich-type isotherm and show a marked linear behaviour for this range of concentrations. The results are presented below:

Table B.8.2-13: Freundlich adsorption/desorption constants of glyphosate in three different soils

Soil	OM (%)	OC (%)	pH	Adsorption				First desorption			
				K_f 1)	K_{foc} 1)	1/n	r^2	K_f 1)	K_{foc} 1)	1/n	r^2
Speyer 2.1	1.1	0.62	5.9	29.52	4762	0.843	0.997	50.7	8178	0.910	0.999
Speyer 2.2	4.0	2.32	5.6	71.72	3091	0.840	0.997	118.1	5089	0.878	0.999
Speyer 2.3	2.1	1.22	6.4	37.72	3092	0.837	0.997	39.59	3245	0.872	0.999

1) mL/g

The calculated K_{foc} values were 4762, 3091 and 3092 for Speyer 2.1, Speyer 2.2 and Speyer 2.3 soil, respectively, yielding a mean K_{foc} value of 3648 ± 964 (± 26 %). The linearity of K_{foc} values indicate that adsorption must have taken place predominantly onto the organic fraction of these soils i.e. humic and fulvic acids. However, a variation of 26 % indicates that clay fraction and iron oxides also play a relevant role.

The mobility of glyphosate was obtained by applying the approach reported by Hamaker (1975) and the calculated R_f-values are as follows:

Table B.8.2-14: R_f values and Tentative classes according to Hamaker (1975)

Soil	R_f-value (corrected)	Tentative class
Speyer 2.1 (sand)	0.16	2
Speyer 2.2 (loamy sand)	0.15	2
Speyer 2.3 (loamy sand)	0.16	2

For the three soils and the range of concentrations tested, results indicate that glyphosate has little to no mobility according to the approach reported by Hamaker.

Conclusions

The adsorption of glyphosate reaches equilibrium after about 5 hours. Under the chosen experimental conditions the adsorption of glyphosate increased linearly with increasing initial concentration in solution and organic carbon content. Hence, extrapolation to soils with similar organic carbon content is possible for the range of concentration used.

Based on the results obtained (mean adsorption K_{oc} for 3 soils: 3648 L/kg), glyphosate can be classified as practically immobile if adsorption to soil is considered as the sole criterion for an assessment of pesticide movement on soil. Glyphosate desorption in soils is very low.

RMS Comment

As outlined in OECD 106, a preliminary study was conducted in order to determine the time to reach equilibrium. Thereby, 3 soils and the highest concentration of glyphosate (5.0 mg/L (nominal), 4.69 mg/L (measured) were used. The adsorption process reaches an apparent equilibrium up to 24 hours. HPLC analysis showed that no glyphosate had degraded within the first five hours in any of the soils. Degradates were found in the Speyer 2.2 and Speyer 2.3 soil after 24 hours. Based on these results the time selected to equilibrate the soil samples was 5 hours, to avoid test substance degradation. The RMS agrees to the decision.

In the main study, the amount of test substance adsorbed onto soil was obtained from the difference between the initial and final amount of the test substance in the aqueous phase (indirect method). This is also in line with OECD 106, among other things, in cases where the test substance is considered to be stable in the time and the scale of the test.

However, HPLC analysis of the 4.66 mg/I supernatants showed that in the Speyer 2.3 soil, degradates were present after 5 hours of the advanced isotherm test (adsorption). Furthermore, HPLC analysis of supernatant of the 4.66 mg/L test solutions showed that after the first desorption step and even more after the second desorption step, most of the radioactivity present in the Speyer 2.3 sample consisted of degradates (mainly AMPA). At the first desorption step 65.9 % of the radioactivity found by HPLC in the Speyer 2.3 soil supernatant were degradates. At the second desorption step it was 71.8 %. In this case, the direct method should have been chosen in order to quantify adsorption and desorption.

Nevertheless, the RMS proposes not to exclude the results of Speyer 2.3 soil from further risk assessment, since K_f and K_{foc} for Speyer 2.3 are in the same order of magnitude as those for the other soils tested in the present study.

KIIA 7.4.1/4 (Schneider, 1993, BVL no 1027844)

Annex point:	KIIA 7.4.1/4
Author(s):	Schneider, E.
Title:	Glyphosate isopropylaminesalt adsorption/desorption
Date:	June 17, 1993
Guideline(s):	OECD Guideline no. 106
Deviations:	The study was only performed as screening test. Neither a preliminary study nor a determination of Freundlich adsorption isotherm was conducted. In addition, the mass balance was not conducted in accordance with OECD 106.
GLP:	Yes
Acceptability:	Yes

Material and methods

The adsorption/desorption properties of glyphosate isopropylaminesalt (98 %, 228 g/mol) were determined with a screening test in three soils 2.1, 2.3 and F3. A solution of 5 mg test substance in 1 L of 0.01 m $CaCl_2$-solution was used for the adsorption experiment. The soil characteristics are summarised in Table B.8.2-15.

Table B.8.2-15: Soil characteristics

Soil	Soil 2.1	Soil 2.3	Soil F3
Type of soil*	-	-	-
Cation exchange capacity (mval/100g)	4.9	9.5	13
Organic carbon (%)	0.7	1.34	1.2
Clay (%)	3.5	8.3	15.2
Silt (%)	4.4	11.2	14.7
Sand (%)	92.1	80.5	70.1
pH	5.9	6.3	7.3

* not reported in the study

Study design

Experimental conditions

a) Screening adsorption

2 g of each soil were weighed into a 50 mL centrifuge tube. 10 mL of the prepared solution of the test solution were pipetted onto the soil. The samples were prepared in double for each soil. From each soil type one blank was prepared using 2 9 of soil and 10 mL of 0.01 m $CaCl_2$-solution. Three control samples were prepared using just 10 mL of the test solution without soil. Samples, blanks and controls were stirred by means of a magnetic stirrer for 16 hours.

After 16 hours the samples were centrifuged. The clear solution was taken off, the volume V was measured and the solution was analysed. Controls and blanks were treated in the same manner as the samples.

b) Screening desorption

To each soil derived from the adsorption step described above volume V (see above) was added. The samples again were agitated for 16 hours in the same way as described. After this time all samples were centrifuged. The clear solution was taken off, the volume V was meas-

ured and the solution was analysed by means of HPLC-UV. The desorption step was repeated once.

c) Mass balance
After the two desorption steps the concentration of the test substance in the soil was determined for the purpose of mass balance.

Analytical procedures
Analysis of the aqueous phase was performed according to standard operation procedure iCD033E. Analysis of the soils was performed according to standard operation procedure DrK037E.

Results and discussion

Mass balance
Even after two extractions (each with two extraction steps) between 32.6 and 75.2 % of the original amount could be reextracted from the soils.

Findings
A percentage of 89.9 % - 94.6 % of glyhosate isopropylamine salt is adsorbed onto the soils. Only small amounts (< 10 %) of test substance were desorbed in the desorption part of the test.

Table B.8.2-16: K_d, K_{doc} values for glyphosate isopropylamine salt

Soil Type	OC (%)	pH (-)	K_d (mL g^{-1})	K_{doc} (mL g^{-1})	1/n (-)
2.1	0.7	5.9	66.4	9486	-
2.3	1.34	6.3	76.5	5709	-
F3	1.2	7.3	54.4	4533	-

Conclusions
Glyphosate isopropylaminesalt is adsorbed very strong by the soils under investigation.

RMS Comment
Deviating from the OECD 106 specifications, no preliminary study was performed. In addition, the mass balance was not conducted in accordance with the current guideline, since only the concentration of the test substance in the soil was determined after desorption.

With respect to the results of other adsorption/desorption studies, it can be assumed that the adsorption equilibrium was reached after 16 hours that were chosen for the screening test. It can also be expected that an accurate determination of K_d was possible, because the value which results by multiplying K_d with the soil/solution ratio is always > 0.1 (see also OECD 106, point 71). Therefore, the RMS proposes to use the K_d/K_{doc} values determined in the study for further risk assessment.

Due to the fact that no investigations of the relationship between soil solution concentration and adsorption behaviour were conducted by the Notifier, the RMS is of the opinion to use default 1/n values of 1.0 according to the ´Clarification note from EFSA concerning data re-

quirements, study guideline, guidance and consensus of member state fate and behaviour experts on soil adsorption measurements and selection of the slope (1/n value) that describe the Freundlich coefficient (2010)´.

B.8.2.2 Adsorption and desorption of all relevant metabolites

The adsorption and desorption behaviour of AMPA in soil was evaluated during the 2001 EU evaluation of glyphosate. The adsorption/desorption characteristics of AMPA derived from one acceptable study (Weeden, 1993, BVL no 2325586) have been summarised in the glyphosate Monograph. K_f and K_{foc} values for AMPA from this study ranged from 15 to 1554 and 1160 to 24800 mL/g, respectively. One additional AMPA adsorption and desorption study (Muller & Lane, 1996, BVL no 2310266), conducted by a GTF member to support its own registrations, has also been evaluated at the EU-Member State level. Details on this study are provided below.

Additionally, two studies (Knoch, 2003, BVL no 2310262; Wittig, 2002, BVL no 2310264) with a wide range of soil characteristics were available from GTF members. Neither of these studies was evaluated during the glyphosate 2001 EU evaluation or at EU Member State level. Details of these studies are provided below and offer a more complete picture of the sorption characteristics of AMPA in soil and allow a more comprehensive evaluation of its environmental fate and behaviour in soil.

Table B.8.2-17 summarises the results of all compliant adsorption/desorption studies available for AMPA. The K_{foc} values for AMPA based on multiple concentration tests range from 1119 to 45900 mL/g (arithmetic mean: 9749 mL/g). The RMS proposes to use 1/n default values of 0.9 in cases where no reliable 1/n value could be derived in the study. The results of all studies show that AMPA has a high adsorption potential.

Table B.8.2-17: Overview of the AMPA adsorption/desorption studies

Reference		Guidelines	Soil	Soil type	OC [%]	Clay [%]	Silt [%]	Sand [%]	CEC [mVal/100g]	pH [-]	K_f [mL/g]	K_{foc} [mL/g]	1/n [-]
Studies from the 2001 evaluation	Weeden, 1993, BVL no 2325586	OECD Test Guideline 106, 1981	SLI Soil #1	Clay loam	2.09	34.7	45.3	20.0	32.8	7.7[1)]	77.1	3640	0.79
			SLI Soil #2[3)]	Sand	18.68[3)]	0.7[3)]	11.3[3)]	88.0[3)]	28.3[3)]	4.7[1,3)]	1570.0[3)]	8310[3)]	0.90[3)]
			SLI Soil #4	Sand	1.33	2.7	5.3	92.0	12.0	7.4[1)]	15.7	1160	0.75
			SLI Soil #5	Clay loam	0.93	28.7	49.3	22.0	31.0	7.6[1)]	53.9	5650	0.79
			SLI Soil #9	Loamy sand	1.57	4.7	19.3	76.0	10.2	6.3[1)]	110.0	6920	0.77
			SLI Soil #11	Sand	0.29	0.7	1.3	98.0	4.8	4.6[1)]	73.0	24800	0.79
Studies not reviewed in the 2001 evaluation or new studies	Muller & Lane, 1996, BVL no 2310266	OECD Test Guideline 106, 1981	Lilly Field	Sand	0.29	4.0	4.0	92.0	1.8	5.7[1)]	133.0	45900	0.86
			Visalia	Sandy loam	0.58	13.0	18.0	69.0	7.3	8.4[1)]	10.0	1720	0.78
			Wisborough Green	Silty clay loam	2.26	32.0	60.0	8.0	11.9	5.7[1)]	509.0	22500	0.91
			Champaign	Silty clay loam	2.15	36.0	52.0	12.0	28.3	6.2[1)]	237.0	11100	0.86
			18 Acres	Sandy loam	1.80	19.0	23.0	58.0	14.4	7.4[1)]	74.2	4130	0.84
	Knoch, 2003, BVL no 2310262	OECD Test Guideline 106, 2000	Schwalbach	Silt loam	1.59	20.9	68.2	10.9	14.6	6.1[1)]	137.4	8642	0.98
			Hofheim	Silt loam	1.24	17.8	52.3	29.9	13.5	6.1[1)]	87.9	7089	0.92
			Bergen-Enkheim	Silty clay	2.25	41.9	41.4	16.7	28.9	8.3[1)]	33.9	1507	0.91
	Wittig, 2002, BVL no 2310264	OECD Test Guideline 106, 2000	Soil 2.1	Sand	0.90	3.8	9.0	87.2	6.0	5.2[2)]	16.7	1861	~~0.90[4)]~~ 0.6650[4)]
			Soil 2.2	Loamy sand	2.30	8.1	16.6	75.3	11.0	5.6[2)]	189.7	8248	~~0.90[4)]~~ 0.5506[4)]
			Soil 3A	Sandy silty loam	2.60	16.9	35.9	47.3	19.0	7.1[2)]	29.1	1119	~~0.90[4)]~~ 0.6710[4)]
Arithmetic mean (n = 16)												**9749**	**~~0.853~~ 0.81**
Min (n = 16)												**1119**	**~~0.75~~ 0.5506**
Max (n = 16)												**45900**	**0.98**
pH dependency												**No**	**-**

1) buffer Solution = H_2O
2) buffer Solution = $CaCl_2$
3) not included for calculation of statistics (mean values, correlations) due to high OC content
~~4) default value, since no reliable 1/n value could be derived in the original study~~

4) the Pesticides Peer Review Meeting 126 concluded that the 1/n values associated with these KFoc values should be the agreed endpoints (and not a default of 0.9).

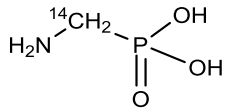
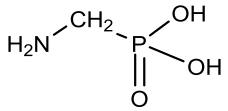
KIIA 7.4.2/1 (Knoch, 2003, BVL no 2310262)

Annex point:	KIIA 7.4.2/1
Author(s):	Knoch, E.
Title:	Aminomethylphosphonic acid: adsorption-desorption
Date:	February 07, 2003
Guideline(s):	OECD Guideline no. 106
Deviations:	No screening test was conducted.
GLP:	Yes
Acceptability:	Yes

Material and methods

The radiolabelled and non-radiolabelled test material is characterised in Table B.8.2-18.

Table B.8.2-18: Test material

	Test material (radiolabelled)	**Test material (non-radiolabelled)**
Common name	Aminomethylphosphonic acid ([^{14}C]-AMPA, specific activity 55 mCi/mmol)	Aminomethylphosphonic acid (AMPA)
Structural formula		
Purity	98.6 % by HPLC; 98.3 % by TLC	99 %
CAS		1066-51-9
Stability of test compound	≤ -18 °C in the dark	+2 to +8 °C in the dark

The adsorption/desorption characteristics of AMPA was determined in three soil types of German origin (Table B.8.2-19) using the batch equilibrium method. Soils were collected from the top 0-20 cm layer in fields with no agricultural use, and no application of agricultural plant protection products for several years. The soils were air dried at room temperature (approximately 20 °C) and stored under aerobic conditions in the dark at ambient temperature (approximately 20 °C).

Table B.8.2-19: Soil physicochemical properties

Soil origin	**Soil type (USDA)**	**pH[1)]**	**CEC (meq/ 100g)**	**OC (%)**	**Sand (%)**	**Silt (%)**	**Clay (%)**	**$CaCO_3$**	**MWHC (%)**	**Bulk density (g/L)**
Schwalbach, DE	Silt loam	5.13/ 6.09	14.6	1.59	10.9	68.2	20.9	< 0.1 %	48.5	1002
Hofheim, DE	Silt loam	5.10/ 6.06	13.5	1.24	29.9	52.3	17.8	< 0.1 %	43.0	1119
Bergen-Enkheim, DE	Silty clay	7.43/ 8.30	28.9	2.25	16.7	41.4	41.9	< 0.1 %	49.4	1060

1) pH in 0.01M $CaCl_2$ /Water

Study design

Experimental conditions

Prior to use, experimental soils were equilibrated overnight using 0.01 mol/L $CaC1_2$ solution. A solution of the test item (radiolabelled AMPA or a mixture of radiolabelled and unlabelled AMPA in reagent water) was mixed with aqueous 0.01 mol/L $CaC1_2$ solution to give various aqueous test item stock solutions of 0.0531, 0.2983, 1.0952, 2.7231 and 5.5366 mg/L of AMPA. Based on the results of the preliminary testing the optimum soil/solution ratio of 1/5 was chosen for the definitive study and an equilibration time of 24 hours at 20 ± 2 °C in the dark were considered appropriate for the experiment. The adsorption isotherms experiment was initiated by adding a known volume of treatment solutions to one gram of pre-equilibrated soil slurries of each soil (two replicates per concentration) in order to provide a test substance concentrations of approximately 0.0531, 0.2983, 1.0952, 2.7231 and 5.5366 mg/L of AMPA. After agitation of the test systems (laboratory shaker: 350 strokes/min) the aqueous phases were analysed only once at the end of the experiment (24 hours).

For the desorption experiment (two desorption steps) the volume of aqueous solution removed after the adsorption step was replaced by an equal volume of 0.01 mol/L $CaCl_2$. The mixture was agitated as described for the adsorption step. The supernatant of desorption steps was analyzed separately. The amount of desorbed test item and desorption constants, respectively, were calculated.

Following desorption steps, the residues of AMPA left over in soil from the highest test concentration were extracted from the soil samples using 1 M aqueous NH_3. The ratio of extraction solvent and soil was approximately 1:1 (volume: soil dry weight). The extractable radioactivity was taken for radioassay.

Analytical procedures

Radioactivity in solution was determined by liquid scintillation counting (LSC) using an Aquasafe 500 Plus scintillation cocktail by Zinsser Analytic. Residual radioactivity in soils was assayed by combustion and radioactivity was assayed by LSC. TLC was performed on precoated plates of Ionex-25 SA-Na. For the separation of specimen aliquots one dimensional thin layer chromatography was used throughout the study. A TLC-solvent system (0.015 M KH2PO4 containing 10 vol. % methanol, adjusted to pH 2.4 by addition of H_3PO_4) was selected for the separation of radioactivity in specimen extracts.

Results and discussion

Mass balance

The material balance from supernatants, soil extracts and combustion of bound residues was in the range of 96.65 % to 100.45 %, of 96.72 to 99.22 % and of 96.55 to 99.89 % for the test systems Schwalbach, Hofheim and Bergen-Enkheim, respectively. The percentage absorbed ranged from 94.94 to 97.85 %, from 94.13 to 97.02 % and from 86.40 to 92.82 %, while the percentage desorbed ranged from 1.41 to 2.27 %, from 1.61 to 5.22 % and from 6.07 to 10.58 % for the test systems Schwalbach, Hofheim and Bergen-Enkheim, respectively.

Findings

The adsorption coefficients assessed with the aid of the Freundlich adsorption isotherm for AMPA ranged from 33.9 – 137.4 mL/g at corresponding K_{foc} values of 1507 - 8642 mL/g for

the three test soils (Table B.8.2-20). The extent of the test item desorption was calculated and expressed in percent of the adsorbed material over the concentration range tested.

Table B.8.2-20: Adsorption/desorption constants and correlation coefficients for AMPA in three test soils

Soil origin	Soil type (USDA)	pH[1)]	OC (%)	Adsorption				Desorption
				K_f (mL/g)	K_{foc} (mL/g)	r^2 (-)	1/n (-)	(% of adsorbed material over the concentration range testes)
Schwalbach	Silt loam	5.13	1.59	137.4	8642	0.978	0.982426	1.41 – 2.27
Hofheim	Silt loam	5.10	1.24	87.9	7089	0.989	0.923385	1.61 to 5.22
Bergen-Enkheim	Silty clay	7.43	2.25	33.9	1507	0.989	0.907390	6.07 – 10.58

1) pH in 0.01M $CaCl_2$

Conclusions

Based on the results obtained (K_{foc} values of 1507 - 8642 mL/g), aminomethylphosphonic acid (AMPA) can be classified as practically immobile if adsorption to soil is considered as the sole criterion for an assessment of a pesticide movement on soil. The test item remained stable during the study.

RMS Comment

In deviation from OECD 106, no screening test was conducted. However, preliminary tests in order to determine soil/solution ration the equilibrium time for adsorption and the stability of the test substance were performed.

The study is considered acceptable by the RMS. Therefore, K_f/K_{foc} values as well as the 1/n values determined in the study can be used for further risk assessment.

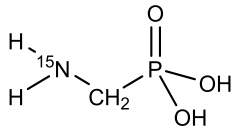
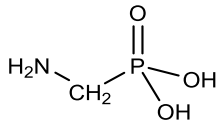
KIIA 7.4.2/2 (Wittig, 2002, BVL no 2310264)

Annex point:	KIIA 7.4.2/2
Author(s):	Wittig, A.
Title:	Adsorption/Desorption of AMPA on Soil according to OECD 106
Date:	June 24, 2002
Guideline(s):	OECD Guideline no. 106
Deviations:	The preliminary test was conducted using only one soil.
GLP:	Yes
Acceptability:	Yes

Material and methods

The radiolabelled and non-radiolabelled test material is characterised in Table B.8.2-21.

Table B.8.2-21: Test material

	Test material (radiolabelled)	**Test material (non-radiolabelled)**
Common name	[15N] Aminomethylphosphonic acid	Aminomethylphosphonic acid (AMPA)
Structural formula		
Purity	98.8 %	100.2 % (S06555, NaOH titration) and 100.3 % (07220ER, NaOH titration)
CAS		1066-51-9
Stability of test compound	At ambient temperature	Stored at room temperature (20 °C)

The study was conducted using three different soil types (sand, loamy sand and sandy silty loam) collected from LUFA Speyer, Germany (Table B.8.2-22).

Table B.8.2-22: Soil physicochemical properties

Soil property	**Soil series name**		
	3A	**2.1**	**2.2**
Sand	47.3 ± 2.3	87.2	75.3 ± 2.0
Silt	35.9 ± 2.2	9.0	16.6 ± 1.4
Clay	16.9 ± 0.1	3.8	8.1 ± 1.2
Classification[1)]	Sandy silty loam	Sand	Loamy sand
Organic carbon (%)	2.6	0.9	2.3
CEC (mval/100g)	19 ± 5	6	11 ± 2
pH[2)]	7.1	5.2	5.6
Maximum water holding capacity (g/100 g TM)	50 ± 7	30	50 ± 5

1) USDA textual class
2) Measured in 0.01 M $CaCl_2$ solution

Study design

Experimental conditions

All experiments were performed at laboratory ambient temperature, typically 22 ± 2 °C.

The soils were air-dried at ambient temperature (20 - 25 °C), disaggregated, homogenised and pre-equilibrated by agitating with 0.01 M $CaCl_2$ for 24 hours. The moisture content of each soil was determined for three aliquots via evaporation at 105 °C until a constant weight was established. For all calculations the mass of soil refers to oven dry mass.

Three stock solutions were prepared by dissolving AMPA (99 %) in defined amounts of water and then mixing with aqueous 0.01 M $CaCl_2$ or water to provide concentrations of 99.99 mg/L (in 0.01 M $CaCl_2$, stock solution I), 0.998 mg/L (in water, stock solution III) and 100.39 mg/L (in 0.01 M $CaCl_2$, stock solution IV). Another stock solution was prepared by dissolving 15N-AMPA in water to a concentration of 0.996 mg/L (stock solution II). The study was performed according to the tiered approach which is described in OECD guideline 106.

As Tier 1 a preliminary study was conducted with one of the 3 soils (soil 2.2). In this preliminary step the optimal soil/solution ratio was determined and the equilibration time for the adsorption was investigated. Moreover the stability of the test substance during the test period was checked. It was found that the optimal soil/solution ratio was 1:50 and the time needed for equilibration was 48 hours. The stability of the test substance during the test period was confirmed by the analysis of control samples and the mass balance was determined (analysis of soil phase and aqueous phase).

As Tier 2 the adsorption kinetics (screening test) was performed. Two additional soils (2.1 and 3A) were investigated, using a soil/solution ratio of 1:50 and a maximum equilibration time of 48 hours. For soil 2.1 however a soil/solution ration of 1:25 was chosen to receive a higher concentration decrease from the aqueous phase during the test.

The adsorption kinetics test was performed in that way that air dried soil samples (either 2 g or 4 g) were equilibrated with 90 ml of 0.01 M $CaCl_2$ solution for 24 h. Then 10 mL of the appropriate AMPA stock solution were added, to adjust to a total volume of 100 mL. The samples then were shaken for up to 48 hours (intervals 6, 16, 24, 32, 40, 48 h). For each time point 2 parallel samples were prepared.

In the Tier 3 the Freundlich adsorption isotherms and the desorption behaviour was determined.

Samples were prepared for all 3 soils in the same way as done also for the adsorption kinetics investigations, but this time for each soil samples with five different concentrations, covering a concentration range from about 0.1 to 10 mg/L, were prepared. All samples were prepared in duplicate. Also for each soil a blank sample and a control sample was included in the investigation. Measurements were corrected for the value measured in the control sample.

For the investigation of the desorption kinetics adsorption experiments for one concentration was repeated. After the adsorption phase (6, 16, 32, 40 and 48 h) however the aqueous supernatant was discarded and replaced by the same amount of 0.01 M $CaCl_2$ solution. For the investigation of the desorption isotherms the same procedure was made, but this time choosing all 5 different concentrations and using only the time interval of 48 h.

Analytical procedures

For the analysis an appropriate aliquot from the aqueous phase (normally 1 mL) of the respective sample was taken off.

After sampling of the aqueous 0.01 M $CaCl_2$ phase the internal standard 15N-AMPA was added. Then this aqueous phase was reduced to dryness in a vacuum rotator and one drop phosphoric acid and 1 ml trifluoroacetic acid (TFA) were added. The sample was left to stand for 15 min at room temperature. This was performed for better dissolution of the residue. After this time 2 mL trifluoroacetic anhydride (TFAA) and 1 mL trifluoroethanol (TFE) were added. The sample was heated at 70 °C for 60 min, then allowed to cool down to room temperature and centrifuged. After the derivatisation step, a clean-up step using liquid/liquid extraction was performed. To do this, the clear supernatant was transferred into a 25 mL conical flask and the sample was concentrated to about 1 mL using a rotation evaporator. This solution was transferred into a 50 ml separation funnel. 10 mL dichloromethane and 10 mL of 5 % ammonium hydroxide solution were added and then the sample was shaken vigorously (1 -

2 min). After this, the sample was left to stand for 10 min at room temperature for separation of organic and aqueous phase. The dichloromethane solution containing the derivatised AMPA was dried over sodium sulphate and filtered into a 50 mL conical flask. The Filter was washed with 5 mL dichloromethane, then 5 mL toluene was added and the sample was evaporated to about 0.5 mL using a rotation evaporator.

The resulting sample was subjected to GC-MS measurement [GC: Dani 86.10 with autosampler LS 32; column; Varian 50 m CP-SIL 19 c.b. (corr. OV1701), I.D. 0.25 mm, df = 0.4 µm; carrier gas: Helium (5.0), 1.8 bar; injector: PTV, total, split open after 8 min; MS: HP 5970 MSD with ChemStation Vers. 3.2, SIM mod]. The fragment ion of the derivatised AMPA and 15N-AMPA were detected to be m/z = 302 and 303, respectively.

The limit of quantification (LOQ) for AMPA in water was determined to be 0.0001 µg/mL. The limit of quantification for the analytical method was settled at 0.029 µg/mL, the recoveries in spiked soil extracts were 93.9 to 102.4 % for Lufa 2.1, 2.2 and 3A soils, respectively.

Results and discussion

Mass balance

The mass balance was assessed by the determination of the amount of test substance in the solution and in the soil. The total recoveries for the single soils plus the aqueous phases were 95.8 % (86.9 % in aqueous phase and 8.9 % in soil), 92.3 % (29.3 % in aqueous phase and 62.9 % in soil) and 91.1 % (76.1 % in aqueous phase and 15 % in soil) for L 2.1, 2.2 and 3A soil, respectively.

Findings

The percentage adsorption was calculated on the basis of the initial nominal concentration and measured concentration after 48 hours. The initial concentration was plotted in logarithmic form (Freundlich isotherms) versus the concentration in soil.

The amount of test substance desorbed from the soil samples was calculated as the amount of test substance in the solution corrected for the amount of test substance contained in the solution which had remained in the soil after the first centrifugation.

The following results were derived from the plots of the Freundlich adsorption/desorption isotherms (Table B.8.2-23):

Table B.8.2-23: Adsorption/desorption isotherms for AMPA in three test soils

Soil	%OC	pH	Adsorption				Desorption			
			K_f^{ads}	K_{oc}^{ads}	1/n	r^2	$K_f$des	K_{oc}^{des}	r^2	1/n
2.1 sand	0.9	5.2	16.746	1861	0.6650*	0.9953	21.38	2376	0.9999	0.9747
2.2 Loamy sand	2.3 ± 0.2	5.6	189.714	8248	0.5506*	0.9983	49.48	2151	1.0000	0.9894
3A Sandy silt loam	2.6 ± 0.7	7.1	29.087	1119	0.6710*	0.9995	41.78	1607	0.9995	0.9729

OC = organic carbon content of soil
1/n = 1/slope of linear regression of Freundlich equation
* low Freundlich slope values (< 0.7)

Conclusions
Adsorption /desorption equilibrium of AMPA was determined for three soils. Based on the K_{oc} values, it is concluded that AMPA is considered to be immobile in all three soils. According to the criteria mentioned in OECD 106 (OECD, 2000), the adsorption of AMPA was regarded as irreversible.

RMS Comment
Apart from the fact that the preliminary test was conducted using only one soil, the study was performed according to OECD 106.

The Freundlich slope values should be assessed according to the ´Clarification note from EFSA concerning data requirements, study guideline, guidance and consensus of member state fate and behaviour experts on soil adsorption measurements and selection of the slope (1/n value) that describe the Freundlich coefficient (2010)´. Values for 1/n are typically in the range from 0.7 to 1.1, though in some valid experiments values outside this range do sometimes occur. FOCUS guidance indicates that an expected average 1/n when looking at a database of a large number of different compounds is 0.9. The RMS considers that the recommendation of the FOCUS guidance to use the often referred to 'FOCUS default' Freundlich slope of 0.9 (average value) is applicable when the experiments attempted to investigate the effect of concentration on adsorption, but a reliable 1/n value could not be derived, as is the case here.

Finally, the RMS proposes to use the K_f/K_{foc} values in combination with the FOCUS default' Freundlich slope of 0.9 instead of values determined in the study for the soils 2.1, 2.2 and 3A for further risk assessment.

Outcome of the discussions in the Pesticides Peer Review Meeting 126 (February 2015):

The RMS made reference to: ´Clarification note from EFSA concerning data requirements, study guideline, guidance and consensus of member state fate and behaviour experts on soil adsorption measurements and selection of the slope (1/n value) that describe the Freundlich coefficient´ that is included in FOCUS version control documents. The clarification note and FOCUS documents indicate that the use of 0.9 was envisaged when there are experimental difficulties (for example a labile active substance). The philosophy in the clarification note is that either the 1/n values as measured should be used (when considered reliable) or the experiments should be left out due to „unrealistic/unexpected" (low) 1/n values.
It was noted that desorbed AMPA was not degrading in soil solution within the equilibrium time of batch experiments in the study Wittig. Therefore experts agreed that the Wittig K_{foc} results were reliable. Experts concluded that the 1/n values associated with these K_{foc} values should be the agreed endpoints (and not a default of 0.9).

KIIA 7.4.2/3 (Muller and Lane, 1996, BVL no 2310266)

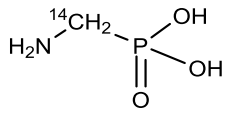
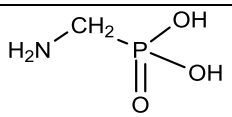
Annex point:	KIIA 7.4.2/3
Author(s):	Muller, K., Lane, M.C.G.
Title:	Glyphosate acid: adsorption and desorption properties of the major metabolite, AMPA, in soil

Date:	August 27, 1996
Guideline(s):	OECD Guideline no. 106, US-EPA Pesticide Assessment Guidelines, Subdivision N. Chemistry: Environmental fate. Series 163-1. Leaching and adsorption/desorption studies (1982)
Deviations:	The preliminary test was conducted using only one soil ('Visalia') as well as one soil/solution ratio.
GLP:	Yes
Acceptability:	Yes

Material and methods

The radiolabelled and non-radiolabelled test material is characterised in Table B.8.2-24.

Table B.8.2-24: Test material

	Test material (radiolabelled)	**Test material (non-radiolabelled)**
Common name	[^{14}C]-Aminomethylphosphonic acid (AMPA): specific activity 1.828 GBq mmol^{-1}	Aminomethylphosphonic acid (AMPA)
Structural formula		
Purity	> 97 % (by TLC)	Not reported
Stability of test compound	Assumed to be stable, as shown by subsequent analysis of the various extracts after equilibrium	Not reported

The adsorption/desorption characteristics of AMPA were determined in five soil types – three UK origin and two USA origin – using the batch equilibrium method. The soils were air dried and passed through a 2 mm sieve. Each soil sample was gamma irradiated (25-40 kGy) to eliminate any living organisms within the soil.

Table B.8.2-25: Soil physicochemical properties

	Soil series name				
Soil property	**'Lillyfield'**	**'Visalia'**	**'Wisborough Green'**	**'Champaign'**	**'18 Acres'**
Origin	**Wishanger Farm, Churt, Surrey, UK**	**WRTC, 498 N. Mariposa Avenue, CA 93292, USA**	**Naldretts Court, Wisborough Green Sussex, UK**	**NRTC, PRI Box 208A, 1300N, IL 61821, USA**	**Jealotts Hill Farm, Bracknell, Berkshire, UK**
Soil type (USDA)	Sand	Sandy loam	Silty clay loam	Silty clay loam	Sandy loam
Sand (%)	92	69	8	12	58
Silt (%)	4	18	60	52	23
Clay (%)	4	13	32	36	19
Organic matter (%)	0.5	1.0	3.9	3.7	3.1
CEC (meq/100g)	1.8	7.3	11.9	28.3	14.4
pH in H_2O	5.7	8.4	5.7	6.2	7.4
Moisture Holding Ca-	3.1	10.4	30.9	22.7	17.1

pacity 1/3 bar (%)					
Moisture Holding Capacity 15 bar (%)	1.1	4.8	19.8	13.5	10.4

Study design

Experimental conditions

All solutions of AMPA were prepared and stored in plastic vials, due to adsorption affinities of AMPA to glass. A stock solution was received in water. The exact radiochemical content was detected using liquid scintillation counting (LSC).

Higher concentration treatment solutions were prepared using a mixture of radiolabelled and non-radiolabelled AMPA. A solution of 10000 µg mL^{-1} was prepared by dissolving AMPA in water in a plastic vial which was stored at -20 ± 5 °C when not in use.

Treatment solutions were prepared by adding appropriate volumes of radiolabelled and non-radiolabelled AMPA solutions to a 0.01 M $CaCl_2$ solution in the way that when aliquots (1 mL) of the treatment solutions were added to the soil slurries, the required concentration of AMPA in the aqueous phase would be achieved (0.05, 0.1, 0.2, 1.0, 2.0 µg mL^{-1}).

All aqueous $CaCl_2$ solutions used in this study were sterilised by autoclaving prior to use.
All soils were sterilised by gamma irradiation prior to use. Soil slurries were prepared with soil:water (0.01M $CaCl_2$) ratios of 1:10.

In a preliminary experiment with 'Visalia' soil, the time taken for ^{14}C-AMPA (0.2 µg mL^{-1}) to equilibrate between soil and solution phases was studied over a period of 48 hours. After 16 hours the approximate equilibrium plateau of the ^{14}C-AMPA distribution between soil and aqueous phases was reached. For convenience a time of 21 hours was chosen as the equilibrium time for both the adsorption and desorption steps.

a) Adsorption experiment
The adsorption studies were carried out with air dried soil samples (equivalent to 2.0 g oven dry weight, four replicates) weighed directly into Teflon centrifuge tubes with self-sealing caps.

Prior to ^{14}C-AMPA treatment, each soil sample was equilibrated for 16 hours in 0.01 M $CaCl_2$ (approximately 19 mL) by continuous mixing on an end-over-end shaker at approximately 1320 revolutions per hour (rph) at 20 ± 2 °C.

After adding 1 mL of the respective treatment solution, the final soil:solution ratio was 1:10. The tubes were transferred to an end-over-end shaker and continuously mixed at 1320 rph for 21 hours at 20 ± 2 °C.

Additional samples were generated as treatment controls (no soil) and soil blanks (no AMPA).

After 21 hours of shaking the slurries were centrifuged at 3500 rpm (1625 g) for 10 minutes. Aliquots of the supernatants (17-18 mL) were decanted. Sub-samples were taken and analysed for radiochemical content by LSC. The remainder of the supernatant was stored at -20 ± 5 °C until further analysis. The remaining soil was also frozen.

b) Soil extraction
AMPA was extracted from the remaining soil after the removal of the adsorption supernatant and subsequent freezer storage (for 2 replicates). To each tube an ammonium phosphate buffer (30 mL) was added. The soil slurry was agitated on an end-over-end shaker for 4 h and then centrifuged at 4000 rpm for 15 minutes. Subsequently, the supernatant buffer solution was decanted. This extraction process was repeated and the supernatants were pooled. In order to ensure that the extract was homogenous for LSC analysis, an emulsion was made. This required the addition of concentrated HCl (2 mL), Optiphase Safe (300 µL) and pentan-1-ol (10 µL). Each extract was then analysed by LSC and, after a concentration step, representative samples were also analysed by TLC. The remaining radioactivity in the residual soil samples was determined by combustion.

c) Desorption experiment
After adsorption, 2 replicates were used for a single desorption step. After the initial centrifugation and decantation (described in a) Adsorption experiment), the volumes of supernatant were replaced with an equivalent volume of fresh sterile 0.01 M $CaCl_2$ solution. The samples were re-equilibrated on an end-over-end shaker (1320 rph) for 21 hours at 20 ± 2 °C. Once again, the samples were centrifuged and the same volume of supernatant was removed. Aliquots were taken for LSC. All supernatant phases were deep frozen at -20 ± 5 °C until further analysis. The soil residues were extracted in the same manner as described for the adsorption samples.

Analytical procedures
All analyses were carried out on individual replicate samples of both the adsorption and desorption supernatants and their respective soil extracts.

Radiochemical content was determined by LSC (LKB 1217 spectrophotometer; LKB Instruments Ltd.) with 'Optiphase Safe' (LKB, Sweden) as scintillation cocktail.

The liquid samples were concentrated by evaporation under a stream of air and an aliquot of the concentrate was analysed by thin layer chromatography (Dowex 50W X8/Cellulose 6/4 plates developed in a 0.1M HCl solvent system). The radioactive areas on the TLC plates were quantified using a Rita 3200 Automatic TLC Analyser. Autoradiographic images of the developed chromatograms were made using a Fuji bas2000 bio-imaging system.

All extracted residual soil samples were dried under a gentle stream of compressed air at room temperature and the radioactivity remaining in the soil determined by combustion (Harvey OX300 Biological Oxidiser). The $^{14}CO_2$ evolved was trapped in 2-methoxyethylamine (part of 'Optiphase Safe'/ 2-methoxyethylamine/distilled water, 50/25/2, v/v/v) and radioassayed by LSC. The recovery of the applied radiochemical was greater than 90 % on all occasions and was used as a correction factor for the combusted samples.

Results and discussion

Mass balance
Recoveries of radioactivity from various samples assessed ranged from 95-106 % and averaged 102 % of that nominally applied.

Transformation of the parent compound
Analysis of aqueous supernatants and soil extracts from the adsorption and desorption steps demonstrated that there had been little or no degradation of AMPA. Greater than 90 % of radiolabelled chemical in aqueous and soil phases was shown to be AMPA.

Findings
a) Adsorption experiment
At adsorption equilibrium, the K_d and K_{oc} values and the percentage of applied radioactivity adsorbed were calculated for each single concentration. Average K_d values in the soils ranged from 19 in the high pH soil 'Visalia' to 881 in the low pH soil 'Wisborough Green'. The K_{oc} values ranged from 3070 in 'Visalia' to 89300 in 'Lillyfield' (Table B.8.2-26).

Adsorption isotherms were calculated by linear regression analysis of adsorption data according to the Freundlich equation. Freundlich adsorption coefficients (K_f) ranged from 10 to 509 and the organic carbon normalised Freundlich coefficients from 1720 to 45900. The Freundlich equation showed a good fit of all soils tested, with r^2 values equal to 1.00. 1/n values ranged from 0.78 to 0.91, demonstrating a small decrease in adsorption with increasing rate of application. No saturation of the adsorption sites was found at the highest rate of application. These data are consistent with a decrease in K_d values with increasing rate of application.

In the majority of soils more than 92 % of the applied chemical was adsorbed, with the exception of soil 'Visalia', in which adsorption was still strong with an average of 62 % adsorbed.

Table B.8.2-26: Adsorption parameters for AMPA in five soils (soil:solution 1:10)

Soil origin	pH[1)]	OM (%)	Adsorption						
			K_d [2)]	K_{oc} [2)]	K_f	K_{foc}	1/n	r^2	% AR adsorbed[2)]
'Lillyfield'	5.7	0.5	259	89300	133	45900	0.86	1.00	96
'Visalia'	8.4	1.0	19	3070	10	1720	0.78	1.00	62
'Wisborough Green'	5.7	3.9	881	39000	509	22500	0.91	1.00	99
'Champaign'	6.2	3.7	516	24000	237	11100	0.86	1.00	98
'18 Acres'	7.4	3.1	146	8110	74	4130	0.84	1.00	93

1) pH in H_2O
2) Mean, rounded values. Calculations were performed with non-rounded values.

b) Desorption experiment
Average K_d values after the desorption step ranged from 28 in the high pH soil 'Visalia' to 1340 in the low pH soil 'Wisborough Green'. The K_d, K_{oc} and K_{foc} values after desorption were all higher than those after the adsorption step. In the majority of soils the percentage of adsorbed chemical desorbed was low (averaging less than 4 %) with soil 'Visalia' again being the exception (24 %).

Table B.8.2-27: Desorption parameters for AMPA in five soils (soil:solution 1:10)

Soil origin	pH[1)]	OM (%)	Desorption			
			K_d [2)]	K_{oc} [2)]	K_{foc}	adsorbed chemical desorbed (%)[2)]
'Lilly-field'	5.7	0.5	388	134000	71500	2
'Visalia'	8.4	1.0	28	4770	2080	24
'Wisborough Green'	5.7	3.9	1340	59100	29600	1
'Cham-paign'	6.2	3.7	699	32600	15000	1
'18 Acres'	7.4	3.1	228	12680	5130	4

1) pH in H_2O
2) Mean, rounded values. Calculations were performed with non-rounded values.

Conclusions
Using the McCall Classification scale to assess a chemical´s potential mobility in soil, AMPA can be classified as having between low mobility and being immobile, based on the adsorption results obtained. However, high K_{foc} desorption values led to the conclusion that adsorption of AMPA was generally not very reversible, resulting in a further reduction of the potential mobility of the compound. Thus, the potential mobility of AMPA in all soils has been reduced to being immobile, except for soil 'Visalia' in which it was classified as slight mobile.

RMS Comment
The preliminary test was conducted using only one soil ('Visalia') as well as one soil/solution ratio. Nevertheless, an accurate determination of K_d according to OECD 106, point 71 was possible and the K_f/K_{foc} values and the 1/n values are reliable. Therefore, the results of the study can be used for further risk assessment.

B.8.2.3 Mobility in soil

B.8.2.3.1 Column leaching studies

This type of study is not required. Reliable adsorption coefficients of the active substance were obtained by adsorption/desorption studies and, consequently, no column leaching studies are to be conducted. However, during the 2001 EU evaluation of glyphosate, two acceptable column leaching studies (Burgener, 1992, BVL no 1932122; Schneider, 1991, BVL no 1062361) and one soil thin layer chromatography study (Brightwell & Malik, 1978, BVL no 1932009) with glyphosate and one column leaching study with glyphosate-trimesium (McGinley, 1992) were submitted . Based on these studies, glyphosate possesses very low potential for leaching. The soil thin layer chromatography study Brightwell & Malik (1978, BVL no 1932009) is only considered as additional information by the RMS since it is not a column leaching study.

B.8.2.3.1 Aged residue column leaching studies

During the 2001 EU evaluation of glyphosate, no acceptable aged residue column leaching study with glyphosate was submitted. An aged column leaching study with glyphosate trimesium (Waring & Purser, 1992, BVL no 1052677) was considered acceptable during the 2001 EU evaluation. Since then, another aged residue column leaching study (McLaughlin,

1996, BVL no 2310268) was provided by the Glyphosate Task Force (GTF) members. The results of this study indicate that aged glyphosate and its major soil metabolite are immobile in the representative sandy soil used in this study.

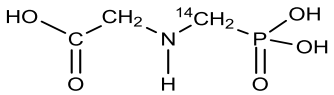
KIIA 7.4.5/1 (McLaughlin, 1996, 1996, BVL no 2310268)

Annex point:	IIA 7.4.5/1
Author(s):	McLaughlin, S.
Title:	[^{14}C]-glyphosate: Determination of the mobility of aged residues in one soil
Date:	June 14, 1996
Guideline(s):	SETAC- Procedures for assessing the environmental fate and ecotoxicity of pesticides, Annex of FAO revised guideline on environmental criteria for the registration of pesticides, BBA Guideline Part IV, 4-2
Deviations:	Differing from BBA Guideline Part IV, 4-2, only one soil was used for the study.
GLP:	Yes
Acceptability:	Yes

Material and methods

The test material is characterised in Table B.8.2-28.

Table B.8.2-28: Test material

	Test material (radiolabelled)
Name	[14C] N-(phosphonomethyl) glycine (glyphosate, specific activity 80.1 µCi/mg)
Identification	
Purity	99.6 % (radiochemical purity)
CAS	1071-83-6
Stability of test compound	The stability was proven by HPLC.

One soil was used for the study: a sand (Speyer 2.1) from Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) Speyer (Germany). Soil physicochemical properties are given in Table B.8.2-29.

Table B.8.2-29: Soil physicochemical properties

Common name	**Soil type**	**Sand (%)**	**Silt (%)**	**Clay (%)**	**pH**	**OC (%)**	**CEC (meq/100g)**	**MWC (g/100 g dry soil)**	**pre-study/ post study microbial biomass (mg microbial carbon/100 g dry soil)**
Speyer 2.1	sand	88.4	9.8	1.9	5.9	0.62	5.0	31	46/71

Study design

The application rate was calculated as 3.33 mg/kg dry soil based on the maximum recommended field application rate of 2.5 kg/ha. The radiolabelled test compound was isotopically diluted with the analytical standard of the test compound. Based on the LSC measurement of the application solution and the weight data of the unlabelled analytical standard, the new specific activity was calculated as 80.1 µCi/mg.

Prior to application, the soil moisture was adjusted to the approximate target moisture. Thereafter, a 200 µL aliquot of the application solution containing 0.335 mg of the diluted ^{14}C-test substance was added drop by drop to each 100 g (equivalent dry weight) soil sample by means of a Hamilton syringe.

The control soil samples were adjusted with deionised water to the target moisture of the respective soils. The aerobic incubation part of the study was carried out in all-glass metabolism flasks equipped with a trapping system. Ethylene glycol was used to trap organic volatiles, 0.5M sodium hydroxide was used to trap $^{14}CO_2$.The metabolism flasks were continuously ventilated with CO_2 free and moistened air.

Samples were taken immediately after dosing and after 5 and 8 days of aerobic incubation. A total of 10 samples were incubated aerobically. Five samples were used to monitor the degradation of the test compound up to its DT_{50}. Aliquots of 3 aged samples were used to confirm the DT_{50} and to conduct the aged leaching experiment. Aliquots from volatility traps for organic compounds and $^{14}CO_2$ were collected on days 1, 2, 4, 5, 7 and 8 post-treatment. Trapped radioactivity was measured by LSC.

The aged leaching part of the study was conducted with 40-cm long all-glass column equipped with a porous glass-filter plate at the bottom. The inner diameter of the column was 4.8 cm. The water supply to the column was by means of a peristaltic pump. The leaching experiment was performed in duplicate. The columns were packed with untreated, preweighed, air-dried soil up to 28 cm. Thereafter, the soil columns were saturated with 0.01 M $CaCl_2$ (approximately 236 mL). Portions of soil samples in which the parent compound was degraded down to 50 % of the initial concentration were removed from the metabolism flasks and air dried. Aliquots of the dried treated soil were then packed on top of the untreated soil columns prepared above. Leaching was performed with a total of 380 mL of 0.01 M $CaCl_2$ solution per column over 2 days. This corresponds to an irrigation rate of 200 mm per 48 hours. Leachates from the daily collections were sampled and their radioactivity was determined by LSC.

Soils were extracted exhaustively with total 125 mL of 0.35 M H_3PO_4/0.09 M $CaCl_2$ per 50 g dry weight soil. Soils were extracted three times at room temperature using the soil to solvent ratio of approximately 1:2.5 (w:v). This procedure was done by shaking the samples on an overhead shaker. After centrifugation of each individual extract, the extraction efficiency was determined by LSC. After exhaustive solvent extraction, extracts were pooled and the extraction efficiency was determined. Extracts were analyzed by HPLC without any further clean-up (direct injection). Non-extractable radioactivity of extracted wet or air dried soil was measured by post-extraction combustion followed by radioassay.

Extractable radioactivity of glyphosate and its radioactive degradation products was qualitatively and quantitatively analysed by HPLC (column: Nucleosil 5 SB 20 cm x 0.4 cm id; flow rate: 1 mL/min) with radiometric detection (RAM). The mobile phase used for the HPLC

analysis was a combination of two solvents - a solution of 0.05 M and 0.75 M KH_2PO_4 adjusted to pH 3.4 with phosphoric acid. The ratio of two solvents was adjusted by a linear gradient programme, which started with 100 % 0.05 M KH_2PO_4 and increased the volume of 0.75 M KH_2PO_4 solution to 75 % within 15 minutes. One dimensional, radio-TLC (Thin-Layer Chromatography on silica gel 60 F 254, 0.25 mm Merck) plates with selected samples helped to tentatively characterise AMPA using solvent system consisting of 40 mL methanol, 20 mL water, and 3 mL of 25 % aqueous ammonia.

Results and discussion

Mass balance

At the initiation of the study, almost complete extraction of radioactivity was observed, since 96.6 % of the applied radioactivities were found in the extracts and 0.3 % in the extracted soil. Thereafter a constant and significant decrease of extractable radioactivity was seen during the eight days of incubation: Extractables accounted for a range of 71.9 % to 72.8 % which corresponds to a mean of 72.7 % of the applied radioactivity. At the same time, non-extractable radioactivity accounted on average at 1.5 %. The total recoveries for all extracted samples used were 94.4 ± 1.7 %.

Findings

The results demonstrated that 101.2 and 101.3 % of the applied radioactivity applied onto the duplicate soil columns was retained by the column. The majority (99.1 % and 97.9 %) of radioactivity was found in the top 0 to 6 cm of the aged soil which occupied the top 2.5 cm. Significantly less radioactivity was found in the 6 to 12 cm soil layer: 2.0 % and 3.3 % of total column A and B radioactivity, respectively. Leached radioactivity did not exceed 0.1 % of the total column radioactivity. Head volatiles contributed 3.2 and 3.1 %. The total recovery for both columns A and B amounted to 104.4 %. This value corresponds to 76.3 % and 77.2 % of the radioactivity which had been applied to each individual metabolism flask.

Vertical distribution of aged soil residues of [^{14}C]-glyphosate in Speyer 2.1 sand after percolation of 200 mm artificial rain, the values were determined by LSC (leachates) and combustion/LSC (soil segments).

Table B.8.2-30: Vertical distribution of aged soil residues of [^{14}C]-glyphosate in Speyer 2.1 soil (sand)

	Speyer 2.1 Column A		**Speyer 2.1 Column B**	
	(%)[1)]	(%)[2)]	(%)[1)]	(%)[2)]
Leachate Day 1	< 0.1	< 0.1	< 0.1	< 0.1
Leachate Day 2	< 0.1	< 0.1	< 0.1	< 0.1
Total leachate	< 0.1	< 0.1	< 0.1	< 0.1
CO_2 Headspace	3.2	2.4	3.1	2.3
Organic volatiles headspace	< 0.1	< 0.1	< 0.1	< 0.1
Total volatiles headspace	3.2	2.4	3.1	2.3
Column segment 1 (top)	99.1	72.4	97.9	72.4
Column segment 2	2.0	1.5	3.3	2.5
Column segment 3	< 0.1	< 0.1	< 0.1	< 0.1
Column segment 4	< 0.1	< 0.1	< 0.1	< 0.1
Column segment 5 (bottom)	< 0.1	< 0.1	< 0.1	< 0.1

Total column segments	101.2	73.9	101.3	74.9
Recovery	104.4	76.3	104.4	77.2

1) Values were calculated in percent of radioactivity applied to each column.
2) Values were calculated in percent of radioactivity applied to each soil sample prior to aging and leaching.

Table B.8.2-31: Radioactive residues of the duplicate soil columns

Radioactive residues (%)		**Column A Segment 1 (Top)**			**Column B Segment 1 (Top)**		
		(%)[1)]	(%)[2)]	(%)[3)]	(%)[1)]	(%)[2)]	(%)[3)]
Extractables	**Glyphosate**	72.5	71.8	52.5	71.0	69.6	44.7
	AMPA	25.2	24.9	18.2	24.7	24.2	24.7
	Total	97.6	96.7	70.7	95.7	93.7	69.3
Non-extractables		2.4	2.4	1.7	2.2	2.2	1.6
Recovery		100.0	99.1	72.4	97.9	95.9	70.9

1) Sum of extractable and non-extractable radioactivity per sample.
2) Values were calculated in percent of radioactivity applied to each column.
3) Values were calculated in percent of radioactivity applied to each soil sample prior to aging and leaching.

The two top segments of each column (0 to 6 and 6 to 12 cm depth) were extracted and the extracts were analyzed by HPLC in order to characterise the extracted radioactivity. The dpm level of the 6 to 12 cm layers of both columns was very low. As a consequence, a characterization was not feasible. However, almost the entire 6-12 cm layer radioactivity was extractable, since the non-extractable portion of radioactivity was below 0.1 %.

For the 0 to 6 cm soil layers, it was determined that 97.6 % and 95.7 % of the segment radioactivity (column A and B, respectively) was extractable. Non-extractable radioactivity amounted to 2.4 % (column A) and 2.2 % (column B). 72.5 % and 71.0 % of the extractable segment radioactivity was characterised as glyphosate. These values correspond to 52.5 % and 44.7 % of total applied radioactivity to one metabolism system. AMPA appeared at 25.2 % and 24.7 % of the column layer radioactivity corresponding to 18.2 and 24.7 % of the total applied radioactivity.

Conclusions
The results indicate that glyphosate and its major soil metabolite are immobile in the representative sandy soil used in this study. No residues penetrated deeper than 12 cm into the soil column and radioactivity in the leachates did not exceed 0.1 % of the applied radioactivity.

RMS Comment
Differing from BBA Guideline Part IV, 4-2, only one soil (Speyer 2.1) was used for the study.

The study is not required for the leaching assessment for glyphosate or its metabolite AMPA; however it can be classified as supporting information.

B.8.2.3.2 Lysimeter studies or field leaching studies

The 2001 EU evaluation concluded that a lysimeter study for glyphosate is not required. No lysimeter studies were therefore conducted by the Notifier. The 2001 EU evaluation conclud-

ed that a field leaching study for glyphosate is not required. In the 2001 evaluation it was concluded, that glyphosate and AMPA have a low propensity to leach in soils.

However, three lysimeter studies conducted close to the BBA test guideline are available in the open literature (Stadlbauer et al., 2005; Grundmann et al., 2008 and Fomsgaard et al., 2003) and are summarised under B.8.2.4.3.

B.8.2.4 Other studies on adsorption, desorption and mobility in soil (Review of open literature)

B.8.2.4.1 Adsorption and desorption of the active substance and all relevant metabolites (open literature)

~~62~~ 68 publications are available in open literature focusing on sorption and desorption properties of glyphosate. A reliability and validity assessment of the articles clarified that 16 articles were plausible but important raw data, e.g. on mass balances and test item concentrations in the aqueous and solid phases, were missing. Consequently, the validity of these studies could not be proven and therefore, results were not used to derive endpoints or to calculate PEC's. Besides, there are numerous OECD-studies available that are considered to be plausible and valid. Thus, the existing endpoint calculation (k_{oc}, 1/n) is based on a comprehensive data base. In general the results of the studies named above are in agreement with those obtained by the OECD-studies; they confirm the strong binding potential of glyphosate to agriculturally used soils in the EU.

~~21~~ 27 articles focus on dependencies of glyphosate sorption on environmental parameters such as the amount of phosphate, copper, iron and aluminum oxide. Such basic research results give a more in-depth insight into sorption processes and furthermore are of importance for the discussion of the behaviour of glyphosate in soils. Last but not least such results give guidance on the interpretation of OECD studies as well as on a possible improvement of data requirements. Some of these articles also use material other than soil, e.g., charcoal, chitin, goethite and building material.

~~17~~ 19 studies have been performed using non-European soils. Therefore, their results are not taken into account to derive endpoints or to calculate PEC's. 7 publications are review articles and letters to the editor; they do not present single data and thus were not used. One of the articles focuses on risk mitigation rather than on results of a laboratory sorption study.

In detail, OECD-studies as well as publications obtained from open literature show that glyphosate sorption in soil is not influence by a single or a dominating soil property only but by many of these physico-chemical properties. Among these, of course the soil organic matter has to be mentioned. The adsorption to soil organic matter can be explained by hydrogen bonding among the various acidic and oxygen-containing groups in glyphosate and humic material (Albers et al., 2009).

Besides soil organic matter the adsorption of glyphosate is influenced by the clay content. The phosphonic moiety of the compound forms strong complexes with metal cations of clay minerals. Also, other metals such as Fe^{3+} and Al^{3+} increase glyphosate sorption (Gimsing et al., 2004), and a positive correlation between the chemical and the iron content in the soil can be observed (Jacobsen et al., 2003).

An inverse correlation between soil pH-value and the sorption of glyphosate is described by Gimsing et al., 2004; Wang et al., 2005; Al-Rajab et al., 2008 and Zhao et al., 2009. This might be due to more net negative charge and the occurrence of electrostatically unfavourable conditions between negatively charged soil oxides and the negatively charged ionized glyphosate molecule. On the other hand De Jonge et al. (2001) described an increase of glyphosate sorption when the soil pH-value was increased by liming.

Many of publications (in particular: De Jonge et al., 2001; Dion et al., 2001; Prata et al., 2003; Wang et al., 2005; Laitinen et al., 2008 and Zhao et al., 2009) focus on the competitive adsorption between glyphosate and phosphate. Competition is plausible because of the similarity of their chemical structures and the fact, that both chemicals are strongly adsorbed by variable charge soil minerals such as aluminum and iron oxides. Generally it can be stated, that a reduction of glyphosate sorption can be observed in phosphate rich soils. However, extend of reduction depends on the soil.

This study published by De Jonge et al. (2001) quantified the variation in glyphosate sorption and desorption to a coarse sandy soil and to a sandy loam soil with varying phosphorus content and pH values. Using batch experiments, glyphosate adsorption and desorption isotherms were determined on soil samples taken from long-term field experiments that received different additions of phosphorus and lime. The isotherms were best fitted with an extended Freundlich model. The phosphate content in the soils had a significant influence on the sorption of glyphosate. With 0.5 M bicarbonate extractable P (pH 8.5) increasing from 6.2 to 58.7 in the loamy sand and 9.1 to 87.4 in the coarse sand, the extended Freundlich adsorption coefficient (K_f, MF, ads) decreased from 214.7 to 106 and from 154.0 to 83.5, respectively.

The paper of Wang et al. (2005) studied the effects of phosphate on the adsorption of glyphosate on three different types of Chinese soils including two variable charge soils and one permanent charge soil. The results indicated that Freundlich equations used to simulate glyphosate adsorption isotherms gave high correlation coefficients (0.990-0.998) with K values of 2751, 2451 and 166 for the three soils. The more the soil iron and aluminum oxides and clay contained, the more glyphosate adsorbed. The presence of phosphate significantly decreased the adsorption of glyphosate to the soils by competing with glyphosate for adsorption sites of soils.

Rampazzo et al. (2012) studied adsorption of glyphosate and aminomethylphosphonic acid in soils. The results showed that glyphosate is initially adsorbed mostly in the upper 2 cm. It is then transported and adsorbed after few days in deeper soil horizons with concomitant increasing content of its metabolite aminomethylphosphonic acid. Moreover, Fe-oxides seem to be a key parameter for glyphosate and aminomethylphosphonic adsorption in soils. This study confirmed previous studies: the analysis showed lower contents of dithionite-soluble and Fe-oxides for the Chernozem, with consequently lower adsorption of glyphosate and aminomethylphosphonic.

Ghafoor et al. (2012) investigated glyphosate sorption in the surface and subsurface soils of an agricultural catchment in southern Sweden. The authors showed that interactions between organic and inorganic sorbents affected glyphosate sorption and concluded that information on clay, iron and aluminium oxides and soil pH, in addition to organic carbon, is needed for accurate prediction of pesticide leaching. The variables f_{oc}, f_{clay} and pH are generally available, whereas measurements of oxides of Al and Fe are rarely reported. The authors therefore

emphasise the need to measure and report contents of oxides of Al and Fe in soil survey databases, because small variations in their concentrations may contribute significantly to large variations in sorption, especially of ionisable pesticides.

Competitive adsorption of glyphosate and phosphate on goethite and gibbsite and on illite, montmorillonite and two kaolinites differing in surface area was evaluated by Gimsing et al. (2004). The results show that glyphosate and phosphate are competing for the adsorption sites, but the degree of competition depends on the adsorbent. On goethite the competition is very much in favor of phosphate, on gibbsite the competition is closer, but still phosphate is favored, while on illite, montmorillonite and kaolinite the competition is almost equal. Changes in the surface area did not affect the competition between glyphosate and phosphate for adsorption sites. The results indicate that differences among soils of different mineralogical composition regarding the adsorption of glyphosate and phosphate can be expected.

Zhao et al. (2009) showed that glyphosate adsorption consistently decreased with increase in system pH value. The effect of phosphate application on glyphosate mobility varied with soil type. The authors concluded that phosphate application can cause system pH value change with various extents in the soil, which subsequently contribute to glyphosate mobility in different degree.

Borggaard (2011) concluded in his review that although several factors may control transport of glyphosate (and AMPA) from the terrestrial to the aquatic environment, the similarity between glyphosate and phosphate in relation to sorption processes strongly indicates competition between the two species for sorption sites on soil solids. This may lead to glyphosate leaching in phosphate-rich soils, where sorption sites are occupied by phosphate provided the sorption mechanisms of the two sorbates are identical. On the other hand, while sorption may protect the herbicide against microbial degradation, soil solution glyphosate is bioavailable and can be biodegraded. In addition to this indirect effect, phosphate may directly interfere with the microbial glyphosate degradation.

Literature data published on soil sorption and desorption properties of AMPA is rather scarce (Huang et al., 2004; Gjettermann, 2009; Mamy et al., 2010). However, published data show that the substance is strongly adsorbed to the soil. Published K_d-values are between 32 and 496 mL/g. This is in the same range as obtained from OECD-studies.

For a more detailed descriptions and the evaluation of the open literature studies, please refer to B.8.10. The respective references are listed there as well.

B.8.2.4.2 Coloumn leaching studies (open literature)

Several articles on soil column leaching can be found in literature. The results show that glyphosate and AMPA can only be found in groundwater under exceptional cases, usually in soil prone to preferential flow. Furthermore, it can be concluded from a soil column leaching study that glyphosate sorption does not necessarily result in phosphate release from soil.

In detail, 12 articles are available from open literature presenting information on glyphosate leaching in laboratory or outdoor soil columns. Either structured or undisturbed soil columns were used. Three representative articles out of the 12 publications are discussed:

Al-Rajab et al. (2008) performed a leaching experiment in undisturbed soil columns under outdoor conditions for 11 months. The authors suggest that non-extractable residues, which are formed at the beginning of the study, become available and take part in biodegradation and leaching. The amounts of ^{14}C-glyphosate derivatives leached were less than 0.28 % of the initially applied glyphosate. AMPA metabolite generally represented up to 100 % of the residues present in the leachates. The results of leaching were highly influenced by the hydrodynamic properties and the biodegradation capacities of the soils.

The leaching of glyphosate through structured soil columns was studied by Dousset et al. (2004) using a loamy sand and two sandy loams from sites currently under Christmas tree cultivation in the Morvan (France). After 160 mm of simulated rainfall applied over 12 days, less than 0.01 % of applied glyphosate appeared in the leachate. The mobility was greater in the soils with higher gravel contents, coarser textures, and lower organic carbon contents. Moreover, glyphosate migration seems negatively correlated not only to soil organic carbon, but also to aluminum and iron contents of soils.

Furthermore, Barrett et al. (2007) concluded that glyphosate sorption does not necessarily result in phosphate release from soil. The authors suggested that there is only limited competition between glyphosate and phosphate for sorption sites.

For a more detailed descriptions and the evaluation of the open literature studies, please refer to B.8.10. The respective references are listed there as well.

B.8.2.4.3 Lysimeter studies or field leaching studies (open literature)

Several articles on lysimeter and field leaching can be found in open literature. The results show that glyphosate and AMPA can only be found in groundwater under exceptional cases, usually in soil prone to preferential flow.

Three lysimeter studies were conducted close to the BBA test guideline and are described here in more detail (Stadlbauer et al., 2005; Grundmann et al., 2008 and Fomsgaard et al., 2003). The main results of these studies are summarised below (see also Table B.8.2-32).

Table B.8.2-32: Summary of experimental conditions in lysimeter studies

Authors	Study design	Soil/Crop/Location	Application rate	Sampling	Precipitation	Detections of glyphosate and AMPA in leachate
Stadlbauer et al. 2005	2 field plots, soil was saturated with water before application	Quaternary substrates of the "Mur" valley, Austria (spatially heterogeneous) Crops: maize monoculture or crop rotation Steiermark, Austria	3.872 L Roundup/ha in 242 L water (one application)[1)]	Leaching (suction caps and leaching collectors in 0.8 and 1.05 m depths) and mixed soil samples (0-90 cm depth, taken up to three month after application) Duration: about 2 years	2002: average annual precipitation (dry spring and autumn; high rainfall in December) 2003: 68 % of the mean annual precipitation were observed (650 mm)	Glyphosate: no detection AMPA: up to about 55 ng/L
	2 lysimeters, soil was saturated with water before application		8.8 and 10.08 L of Roundup solution (2 applications, see above)	Leachate and monolith lysimeters (down to of 3 m depth) Duration: about 2 years		Glyphosate: no detection AMPA: breakthrough with a maximum concentration of 70 ng/L
Grundmann et al. 2008	2 lysimeters which allowed the trapping of gaseous ^{14}C losses using soil and plant chambers (surface: 1 m², depth: 2 m)	Sandy soil Crop: transgenic soybeans Germany	1 kg glyphosate/ha, (3 applications), ^{14}C labelled glyphosate was mixed with Roundup	Leachate and soil cores Duration: about 15 month	Precipitation was not reported	No detections of glyphosate and AMPA
Fomsgaard et al. 2003	2 lysimeters of a low tillage field (surface: 0.5 m², depth 1.1 m)	Sandy loam soil, not ploughed in the last 20 – 30 years Crops: spring barley and winter wheat Denmark	0.8 kg glyphosate/ha, (one application), ^{14}C-labelled and unlabelled glyphosate (Roundup 2000) were mixed	Leaching, combustion and extraction of soil samples Duration: about 2 years	Precipitation was measured but not reported, Mean volume of leachate: 260 L	Glyphosate: max. annual mean concentration: 0.04 µg/L, AMPA: max. annual mean concentration: 0.02 µg/L,
	2 lysimeters of normal tillage field (surface: 0.5 m², depth 1.1 m)	Sandy loam soil, ploughed according to normal treatment Crops: spring barley and winter wheat Denmark	0.8 kg glyphosate/ha, (one application), unlabelled glyphosate (Roundup 2000)	Leaching and extraction of soil samples Duration: about 2 years	Precipitation was measured but not reported, Mean volume of leachate: 375 L	Glyphosate: max. annual mean concentration: 0.04 µg/L, AMPA: max. annual mean concentration: 0.02 µg/L

The overall risk for the leaching of glyphosate to groundwater in these studies was assessed to be low. Glyphosate was either not detected in the leachate (Stadlbauer et al., 2005; Grundmann et al., 2008) or the mean annual concentrations were significantly below 0.1 µg/L (Fomsgaard et al., 2003). A similar pattern was observed for its metabolite AMPA.

Stadlbauer et al. (2005) investigated the leaching behaviour of glyphosate and its degradate AMPA in two field plots and two lysimeters on which either maize was grown (monculture) or which were subjected to crop rotation.

Soil samples and leachate samples (using suction cups) were taken from the field plots and analysed for glyphosate and AMPA. Concentrations of glyphosate and AMPA in the leachate were also determined using the percolate water samples from the lysimeters. The test sites were located in the Mur valley at the research station Wagna (Steiermark, Austria) where the soil was spatially very heterogeneous concerning structure, texture and organic carbon. Non-radiolabelled glyphosate was applied as Roundup according to usual agricultural practice (field plots, 3.872 L/ha) and with double dose (lysimeters, 8.8 and 10.08 L/ha). The experiments were exposed to natural meteorological conditions and local agricultural practice. The water flow was characterised using water with isotopically enriched deuterium which was applied via irrigation four days prior to application. The supply of water led to a saturation of the soil with water in order to create “worst-case” conditions for the experiment. Leachate samples were collected from different depths through suction cells, and at the bottom of the lysimeters.

Overall the transport of glyphosate and AMPA was regarded as heterogeneous. While trace amounts of glyphosate were translocated into the 60-90 cm soil layer of the field plots during the first three weeks (up to 12 µg/kg dry matter), glyphosate was not detected in any leachate of the field plots or the lysimeters. Its degradate AMPA was found at several sampling intervals in the leachate with a maximum concentration of 70 ng/L. The experiment was conducted in a period with low rainfall and groundwater recharge and, thus, low amounts of percolate water. Although none of the analyzed leachate water samples contained glyphosate or AMPA above 0.1 µg/L, the authors concluded that a possible groundwater contamination cannot be excluded under extreme hydro-meteorological conditions and extensive glyphosate application.

Grundmann et al. (2008) investigated the fate of glyphosate in a two-chamber-lysimeter-test-system which allows the detailed investigation of degradation, transport and transfer processes of 14C-labelled substances in soil–plant–atmosphere-systems under outdoor conditions.

The study was conducted in a sandy soil collected from Neumarkt, Germany (clay: 4 %, silt: 8 %, sand: 88 %, pH 5.8 ($CaCl_2$), Corg: 0.97 %) which was filled in the lysimeters according to its natural soil density. Transgenic soybeans were planted. A few weeks before application the soil chambers with open top were placed on the lysimeters and soil humidity and temperature sensors were installed. ^{14}C-glyphosate was mixed with the commercial formulation of Roundup according to the instructions of the manufacturer (Monsanto Europe, Brussels, Belgium). The herbicide formulation was applied with a semi-automatic sprayer to ensure an even distribution of the pesticide on the lysimeter surface. Glyphosate was applied three times, in spring 2004 and in spring and autumn 2005 in an amount of 1 kg as/ha (1.92 MBq/mg). Immediately after application the soil chambers were covered and the plant chambers were placed on the lysimeters. The two lysimeters were equipped with two soil

chambers and two plant chambers each. During the experiment, mineralisation and volatilisation from soil and plants were measured during a time period of about 2–3 months after application. Soil temperature and soil humidity were measured inside and outside the soil chambers. Soil and plant samples were taken at different time intervals and analysed for parent compound, metabolites and non extractable residues. At each sampling date five soil cores (3 cm diameter, 20 cm depth) were taken per lysimeter. Leachate was sampled weekly and checked for radioactivity, parent compound and degradation products.

Mineralization in soil was the main elimination path of glyphosate ($^{14}CO_2$ in soil chambers accounted for 38.55 and 36.08 % of applied ^{14}C-glyphosate after the first application). After each of the three applications similar mineralisation rates were measured. No accelerated mineralization of the herbicide and therefore, no adaptation of the microbial community were detected. Glyphosate volatilization from soil and plant surfaces was negligible due to its low vapour pressure. The ^{14}C-residues in plants were due to foliar application since plant uptake of glyphosate from soil is rather unlikely. Glyphosate, applied to the plant surface was rapidly adsorbed by the plants and subsequently metabolized to a considerable extent as can be seen from the relative high amount of $^{14}CO_2$ (2.10 and 2.09 % of applied ^{14}C-glyphosate after the first application) compared with the total plant residues (5.71 and 6.47 % of applied ^{14}C-glyphosate after the first application).

Leaching was negligible since no glyphosate or AMPA could be detected in the leachate. After 15 months and three applications, more than 90 % of the glyphosate residues were found in the upper 5 cm of the soil with the highest concentration in a depth of 0–2 cm.

Fomsgaard et al. (2003) studied the leaching of glyphosate and/or its metabolite AMPA in four lysimeters.

Two of the lysimeters were replicates from a low tillage field (lysimeter 3 and 4) and the other two were replicates from a normal tillage field (lysimeter 5 and 6). On both sets of lysimeters spring barley and winter wheat were grown. The soil was a sandy loam soil with 13–14 % clay. The lysimeters had a surface area of 0.5 m2 and a depth of 110 cm. Lysimeter 3 and 4 were sprayed with a mixture of ^{14}C-labelled glyphosate and unlabelled glyphosate (Roundup 2000 together with the additive Team Up), while lysimeters 5 and 6 were sprayed with unlabelled glyphosate. The spraying took place on September 18, 1997. The nominal amount of glyphosate sprayed onto each lysimeter was 40 mg, corresponding to 0.8 kg as/ha. The lysimeters were installed in an outdoor system in Research Centre Flakkebjerg (Denmark) and were thus exposed to the normal climatic conditions of the area. A mean of 260 litres drainage water was collected from lysimeter 3 and 4 and a mean of 375 litres was collected from lysimeters 5 and 6. The mean yearly concentration of leached glyphosate and/or AMPA was significantly below 0.1 µg/L in both sets of lysimeters, and thus no significant difference between the two lysimeter sets was shown. However, in both sets of lysimeters several single findings at concentrations above 0.1 µg/L were seen (glyphosate: up to 0.52 µg/L, AMPA: up to 0.22 µg/L), which might be due to the leaching of particle bound compounds. A significant difference between the soil residual concentrations of AMPA was seen, the higher concentration was found in the set of lysimeter where low-tillage had been practiced. This might be due to differences in extraction efficiencies or due to residues resulting from earlier, more frequent sprayings with Roundup in the low tillage soil.

Besides the studies discussed in detail (Stadlbauer et al., 2005; Grundmann et al., 2008 and Fomsgaard et al., 2003) there are some other studies available:

Malone et al. (2004) used four monolith lysimeters to investigate leaching of glyphosate. A high-intensity rainfall was applied shortly after herbicide application. Even with preferential flow, glyphosate was not transported to 2.4 m at concentrations approaching environmental concern.

The transport of glyphosate and AMPA has been studied by Candela et al. (2010) in the north of Barcelona, where groundwater is located at a depth of 5.5 m. After 69 days of application residues of glyphosate up to 73.6 µg/1 were detected till a depth of 0.5 m under irrigated conditions, AMPA, analysed only in the irrigated plot was detected till a depth of 0.5 m. Such migration could be related to the low content of organic matter and clays in the soils; recharge generated by irrigation and heavy rain, and possible preferential solute transport and/or colloidal mediated transport.

Aronsson et al. (2011) conducted a field leaching study over a period of 2-year on two different soil types located at different sites in southwest Sweden. The results of the study confirmed that soil texture was the dominant factor for leaching of glyphosate. The immediate detection of glyphosate in the drain flow after application could be attributed to particle-facilitated preferential flow contributions, although the study did not distinguish between particle-bound and dissolved glyphosate.

For a more detailed descriptions and the evaluation of the open literature studies, please refer to B.8.10. The respective references are listed there as well.

B.8.3 Predicted environmental concentrations in soil (PECSoil, Annex IIIA1 9.4)

B.8.3.1 Predicted environmental concentrations in soil (PECSoil) for the active substance glyphosate (Annex IIIA1 9.4)

KIIIA 9.4/01 & KIIIA 9.5/01(Anyusheva, 2012, BVL no 2315997)

Annex point:	IIIA1 9.4/01 & IIIA1 9.5/01
Author(s):	Anyusheva, M.
Title:	Predicted environmental concentrations of glyphosate and its metabolite AMPA in soil (PECs) following application to various crops in the EU
Date:	April 25, 2012
Guideline(s):	FOCUS (1997): Soil persistence models and EU Registration. The final report of the work of the Soil Modelling Work group of FOCUS. February 1997. FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the Work Group on Degradation Kinetics of FOCUS. EC Document Reference SANCO/10058/2005 version 2.0, June 2006. FOCUS (2011a): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.0.

Deviations: None
GLP: Not relevant
Acceptability: Due to the fact that RMS has corrected the endpoints for modelling provided by the applicant, the study is not acceptable. Therefore, new PECsoil calculations were provided by the RMS.

Material and methods
Not applicable, no materials were used as this study is a computer simulation.

ModelMaker™ (version 4.0; Cherwell Scientific Publishing, The Magdalene Centre, Oxford OX4 4 GA) and Microsoft Excel™ 2003 were used for calculation of PEC_{Soil}.

The exposure assessment was based on a worst-case use pattern derived from the representative GAP information. Following a conservative approach, a single application at the maximum total yearly rate of 4320 g glyphosate acid/ha was used which is protective for all glyphosate uses included in the representative GAP (Table B.8.3-1). A worst-case crop interception of zero was assumed in all calculations.

Table B.8.3-1: GAPs for use of glyphosate

Indication	Crop	Application area	Number of applications	Maximum use rate [g as/ha]	Application timing (BBCH)	Crop interception [%]	Effective soil loading [g as/ha]	Remarks
001	All crops	Field (spray)	1-2 (min. interval 21 d)	360 – 2160	0 pre-planting	0	720 - 4320	maximum application rate 4.32 kg as/ha/year
002	All crops	Field (spray)	1	360 - 1080	0 post-planting, pre-emergence	0	360 - 1080	
003	Cereals wheat, rye, triticale	Field (spray)	1	720 - 2160	80 pre-harvest	90	720 - 2160	
004	Cereals barley, oats	Field (spray)	1	720 - 2160	80 pre-harvest	90	720 - 2160	
005	Oilseed rape	Field (spray)	1	720 - 2160	80 pre-harvest	80	720 - 2160	
006	Orchard crop, vines, citrus & tree nuts	Field (spray)	1-3	720-2880*	0 post emergence of weeds	0	720-8640* (only 240 – 2880 g/ha, because only 33 % of treated area)	
007	Orchard crop, vines, citrus & tree nuts	Field (ULV) spot treatment	1-3 (min. interval 28 days)	720-2880*	0 post emergence of weeds	0	720-8640** (only 360 – 4320 g/ha, because of max. 50 %	

							treated area)	

*Because applications are made to the intra-rows (inner strips between the trees within a row) application rates per ha are expressed per "unit of treated surface area" the actual application rate per ha orchard or vineyard will roughly only be 33 %
**Because applications are made round base of trunk and to the intra-rows (inner strips between the trees within a row) application rates per ha are expressed per "unit of treated surface area" the actual application rate per ha orchard or vineyard will roughly only be 33 % - 50 %

The initial PEC_{Soil} values (initial PEC_{Soil}, 5), i.e. maximum values after single application to the soil surface were calculated for the soil depth of 5 cm (FOCUS, 1997). The accumulation of glyphosate and AMPA was considered by calculating the concentration in soil for two depths. For the field crops, annual field mixing by ploughing was assumed and therefore, the accumulation was considered at 20 cm soil depth. In order to consider scenarios without regular ploughing (e.g. perennial crops), the accumulation was calculated for 5 cm depth. The long-term concentrations for both soil depths were calculated for a period of 30 years. The TWA values were derived based on a moving time-frame approach applied to the ModelMaker™ results. In all instances, the soil bulk density was 1.5 kg/L.

Parameters for degradation of glyphosate and formation and degradation of AMPA were derived from field dissipation studies evaluated according to FOCUS degradation kinetics (2006, 2011a).

Two compartment models were set up for parent and metabolite separately. For the calculation of glyphosate PEC_{Soil}, a maximum DT_{50} value of 143.3 days was used for glyphosate (DFOP kinetics) derived from field dissipation studies (Oppenhuizen, 1993; Kreschnak, 2012, BVL no 2315993). To represent the biphasic degradation pattern two compartments were installed representing fast (compartment 1) and slow (compartment 2) degradation of glyphosate. The actual concentrations are calculated by summing up the concentrations of each compartment for each time step. The time step was set to one day. Time weighted-average concentrations were determined from the actual concentrations using a moving time-frame approach in Microsoft Excel.

AMPA PEC_{Soil} values were calculated considering the simultaneous formation of the metabolite from the parent as well as its degradation. Since the degradation from glyphosate to AMPA follows DFOP kinetics while the degradation of AMPA follows SFO kinetics, the glyphosate parameterisation differs from the one chosen for calculation of glyphosate PEC_{Soil}: For the calculation of AMPA PEC_{Soil}, the maximum DT_{50} value of 514.9 days and the corresponding formation fraction of 0.51 (SFO kinetics) derived from field dissipation studies (Hill, 1992, BVL no 2154352, Kreschnak, 2012, BVL no 2315993) were chosen as a model input. The corresponding glyphosate half-life of 38.3 days (DFOP kinetics) was used in the calculation of PEC_{Soil} for AMPA *i.e.* the value determined for the same soil (Hill, 1992, BVL no 2154352, Kreschnak, 2012, BVL no 2315993). A summary of the relevant substance related model input data is given in Table B.8.3-2.

Table B.8.3-2: Model input data of glyphosate and its metabolite AMPA used for PEC_{Soil} simulations

Compound	DT_{50} soil, field (days)	Degradation parameters (-)	Model
Glyphosate[1)]	143.3	k1: 0.1305[3)] k2: 0.0029[4)] g: 0.2470[5)]	DFOP
Glyphosate[2)]	38.3	k1: 0.0384[3)] k2: 0.0037[4)] g: 0.5753[5)] formation fraction AMPA: 0.51	DFOP
AMPA	514.9	k: 0.0013[6)]	SFO

1) Worst-case regarding parent compound
2) Worst-case regarding formation of AMPA
3) Degradation constant in the fast phase (DFOP model)
4) Degradation constant in the slow phase (DFOP model)
5) Glyphosate fraction assigned to the fast degrading compartment
6) AMPA degradation constant (SFO model)

Results and discussion

The initial PEC_{Soil} values of glyphosate acid and AMPA after application to the soil surface calculated for a soil depth of 5 cm are shown in the table below (see Table B.8.3-3).

Table B.8.3-3: Initial PEC_{Soil} of glyphosate acid and AMPA (at 5cm depth)

Use pattern	Glyphosate acid (mg/kg)	AMPA (mg/kg)
Worst-case application scheme	5.76	1.48

The results of the accumulation investigation for glyphosate acid and AMPA for both the 0-5 cm and 0 – 20 cm soil layer are shown in Figure B.8.3-1 to Figure B.8.3-4. The plateau maximum PEC_{Soil} values for glyphosate acid were 8.07 and 2.02 mg/kg at 5 and 20 cm depth, respectively. The corresponding plateau maximum PEC_{Soil} values for AMPA were 5.35 and 1.34 mg/kg.

Table B.8.3-4: Plateau PEC_{Soil} of glyphosate acid and AMPA for tillage and no-tillage systems (maximum in bold)

PEC_{Soil}	Glyphosate acid (mg/kg)	AMPA (mg/kg)
0 - 5 cm		
Plateau minimum PEC_{Soil}	2.31	4.32
Plateau maximum PEC_{Soil}	**8.07**	**5.35**
0 - 20 cm		
Plateau minimum PEC_{Soil}	0.578	1.08
Plateau maximum PEC_{Soil}	2.02	1.34
Plateau minimum PEC_{Soil} 1)+ initial PEC_{Soils} 2)	6.34	2.56
1) calculated at 20 cm soil depth 2) calculated at 5 cm soil depth		

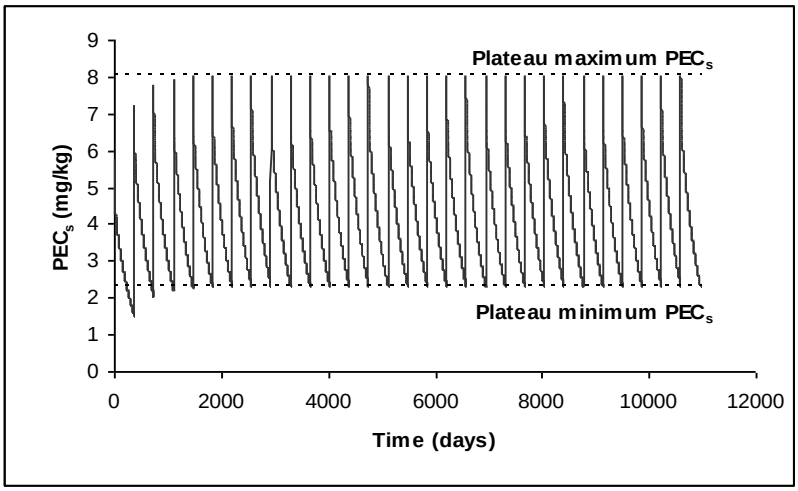


Figure B.8.3-1: Glyphosate acid concentration in soil for 30 years at 5 cm soil mixing depth. The dotted lines represent plateau maximum and minimum PEC_{Soil}.

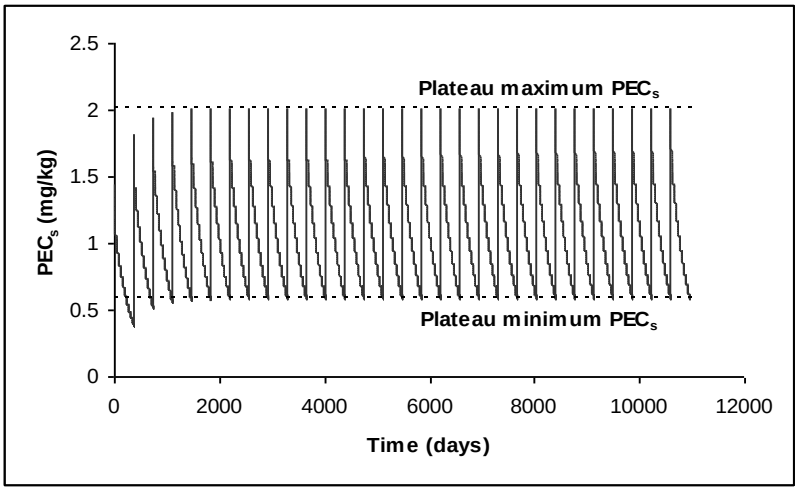


Figure B.8.3-2: Glyphosate acid concentration in soil for 30 years at 20 cm soil mixing depth. The dotted lines represent plateau maximum and minimum PEC_{Soil}.

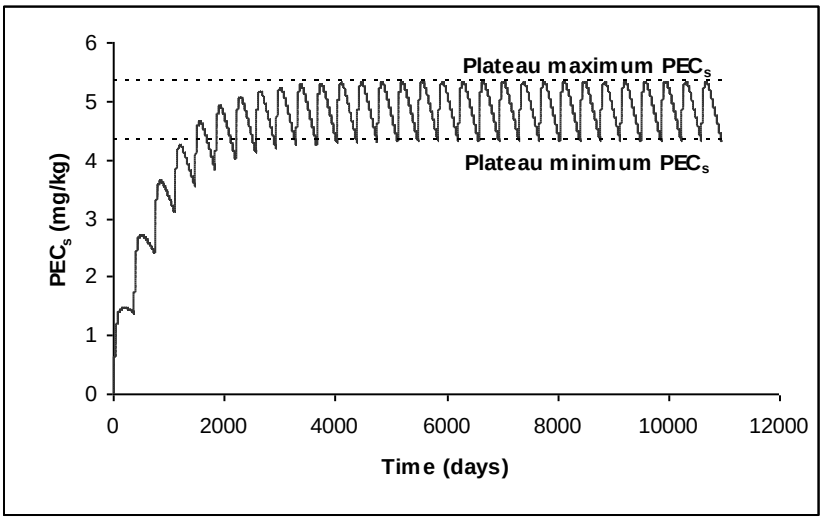


Figure B.8.3-3: AMPA concentration in soil for 30 years at 5 cm soil mixing depth. The dotted lines represent plateau maximum and minimum PEC_{Soil}.

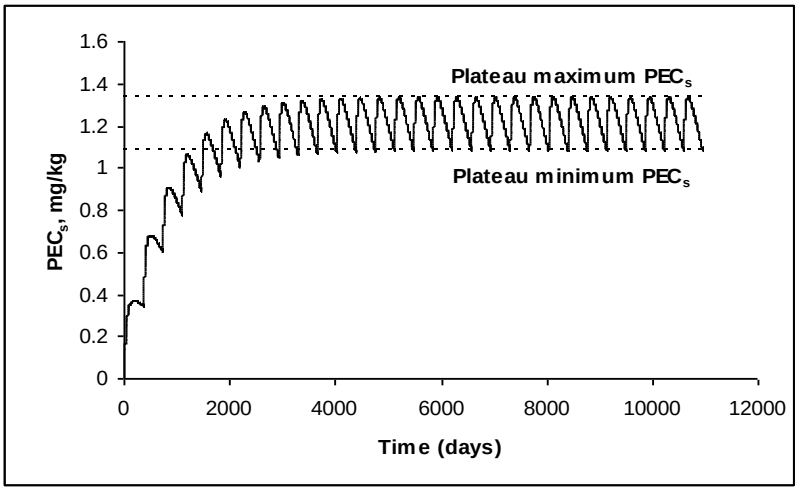


Figure B.8.3-4: AMPA concentration in soil for 30 years at 20 cm soil mixing depth. The dotted lines represent plateau maximum and minimum PEC_{Soil}.

Maximum actual and time-weighted average (TWA) PEC_{Soil} values are shown in Table B.8.3-5 and Table B.8.3-6. Only the values for the maximum initial PEC_{Soil} (5.76 mg/kg for single-year application and 5 cm soil depth) and the maximum plateau PEC_{Soil} (8.07 mg/kg for 30 years application and 5 cm soil depth) are reported representing a worst-case for all other cases (distribution over 20 cm soil depth).

Table B.8.3-5: Maximum actual and time weighted average PEC_{Soil}, of glyphosate acid and AMPA for single-year application (5 cm soil depth)

		Glyphosate acid		AMPA	
		Actual PEC_{Soil}	TWA PEC_{Soil}	Actual PEC_{Soil}	TWA PEC_{Soil}
Days after maximum		(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
Initial	**0**	5.76	-	1.48	-
Short term	**1**	5.57	5.67	1.48	1.48
	2	5.41	5.58	1.48	1.48
	4	5.13	5.43	1.48	1.48
Long term	**7**	4.82	5.24	1.48	1.48
	14	4.39	4.92	1.48	1.48
	21	4.17	4.71	1.47	1.48
	28	4.04	4.56	1.47	1.47
	50	3.75	4.27	1.47	1.47
	100	3.25	3.88	1.44	1.46

Table B.8.3-6: Maximum plateau and time weighted average PEC_{Soil}, of glyphosate acid and AMPA for 30 years application (5 cm soil depth)

		Glyphosate acid		AMPA	
		Actual PEC_{Soil}	TWA PEC_{Soil}	Actual PEC_{Soil}	TWA PEC_{Soil}
Days after maximum		(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
Initial	**0**	8.07	-	5.35	-
Short term	**1**	7.87	7.97	5.35	5.35
	2	7.70	7.88	5.35	5.35
	4	7.41	7.72	5.34	5.35
Long term	**7**	7.08	7.52	5.34	5.35
	14	6.61	7.18	5.33	5.34
	21	6.34	6.94	5.32	5.34
	28	6.16	6.77	5.31	5.34
	50	5.75	6.41	5.25	5.33
	100	4.97	5.88	5.07	5.30

Conclusion

Predicted environmental concentrations for glyphosate acid and its metabolite AMPA in soil (PEC_{Soil}) were calculated for the use on various crops in Europe in accordance with FOCUS guidelines (FOCUS, 1997, 2006). Soil accumulation was investigated for both the 0 - 5 cm and 0 – 20 cm soil layer, based on a single application of MON 52276 formulation at a maximum rate of 4320 g glyphosate acid/ha.

The initial PEC_{Soil} values for glyphosate acid and AMPA were 5.76 and 1.48 mg/kg, respectively. Maximum plateau PEC_{Soil} values of glyphosate acid and AMPA were calculated to be 8.07 and 5.35 mg/kg, respectively, for the 5 cm soil depth. For distribution over the 20 cm soil depth, the respective values were predicted to be 2.02 and 1.34 mg/kg.

RMS comment

Because of deviations in the persistence endpoints and input parameters of provided PEC soil calculations RMS considers the approach of the Notifier not acceptable. The US-field dissipation studies are not appropriate to derive the relevant maximal half life value for glyphosate discussed in B.8.1.2.2. The PEC soil calculation of AMPA using the pathway approach is not conservative for the metabolite AMPA. The RMS proposes to calculate the estimated soil concentration of AMPA based on its maximum observed occurrence in soil.

PEC_{Soil} calculations by the RMS

RMS recalculated the predicted environmental concentrations in soil (PEC_{Soil}) of glyphosate and its major soil metabolite aminomethylphosphonic acid (AMPA) using the program ESCAPE 2.0. PEC_{Soil} calculations are based on the recommendations of the FOCUS workgroup on degradation kinetics. A soil bulk density of 1.5 g/cm^3, a soil depth of 5 cm and a tillage depth of 20 cm (arable crop)/ 5 cm (permanent crops) were assumed. The PEC_{Soil} calculations were performed with ESCAPE 2.0 based on the input parameters for glyphosate and its metabolite AMPA as presented in Table B.8.3-7.

Table B.8.3-7: Summary of the input parameter of glyphosate and its metabolite AMPA used for the PEC_{Soil} simulations with ESCAPE 2.0

Compound	Maximal occurence in field dissipation studies (%)	DT_{50} soil, (days)	DT_{90} soil, (days)	Degradation parameters (-)	Model	Remarks
Glyphosate	-	38.3[1)]	386.6[2)]	k_1: 0.0384[3)] k_2: 0.0037[4)] g: 0.5753[5)]	DFOP	worst case best-fit-kinetics, unnormalised field studies, trial Kleinzecher/Germany
AMPA	53.8	633	>1000	k: 0.0013[6)]	SFO	maximum DT_{50}, unnormalised field studies, trial Unzhorst/Germany

1) Overall DT_{50} of the DFOP model
2) Overall DT_{90} of the DFOP model
3) Degradation constant in the fast phase (DFOP model)- input parameter in ESCAPE 2.0
4) Degradation constant in the slow phase (DFOP model)- input parameter in ESCAPE 2.0
5) Glyphosate fraction assigned to the fast degrading compartment- input parameter in ESCAPE 2.0
6) AMPA degradation constant (SFO model)- input parameter in ESCAPE 2.0

PEC_{Soil} immediately after the first application was calculated using FOCUS guidance recommendations with the following equation:

$$PEC_{Soil,act}\ (mg/kg) = \frac{A[g/ha] \times (1 - F)}{100 \times d\ [cm] \times \rho\ [g/cm^3]}$$

where:
A = Application rate
F = Fraction intercepted by crop
d = Depth of field soil layer (5 cm)
ρ = Dry bulk density (1.5 g/cm^3)

Due to the slow degradation of glyphosate and its metabolite AMPA in soil (DT_{90} > 365 d, field data) the accumulation potential of both substances needs to be considered. Therefore an accumulated soil concentration ($PEC_{Soil,accu}$) is used for risk assessment which comprises background concentration in soil ($PEC_{Soil,bkgd}$) considering a tillage depth of 20 cm (arable crop) or 5 cm (permanent crops) and the maximum annual soil concentration $PEC_{Soil,act}$ for a soil depth of 5 cm:

$$PEC_{Soil,accu} = PEC_{Soil,act} + PEC_{Soil,bkgd}\ [mg/kg]$$

where:

$PEC_{Soil,act}$ = maximum annual soil concentration
$PEC_{Soil,bkgd}$ = Plateau concentration in soil after many years considering a tillage depth of 20 cm (arable crop) or 5 cm (permanent crops)

Beside $PEC_{Soil,act}$ values $PEC_{Soil,twa}$ values are also required for risk assessment. $PEC_{Soil,act}$ and $PEC_{Soil,twa}$ values after a single maximum application of of 4320 g glyphosate/ha to all crops are presented in Table B.8.3-8 and Table B.8.3-9.

Table B.8.3-8: Results of PEC_{Soil} calculation (soil bulk density 1.5 g/cm^{-3}, soil depth 5 cm) after maximal application of 4320 g glyphosate/ha to all crops

plant protection product:			Mon 52276				
use:			all crops – worst case approach				
Number of applications/intervall			1				
application rate:			4320 g as/ha				
crop interception:			0 %				
active sub-stance	application	soil relevant application rate (g/ha)	PECact (mg/kg)	PECtwa 21 d (mg/kg)	tillage depth (cm)	PECbkgd (mg/kg)	PECaccu = PECact + PECbkgd* (mg/kg)
glyphosate	1 x every year annual crops	4320	5.76	4.6290	20	0.2140	5.974
	1 x every year permanent crops	4320	5.76	4.6290	5	0.8562	6.6162

* a tillage depth of 20/5 cm was considered for calculating the background concentrationof annual/permanent crops

Table B.8.3-9: Results of $PEC_{Soil,twa}$ calculation (soil bulk density 1.5 g/cm^{-3}, soil depth 5 cm) after maximal application of glyphosate to all crops

Time (d)	**PECact* (mg/kg)**	**PECtwa (mg/kg)**	**Begin TWA-frame (d)**	**End TWA-frame (d)**
1	5.6262	5.6931	0	1
2	5.4971	5.6274	0	2
4	5.2524	5.5005	0	4
7	4.9167	5.3211	0	7
14	4.2591	4.9479	0	14
21	3.7436	4.6290	0	21
28	3.3372	4.3549	0	28
42	2.7558	3.9117	0	42
50	2.5201	3.7072	0	50
100	1.7621	2.8902	0	100

$PEC_{Soil,act}$ and $PEC_{Soil,twa}$ values for the seven different intended uses of glyphosate summarised in Table B.8.3-1 are provided in Table B.8.3-12 to Table B.8.3-21.

Table B.8.3-10: Results of PEC_{Soil} calculation (soil bulk density 1.5 g/cm^{-3}, soil depth 5 cm) after maximal application of 2 x 2160 g glyphosate/ha to all crops - indication 001

plant protection product:			Mon 52276				
use:			all crops – indication 001				
Number of applications/intervall			2, interval 21 days				
application rate:			2160 g as/ha				
crop interception:			0 %				
active sub-stance	application	soil relevant application rate (g/ha)	PECact (mg/kg)	PECtwa 21 d (mg/kg)	tillage depth (cm)	PECbkgd (mg/kg)	PECaccu = PECact + PECbkgd* (mg/kg)
glyphosate	1 x every year annual crops	2 x 2160	4.7514	3.9135	20	0.2058	4.9572
	1 x every year permanent crops	2 x 2160	4.7514	3.9135	5	0.8232	5.5746

* a tillage depth of 20/5 cm was considered for calculating the background concentrationof annual/ permanent crops

Table B.8.3-11: Results of $PEC_{Soil,twa}$ calculation (soil bulk density 1.5 g/cm^{-3}, soil depth 5 cm) after maximal application of glyphosate to all crops- indication 001

Time (d)	**PECact* (mg/kg)**	**PECtwa (mg/kg)**	**Begin TWA-frame (d)**	**End TWA-frame (d)**
1	4.6524	4.7019	0	1
2	4.5568	4.6533	0	2
4	4.3755	4.5593	0	4
7	4.1263	4.4263	0	7
14	3.6358	4.1489	0	14
21	3.2487	3.9135	0	21
28	2.9408	3.7186	0	28
42	2.4935	3.3904	0	42
50	2.3084	3.2353	0	50
100	1.6779	2.7075	0	100

Table B.8.3-12: Results of PEC_{Soil} calculation (soil bulk density 1.5 g/cm^{-3}, soil depth 5 cm) after application of 2 x 2160 g glyphosate/ha to all crops - indication 002

plant protection product:			Mon 52276				
use:			all crops – indication 002				
Number of applications/intervall			1				
application rate:			1080 g as/ha				
crop interception:			0 %				
active substance	application	soil relevant application rate (g/ha)	PECact (mg/kg)	PECtwa 21 d (mg/kg)	tillage depth (cm)	PECbkgd (mg/kg)	PECaccu = PECact + PECbkgd* (mg/kg)
glyphosate	1 x every year annual crops	1080	1.440	1.1571	20	0.0535	1.4935
	1 x every year permanent crops	1080	1.440	1.1571	5	0.2138	1.6538

* a tillage depth of 20/5 cm was considered for calculating the background concentrationof annual/ permanent crops

Table B.8.3-13: Results of $PEC_{Soil,twa}$ calculation (soil bulk density 1.5 g/cm^{-3}, soil depth 5 cm) after application of of 2 x 2160 g glyphosate/ha to all crops- indication 002

Time (d)	**PECact* (mg/kg)**	**PECtwa (mg/kg)**	**Begin TWA-frame (d)**	**End TWA-frame (d)**
1	1.4065	1.4233	0	1
2	1.3742	1.4068	0	2
4	1.3131	1.3751	0	4
7	1.2291	1.3302	0	7
14	1.0646	1.2369	0	14
21	0.9357	1.1571	0	21
28	0.8340	1.0886	0	28
42	0.6887	0.9777	0	42
50	0.6297	0.9266	0	50
100	0.4402	0.7223	0	100

Table B.8.3-14: Results of PEC_{Soil} calculation (soil bulk density 1.5 g/cm^{-3}, soil depth 5 cm) after application of 2160 g glyphosate/ha to cereals (pre-harvest)- indication 003 and 004

plant protection product:			Mon 52276				
use:			cereals – indication 003 and 004				
Number of applications/intervall			1				
application rate:			2160 g as/ha				
crop interception:			BBCH 80 pre-harvest (90 %)				
active sub-stance	application	soil relevant application rate (g/ha)	PECact (mg/kg)	PECtwa 21 d (mg/kg)	tillage depth (cm)	PECbkgd (mg/kg)	PECaccu = PECact + PECbkgd (mg/kg)
glyphosate	1 x every year cereals	216	0.2880	0.2314	20	0.0107	0.2987

Table B.8.3-15: Results of $PEC_{Soil,twa}$ calculation (soil bulk density 1.5 g/cm^{-3}, soil depth 5 cm) after application of 2160 g glyphosate/ha to cereals (pre-harvest)- indication 003 and 004

Time (d)	**PECact* (mg/kg)**	**PECtwa (mg/kg)**	**Begin TWA-frame (d)**	**End TWA-frame (d)**
1	0.2813	0.2847	0	1
2	0.2748	0.2814	0	2
4	0.2626	0.2750	0	4
7	0.2458	0.2660	0	7
14	0.2129	0.2474	0	14
21	0.1871	0.2314	0	21
28	0.1668	0.2177	0	28
42	0.1377	0.1955	0	42
50	0.1259	0.1853	0	50
100	0.0880	0.1445	0	100

Table B.8.3-16: Results of PEC_{Soil} calculation (soil bulk density 1.5 g/cm^{-3}, soil depth 5 cm) after application of 2160 g glyphosate/ha to oil seed rape (pre-harvest)- indication 005

plant protection product:			Mon 52276				
use:			oil seed rape– indication 005				
Number of applications/intervall			1				
application rate:			2160 g as/ha				
crop interception:			BBCH 80 pre-harvest (80 %)				
active sub-stance	application	soil relevant application rate (g/ha)	PECact (mg/kg)	PECtwa 21 d (mg/kg)	tillage depth (cm)	PECbkgd (mg/kg)	PECaccu = PECact + PECbkgd (mg/kg)
glyphosate	1 x every year oil seed rape	432	0.576	0.4629	20	0.0214	0.5974

Table B.8.3-17: Results of $PEC_{Soil,twa}$ calculation (soil bulk density 1.5 g/cm^{-3}, soil depth 5 cm) after application of 2160 g glyphosate/ha to oil seed rape (pre-harvest)- indication 005

Time (d)	PECact* (mg/kg)	PECtwa (mg/kg)	Begin TWA-frame (d)	End TWA-frame (d)
1	0.5626	0.5693	0	1
2	0.5497	0.5627	0	2
4	0.5252	0.5500	0	4
7	0.4916	0.5321	0	7
14	0.4258	0.4948	0	14
21	0.3743	0.4629	0	21
28	0.3336	0.4354	0	28
42	0.2755	0.3911	0	42
50	0.2519	0.3706	0	50
100	0.1761	0.2889	0	100

Table B.8.3-18: Results of PEC_{Soil} calculation (soil bulk density 1.5 g/cm^{-3}, soil depth 5 cm) after application of 3 x 2880 g glyphosate/ha to orchard crop, vines, citrus & tree nuts - indication 006 (33 % of area treated)

plant protection product:			Mon 52276				
use:			orchard crop, vines, citrus&tree nuts – indication 006				
Number of applications/intervall			3, interval 28 days				
application rate:			2880 g as/ha*				
crop interception:			0 %				
active sub-stance	application	soil relevant application rate (g/ha)	PECact (mg/kg)	PECtwa 21 d (mg/kg)	tillage depth (cm)	PECbkgd (mg/kg)	PECaccu = PECact + PECbkgd (mg/kg)
glyphosate	1 x every year orchard crop, vines, citrus&tree nuts	3 x 960*	2.5490	2.1595	5	0.5159	3.0648

*Because applications are made to the intra-rows (inner strips between the trees within a row) application rates per ha are expressed per "unit of treated surface area" the actual application rate per ha orchard or vineyard will roughly only be 33 %

Table B.8.3-19: Results of $PEC_{Soil,twa}$ calculation (soil bulk density 1.5 g/cm^{-3}, soil depth 5 cm) after application of 3 x 2880 g glyphosate/ha to orchard crop, vines, citrus&tree nuts - indication 006 (33% of area treated)

Time (d)	PECact* (mg/kg)	PECtwa (mg/kg)	Begin TWA-frame (d)	End TWA-frame (d)
1	2.5031	2.5260	0	1
2	2.4587	2.5035	0	2
4	2.3744	2.4599	0	4
7	2.2582	2.3980	0	7
14	2.0281	2.2684	0	14
21	1.8445	2.1595	0	21
28	1.6966	2.0670	0	28
42	1.4771	1.9099	0	42
50	1.3837	1.8440	0	50
100	1.0422	1.6473	0	100

Table B.8.3-20: Results of PEC_{Soil} calculation (soil bulk density 1.5 g/cm^{-3}, soil depth 5 cm) after application of 3 x 2880 g glyphosate/ha to orchard crop, vines, citrus&tree nuts - indication 007 (50% of area treated,)

plant protection product:			Mon 52276				
use:			orchard crop, vines, citrus&tree nuts – indication 007 (UVL)				
Number of applications/intervall			3, interval 28 days				
application rate:			2880 g as/ha*				
crop interception:			0 %				
active sub-stance	application	soil relevant application rate (g/ha)	PECact (mg/kg)	PECtwa 21 d (mg/kg)	tillage depth (cm)	PECbkgd (mg/kg)	PECaccu = PECact + PECbkgd (mg/kg)
glyphosate	1 x every year orchard crop, vines, citrus&tree nuts	3 x 1440*	3.8235	3.2392	5	0.7738	4.5973

*Because applications are made round base of trunk and to the intra-rows (inner strips between the trees within a row) application rates per ha are expressed per "unit of treated surface area" the actual application rate per ha orchard or vineyard will roughly only be 33 % - 50 %

Table B.8.3-21: Results of $PEC_{Soil,twa}$ calculation (soil bulk density 1.5 g/cm^{-3}, soil depth 5 cm) after application of 3 x 2880 g glyphosate/ha to orchard crop, vines, citrus&tree nuts - indication 007 (50% of area treated)

Time (d)	PECact* (mg/kg)	PECtwa (mg/kg)	Begin TWA-frame (d)	End TWA-frame (d)
1	3.7546	3.7890	0	1
2	3.6881	3.7552	0	2
4	3.5617	3.6898	0	4
7	3.3873	3.5970	0	7
14	3.0421	3.4026	0	14
21	2.7667	3.2392	0	21
28	2.5449	3.1005	0	28
42	2.2157	2.8648	0	42
50	2.0755	2.7661	0	50
100	1.5633	2.4709	0	100

In Table B.8.3-22 the RMS provides the summary of the results of the PEC_{Soil} calculations of glyphosate for all intended uses shown in Table B.8.3-1.

Table B.8.3-22: Summary of the results of the PEC_{Soil} calculations of glyphosate for: all intended uses

Indication	Intended use	Application rate (g/ha) interception (%)	PECact (mg/kg)	PECaccu (mg/kg)*
worst case	all crops annual	1 x 4320 (0%)	5.7600	5.974
	all crops permanent	1 x 4320 (0%)	5.7600	6.6162
001	all crops annual	2 x 2160 (0%)	4.7514	4.9572
	all crops permanent	2 x 2160 (0%)	4.7514	5.5746
002	all crop annual	1 x 1080 (0 %)	1.4400	1.4935
	all crop permanent	1 x 1080 (0 %)	1.4400	1.6538
003, 004	cereals	1 x 2160 (90%)	0.2880	0.2987
005	oil seed rape	1 x 2160 (80%)	0.5760	0.5974
006	orchard crop, vines, citrus&tree nuts	3 x 2880 (0%) only 33% of area treated	2.5490	3.0648
007	orchard crop, vines, citrus&tree nuts	3 x 2880 (0%) only 50% of area treated	3.8235	4.5973

B.8.3.2 Predicted environmental concentrations in soil (PECSoil) for soil relevant metabolite AMPA (IIIA 9.1)

The study of Anyusheva (2012, BVL no 2315997) provided the results of PEC_{Soil} calculations for the active substance glyphosate and its metabolite AMPA (see B.8.3.1). Because of deviations in the persistence endpoints and input parameters of provided PEC soil calculations RMS considers the approach of the Notifier not acceptable. The PEC soil calculation of AMPA using the pathway approach is not conservative for the metabolite AMPA. The RMS proposes to calculate the estimated soil concentration of AMPA based on its maximum observed occurrence in soil.

The PEC_{Soil} calculations of RMS for glyphosate acid and AMPA were based on their respective longest half-lives derived from the relevant field dissipation studies. The PEC_{Soil} values for AMPA were calculated for itself considering the maximal observed occurrence (%) in the field of 53.8 % and the molecular weight ratio of the parent and the metabolite of 0.65 to ensure a worst case

For the metabolite AMPA the PEC in soil (PEC_{Soil}) is calculated using the maximum parent PEC_{Soil} after single maximal application of glyphosate to all crops according to the following equation:

PEC_{Soil} [mg/k] = max. parent PEC_{soil} × max. metabolite in soil × Mol. weight ratio

where:
max. metabolite in soil = maximum percentage (as a fraction) observed in soil metabolism studies
Mol. weight ratio = ratio of molecular weights of metabolite to the parent

Due to the slow degradation of metabolites AMPA in soil (DT_{90} > 365 d, field data) the accumulation potential of these metabolites needs to be further considered. The maximum half life value of AMPA from un-normalised field study of the trial Unzhorst/Germany is 633 days following SFO kinetics (see Table B.8.3-7).

A corrected $PEC_{Soil,accu}$ calculation is provided by the RMS due to different degradation end-point evaluation for this metabolite and the conservative approach based on its maximal occurrence in field dissipation studies in Table B.8.3-23 and Table B.8.3-24.

Table B.8.3-23: Results of PEC_{Soil} calculation (soil bulk density 1.5 g/cm^{-3}, soil depth 5 cm) of AMPA after maximal application of 4320 g glyphosate/ha to all crops

plant protection product:			Glyphosate SL (360 g as/L)				
use:			all crops -				
Number of applications/intervall			1				
application rate:			4320 g ai/ha				
crop interception:			0 %				
Metabolite			AMPA				
maximal occurrence in field studies (%)			53.8 (Schulz1992, BVL no 1932133, trial Menslage/Germany)				
Molecular weight ratio			0.657				
active substance	application	soil relevant application rate (g/ha)	PECact (mg/kg)	PECtwa 21 d (mg/kg)	tillage depth (cm)	PECbkgd (mg/kg)	PECaccu = PECact + PECbkgd* (mg/kg)
AMPA	1 x every year annual crops	1527	2.0360.	2.0128	20	1.0359	3.0719
	1 x every year permanent crops	1527	2.0360.	2.0128	5	4.1437	6.1797

* a tillage depth of 20 cm was considered for calculating the background concentration

Table B.8.3-24: Results of $PEC_{Soil,twa}$ calculation (soil bulk density 1.5 g/cm^{-3}, soil depth 5 cm) after maximal application of AMPA to all crops

Time (d)	PECact* (mg/kg)	PECtwa (mg/kg)	Begin TWA-frame (d)	End TWA-frame (d)
1	2.0338	2.0349	0	1
2	2.0315	2.0338	0	2
4	2.0271	2.0315	0	4
7	2.0205	2.0282	0	7
14	2.0050	2.0205	0	14
21	1.9897	2.0128	0	21
28	1.9745	2.0051	0	28
42	1.9445	1.9899	0	42
50	1.9275	1.9813	0	50
100	1.8248	1.9285	0	100

B.8.4 Fate and behaviour in water (Annex IIA 7.5 to 7.8)

B.8.4.1 Hydrolytic degradation

Two hydrolysis studies with glyphosate and glyphosate-trimesium (Burgener (1990, BVL no 2442046), Myers et al, (1983, BVL no 1031582)) were assessed as acceptable during the EU review of glyphosate (2001). The results are summarised in the monographes of glyphosate and glyphosate-trimesium. Two additional studies that were not previously reviewed are available from GTF members (see IIA 2.9.1). In the studies glyphosate was found to be stable to hydrolysis. No significant degradation products have been found in these studies. Therefore, no hydrolysis study for AMPA was conducted. However, because of chemical structure similarity of glyphosate and AMPA and the general observation of the prolonged stability of AMPA in highly alkaline (*e.g.* 0.1 N NH_4OH solvent commonly used to extract glyphosate and AMPA from soil) and acidic aqueous solutions (*e.g.* 6 N HCl elution solvent in AMPA crop method), AMPA also could also be characterized as stable toward hydrolysis.

B.8.4.2 Photochemical degradation

The photochemical degradation of glyphosate and glyphosate trimesium were investigated during the 2001 EU approval of glyphosate. The results of the acceptable studies with glyphosate and glyphosate trimesium (van Dijk, 1992, BVL no 2252558, Ericson, 1992, BVL no 1052518) are summarized in the Monographes of glyphosate and glyphosate-trimesium.
The metabolite aminomethylphosphonic acid (AMPA) does not absorb light significantly at wavelengths longer than 230 nm. Thus, in highly purified sterile water, in which direct photolysis is the only mechanism for photo-transformation, AMPA is expected to be photo-stable. Together with the results of the photochemical degradation studies for glyphosate and glyphosate-trimesium presented in Annex Point IIA 2.9.2, it can be concluded that both glyphosate and AMPA should be stable to direct phototransformation in the purified sterile water.

B.8.4.3 Biological degradation

In the 2001 EU evaluation of glyphosate, several studies assessing glyphosate's ready biodegradability have been reviewed (Henshall et al., 1972, BVL no 1934355; Brightwell et al., 1978, BVL no 1932009; Wüthrich, 1990, BVL no 1934369; Carrick, 1991, BVL no 2325628; Anonymous, 1990, BVL no 1934372; Neven, 1990a; Neven, 1990b). Two out of these reviewed studies were conducted according to the OECD guideline 302 for test on inherent biodegradability (Wüthrich, 1990, BVL no 1934369; Carrick, 1991, BVL no 2325628). One addition study according to OECD guideline 301 F (Mamometric Respirometry Test) was prepared by a Glyphosate Task Force (GTF) member (Feil, 2009).

In all studies, glyphosate did not show mineralisation of more than 60 % within 28 days. Therefore, the active substance is classified as not ready biodegradable. Table B.8.4-1 summarizes all the available compliant studies mentioned above.

The study of Feil (2009) was not presented to the RMS and therefore could not be checked. However, the results presented in the dossier of the notifier are in line with the available studies and therefore are plausible.

Table B.8.4-1: Overview of the glyphosate biodegradability studies

Reference		Guideline	Inocolum	Conc. (g dry material/L)	Test Conc. (mg/L)	Fraction of CO_2 produced from parent	
						Functional control	Glyphosate
Studies from the 2001 Evaluation	Wüthrich, 1990, BVL no 1934369	OECD 302 B, 1981	1. Sludge from domestic WTP (CH) 2. Sludge from WTP of Cheminova (DK)	0.2	620	88 % and 89 % within 7 days	0 % after 28 days for both systems
	Carrick, 1991, BVL no 2325628	OECD 302 B, 1981	Activated sludge from Kendal WTP	0.2	250	100 % within 2 days	2 % after 28 days
New study	Feil, 2009	OECD 301 F	Activated sludge from Darmstadt (Germany) WTP	1.5	103	98 % after 28 days	< 60 % after 28 days

Conc. = concentration; WTP = waste water treatment plant

B.8.4.4 Water/sediment study

The fate of glyphosate in several different water/sediment systems was evaluated during the 2001 EU evaluation of glyphosate. The results of these studies (Möllerfeld and Römbke, 1993, BVL no 1934113; Muttzall, 1993, BVL no 1982136; Steginsky and Powell, 1995, BVL no 1934389; Henshall and Brightwell, 1972, BVL no 1934355; Kesterson and Jackson, 1990; Honegger, 1992; Brightwell, 1978, BVL no 1932052) are summarised in the monograph – volume 3, 11th Dec. 1998, Part A, Glyphosate. The results of Möllerfeld and Römbke (1993, BVL no 1934113) and Muttzall (1993, BVL no 1982136) demonstrated the degradation of glyphosate to AMPA and carbon dioxide and the subsequent degradation of AMPA to carbon dioxide, similar to the pathway in soil. In one of the water/sediment studies (Möllerfeld and Römbke, 1993, BVL no 1934113), AMPA was seen in the water phase at maximum of 16 % after 14 days and an additional metabolite was also detected in the water phase, with maxi-

mum amount of about 10 % of the dose after 61 days. This metabolite was identified as hydroxymethylphosphonic acid (HMPA) in an additional study (Steginsky and Powell, 1995, BVL no 1934389). In the other study (Muttzall, 1993, BVL no 1982136), HMPA was not identified as a significant degradation product. The studies of Henshall and Brightwell (1972, BVL no 1934355), Kesterson and Jackson (1990), Honegger (1992), Brightwell (1978, BVL no 1932052) were not considered in the EU renewal since they were not accepted in the 2001 EU evaluation.

There is one additional water/sediment study (Bowler and Johnson, 1999, BVL no 2154357) conducted with ^{14}C-glyphosate-trimesium. The results from this study are summarised in an Addendum III to the glyphosate–trimesium monograph (Addendum to the monograph – volume 3, 11th Dec. 1998, Part B, glyphosate-trimesium, 16th October 2000). The results of this study are also consistent with previous studies showing degradation of glyphosate to AMPA and CO_2. However, HMPA was not identified as a significant metabolite in this study.

In the initial Annex I submission, an AMPA water/sediment study was not provided by any Notifier; however, one study (Knoch and Spirlet, 1999, BVL no 1934122) was provided later. This study is evaluted in the Addendum to the monograph – volume 3, 11th Dec. 1998, Part A, glyphosate, 16th October 2000). In this study (Knoch & Spirlet, 1999, BVL no 1934122) the fate and behaviour of ^{14}C-AMPA was assessed in two water/sediments systems freshly collected from two different creeks in Germany. During the course of the 100-day incubation period, 38 % and 29 % of applied radioactivity was mineralised to ^{14}C-carbon dioxide in the two water/sediment systems. The two thin layer chromatography (TLC) systems utilised in this study separated the water samples and sediment extracts into AMPA and up to three radioactive metabolite zones. Additional work on the characterisation of unknown metabolites in sediment extracts using ion-exchange chromatography was unsuccessful.

In addition to the above studies one other glyphosate and three other ^{14}C-AMPA water/ sediment studies are available which were not reviewed during the 2001 EU glyphosate evaluation. These studies (Heintze, 1996, BVL no 1939626; Feser-Zügner, 2002, BVL no 2310270; McEwen, 2004, BVL no 2310275 and Knoch, 2003, BVL no 2310273) will be summarised below. The results of Heintze (1996, BVL no 1939626) showed that the decrease of the glyphosate concentration is caused by metabolism and degradation of the compound as well as by transfer process from the water to the sediment, probably caused by sorption processes. AMPA was seen in the water phase at maximum of 10.3 % after 58 days and in the sediment at a maximum of 15.9 % after 120 days. HMPA was not identified as a significant degradation product. The results of Feser-Zügner (2002, BVL no 2310270), McEwen, (2004, BVL no 2310275) and Knoch, (2003, BVL no 2310273) provide supplementary/supporting information on the water/sediment behaviour of AMPA. The results of these studies are also consistent with the previous studies showing rapid dissipation of AMPA from water phase by adsorption to the sediment followed by microbial degradation to CO_2. In Feser-Zügner (2002, BVL no 2310270) other metabolites were detected in the water/sediment system. The major metabolite of AMPA (designated as M3.3 metabolite) which was found mainly in sediments was identified as 1-oxo AMPA (for structure see Figure B.8.4-1) in which the sole carbon of AMPA was oxidized to a carboxyl moiety. Another transient degradation product which was designated as the metabolite M7 in the study report was mostly detected in the water phases of both aquatic systems. This metabolite was characterised as acid labile in the study report. Although this metabolite was not identified in the study report but based on ready mineralisation of AMPA to CO_2, one logical metabolic transformation expected is further oxidation of

the carbon of AMPA and/or 1-oxo AMPA to form phosphonoformic acid as a possible structure for metabolite M7 (for structure see Figure B.8.4-1). Phosphonoformic acid has been reported in the literature to be acid labile and readily degrades to carbon dioxide and phosphoric acid under mild acidic conditions. In McEwen (2004, BVL no 2310275) and Knoch (2003, BVL no 2310273) other unidentified metabolites (P1a, P3, unknown) were also detected in sediments or in total systems, respectively. No risk assessment is necessary for the potential minor transient degradation products named above since they were only seen in AMPA water/sediment studies and were never detected in any of the available glyphosate water/sediment studies.

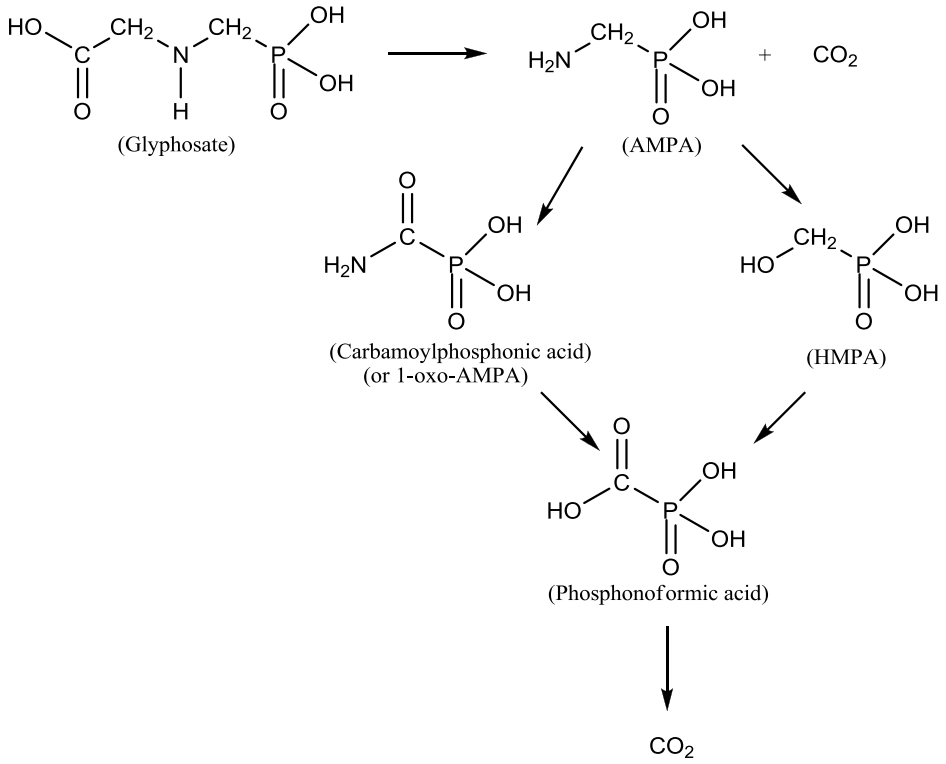


Figure B.8.4-1: Proposed degradation pathway of glyphosate and AMPA in water/sediment systems

In summary, the results of all water/sediment studies show that, in addition to microbial degradation, a major contributor to the aquatic dissipation of glyphosate is adsorption to the sediment. They also demonstrated that from approximately 6 % to 48 % of the applied glyphosate is mineralised to carbon dioxide during 91 or 100 days of incubations. Radioactivity associated with non-extractable residue was between 8 % and 35 % of the applied glyphosate during 97 or 91 days of incubation. The principal metabolite of glyphosate in water/sediment system is AMPA. The maximum amounts of AMPA detected were 16 % (water phase), 19 % (sediment) and up to 27 % (total system) of the total glyphosate applied. These studies that were independently conducted with 14C-AMPA as a test substance also established that AMPA quickly dissipates from the water phase by both adsorption to the sediment and by degradation by the sediment micro-flora. Studies demonstrated that from 8 % to 40 % of the applied AMPA is mineralised to carbon dioxide. Several other minor components were also detected in these studies. Based on degradation products identified a degradation pathway for

glyphosate and AMPA in water/sediment system is proposed. Potential minor transient degradation products of AMPA have also been depicted in the proposed pathway in Figure B.8.4-1 in order to postulate the mechanism of mineralisation of AMPA to CO_2.

The end points as reported in the original reports are not appropriate for risk-assessment and exposure modelling of the environmental fate of glyphosate and AMPA in the EU. Therefore, the degradation and dissipation rates of glyphosate and AMPA from both old and new water/sediment studies were re-calculated by Partsch (2012, BVL no 2316005) and the RMS in accordance with the kinetic approaches recommended in the latest guidance (FOCUS, 2006, 2011). Table B.8.4-69 and Table B.8.4-70 show the re-calculated persistence and modelling degradation values of glyphosate and AMPA, respectively, in accordance with the kinetic approaches recommended in the latest guidance (FOCUS, 2006, 2011) to support the aquatic risk assessment.

The recalculated half-life values of glyphosate for the total system ranged between 13.8 and 329.9 days leading to a geometric mean of 67.7 days (n = 6). Water phase DT_{50} values of glyphosate varied between 6.8 and 21.8 days. A geometric mean value of 9.6 days resulted for the water phase. Sediment DT_{50} values of glyphosate ranged between 34.1 and 75.6 days.

The recalculated half-life values of AMPA for the total systems ranged between 69.3 and 102.9 days leading to a geometric mean value of 86.1 days (n = 4). Water phase DT_{50} values of AMPA varied between 2.1 and 15.5 days. A geometric mean value of 5.5 days resulted for the water phase. Sediment DT_{50} values of AMPA could not be derived.

Table B.8.4-2: Overview of the glyphosate and glyphosate-trimesium aerobic laboratory water-sediment studies

	Reference	**Guideline**	**Application rate**	**Temp. (°C)**	**Duration of test (Days)**	**pH water**	**Sediment characteristics**								**Metabolites (maximum, day, water-sediment-system)**
							Sediment type	OC (%)	pH	Clay (%)	Sand (%)	Silt (%)	CEC (mval/ 100 g)	Water: sed ratio	
Studies from the 2001 evaluation	Heintze, 1996, BVL no 1939626	BBA, part IV, 5-1 (1990)	8.375 µg/cm^3	20±2	120	7.9/ 8.3	Sandy creek	0.11	7.85	1.1	97.2	1.7	4.3 mval/ 100 g	2.4:1	AMPA (water 10.3 % at day 58, sediment 15.9 % at day 120, sandy creek)
							Silty pond	3.31	6.64	11	9.8	79.1	22.1 mval/ 100 g		
	Muttzall, 1993, BVL no 1982136	Dutch guideline, section G.2.1 (1991)	0.2 mg/flask	20±2	13 weeks	9.3/ 7.7	Zuidpolder	7.2[1)]	7.3[2)]	26.8	39.0	34.2	35.0 meq/ 100 g	20-30 g : 200 mL	-
							Kromme Rijn	1.45[1)]	7.4[2)]	9.3	79.6	11.1	7.8 meq/ 100 g		
	Möller-feld & Römbke, 1993, BVL no 1934113	German BBA Guideline Part IV, Section 5-1 (Dec. 1990)	230 µg/test container	20±2	100	8.6/ 8.6	Sand	0.68[1)]	7.80	5.9	82.3	11.8	762 mval/ kg	3:1 – 2.4:1	AMPA (water 15.7 % at day 14, sand) HMPA (water 10 % at day 61 and 7.5 % at day 100, sand)
							Loam	4.2[1)]	7.68	10.0	15.0	75.0	1030 mval/ kg		
	Bowler & Johnson, 1999, BVL no 2154357	German BBA Guideline Part IV Section 5-1 (Dec. 1990) and SETAC 8.2, Aerobic Aquatic	3.3 mg/L	20	100	7.6/ 7.7	Sandy loam (Cache)	0.34[1)]	8.1	2	76	22	11.7 meq/ 100g	2:1	AMPA (water 10.3 % at day 30, sediment 18.7 % at day 58, total system 27.1 % at day 30, Cache)
							Silty	1.2[1)]	7.5	20	26	54	22.0		

		Degradation (March 1995)					loam (Putah)						meq/ 100g		

1) calculated from OM content of 0.6 % and 2.1 %, respectively
2) KCl

Table B.8.4-3: Overview of the AMPA aerobic laboratory water-sediment studies

	Reference	Guide-line	Appli-cation rate	Temp. (°C)	Dura-tion of test (days)	pH wa-ter	Sediment characteristics								Metabolites (maximum, day, water-sediment-system)
							Sedi-ment type	OC (%)	pH	Clay (%)	Sand (%)	Silt (%)	CEC (mval /100 g)	Water: sed ratio	
Studies from the 2001 evaluation	Knoch & Spirlet, 1999, BVL no 1934122	SETAC, Part 1, 8.2 (1995)	470 µg/L	20±2	100	8.1/ 8.4	Sand	0.52	7.7	0.6	99.3	3.7	16.1 mval/ kg	2.4:1	≥ 4 unknown (water 10 % at day 14, sediment 12 % at day 30, silty-sandy loam)
							Silty-sandy loam	3.83	7.6	17.5	38.5	45.7	137 mval/ kg		
Studies not reviewed in the 2001 evaluation	Feser-Zügner, 2002, BVL no 2310270	BBA part IV, 5-1; 91/414/ EWG; SETAC 1995	0.197 mg/L	20±2	119	7.1/ 7.3	Rück halte-beck-en	1.3	n.s.[1)]	5.7	10.6	83.7	142.3 µmol/ g	3:1 – 4:1	M2.5 (sediment 7.0 % at day 13 and 5.7 % at day 31, Schäphysen) M3.3 (sediment 22.9 % at day 13, Schäphysen) M7 (water 8.0 % at day 31 and 7.6 % at day 60, Rückhaltebeck-en)
							Schäp hysen	4.2	n.s.[1)]	4.5	86.2	9.3	172.7 µmol/ g		
	McEwen, 2004, BVL no 2310275	91/414/ EEC; SETAC (1995)	3.6 kg/ha	20±2	103	7.2/ 7.1	Clay loam (A)	3.25	7.6	23	48	29	14.7 mEq/ 100 g	2:1	P1a (sediment 53.0 % at day 14, A) P3 (sediment, 33.2 % at day 103, B) Others (sediment 22.0 % at day 103, A)
							Clay loam (B)	3.48	6.3	24	48	28	17.0 mEq/ 100 g		
	Knoch, 2003, BVL no 2310273	SETAC, Part 1, 8.2 (1995)	0.958 mg/L	20±2	104	8.1	Sand	0.64	7.9	0.2	93.8	6.2	28.7 mval/ kg	4:1	Unknown (total system 11.2 % at day 7)

1) n.s.: not stated in report

KIIA 7.8.3/1 (Feser-Zügner, 2002, BVL no 2310270)

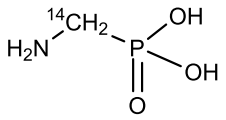
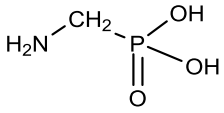
Annex point:	KIIA 7.8.3/1
Author(s):	Feser-Zügner, W.
Title:	Aminomethylphosphonic acid: fate and behaviour in water-sediment
Date:	November 12, 2002
Guideline(s):	Richtlinen für die Prüfung von Pflanzenschutzmitteln im Zulassungsverfahren, part IV, 5-1of the 'Biologische Bundesanstalt für Land- und Forstwirtschaft', Germany and 91/414/EWG, SET AC-Society of Environmental Toxicology and Chemistry, 1995: "Procedures for Assessing the Environmental Fate and Ecotoxicology of Pesticides", OECD guidelines for the testing of chemieals, draft proposal for a new guideline 308, Aerobic and Anaerobic Transformation in Aquatic Sediment Systems
Deviations:	The sediments were filled into flasks with up to a height of 1.5 - 2.0 cm. According to OECD 308 the test should be performed with a sedimet layer of 2.5 cm (± 0.5 cm). Scine the requirement ragarding the water:sediment volume ratio between 3:1 and 4:1 (OECD 308) was met, the variations in the height of the sediment layer that may have occured are acceptable.
GLP:	Yes
Acceptability:	Yes

Material and methods

Test item

The radiolabelled and non-radiolabelled test item is characterised in Table B.8.4-4.

Table B.8.4-4: Test item

	Test material (radiolabelled)	**Test material (non-radiolabelled)**
Common name	Aminomethylphosphonic acid ([^{14}C]-AMPA, specific activity 2.11 GBq/mmol)	Aminomethylphosphonic acid (AMPA)
Structural formula		
Purity	98.1 % (supplier); 98.7 % (A & M)	> 99 % (supplier); 100 % (A & M)
CAS	1066-51-9	1066-51-9
Stability of test compound	≤ -18 °C, protected from light	At room temperature, protected from light

Water/sediment systems

Two independent water/sediment systems were used, one from a pond near 'Schaephysen' (Germany) and one from the 'Rückhaltebecken/Selbeckerbach' at the 'Angertal' (Germany). Sampling was performed 0.5 - 2 m off-shore. About 10 L of water and 10 L of sediment (maximal depth: 20 cm) were taken and stored well ventilated at 2 - 8 °C in the dark for 4 days after collection. Prior to use the water was filtered through a 0.1 mm sieve and the sedi-

ment was sieved through a 2.0 mm sieve. The physiochemical parameters of the water/sediment systems are given in Table B.8.4-5.

Table B.8.4-5: Physiochemical parameters of the water/sediment systems

System	Parameter	Rückhaltebecken			Schaephysen		
		Post handling	Start of test	End of test	Post handling	Start of test	End of test
Water phase	Total OC (mg/L)	< 1	4 (n = 2)	6 (n = 2)	< 1	5 (n =2)	6 (n=2)
	Total N (mg/L)	5.0	-	< 5	< 5	-	< 5
	Total P (mg/L)	0.07	-	0.28	0.06	-	0.05
	pH (water)	7.1	8.93/8.40	9.02/8.41	7.3	8.05/8.00	7.96/7.49
	CEC (µmol/g)	142.3	-	-	172.7	-	-
	Oxygen content (mg/L)	8.8/8.8[3)]			9.1/9.0[3)]		
	Oxygen water (% saturation)		93	95/92		87/86	90/85
	Redox potential (mV)	270	280	248	254	298	303
Sediment phase	pH (sediment)	-	7.63/7.65	7.97/7.97	-	7.35/7.32	6.92/6.87
	Total OC (mg/L)	1.3 % (dry weight)	1.3 % (dry weight)	1.4 % 1.3 % (dry weight)	4.2 % (dry weight)	3.4 % / 2.9 % (dry weight)	3.2 % (dry weight) (n=2)
	Total N (mg/L)	< 0.1 % (dry weight)	-	0.08 % (dry weight)	< 0.1 % (dry weight)	-	0.08 % (dry weight)
	Total P (mg/kg)	694 (dry weight)	-	687 (dry weight)	377 (dry weight)	-	340 (dry weight)
	Sand	10.6	-	-	86.2	-	-
	Silt	83.7	-	-	9.3	-	-
	Clay	5.7	-	-	4.5	-	-
	Biomass (mg BioC/100 g dry weight)	13.9	11.21 10.89	11.81/11.71[1)] 10.16/14.4[2)]	20.25	15.85/11.70	13.05/14.04[1)] 11.60/13.93[2)]
	Redox potential (mV)	-	-185	-190	-	-390	-193

1) 119/123 days, incubation with AMPA
2) 122/124 days, control
3) Value below the water surface/approx. 5 cm above sediment surface

Study design

Experimental conditions

Portions of 110 g of the sediment 'Rückhaltebecken' (63.5 % dry weight) and 110 g of the sediment 'Schaephysen' (64.1 % dry weight) were filled into 500-mL-flasks with NS29 up to a height of 1.5 - 2.0 cm. 220 g of the corresponding water phase was added to a height of about 6 cm. The systems were left at 20 ± 2 °C to reach a steady state in pH, redox potential, oxygen content and clearing of the water phase.

The soil/water mixtures used as sterile control were sterilised by heating the water/sediment systems on two consecutive days in an autoclave for 2 h.

Radiolabelled AMPA solution was prepared and used at an application rate of 0.197 mg AMPA/L water corresponding to a maximum rate of 1.8 kg glyphosate/ha applied in the field under the assumption that glyphosate was metabolised to an extent of 50 % to AMPA. An unlabelled AMPA stock solution was also prepared at the same rate and used to determine the microbial biomass at the end of the study (day 119/123).

The absorption/ventilation device consisted of a glass tube (total length 14.5 cm, NS 29) with a gas inlet tube filled with (from inside to outside):1 g paraffin-covered glass wool for adsorption of volatile organic compounds (moistened with 2 % paraffin-oil in hexane); 0.2 g glass wool; 10 g soda lime for absorption of carbon dioxide from the incubation mixture; 0.2 g glass wool; 4 g soda lime for absorption of atmospheric carbon dioxide and 0.2 g glass wool.

The experiments were prepared in duplicate for each time point at 20 ± 2 °C in the dark and passively ventilated with CO_2-free air for 119 days. For each test system, 2 x 14 incubation flasks were set up and used for the following experiments of the test item: biomass and TOC (without AMPA) before start of the incubation, blank (without AMPA), sterile control, biomass after incubation and control biomass.

Sampling
Duplicate samples for analysis were taken on day 0, 3, 7, 14, 31, 60, 89, 119 and 122 (sterile controls) of the incubation period for system ‘Rückhaltebecken’ and on 0, 3, 5, 13, 31, 60, 90, 119 and 122 (sterile controls) of the incubation period for system ‘Schaephysen’.

Analytical procedures
At each processing time of the incubation period oxygen content of the water, pH of the water and sediment, redox potential of the water and redox potential of the sediment were determined.

After sampling and before opening, the flasks were flushed for 10 minutes with CO_2-free air (approximately 100 mL/min) to absorb volatile organic compounds and carbon dioxide from the gas phase. Then sediment, water and compartments of absorption/ventilation devices were analysed separately.

After adding 1 ml 0.1 M NaOH to the test system, the water phase was decanted from the sediment using a folded filter. After measuring the volume, the water was filled into polyethylene flasks and stored in the dark at ≤ -18 °C. The radioactivity was determined by mixing 1 mL aliquots with 4 mL of Pico-FluorTM 40 cocktail and scintillation counting for 10 minutes.

For the samples of two test systems which exhibited a formation of carbon dioxide > 20 %, water phases were analysed for water dissolved carbon dioxide. The corresponding water phases were thawed and 50 mL were used for the liberation of carbon dioxide.

Sediment was extracted with 80 mL 0.1 M NaOH and then shaken for 5 minutes followed by centrifugation for 15 minutes at 13000 rpm. The supernatant was decanted using a folded filter. The sediment was extracted for a second time using 80 mL 0.1 M NaOH as described above. The post NaOH-extracted sediment was exhaustively extracted in a Soxhlet apparatus with 70 mL methanol for approximately 2 h (Soxhlet-extract).

The air dried extracted residues were combusted in a Sample Oxidiser. Samples were combusted under a continuous flow of oxygen to trap the CO_2 of the incineration gas in a specially designed reaction column. It was filled with 10 mL of 3- methoxypropylamine as carbon dioxide absorbent (Carbosorb E). As liquid scintillation cocktail 10 mL of Permafluor E+ was added automatically.

Prior to HPLC analysis, the water fractions were centrifuged for 10 minutes at 12000 rpm and 20 °C. The NaOH-extracts were acidified, vortexed and centrifuged for 10 minutes at 12000 rpm and 4 °C. The MeOH-extracts were evaporated at 35 °C in a blow-off device under nitrogen current. The residues were dissolved in water and vortexed for 1 min. Then, each sample was acidified, homogenised and centrifuged for 10 minutes at 12000 rpm and 4 °C. Then the supernatant of these fractions were subjected to analysis.

A HPLC system I (Schimadzu; column: Hamilton PRP-X400, 250 mm x 4.1 mm, 7 µm; column oven temperature of 20 °C; flow rate of 0.8 mL/min (0 – 4.9 min) and 1.2mL/min (5.0 – 11.0 min); detection: Radiomatic FLO-ONE®\beta] and eluent A of 5mM NH4OAc pH 1.9 (with HCOOH)) was used for the determination of the radioactivity of water, of NaOH-extracts and MeOH-Soxhlet-extracts.

The characterisation of the metabolites was performed by HPLC-MS or LC-MS analysis.

A HPLC-MS system II (Agilent; flow 0.05mL/min or 0.1mL/min; ionisation interface: chemical ionisation (APCI); ionisation mode: negative; vaporiser temperature: 500 °C) was used to analyse the metabolites of MeOH-Soxhlet-extracts and of a solution of oxidised unlabelled AMPA. The mobile phase consists of two eluents (eluent A (water + 0.2 % HCOOH) and 20 % eluent B (MeOH + 0.2 % HCOOH).

The metabolites of NaOH-extracts was characterised by LC-MS analysis (column: HyPurity Elite C18, 50 mm x 2.1 mm, 5 µm; flow rate of 0.3 mL/min; ionisation interface: chemical ionisation (APCI); ionisation mode: negative; vaporiser temperature: 500 °C).

The microbial biomass of sediments was determined according to the method developed by Anderson and Domsch by substrate induced respiration.

Results and discussion

Mass balance

The total radioactivity (expressed as percent) of applied radioactivity of the extracts, carbon dioxide, volatile compounds, and bound residue were in the range of 95.7 % to 100.3 % for the system 'Rückhaltebecken', in the range of 93.9 % to 99.1 % for the system 'Schaephysen'.

Findings

A summary of recoveries and distribution of residues expressed as percentage of applied radioactivity at each sampling time interval is provided in Table B.8.4-6 for the system 'Rückhaltebecken' and in Table B.8.4-7 for the system 'Schaephysen'.

Table B.8.4-6: Distribution of radioactivity (% of applied radioactivity, mean values) in the system 'Rückhaltebecken'

Incubation time (day)	0	3	7	14	31	60	89	119	122 sterile
Water	95.5 94.2	42.3 48.8	30.0 30.8	26.7 21.0	16.1 17.7	12.5 11.8	4.5 12.1	9.1 2.0	4.6 4.8
Sediment (sum)[1)]	4.7 6.2	54.8 48.8	66.1 65.7	68.1 73.7	71.7 70.9	64.8 66.9	67.4 66.2	66.4 61.8	84.9 82.0
"Bound residue"	0.3 0.5	5.2 5.2	10.6 10.2	13.6 12.7	17.1 17.4	18.4 20.9	27.9 32.3	24.2 26.1	22.9 21.0
Carbon dioxide[3)]	n.t. n.t.	0.3 0.2	1.0 0.9	2.7 3.4	8.1 9.2	17.7 18.1	25.8 18.3	27.0 28.2	7.3 7.9
Volatile compounds	n. t. n.t.	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Recovery	100.2 100.4	97.3 97.9	97.2 97.4	97.6 98.2	96.0 97.8	95.0 96.7	97.7 96.6	102.4 92.0	96.8 94.6
Distribution in water fraction									
AMPA	93.36 91.39	39.27 46.05	24.96 25.39	19.17 11.94	7.36 8.37	4.04 3.27	0.88 3.18	2.06 0.75	2.40 2.34
M 2.5	1.00 0.97	1.19 0.91	0.69 0.58	0.65 0.85	0.73 0.69	0.60 -	- 0.98	0.64 -	1.41 1.55
M 3.3	0.36 0.73	- -	- -	- -	- -	- -	- -	- -	0.18 -
M 7	- -	0.68 1.12	3.42 3.32	6.63 7.80	7.77 8.28	7.57 7.58	3.30 7.36	6.20 1.21	0.47 0.72
non classified radioactivity	0.8 1.1	1.2 0.7	1.0 1.5	0.3 0.5	0.3 0.4	0.3 0.9	0.4 0.6	- -	- -
Distribution in extracts of sediment									
AMPA	2.85 3.91	29.82 26.90	34.46 34.19	32.73 36.47	32.04 29.05	26.62 27.94	27.91 21.21	17.80 27.10	40.72 45.91
M 2.5	- -	3.37 2.94	3.32 4.52	3.53 5.13	4.16 3.97	3.40 3.18	2.09 2.30	1.19 1.86	3.26 5.61
M 3.3	0.51 0.66	7.97 5.98	7.58 7.29	8.46 9.18	6.93 7.89	5.34 5.59	3.96 2.49	2.37 3.33	6.37 5.38
M 7	- -	- 0.34	- -	0.60 -	1.46 2.08	0.42 0.85	0.60 1.71	0.38 0.48	1.81 -
Non classified radioactivity[2)]	1.0 1.1	9.4 7.5	10.2 9.6	9.1 10.2	10.1 10.6	10.7 8.4	4.9 6.2	20.4 2.9	9.9 4.1
Sum complete system (water f actions + extracts of sediment)									
AMPA	96.21 95.30	68.08 72.94	59.42 59.59	51.90 48.41	39.40 37.43	30.67 31.21	28.78 24.38	19.86 27.86	43.12 48.25
M 2.5	1.00 0.97	4.57 3.85	4.01 5.10	4.18 5.98	4.89 4.66	3.99 3.18	2.09 3.28	1.83 1.86	4.67 7.16
M 3.3	0.87 1.40	7.97 5.98	7.58 7.29	8.46 9.18	6.93 7.89	5.34 5.59	3.96 2.49	2.37 3.33	6.55 5.38
M 7	- -	0.68 1.46	3.42 3.32	7.23 7.80	9.23 10.36	7.99 8.44	3.91 9.07	6.58 1.69	2.29 0.72
Non classified radioactivity	1.8 2.3	10.5 8.2	11.1 11.0	9.4 10.7	10.3 10.9	10.9 9.4	5.3 6.8	11.5 0.9	5.4 -0.5

1) calculated as sum from the values of the 0.1 M NaOH extraction and the Me0H Soxhlet extraction of the sediment and the 'bound residue'
2) the non classified radioactivity is the sum of the non classified radioactivity from the HPLC-analysis and the humic acid associated radioactivity which was not available for HPLC-analysis
3) the formation of carbon dioxide in the sterile controls may be caused by the use of non sterilised water of the application solution

-: <LLOQ, not detected or not tested
n.t.: not tested

Table B.8.4-7: Distribution of radioactivity (% of applied radioactivity, mean values) in the system 'Schaephysen'

Incubation time (day)	0	3	5	13	31	60	90	119	122 sterile
Water	85.7 83.0	21.9 26.9	16.7 16.3	6.0 4.9	1.9 0.7	0.5 3.2	0.5 2.1	0.5 0.4	1.1 1.4
Sediment (sum)[1)]	11.7 16.6	71.8 66.9	79.0 79.7	90.7 91.6	90.4 86.3	92.5 80.2	87.3 84.2	84.9 88.1	85.2 73.7
"Bound residue"	1.7 3.0	15.5 11.8	15.3 16.8	18.9 18.7	26.3 28.3	31.1 29.8	31.7 33.8	37.4 40.8	40.4 35.0
Carbon dioxide[3)]	n.t. n.t.	0.1 0.1	0.5 0.5	2.5 2.5	6.5 5.6	6.5 12.2	10.5 10.7	14.4 9.2	12.1 21.5
Volatile compounds	n. t. n.t.	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Recovery	97.4 99.7	93.8 94.0	96.2 96.4	99.3 99.0	98.8 92.6	99.4 95.7	98.3 97.1	99.9 97.7	98.5 96.5
Distribution in water fraction									
AMPA	82.97 80.53	18.91 24.94	15.80 13.95	3.69 3.46	0.82 0.30	- 0.88	n.c. 0.36	0.24 n.c.	0.61 0.85
M 2.5	1.11 0.50	0.55 0.87	0.45 0.46	- -	- -	- -	- -	- -	- -
M 3.3	- -	0.57 -	- -	- -	- -	- -	- -	- -	- -
M 7	0.87 -	1.35 0.56	n.c. 1.59	1.69 1.25	0.68 0.31	- 1.90	n.c. 1.32	0.19 n.c.	0.29 0.39
non classified radioactivity	0.7 2.0	0.5 0.6	0.5 0.3	0.6 0.2	0.4 0.1	0.5 0.4	0.5 0.4	- -	- -
Distribution in extracts of sediment									
AMPA	3.76 6.51	19.58 19.73	19.48 19.43	21.47 29.49	19.04 18.35	27.28 23.48	20.54 19.18	22.94 23.55	25.90 22.13
M 2.5	1.07 1.27	5.63 6.73	6.28 5.53	7.23 6.79	5.78 5.58	4.81 3.90	5.11 3.62	3.46 3.60	3.74 4.44
M 3.3	0.63 0.44	14.80 12.56	19.24 19.37	22.80 22.92	22.18 19.41	14.35 9.26	15.96 11.84	8.36 8.57	6.66 5.47
M 7	0.55 0.57	0.70 0.42	0.60 0.74	- -	1.71 0.62	0.55 -	- 0.43	0.41 0.56	0.48 -
non classified radioactivity[2)]	4.0 4.8	15.5 15.7	18.1 17.8	20.3 19.7	15.4 14.0	14.4 13.7	14.0 15.3	12.4 11.1	6.7 6.6
Sum complete system (water f actions + extracts of sediment)									
AMPA	86.73 87.04	38.49 44.67	35.28 33.38	25.16 26.95	19.87 18.65	27.28 24.36	20.54 19.54	23.18 23.55	26.51 22.97
M 2.5	2.17 1.77	6.18 7.61	6.73 5.99	7.23 6.79	5.78 5.58	4.81 3.90	5.11 3.62	3.46 3.60	3.74 4.44
M 3.3	0.63 0.44	15.37 12.56	19.24 19.37	22.80 22.92	22.18 19.41	14.35 9.26	15.96 11.84	8.36 8.57	6.66 5.47
M 7	1.42 0.57	2.05 0.98	0.60 2.32	1.69 1.25	2.39 0.93	0.55 1.90	- 1.76	0.60 0.56	0.78 0.39
non classified radioactivity	4.7 6.8	16.0 16.2	18.6 18.1	21.0 19.9	15.8 14.1	14.9 14.2	14.5 15.8	11.9 11.1	5.8 5.4

1) calculated as sum from the values of the 0.1 M NaOH extraction and the Me0H Soxhlet extraction of the sediment and the 'bound residue'
2) the non classified radioactivity is the sum of the non classified radioactivity from the HPLC-analysis and the humic acid associated radioactivity which was not available for HPLC-analysis
3) the formation of carbon dioxide in the sterile controls may be caused by the use of non sterilised water of the application solution

-: <LLOQ, not detected or not tested
n.t.: not tested
n.c.: <LLOQ (was not considered as metabolite specific)

Up to 3 different metabolites were detected which were assigned to M2.5, M3.3 and M7. The first two of them eluted earlier than the parent compound AMPA. The major metabolites of AMPA were M3.3 and M7 which amounted to 8.8 % and 9.8 % in the system 'Rückhaltebecken' and to 22.9 % and 1.8 % in the system 'Schaephysen', respectively. The occurrence of M3.3 and M7 differed. The metabolite M3.3 was found mainly in sediments, whereas the metabolite M7 was the major one in water phases of both aquatic systems. Based on the comparable retention time of radioactive oxidation product of AMPA (oxidation of [^{14}C]-AMPA performed chemically with H_2O_2), metabolite M3.3 could be characterised as 1-oxo-AMPA. The metabolites M2.5 and M7 were not identified/characterised in the study, however it was found the M7 is acid labile.

As a part of the study the degradation rate constants were calculated according to Timme et al. (1986). Since Timme et al. (1986) is not the latest guidance for kinetic approaches, the results are not reported here.

Conclusions
The fate and behaviour of aminomethylphosphonic acid (AMPA) was investigated in two independent aquatic sediment systems. AMPA was found at a relatively high extent in the sediment after three days. The decrease of AMPA from the water phase was rapid. After 13 days (for the system 'Schaephysen') and after 31 days (for the system 'Rückhaltebecken') less than 10 % of the initial applied AMPA was found in the water phase. The mineralisation to carbon dioxide was three fold higher in the system 'Rückhaltebecken' (28 %) when compared to 'Schaephysen' (11 %).

The major metabolites of AMPA were M3.3 and M7 which amounted to 8.8 % and 9.8 % in the system 'Rückhaltebecken' and to 22.9 % and 1.8 % in the system 'Schaephysen'.

RMS Comment
The sediments were filled into flasks with up to a height of 1.5 - 2.0 cm. According to OECD 308 the test should be performed with a sediment layer of 2.5 cm (± 0.5 cm). Since the requirement regarding the water/sediment volume ratio between 3:1 and 4:1 (OECD 308) was met, the variations in the height of the sediment layer that may have occurred are acceptable.

The degradation rate constants of AMPA in the water/sediment systems of the study were recalculated in accordance with the kinetic approaches recommended in the latest guidance (FOCUS, 2006, 2011).

The study can be accepted and the endpoints may be used for further risk assessment.

KIIA 7.8.3/2 (Knoch, 2003, BVL no 2310273)

Annex point:	KIIA 7.8.3/2
Author(s):	Knoch, E.
Title:	Aerobic aquatic degradation of aminomethylphosphonic acid accord-

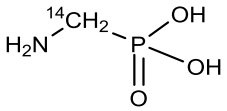
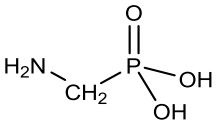
	ing to SETAC, part 1,8.2 (March 1995)
Date:	February 07, 2003
Guideline(s):	SETAC ‘‘Procedures for assessing the environmental fate and eco-toxicity of pesticides’’, part 1, 8.2 (March 1995).
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Material and methods

Aminomethylphosphonic acid (AMPA), was tested in two different water/sediment systems. The test concentration for the metabolism experiment was 0.958 mg/L. The study was conducted with a mixture of radiolabelled/unlabelled AMPA.

Test item

Table B.8.4-8: Test item

	Test material (radiolabelled)	**Test material (non-radiolabelled)**
Common name	Aminomethylphosphonic acid ([^{14}C]AMPA, specific activity 55 mCi/mmol)	Aminomethylphosphonic acid (AMPA)
Structural formula		
Purity	98.6 % (by HPLC, supplier); 100 % (by TLC/HPLC, Institut Fresenius)	99 % (supplier)
Stability of test compound	≤ -18°C in the dark	+2 to + 8 °C in dark

Water/sediment systems

Two independent water/sediment systems collected at different locations in Germany were used, one from Bickenbach and the other from Unter-Widdersheim. The sediment (layers of approximately 2 to 5 cm) of location Bickenbach was collected from 15 cm below the water surface down to a depth of 30 cm. The sediment of location Unter-Widdersheim was collected from 3 cm below the water surface down to a depth of 15 cm. The distance to the bank was 10 cm minimum. The water used in the study was collected in line with the sediment. The water and sediment were transported separately under ambient conditions and then stored separately in the dark at approximately 6 °C under aerobic conditions. Prior to use, the water was filtered (≤ 0.2 mm) and the sediment was sieved through a 2.0 mm screen.

Table B.8.4-9: Physiochemical parameters of the water/sediment systems

System	Parameter	Bickenbach			Unter-Widdersheim		
		At the time of sampling	After sampling	End of test	At the time of sampling	After sam-pling	End of test
Water phase	Temperature (°C)	7.8	19.5	20 ± 2	11.5	19.5	20 ± 2
	Dissolved O_2 (below sur-face/at 5 cm above sedi-ment) (mg/L)	12.7/12.7	6.6	39 to 45 % satura-tion	12.2 /12.2	6.6	38 to 42 % saturation
	Total OC (mg/L)	NA	1.78	12.6	NA	2.50	16.9
	Total N (mg/L)	NA	4.3	3.2	NA	6.05	5.3
	Total P (mg/L)	NA	0.06	0.13	NA	< 0.06	1.03
	pH	8.1	8.5	7.4 to 7.7	8.1	8.5	7.6 to 7.8
	Redox (E_h, mV)	430	137	154 to 157	350	134	102 to 106
Sediment phase	Temperature (°C)	7.8	18.4	20 ± 2	11.5	18.3	20 ± 2
	pH	7.9	8.5	6.9 to 7.2	7.8	8.5	7.2 to 7.4
	OC (%)	NA	0.64	NA	NA	2.96	NA
	OM (%)	NA	1.10	NA	NA	5.10	NA
	Dry weight (%)	NA	70.7	NA	NA	57	NA
	Total N (mg/kg)	NA	400	NA	NA	1700	NA
	Total P (mg/kg)	NA	459	NA	NA	1250	NA
	MWHC (g water/100 g)	NA	41.4	NA	NA	75.4	NA
	Sand		93.8			34.0	
	Silt		6.2			42.9	
	Clay		0.2			23.1	
	Classification (DIN)		Sand			Slight sandy loam	
	CEC (mval/kg)	NA	28.7	NA	NA	123	NA
	Microbial activity „An-derson and Domsch'' at a moisture con-tent of 40% MWHC (mg C/100g)	NA	23	14	NA	24	15
	Redox poten-tial (mV)	280	132	-142 to -187	120	130	-205 to -210

Study design

Experimental conditions

Flasks of water/sediment system samples were placed in a temperature controlled incubation chamber at 20 ± 2 °C and pre-incubated in the dark until equilibrium based on redox potential

of water and sediment, oxygen concentration of water and pH-value of water and sediments was reached after 4 days.

The study was performed by using metabolism flasks (500 mL Woulff'sche flask, 8 cm inner diameter) containing sediment layers (water saturated) equivalent to 75 g dry weight of either the 'Bickenbach' or 'Unter-Widdersheim' sediments and 300 mL reagent water (ratio of 1:4). After application of test item the metabolism flasks were placed into a flow through system with a series of traps. Test systems were aerated (air flow approximately 15 - 30 mL/min) and slightly agitated from the top by means of a suspended magnetic stirrer (with exception of the specimen taken at zero time). For maintenance of aerobic conditions during the experiment, the oxygen concentration of water was at least > 20 % of its saturation. A flow through system with traps for carbon dioxide and volatile organics was used for metabolism flasks containing an untreated test matrix (hydrographic analysis of aged sediment surface water) and reserved test matrix for the determination of aged microbial activity of sediments.

The ratio of AMPA stock solution ^{14}C:^{12}C-AMPA was approximately 1:3, equivalent to 26.14 mg AMPA in total dissolved in 10 mL reagent water. The radiocarbon concentration of the application solution (dosing of 110 µL portions equivalent to 0.958 mg/L) remained stable during the application procedure. The test item application rate is equivalent to a use rate of 2874 g/ha, assuming a direct overspray of water bodies and a homogeneous distribution of the test item at the top 30 cm of the water column. The stability of ^{14}C-AMPA in the application solution was established by radio-chromatography before and after application procedure.
Reserved test matrix for the determination of aged microbial activity was treated simultaneously to the kinetic specimens, but with fortification of the unlabelled test item (dosing of 52 µL equivalent to 0.952 mg/L). The study was conducted in the dark for a 104 day incubation period.

The test item was applied in reagent water onto the surface of the water phase.

Sampling
Duplicate samples for analysis were taken at the following times: zero time, 6 hours, 1, 2, 7, 14, 30, 62 and 104 days from both water/sediment systems.

The redox potential of water and sediment, oxygen concentration of water and pH-values of water and sediments were determined at each sampling interval or at about 14 day intervals using untreated test systems. Also aliquots of the volatile traps were radioassayed at each sampling time (excluding zero time) or at about 14 day intervals, whichever came first.

Analytical procedures
After decanting the water phase of the test system, water and sediments were assayed separately for their radiocarbon concentration and composition. Analyzed specimen extracts were stored in tightly closed glass storage containers at < -18 °C in the dark.

a) Sediment
The sediments were transferred quantitatively into 750 mL all-plastic centrifuge vessels and extracted several times by shaking (laboratory shaker: 350 strokes/min for 60 min) with 100 mL portions of 1 M NH3-solution. The extraction solvent was separated from the soil by centrifugation (10 minutes at 4500 rpm).

The sequential extractability of radioactivity of each individual extract as well as the combined extraction solutions were radioassayed by liquid scintillation counting (LSC). The combined extraction solutions were adjusted by the addition of HCl to pH 2 and centrifuged again.

After exhaustive soil extraction, the residual radioactivity in soils was assayed by combustion. Remaining soil was stored at room temperature in tightly closed storage containers.

The extracted soils of the 104 day samplings (air dried and ground) were subjected to further characterization of soil radioactivity which remained bound to the humic and fulvic acids and the humin fraction. The remaining soil was transferred quantitatively into 750 mL all-plastic centrifuge vessels and suspended with 120 mL of 0.5 M NaOH. The slurry was shaken for 16 hours at 350 strokes/min (laboratory shaker) and afterwards centrifuged for 10 minutes at 3200 rpm. The supernatant was separated and the remaining soil was extracted again (120 mL of 0.5 M NaOH/shaken: 1 h at 350 strokes/min/centrifuged: 10 minutes at 3200 rpm). The extractable radioactivity was assayed by LSC. The liquid phase was then acidified to approximately pH 1 by addition of concentrated HCl. The resulting suspension was stored for 24 hours at room temperature for precipitation. The suspension was separated into soluble fulvic acids and precipitated humic acids by centrifugation for ten minutes at 3200 rpm. The liquid phase containing fulvic acids was assayed by LSC. The precipitate, containing humic acids was re-dissolved with 0.5 M NaOH and assayed by LSC.

b) Water
After separation from the sediment phase, the volume of the water phase was measured and aliquots analysed by LSC. When required, water phases were concentrated by freeze drying. Specimen residues were reconstituted with reagent water. The volume of the specimen extract concentrate was measured and subjected to LSC for confirmation that there was no loss of radioactivity during specimen concentration.

c) Analysis
The specimen extracts of sediment and water were analysed in duplicate by two chromatographic systems (HPLC and TLC).

Thin Layer Chromatography (TLC) was performed on pre-coated plates of Ionex-25 SA-Na, which was developed under chamber saturation conditions with a general target development distance of approximately 16 cm. The radioactivity in specimen extracts of water and sediment was separated by using a solvent system: 0.015 M KH_2PO_4 containing 10 vol. % methanol, adjusted to pH 2.4 by addition of H_3PO_4.

HPLC analysis was based on glyphosate cation-exchange columns. The mobile phased used for the HPLC analysis was 5 M KH_2PO_4 containing 4 vol. % methanol, adjusted to pH 2 by the addition of H_3P0_4. After HPLC separation of specimen aliquots, radioactive signals were detected by means of a radioactivity monitor/UV-photometer.

The residual radioactivity in post extracted sediment was assayed in triplicate by combustion analysis. Sub-specimens, air dried and ground, were combusted in a stream of oxygen (350 cc/min) for 4 minutes at 900 °C, supported by three catalysts. Liberated $^{14}CO_2$ was absorbed in a suitable scintillation cocktail (Oxysolve C 400 by Zinsser Analytics) and the radioactivity was assayed by LSC.

Results and discussion

Mass balance

Over a period of 104 days recoveries of applied radioactivity at each sampling time ranged from 93.8 % to 103.6 % and from 98.6 to 106.5 % for Bickenbach and Unter-Widdersheim systems, respectively.

Findings

The mass balance and the distribution of residues expressed as percentage of applied radioactivity at each sampling time interval is provided in Table B.8.4-10 for the Bickenbach system and in Table B.8.4-11 for the Unter-Widdersheim system. The proportions of radioative metabolites in water and sediment extracts are presented in Table B.8.4-12 (Bickenbach system) and in Table B.8.4-13 (Unter-Widdersheim system). Additional atetppts for structure characterisation of the unknown fractions by LC/MS failed.

Table B.8.4-10: Distribution of radioactivity in the Bickenbach water/sediment system

Time point		Radioactivity recovered (% of applied radioactivity)					
	Replicate	Total water	Sediment (extractable)	Sedimen (non-extractable)	CO_2	Organic volatiles	Mass balance
Day-0	1	94.4	2.5	0.4	n.t.	n.t.	97.3
	2	99.9	1.4	0.4	n.t.	n.t.	101.7
6-hour	1	88.4	11.6	0.9	0.1	<0.1	101.0
	2	87.4	10.5	0.8	0.1	<0.1	98.8
1-day	1	69.7	23.0	1.9	0.2	<0.1	94.8
	2	72.0	25.4	2.2	0.2	<0.1	99.8
2-day	1	65.3	28.4	3.2	1.7	<0.1	98.6
	2	65.5	29.7	2.7	1.7	<0.1	99.6
7-day	1	41.5	32.2	10.6	10.1	<0.1	64.4
	2	41.4	33.5	10.7	10.1	<0.1	95.7
14-day	1	27.4	32.5	16.4	18.0	<0.1	94.3
	2	23.3	33.1	19.4	18.0	<0.1	93.8
30-day	1	13.2	33.5	27.4	24.4	1.5	100.0
	2	17.7	33.0	20.9	24.4	1.5	97.5
62-day	1	12.3	24.9	31.8	32.7	1.5	103.2
	2	12.4	25.4	31.6	32.7	1.5	103.6
104-day	1	6.8	18.3	30.0	40.1	1.5	96.7
	2	8.2	15.3	32.5	40.1	1.5	97.6

n.t.: not tested

Table B.8.4-11: Distribution of radioactivity in the Unter-Widdersheim water/ sediment system

Time point	Replicate	Radioactivity recovered (% of applied radioactivity)					
		Total water	Sediment (extractable)	Sediment (non-extractable)	CO_2	Organic volatiles	Mass balance
Day-0	1	99.1	1.1	0.5	n.t.	n.t.	100.7
	2	98.9	2.5	1.0	n.t.	n.t.	102.4
6-hour	1	83.7	14.3	2.9	<0.1	<0.1	100.9
	2	85.5	14.1	2.9	<0.1	<0.1	102.5
1-day	1	67.4	30.2	3.2	0.1	<0.1	100.9
	2	71.0	30.5	4.3	0.1	<0.1	105.9
2-day	1	57.9	40.3	6.3	0.3	<0.1	104.8
	2	58.4	38.0	4.7	0.3	<0.1	101.4
7-day	1	33.5	55.5	8.0	1.6	<0.1	98.6
	2	37.0	53.6	6.5	1.6	<0.1	98.7
14-day	1	17.4	65.1	15.5	3.4	<0.1	101.4
	2	19.4	60.4	15.4	3.4	<0.1	98.6
30-day	1	12.2	64.4	14.6	9.3	0.1	100.6
	2	12.0	65.8	15.9	9.3	0.1	103.1
62-day	1	2.7	60.9	26.9	15.9	0.1	106.5
	2	3.0	57.1	28.5	15.9	0.1	104.6
104-day	1	2.5	39.4	36.0	21.2	0.1	99.2
	2	2.2	46.6	29.5	21.2	0.1	99.6

n.t.: not tested

Table B.8.4-12: Proportions of AMPA and unknown metabolites according to TLC and HPLC in the Bickenbach water/sediment system

Time point	Replicate	TLC		HPLC			
		AMPA (water)	AMPA (sediments)	AMPA (total system)	Unknown 1 (total system)	Unknown 2 (total system)	All unknown (total system)
Day-0	1	94.4	n.t.	92.6	1.9	n.d.	1.9
	2	99.9	n.t.	97.7	2.2	n.d.	2.2
6-hour	1	87.8	11.0	97.8	n.d.	2.2	2.2
	2	87.4	10.1	97.9	n.d.	n.d.	n.d.
1-day	1	63.9	23.0	84.5	3.1	3.1	8.3
	2	62.5	25.4	85.3	7.0	1.4	12.2
2-day	1	58.5	26.1	71.5	11.7	7.6	22.4
	2	59.5	27.6	80.6	5.3	6.1	14.7
7-day	1	27.9	29.0	56.4	6.2	11.2	17.4
	2	28.6	30.7	54.1	9.9	11.1	21.0
14-day	1	16.7	29.3	46.6	8.4	5.0	13.4
	2	15.4	31.3	46.7	5.0	4.8	9.8
30-day	1	9.7	32.0	41.1	3.5	2.3	5.8
	2	13.8	31.7	44.3	3.9	2.8	6.7
62-day	1	7.6	22.5	30.5	4.5	2.3	6.8
	2	7.6	23.4	29.1	4.5	4.5	9.0
104-day	1	4.7	17.7	23.0	1.9	0.3	2.2
	2	5.8	14.2	16.6	7.0	n.d.	7.0

Results expressed as % applied radioactivity
n.t.: not tested
n.d.: not detected

Table B.8.4-13: Proportions of AMPA and unknown metabolites according to TLC and HPLC in the Unter-Widdersheim water/sediment system

		TLC		HPLC			
Time point	**Replicate**	**AMPA (water)**	**AMPA (sediments)**	**AMPA (total system)**	**Unknown 1 (total system)**	**Unknown 2 (total system)**	**All unknown (total system)**
Day-0	1	99.1	n.t.	97.4	1.7	n.d.	1.7
	2	98.9	n.t.	96.8	2.2	n.d.	2.2
6-hour	1	80.6	13.7	93.1	2.2	2.9	5.1
	2	82.1	13.2	95.3	1.8	2.5	4.3
1-day	1	60.2	30.2	92.5	2.3	2.9	5.2
	2	63.1	30.5	96.6	2.5	2.5	5.0
2-day	1	52.7	37.9	91.2	3.5	3.7	7.2
	2	54.4	35.9	91.0	2.4	2.9	5.5
7-day	1	28.9	53.7	83.8	1.9	3.4	5.3
	2	32.0	51.5	86.0	2.1	2.6	4.7
14-day	1	12.2	62.7	73.3	4.7	4.6	9.3
	2	14.4	58.3	72.3	3.5	4.1	7.6
30-day	1	7.2	61.3	70.0	1.9	4.8	6.7
	2	6.8	61.8	69.1	4.4	4.0	8.9
62-day	1	1.3	55.2	58.4	3.4	1.9	5.3
	2	1.5	50.7	53.7	3.9	2.6	6.5
104-day	1	1.8	37.0	36.9	5.0	0.2	5.2
	2	0.8	43.2	45.4	3.3	0.2	3.5

Results expressed as % applied radioactivity
n.t.: not tested
n.d.: not detected

The sediments of the 104 day samplings (ambient extracted, air dried and ground) were subjected to further characterisation of sediment radioactivity which remained bound to humic and fulvic acids, and humin fraction (Table B.8.4-14).

Table B.8.4-14: Characterisation of residual radioactivity in the Bickenbach and Unter-Widdersheim sediment

All values in % AR	**Bickenbach**		**Unter-Widdersheim**	
	ABB17[1)]	**ABB18[1)]**	**AUW17[1)]**	**AUW18[1)]**
Combustion	30.0	32.5	36.0	29.5
Pool[2)]	12.9	11.9	9.5	12.8
Fulvic acids	4.4	4.2	2.9	3.9
Humic acids	8.5	7.2	6.2	8.9
Sum of fulvic acids and humic acids	12.9	11.4	9.1	12.8
Humin[3)]	17.1	20.6	26.5	16.7
Sum of fulvic acids, humic acids and humin in % of nominal[4)]	100.0	98.5	98.9	100.0

[1)] 104 day samplings
[2)] previous measurement of summed % AR of fulvic acids and humic acids (pool of two extraction steps)
[3)] calculated: % AR combustion - % AR pool
[4)] (fulvic acids + humic acids + humin) (in % AR)/combustion (% AR) x 100 %

Conclusions

Radioactivity in the water phase declined steadily up to day 104 with final levels ranging from 2.2 to 8.2 % AR. Corresponding levels in the sediments ranged from 47.8 to 76.1 % AR on day 104. Non-extractable radioactivity in the sediments accounted for 29.5 to 36.0 % AR on day 104.

AMPA dissipated rapidly in the water phases to an average of 5 % AR and 1 % AR at day 104. Corresponding levels of AMPA in the sediment showed a peak concentration of 32 % AR at day 30, followed by a decline to an average of 16 % AR at day 104 in the Bickenbach system, and a peak concentration of 63 % AR at day 14, followed by a decline to an average of 40 % AR at day 104 in the system Unter-Widdersheim.

AMPA was converted in both compartments of the two test matrices into two unknown degradates, but predominantly in the aerobic water phase (> 10 % AR). The degradation of AMPA was reflected by the formation of residues and a maximum formation of CO_2 of 40 % AR.

RMS Comment

In the study report, amounts of AMPA in the sediment reported in the text (p. 30 of study report) differ from amounts reported in the tables (p. 51 of study report). ~~The amounts reported in the table are assumed to be correct and are reported above.~~ On page 30 only the values of AMPA as percent of applied radioactivity for 6 h and 104 d are given in text form: "AMPA could be found at the 6 hours sampling time in the processed sediment extractable radioactivity with average values of 11.1 (Bickenbach) and 13.7 % AR (Unter-Widdersheim). It's level accounted for 14.0 and 18.3 % AR for Bickenbach sediment and 36.3 and 44.2 % AR for the sediment of location Unter-Widdersheim by the final samplings at day 104." On page 51 of the study report the course of AMPA in the test system on basis of TLC chromatographie results (percent of applied radioactivity) is shown in a table. According to page 51 of the study report the values of AMPA in sediment are: 6 hours: Bickenbach: 11.0 and 10.1 % AR, Unter-Widdersheim: 13.7 and 13.2 % AR, 104 days: Bickenbach: 17.7 and 14.2 % AR, Unter-Widdersheim: 37.0 and 43.2 % AR. Since it is not clear, which values are correct, the sediment systems Bickenbach and Unter-Widdersheim should be excluded from kinetic evaluation.

Apart from that, the study can be accepted and the end points may be used for further risk assessment.

KIIA 7.8.3/3 (McEwen, A., 2004, BVL no 2310275)

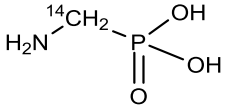
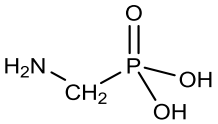
Annex point:	KIIA 7.8.3/3
Author(s):	McEwen, A.
Title:	[^{14}C]-AMPA: Degradation and fate in water/sediment systems
Date:	June 07, 2004
Guideline(s):	Guidelines concerning the inclusion of Active Substances in Annex I to EEC Council Directive 91/414/EEC as amended by Commission Directive 96/68/EC; Society of Environmental Toxicology and Chemistry.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Material and methods

Test item

The radiolabelled and non-radiolabelled test item is characterised in Table B.8.4-15.

Table B.8.4-15: Test item

	Test material (radiolabelled)	**Test material (non-radiolabelled)**
Common name	Aminomethylphosphonic acid (AMPA) (Specific activity: 17.65 mCi/mmol or 159 µCi/mg)	Aminomethylphosphonic acid (AMPA)
Structural formula		
Purity	97.8 %	99 %
CAS	1066-51-6	
Stability of test compound	not determined	Storage under ambient temperature

Water/sediment systems

Two water/sediment systems were obtaind for the study. Water and sediment were obtained immediately prior to the start of the study from AgroChemex, Manningtree, UK. The physiochemical parameters of the water/sediment systems are presented in Table B.8.4-16.

Table B.8.4-16: Physiochemical parameters of the water/sediment systems

	Sand (%)	**Silt (%)**	**Clay (%)**	**pH**	**Organic matter (Walkley-Black) (%)**	**Biomass (µg C/g)**
Sediment A (Manningtree A)	48	29	23	7.6	5.6	Day 0: 338.6 Day 103: 296.1
Sediment B (Manningtree B)	48	28	24	6.3	6.0	Day 0: 316.3 Day 103: 143.9

Study design

Experimental conditions

The [^{14}C]-labelled test substance was added at a rate of 4.8 mg/kg (approximately equivalent to an agricultural use rate of 3.6 kg/ha) to test vessels containing acclimatised water/sediment. The test vessels were incubated in the dark at 20±2 °C. Water/sediment samples were taken for analysis at appropriate time intervals of up to 103 days after test substance application. The sediment and water in each test system were analysed separately. The sediment was extracted with aqueous solvents and the proportions of test substance and its major degradation products in water and sediment were determined. Radiolabelled volatile metabolites and CO_2 were trapped and quantified. The test substance and major degradation products in water and sediment were identified by comparison to reference substances.

Sampling

For each sediment type, a single vessel treated with [^{14}C]-test substance was subjected to analysis at zero time and on or at about the following times after test substance application: 2, 7, 14, 30, 60 and 100 days.

Trapping media was taken for analysis and replaced with fresh media at each sampling interval then at about weekly intervals during the first month of the study and about every ten days thereafter.

Analytical procedures

Sediment and water in each test vessel were separated by decanting the water from the test vessel. The total volume of the water layer and concentration of radioactivity in the water was measured. The water was then stored at <-15 °C prior to chromatographic analysis. Soil residues were air-dried and analysed by combustion/LSC.

Sediment was then extracted as follows:

Extract 1: Sediment was shaken for 2 hours at room temperature with ca 100 mL of 0.5M ammonium hydroxide. The extract solution was separated from the soil solids by centrifugation and the total extract volume recorded. Duplicate aliquots were taken for LSC analysis.

Extract 2-3: As extract 1

Extract 4-5: As extract 1 using 1M hydrochloric acid

Soil residues were air-dried and analysed by combustion/LSC.

The radioactivity associated with dosing formulations, water and soil extracts was determined directly by liquid scintillation counting of known weights of samples. Samples were mixed with Ultima Gold XR scintillant and counted using a Canberra Packard scintillation counter. Radioactive content in soil residues was determined by combustion of aliquots and the $^{14}CO_2$ trapped by carbon dioxide absorbent Carbosorb E+, which was mixed with the scintillant Permafluor E+ prior to counting. Radioactivity in samples was quantified directly by liquid scintillation counting (LSC) using a Canberra Packard liquid scintillation counter with automatic external standard quench correction.

Thin-layer chromatography was carried out on pre-layered cellulose TLC plates, layer thickness of 0.25 mm. The developing system was: Butanol:Water:Acetic acid (6:5:2 by volume). Radiolabelled components on thin-layer chromatograms were detected and quantified using a Fuji BAS 1500 Bio-image analyser.

HPLC analysis of the study samples was performed using the following conditions:

Mobile Phase A: Aqueous Buffer (5 mM Potassium phosphate adjusted to pH 1.9 with HNO_3) – Isocratic

UV Wavelength: 254 nm

Flow Rate: 0.5 mL/min

Run Time: 20 minutes

Results and discussion

Mass balance

In Sediment A, recovery of radioactivity over 103 days was generally within a range of 89.3 – 96.6 % AR, the exception being vessel A7, day 61 (recovery 86.5 % AR). In Sediment B, recovery of radioactivity over 103 days was generally within a range of 87.7 – 97.2 % AR, the exception being vessel B6, day 61 (recovery 81.9 % AR).

Findings

A summary of recoveries and distribution of residues expressed as percentage of applied radioactivity at each sampling time interval is provided in Table B.8.4-17 for Sediment A and in Table B.8.4-18 for Sediment B. The proportions of radioative metabolites in water and sediment extracts are presented in Table B.8.4-19 (Sediment A) and in Table B.8.4-20 (Sediment B).

Table B.8.4-17: Recovery of radioactivity in Sediment A following application of [14C]-AMPA at a nominal rate of 3.6 kg/ha

Day	Vessel	Water	Total extracts (%)	Residue (%)	Air traps (%)	Total recovery (%)
0	A1	94.7	1.5	0.4	ns	96.6
1	A2	38.4	39.2	13.4	0.3	91.3
7	A3	11.3	55.1	24.5	0.5	91.4
14	A4	6.8	66.9	16.7	0.6	91.0
29	A6	5.3	64.7	16.0	3.3	89.3
61	A7	2.8	67.7	7.9	8.1	86.5
103	A8	1.8	65.3	13.0	9.8	89.9

Results expressed as % applied radioactivity
ns: no sample

Table B.8.4-18: Recovery of radioactivity in Sediment B following application of [^{14}C]-AMPA at a nominal rate of 3.6 kg/ha

Day	Vessel	Water	Total extracts (%)	Residue (%)	Air traps (%)	Total recovery (%)
0	B1	96.7	0.3	0.2	ns	97.2
1	B2	53.5	25.8	13.0	0.2	92.5
7	B3	9.0	48.0	35.3	0.6	92.9
14	B4	6.6	50.8	31.6	2.4	91.4
29	B5	2.3	47.4	40.7	3.0	93.4
61	B6	0.3	62.9	10.7	8.0	81.9
103	B7	0.3	56.2	23.0	8.2	87.7

Results expressed as % applied radioactivity
ns: no sample

Table B.8.4-19: Proportions of radioactive metabolites in water and sediment extracts following application of [^{14}C]-AMPA at a nominal rate of 3.6 kg/ha to Sediment A

		Water			**Sediment**		
Day	Vessel	P1a (5 mins)	P2 (AMPA) (11 mins)	Others[1)]	P1a (5 mins)	P2 (AMPA) (11 mins)	Others[1)]
0	A1	<0.1	90.5	4.2	0.3	1.1	0.1
1	A2	0.3	37.7	0.5	21.7	15.4	2.1
7	A3	0.4	10.7	0.2	34.8	16.4	3.9
14	A4	0.7	5.9	0.2	53.0	3.5	10.4
29	A6	0.5	4.7	0.1	24.5	29.6	10.6
61	A7	0.5	2.3	0.1	31.6	30.2	5.9
103	A8	0.7	0.8	0.4	31.0	12.3	22.0

Results expressed as % applied radioactivity
Components separated by ionic exchange HPLC
1) Represents regions of radioactivity which cannot be assigned to a designated peak

Table B.8.4-20: Proportions of radioactive metabolites in water and sediment extracts following application of [^{14}C]-AMPA at a nominal rate of 3.6 kg/ha to Sediment B

		Water			Sediment			
Day	**Vessel**	**P1a (5 mins)**	**P2 (AM-PA) (11 mins)**	**Others1)**	**P1a (5 mins)**	**P2 (AMPA) (11 mins)**	**P3 (11–15 mins)**	**Others[1)]**
0	B1	<0.1	91.2	5.5	0.1	0.1	<0.1	0.1
1	B2	0.3	52.7	0.5	1.4	22.3	<0.1	2.1
7	B3	0.3	8.6	0.1	44.1	2.6	<0.1	1.3
14	B4	0.3	6.2	0.1	49.2	0.2	<0.1	1.4
29	B5	0.2	1.9	0.2	26.6	<0.1	6.1	14.7
61	B6	0.1	0.1	0.1	41.8	<0.1	16.6	4.5
103	B7	<0.1	0.2	0.1	18.0	<0.1	33.2	5.0

Results expressed as % applied radioactivity
Components separated by ionic exchange HPLC
[1)] Represents regions of radioactivity which cannot be assigned to a designated peak

In Sediment B severe problems were encountered in obtaining chromatography for the extracts. This is believed to be due to the presence of co-extracted endogenous material affecting the ion-exchange chromatography.

Two unidentified metabolites were detected and accounted for 53 % AR (P1a) up to 14 days in Sediment A and 33 % AR (P3) up to 103 days in Sediment B. Metabolite P3 was observed as a broad peak and could well be composed of several components that were unresolved due to chromatographic interference from endogenous material. Several unsuccessful attempts were made to improve the chromatography, including solid phase extraction dilution of the extracts with mobile phase and concentration/re-suspension in mobile phase.

Bound residue analysis of representative samples indicated that the majority of the radioactivity was associated with the humic acid fraction (Table B.8.4-21).

Table B.8.4-21: Recovery of radioactivity in representative bound residues following application of [^{14}C]-AMPA at a nominal rate of 3.6 kg/ha

Fraction	**Sample**	
	A9	B9
Humin	1.8	3.4
Fulvic acid	38.8	28.2
Humic acid	59.5	68.4

Results expressed as % applied radioactivity

Conclusions

The degradation of AMPA has been demonstrated in two aerobic water/sediment systems. AMPA is mineralised to CO_2.

One major radioactive component (P1a) was observed in sediments after 103 days. This component was more acidic in the chromatographic system employed than AMPA. In system B sediment extracts an additional radioactive component was observed which accounted for around 33 % AR after 103 days. Due to the broad nature of the chromatographic peak it is believed that the radioactivity is associated with more than one component.

RMS Comment

Due to problems analysing extracts obtained from Sediment B, believed to be caused by endogenous co-extracted material disrupting the ion-exchange chromatography, Sediment B and the total system B should be excluded from kinetic evaluation. Apart from that, the study can be accepted and the end points for system A (total system, water phase and sediment) as well as for the water phase of system B may be used for further risk assessment.

KIIA 7.8.3/4 (Heintze, A., 1996, BVL no 1939626)

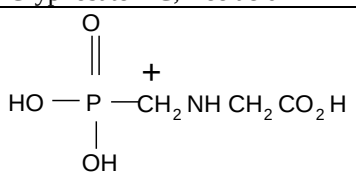
Annex point:	KIIA 7.8.3/04
Author(s):	Heintze, A.
Title:	Degradation and metabolism of glyphosate in two water/sediment systems under aerobic conditions - Laboratory test
Date:	December 16, 1996
Guideline(s):	BBA IV 5-1 (1990) “Abbaubarkeit und Verbleib von Pflanzenschutzmitteln im Wasser/Sediment-System”
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Material and methods

Test item

The rate of degradation and the metabolic pathway of glyphosate in two water/sediment systems under aerobic conditions were determined according to BBA IV 5-1 (1990) ‘Abbaubarkeit und Verbleib von Pflanzenschutzmitteln im Wasser/Sediment-System’. The test systems (flaks) were treated on surface with 691.2 µg glyphosate/80 cm², equivalent to 4.32 kg/ha. Further information about the test item is presented in Table B.8.4-22.

Table B.8.4-22: Test item

	Test material (radiolabelled)	**Test material (non-radiolabelled)**
Common name	Glyphosate-^{14}C, free acid	Glyphosate technical
Structural formula	 + Labelled position	-
Purity	98.3 %	-

Water/sediment system

The water-sediment systems tested were Pond and Creek, which preliminary differ in their organic carbon content and therefore in their microbial activity. The water-sediment systems are further characterized in Table B.8.4-23.

Table B.8.4-23: Characterisation of the water/sediment systems

	Pond	**Creek**
Origin	Bauschlott	Ottenhofen
Water		
pH	8.26	7.85
Oxygen concentration (mg/L)	15.6	11.3
Redox potential (mV)	88	90
Sediment		
pH	6.64	7.85
Organic carbon (%)	3.31	0.11
Particle size distribution (Clay/silt/sand) (%)	11/79.1/9.8	1.1/1.7/97.2
Sediment classification	Loamy silt	Sand
Cation exchange capacity (mval/100 g)	22.1	4.3
Redox potential (mV)	- 192	208
Microbial biomass (µg C/g dry matter) Beginning of the study/ End of the study	1017 ± 25/ 1024 ± 24	121 ± 3/ 214 ± 5

Water was sampled down to a depth of 10 to 30 cm and the sediment was sampled from the top 20 cm of each system. The water sediment systems were stored in the laboratory for 14 d at approximately 4 ºC and afterwards prepared for acclimatization. Prior to use the sediment was sieved through a 2 mm sieve and the water through a 0.2 mm sieve. Approx. 360 g Creek sediment and 230 g wet Pond sediment were transferred into flasks. The water/ sediment system was incubated at 20 ± 2 ºC until an equilibrium based on measured variables was reached. The oxygen concentration, pH and redox potential were measured regularly. A visual settling of colloid particles took place. Bach system was aerated four times for one hour each day.

Study design

Experimental conditions

The study was performed with a closed gas flow system using 1000 mL all-glass metabolism flasks (inner diameter: ≈ 10.1 cm surface: ≈ 80 cm²) containing about 500 mL ± 40 mL water and 300 g ± 100 g sediment. These amounts were obtained by assuming a height of the water column of about 6 cm and an approx. 2.5 cm thick sediment layer with a bulk density of approx. 1.5 cm³. The system was ventilated discontinuously (at least 60 min each day) with CO_2-free (liquid carbon dioxide trap) moistened air (bubbled through water). The out-coming air was passed through a trapping system for organic volatiles (ethylene glycol) and two solid phase carbon dioxide traps and one liquid carbon dioxide trap (NaOH). Aeration took place at least until the air has been exchanged for a minimum of 5 times. Each test system 24 flasks, each sampling interval in duplicate, was set up comprising 20 treated flasks and four non treated controls, which were used to determine biomass at 0 d and at the end of the study. The samples were incubated at 20 ± 2 ºC protected from light. Redox potential, pH and oxygen concentration were determined one day prior to the treatment in all flasks in the water.

Sampling

At the following times, two flasks of each system were taken for analysis: 0 h, 6 h, 24 h, 48 h and 7 d, 14 d, 29 d, 58 d, 98 d and 120 d after treatment. The samples were analysed to determine the rate, type and degree of metabolism of 14C-Glyphosate. After the determination of the height of the sediment and water layer, redox potential, pH and oxygen concentration were determined in the water and in the sediment.

Analytical procedures

The radiochemical purity of ^{14}C-glyphosate was supplied by Amersham and confirmed by thin-layer chromatography (TLC). The specific activity of the ^{14}C-glyphosate was determined and quantified by liquid scintillation counting (LSC) and the results proved by high-performance liquid chromatography (HPLC). The mass of glyphosate was determined by the area of the peak and a relative calculation compared to the mass applied.

The radioactivity evolved from the water/sediment system was adsorbed in ethylene glycol traps. These traps were removed and replaced by fresh traps at sampling date or at least after 28 ± 2 days, whichever was shorter, until the end of the incubation period. Usually an aliquot of 1 mL ethylene glycol was taken from the trap, mixed with liquid scintillation cocktail and the rate as well as the degree of ^{14}C-glyphosate degradation was determined by liquid scintillation counting.

Evolved radioactive carbon dioxide was trapped into gas traps filled with 50 mL of 2 M sodium hydroxide solution at the beginning of the study and then with a solid phase system. These traps were removed and replaced by fresh traps at sampling date or at least after 28 ± 2 days. The solid phase system was constructed as follows: A dehumidifying chamber filled with 2 to 3 g of a desiccating agent based on silica gel was followed by 2 chambers of carbon dioxide adsorber filled with sodium hydroxide on support or soda lime pellets. 6 g were used in the first chamber and 2 g were used in the second chamber. Glass wool was used as a separator between the adsorbing agents. The absorbed carbon dioxide was completely transferred to the liquid scintillation cocktail by acidifying the adsorber in a gas generating apparatus followed by a carbon dioxide trap filled with liquid scintillation cocktail used for total carbon combustors. The radioactivity was determined by liquid scintillation counting.

Water and sediment were analysed within 24 h from the samples which were stored in the refrigerator until analysis (≤ 8 ºC). Otherwise the samples were stored in a freezer (< - 18 ºC over almost the whole period) up to analysis.

Sediment (about 30 g d.w.) was extracted by 3 times shaking for 1 h with 50 mL 1 mol/L ammonium solution at room temperature on a flatbed shaker and finally extracted with 50 mL acetone/water for 1 h. The glyphosate and the AMPA content in the ammonium extract were determined following the method described in “Rückstandsanalytik von Pflanzenschutzmitteln” (GAB SOP 12.3.2-1) excluding the analytical chromatography for the determination of AMPA and Glyphosate content which was performed as described in this report. The radioactivity in the sample that did not remain on the columns or precipitate during sample preparation was determined by LSC. The solution was concentrated by evaporation under vacuum and otherwise prepared for an analytical determination of the radioactivity. The radioactivity of acetone/water extract was determined by LSC. Because the activity was less than 5 % at each sampling date, the extract was discarded each time. Partitioning of extractables was performed by HPLC and TLC. Extracts were measured by LSC. Extracts containing more than 5 % of the radioactivity applied were characterized by HPLC or and TLC and co-

chromatography of available reference compounds in each of the two systems. The resolution of TLC was not good enough for determination of the partitioning between glyphosate and AMPA. Therefore, only HPLC results were taken for the calculations of degree of metabolisation. Remaining non-extractables (3 x 1 g) and filters were determined by combustion and the radioactivity determined by LSC. Precipitates during sample preparation were dissolved in water, an aliquot mixed with LSC-cocktail and radioactivity determined by LSC.

The radioactivity in the water was determined by LSC. The glyphosate and the AMPA content in the water phase were determined following the method described in "Rückstandsanalytik von Pflanzenschutzmitteln" (GAB SOP 12.3.2-1) excluding the analytical chromatography for the determination of AMPA and Glyphosate content which was performed as described in this report. The radioactivity in the sample that did not remain on the columns or precipitate during sample preparation was determined by LSC. The solution was concentrated by evaporation under vacuum and otherwise prepared for an analytical determination of the radioactivity. Remaining non-extractables on filters were determined by combustion and the radioactivity determined by LSC. Precipitates during sample preparation were dissolved in water, an aliquot mixed with LSC-cocktail and radioactivity determined by LSC.

Results and discussion

Mass balance

Over a period of 120 days recoveries of applied radioactivity at each sampling time ranged from 90.7 % to 109.3 % for the system Pond and from 86.9 to 101.6 % for the system Creek systems, the exceptions being day 97 and day 120 (recovery 79.9 % and 82.5 %, respectively).

Findings

The distribution radioactivity expressed as percentage of applied radioactivity between water, sediment, volatiles and carbon dioxide is provided in Table B.8.4-24 for the Pond system and in Table B.8.4-25 for the Creek system. The proportions of glyphosate and its metabolite AMPA in water and sediment extracts are presented in Table B.8.4-26 and Table B.8.4-27 (Pond system) as well as in Table B.8.4-28 and Table B.8.4-29 (Creek system).

Table B.8.4-24: Distribution of radioactivity in the Pond water/sediment system in % of the applied radioactivity

Time (days)	Water phase	Sediment	Volatiles	Carbon dioxide	Total
0	84	14.16	0	0	98.16
0.25	73.01	26.77	0.01	0.04	99.83
1	52.78	52.89	0.01	0.04	105.72
2	41.55	62.32	0.01	0.06	103.94
7	37.23	71.87	0.01	0.19	109.3
14	19.27	86.35	0.01	0.55	106.18
29	12.43	91.67	0.01	0.7	104.81
58	1.55	93.13	0.01	6.7	101.4
97	3.08	77.95	0.02	9.63	90.69
120	3.79	17.9	0.03	14.77	96.5

Table B.8.4-25: Distribution of radioactivity in the Creek water/sediment system in % of the applied radioactivity

Time (days)	Water phase	Sediment	Volatiles	Carbon dioxide	Total
0	92.31	8.74	0	0	101.05
0.25	91.71	9.33	0.01	0.03	101.08
1	86.09	12.99	0.01	0.08	99.17
2	81.42	19.88	0.01	0.26	101.57
7	72.11	25.55	0.01	1.26	98.93
14	66.19	26.36	0.01	2.57	95.13
29	49.91	36.2	0.01	7.99	94.11
58	38.75	35.78	0.02	12.33	86.88
97	22.99	30.54	0.04	26.32	79.89
120	20.57	31.76	0.04	30.08	82.45

Table B.8.4-26: Content of glyphosate and its metabolite AMPA in the water phase in % of the applied radioactivity in the Pond water/sediment system

Time (days)	NCR	Glyphosate	AMPA
0	11.84	70.57	1.59
0.25	16.91	55.18	0.93
1	11.72	39.57	1.49
2	8.82	31.36	1.38
7	7.85	27.42	1.97
14	3.29	14.32	1.67
29	2.44	8.2	1.79
58	1.13	0.24	0.12
97	1.55	1.04	0.49
120	1.32	1.83	0.64

Table B.8.4-27: Content of glyphosate and its metabolite AMPA in the sediment in % of the applied radioactivity in the Pond water/sediment system

Time (days)	Bound Residues	NCR	Glyphosate	AMPA
0	2.6	6.8	3.6	1.2
0.25	2.84	12.3	8.8	2.9
1	10.06	18.1	19.9	4.9
2	11.62	11.4	33.4	5.9
7	19.85	14.9	31.6	5.5
14	25.12	19.4	35.5	6.3
29	24.46	16.6	40	10.5
58	29.46	20	33	10.7
97	16.13	20.8	27.1	13.9
120	17.15	15.2	29.8	15.7

Table B.8.4-28: Content of glyphosate and its metabolite AMPA in the water phase in % of the applied radioactivity in the Creek water/sediment system

Time (days)	NCR	Glyphosate	AMPA
0	6.42	85.9	0
0.25	14.2	77.51	0
1	10.68	72.78	2.63
2	7.82	69.89	3.71
7	12.99	52.97	6.16
14	16.2	42.54	7.45
29	24.83	16.69	8.39
58	22.39	6.03	10.34
97	16.62	1.43	4.95
120	12.47	0	8.1

Table B.8.4-29: Content of glyphosate and its metabolite AMPA in the sediment in % of the applied radioactivity in the Creek water/sediment system

Time (days)	Bound Residues	NCR	Glyphosate	AMPA
0	1.1	1.4	5.6	0.6
0.25	1.3	1.5	5.1	1.4
1	2.05	2.1	6.9	2
2	2.65	4.2	9.4	3.7
7	4.83	4.9	8.9	7
14	4.12	6.9	6.6	8.7
29	9.81	7.1	7.2	12.2
58	10.43	6.3	6.3	12.8
97	8.02	7.4	0	15.1
120	9.49	6.4	0	15.9

Disappearance in the water phase of the water/sediment system Pond was primarily caused by a continuous transfer from the water phase to the sediment phase, probably caused by sorption processes. In the sediment, rising amounts of degradation products indicated that the degradation process was still in progress after 120 d in both systems. The main fractions were the uncharacterized group of bound residues (Pond), the soluble or extractable group of non-chromatographable residues and the metabolite AMPA. Another metabolite was detected in the water phase but not identified as it only appeared in samples taken from the water/sediment system Pond (58 d) with an amount of less than 0.1 % of the applied activity.

Conclusions

In the study (Heintze, 1996, BVL no 1939626) the decrease of the glyphosate concentration is caused by metabolism and degradation of the compound as well as by transfer process from the water to the sediment, probably caused by sorption processes.

RMS Comment

The study can be accepted and the endpoints (see B.8.4.4.5) may be used for further risk assessment.

KIIA 7.8.3/5 (Partsch, 2012, BVL no 2316005)

Annex point: KIIA 7.8.3/05
Author(s): Partsch, S.

Title:	Kinetic modelling analysis of the disappearance behaviour of glyphosate and its metabolite AMPA in water-sediment studies
Date:	April 30, 2012
Guideline(s):	FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the Work Group on Degradation Kinetics of FOCUS. EC Document Reference SANCO/10058/2005 version 2.0, June 2006. FOCUS (2011a): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.0.
Deviations:	No
GLP:	No (kinetic evaluation: does not contain laboratory work)
Acceptability:	Generally yes, but the RMS proposes some changes in concerning the studies to consider and endpoint selection.

Material and methods

Studies on the degradation behaviour of glyphosate and its major metabolite AMPA in laboratory water/sediment systems were kinetically evaluated following FOCUS guidance (FOCUS, 2006, 2011a) to derive persistence and modelling endpoints for comparison against trigger values and for use in environmental fate models, respectively. At Level P-I (one-compartment approaches), single first-order and biphasic kinetic models were used to describe the behaviour of glyphosate and AMPA in the water phase, the sediment phase and the total system. No Level P-II was calculated. ModelMaker™ (version 4.0) was used as fitting software. The Microsoft Excel™ 2003 *Degradation Kinetics Spreadsheet* (v2, June 2007), as provided by FOCUS Work Group, was used for statistical evaluation of the optimised parameters.

The degradation of glyphosate and its major metabolite in water and sediment, AMPA, was initially investigated in eight laboratory water/sediment systems under aerobic conditions in the dark between 1993 and 2004 (Bowler & Johnson, 1999, BVL no 2154357; Möllerfeld & Römbke, 1993, BVL no 1934113; Heintze 1996, BVL no 1939626; Muttzall, 1993, BVL no 1982136; Feser-Zügner, 2002, BVL no 2310270; Knoch, 2003, BVL no 2310273; Knoch & Spirlet, 1999, BVL no 1934122; McEwen, 2004, BVL no 2310275). Each study tested two independent aquatic sediment systems with different characteristics. As test item, [^{14}C]-labelled glyphosate (Möllerfeld and Römbke, 1993, BVL no 1934113; Heintze, 1996, BVL no 1939626; Muttzall, 1993, BVL no 1982136), [^{14}C]-labelled glyphosate-trimesium (Bowler and Johnson, 1999, BVL no 2154357, with [^{14}C]-labelled in the glyphosate anion portion of the molecule), [^{14}C]-labelled AMPA (Feser-Zügner, 2002, BVL no 2310270; Knoch & Spirlet, 1999, BVL no 1934122; Knoch, 2003, BVL no 2310273; McEwen, 2004, BVL no 2310275) were used.
The author did exclude the studies of Muttzall (1993, BVL no 1982136) and McEwen (2004, BVL no 2310275) from kinetic evaluation. He stated that they revealed several deviations from OECD Guidelines for Testing of Chemicals No. 308 (OECD, 2002) such as analytical artefacts and insufficient reporting of important parameters.

Replicate samples for residue data were available for all studies on AMPA degradation. However, for the study by Feser-Zügner (2002, BVL no 2310270), mean values of duplicate samples were kinetically evaluated. The study by Knoch and Spirlet (1999, BVL no 1934122) on degradation of AMPA provided replicate samples for each sampling time and each of the sed-

iment systems, which again were analysed with two different TLC systems. These values (derived from the different TLC-systems) were considered to be analytical replicates and were therefore averaged prior to kinetic evaluation to obtain true replicates per sampling time. All other study reports provided true replicates to be used in kinetic analyses without further preprocessing.

All end points were derived from studies where glyphosate and AMPA were applied as test items in separate studies. No pathway fit was evaluated to obtain endpoints for the metabolite AMPA from the glyphosate studies.

As a first step for all datasets, the fit of a SFO kinetic model was tested for the applied substance. For modelling endpoints, in cases where SFO was not appropriate, the decision whether to test only the HS and DFOP models (>10 % AR) or additionally FOMC (<10 % AR) as bi-phasic models depended on the amount of residue in the respective compartment (water, sediment, total system) at the end of the experimental phase. For persistence (best-fit) endpoints the SFO model was compared to the FOMC model. In cases, where SFO was not appropriate as the best-fit model, DFOP and HS were tested as further bi-phasic models.

Results and discussion

Overview

All kinetic endpoints for glyphosate and AMPA, are provided in Table B.8.4-30 and Table B.8.4-31, respectively. No reliable kinetic fit could be achieved for the degradation behaviour of AMPA in the sediment phase as the peak concentration of AMPA in sediment was observed too late to allow the decline phase to be fitted. Consequently, no modelling end points for AMPA could be derived for the sediment phase.

Table B.8.4-30: Persistence and modelling end points of glyphosate in water/sediment systems

Study	System	Persistence end points at Level P-I			Modelling end points at Level P-I	
		Model	DT_{50} 4) (days)	DT_{90} 4) (days)	Model	SFO DT_{50} 4) (days)
Glyphosate (total system)						
Bowler & Johnson (1999), BVL no 2154357	Cache	FOMC	8.47	45.89	FOMC	13.82 1)
	Putah	DFOP	210.66	976.54	DFOP	329.85 2)
Möllerfeld & Römbke (1993), BVL no 1934113	Loamy Sediment	DFOP	116.56	1328.20	- 3)	- 3)
	Sandy Sediment	HS	16.03	346.81	HS	154.19 2)
Heintze (1996), BVL no 1939626	Creek	HS	16.10	65.93	SFO	16.78
	Pond	FOMC	65.86	2053.35	SFO	72.75
Minimum			8.47	45.89		13.82
Maximum			210.66	2053.35		329.85
Geometric mean			**39.03**	**375.31**		**61.19**
Glyphosate (water phase)						
Bowler & Johnson (1999), BVL no 2154357	Cache	HS	4.98	26.84	SFO	6.94
	Putah	FOMC	8.25	72.40	FOMC	21.81 1)
Möllerfeld & Römbke (1993), BVL no 1934113	Loamy Sediment	FOMC	1.06	24.11	FOMC	7.26 1)
	Sandy Sediment	DFOP	2.03	22.63	DFOP	6.82 1)
Heintze (1996), BVL no 1939626	Creek	DFOP	11.95	48.10	SFO	13.15
	Pond	HS	1.00	26.89	HS	8.10 1)
Minimum			1.00	22.63		6.82
Maximum			11.95	72.40		21.81
Geometric mean			**3.19**	**33.33**		**9.63**
Glyphosate (sediment phase)						
Bowler & Johnson (1999), BVL no 2154357	Cache	SFO	34.05	113.10	SFO	34.05
	Putah	- 3)	- 3)	- 3)	- 3)	- 3)
Möllerfeld & Römbke (1993), BVL no 1934113	Loamy Sediment	- 3)	- 3)	- 3)	- 3)	- 3)
	Sandy Sediment	HS	146.26	850.52	HS	303.31 2)
Heintze (1996), BVL no 1939626	Creek	SFO	47.88	159.06	SFO	47.88
	Pond	- 3)	- 3)	- 3)	- 3)	- 3)
Minimum			34.05	113.10		34.05
Maximum			146.26	850.52		303.31
Geometric mean			**62.01**	**248.26**		**79.08**

1) Back-calculated from DT_{90} of bi-phasic model (DT_{90}/3.32)
2) Calculated from slower k-rate
3) no reliable fit achieved
4) DT_{50} = $DegT_{50}$ for total system but DT_{50} for water and sediment phase

Table B.8.4-31: Persistence and modelling end points of AMPA in water/sediment systems

Study	System	Persistence end points at Level P-I			Modelling end points at Level P-I	
		Model	DT_{50} 4) (days)	DT_{90} 4) (days)	Model	SFO DT_{50} 4) (days)
AMPA (total system)						
Feser-Zügner (2002), BVL no 2310270	Rückhaltebecken	FOMC	13.80	1513.00	DFOP	102.87 2)
	Schäphysen	DFOP	2.48	354.99	- 3)	- 3)
Knoch (2003), BVL no 2310273	Bickenbach	HS	10.54	191.25	HS	77.83 2)
	Unter-Widdersheim	HS	77.36	307.19	HS	98.98 2)
Knoch & Spirlet (1999), BVL no 1934122	Bickenbach	HS	44.53	205.21	HS	61.81 1)
	Unter-Widdersheim	HS	18.02	640.66	- 3)	- 3)
Minimum			2.48	191.25		61.8
Maximum			77.36	1513.00		102.9
Geometric mean			**16.79**	**400.85**		**83.7**
AMPA (water phase)						
Feser-Zügner (2002), BVL no 2310270	Rückhaltebecken	FOMC	2.20	22.50	FOMC	6.78 1)
	Schäphysen	FOMC	1.00	7.80	SFO	1.58
Knoch (2003), BVL no 2310273	Bickenbach	DFOP	2.54	47.57	DFOP	14.33 1)
	Unter-Widdersheim	DFOP	1.81	19.29	DFOP	5.81 1)
Knoch & Spirlet (1999), BVL no 1934122	Bickenbach	DFOP	6.59	51.47	DFOP	15.50 1)
	Unter-Widdersheim	HS	2.02	17.15	HS	5.17 1)
Minimum			1.00	7.80		1.58
Maximum			6.59	47.57		15.50
Geometric mean			**2.26**	**22.85**		**6.44**
AMPA (sediment phase)						
Feser-Zügner (2002), BVL no 2310270	Rückhaltebecken	DFOP	184.33	678.38	- 3)	- 3)
	Schäphysen	- 3)	- 3)	- 3)	- 3)	- 3)
Knoch (2003), BVL no 2310273	Bickenbach	- 3)	- 3)	- 3)	- 3)	- 3)
	Unter-Widdersheim	- 3)	- 3)	- 3)	- 3)	- 3)
Knoch & Spirlet (1999), BVL no 1934122	Bickenbach	- 3)	- 3)	- 3)	- 3)	- 3)
	Unter-Widdersheim	- 3)	- 3)	- 3)	- 3)	- 3)
Geometric mean			**184.33**	**678.38**		**-**

1) Back-calculated from DT_{90} of bi-phasic model (DT_{90}/3.32)
2) Calculated from slower k-rate
3) no reliable fit achieved
4) DT_{50} = $DegT_{50}$ for total system but DT_{50} for water and sediment phase

The resluts of the kinetic modelling analysis of the disappearance behaviour of glyphosate and its metabolite AMPA in the water/sediment studies are presented in detail in the following part.

Determination of appropriate kinetic models

Bowler and Johnson (1999, BVL no 2154357), system Cache

Total system:
FOMC was visually more appropriate than SFO; hence, HS and DFOP models were additionally tested. FOMC revealed the best χ^2-error, met M_0 slightly better than HS and DFOP, and gave the most appropriate fit for later sampling points. FOMC was therefore accepted as persistence endpoint. Since 10 % of the initially measured concentration was reached within the

study period, FOMC was also tested as modelling endpoint. There again, FOMC gave a very good visual fit around the $DegT_{50}$. Consequently, the modelling endpoint was derived from FOMC, leading to a conservative SFO half-life back-calculated from FOMC $DegT_{90}$.

Water phase:
As FOMC was more appropriate than SFO, HS and DFOP were tested as further bi-phasic models. DFOP was the best-fit model ($\chi^2 = 6.1$) but added only little to the HS fit. As both k-rates of HS were significantly different from zero and the fit around DT_{90} was slightly better than that of DFOP, preference was given to HS as the best-fit model. The SFO model adequately represented the general dissipation behaviour and passed all required statistical and visual criteria; SFO was therefore accepted as modelling endpoint.

Sediment phase:
SFO gave a slightly better fit compared to FOMC. In addition, FOMC showed unrealistic high standard errors of the optimised parameters. Nevertheless, additional biphasic model (HS, DFOP) were tested as SFO did not perfectly meet later sampling points. Since HS could not be optimised, and DFOP did neither improve the χ^2-error nor the general visual fit, SFO was considered appropriate as persistence and modelling endpoint.

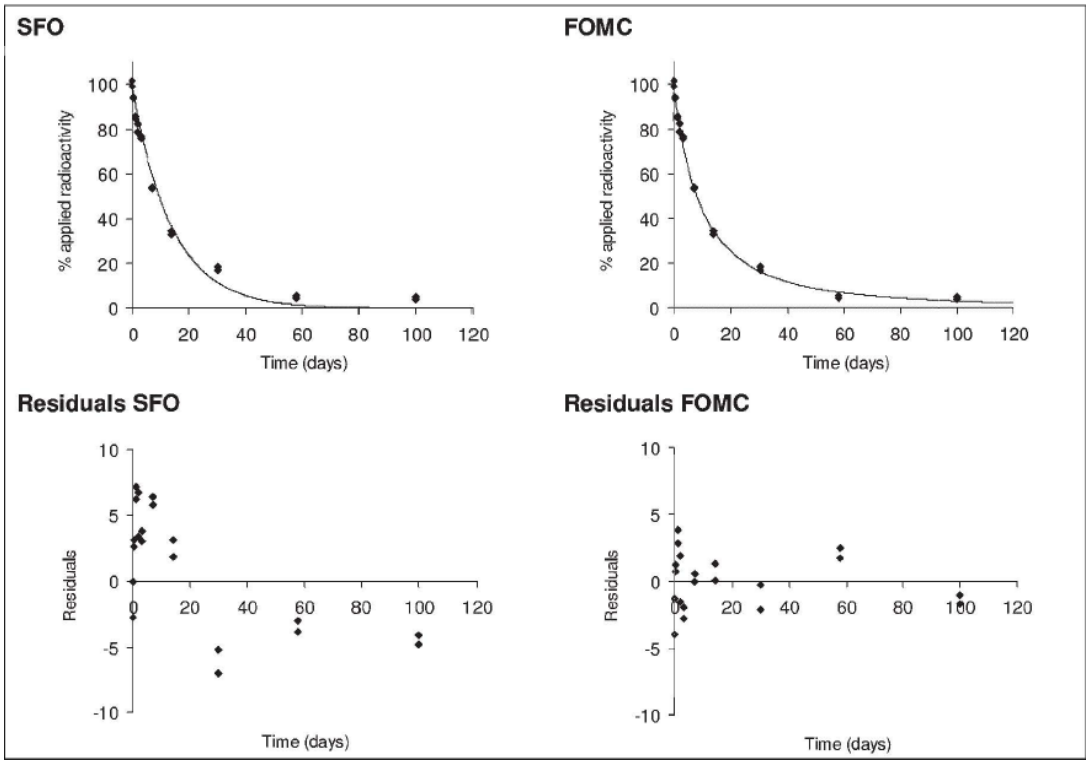


Figure B.8.4-2: Optimised SFO and FOMC kinetic models for degradation of glyphosate in the total system (Bowler and Johnson (1999, BVL no 2154357), system Cache)

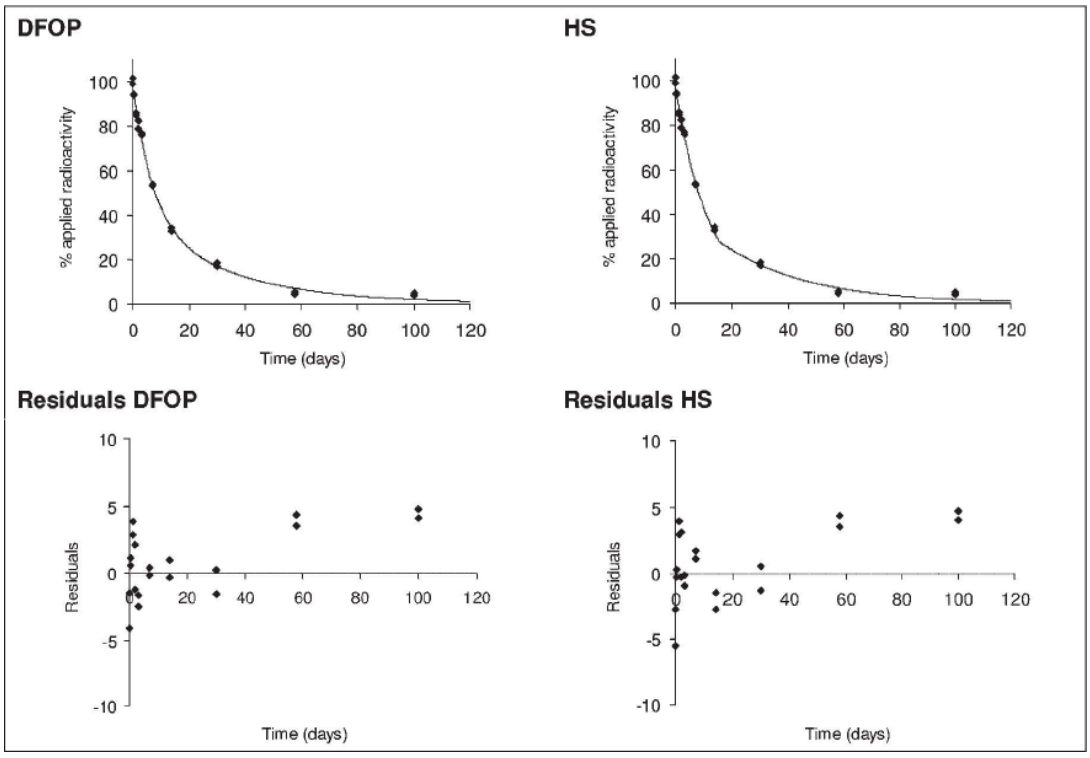


Figure B.8.4-3: Optimised DFOP and HS kinetic models for degradation of glyphosate in the total system (Bowler and Johnson (1999, BVL no 2154357), system Cache)

Note by the RMS: The residual graphs do not to fit to the modelled curves. The data points in the figures of the curves are correct, whereas the data points in the residual plots seem mistaken. Due to the minor importance it is considered neglegible.

Table B.8.4-32: Kinetic evaluation for degradation of glyphosate in the total system (Bowler and Johnson (1999, BVL no 2154357), system Cache)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	99.0000	1.9541	n.r.	6.3	9.70	32.23
	k_SFO	0.0715	0.0052	<0.001			
FOMC	M_0	97.7450	2.2639	n.r.	2.7	8.47	45.89
	α	1.8626	0.5686	n.r.			
	β	18.7870	7.7533	n.r.			
DFOP	M_0	97.5330	2.3978	n.r.	4.0	8.41	47.22
	k1_DFOP	0.1382	0.0612	0.019			
	k2_DFOP	0.0296	0.0171	0.051			
	g	0.5988	0.2781	n.r.			
HS	M0	96.2430	1.2096	n.r.	4.4	8.77	47.74
	k1_HS	0.0790	0.0039	<0.001			
	k2_HS	0.0331	0.0096	0.002			
	tb	15.7220	5.1689	n.r.			

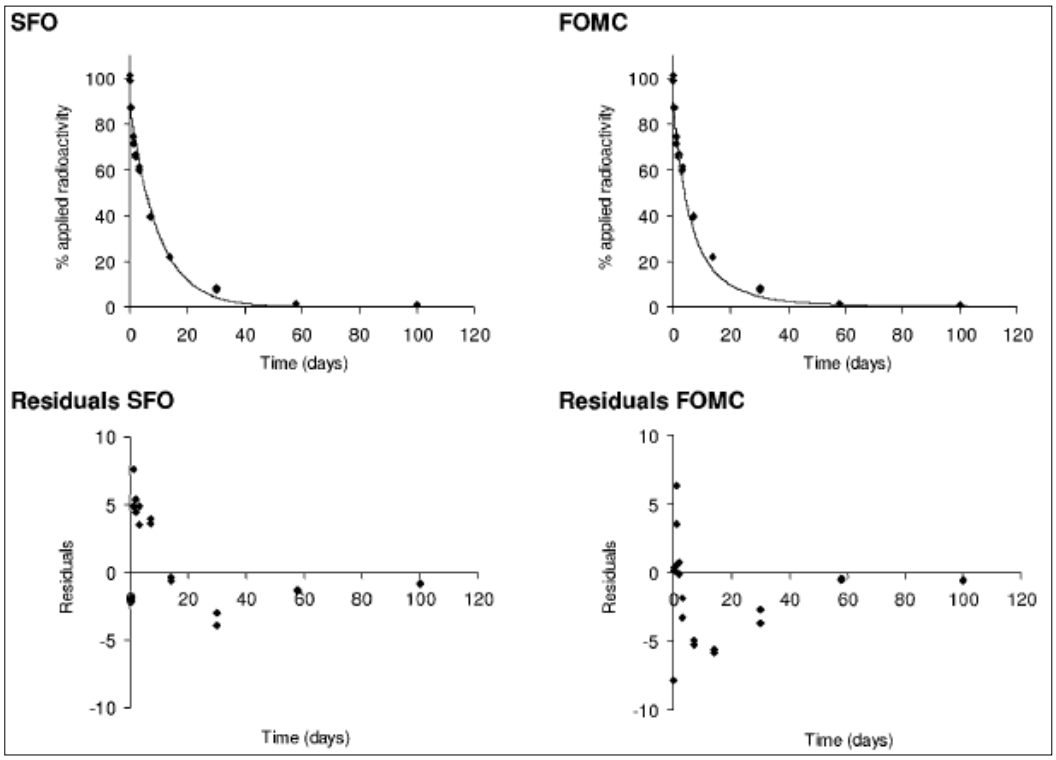


Figure B.8.4-4: Optimised SFO and FOMC kinetic models for dissipation of glyphosate in the water phase (Bowler and Johnson (1999, BVL no 2154357), system Cache)

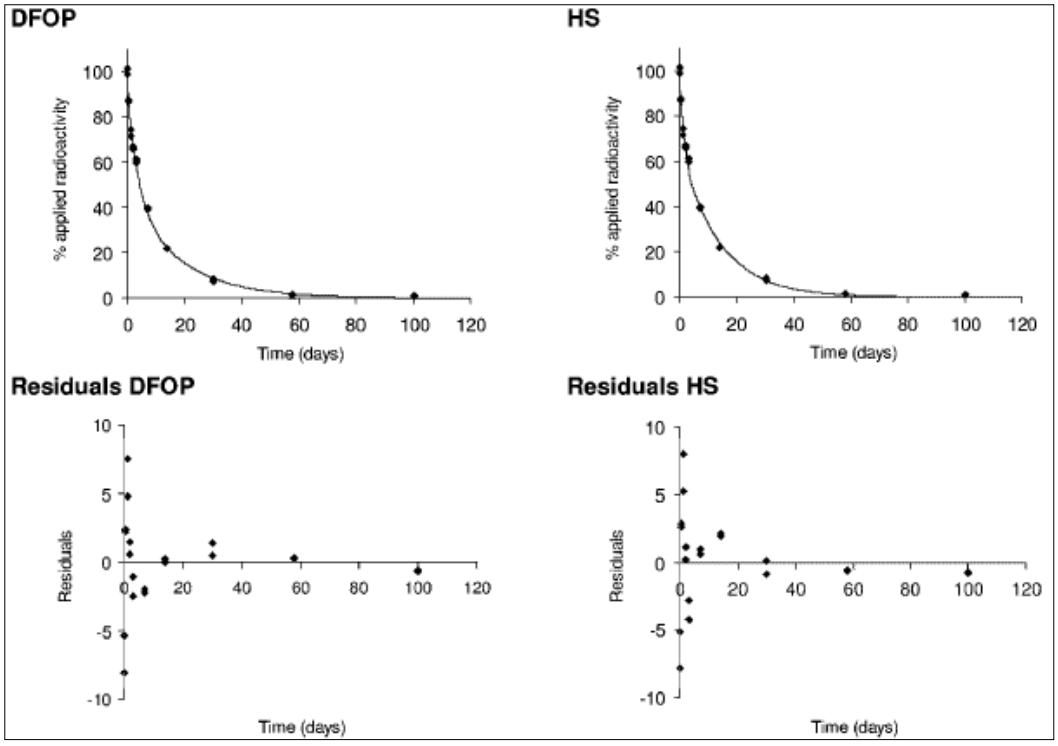


Figure B.8.4-5: Optimised DFOP and HS kinetic models for dissipation of glyphosate in the water phase (Bowler and Johnson (1999, BVL no 2154357), system Cache)

Table B.8.4-33: Kinetic evaluation for dissipation of glyphosate in the water phase (Bowler and Johnson (1999), system Cache, BVL no 2154357)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	87.4730	7.2873	n.r.	9.2	6.94	23.06
	k_SFO	0.0999	0.0288	0.001			
FOMC	M_0	91.1420	8.4842	n.r.	7.9	4.79	20.71
	α	3.2805	0.8410	n.r.			
	β	20.3510	5.6636	n.r.			
DFOP	M_0	93.5990	5.9143	n.r.	6.1	4.85	28.84
	k1_DFOP	0.2988	0.3036	0.170			
	k2_DFOP	0.0561	0.0517	0.147			
	g	0.4965	0.4852	n.r.			
HS	M0	93.8670	2.1330	n.r.	6.5	4.98	26.84
	k1_HS	0.1659	0.0168	<0.001			
	k2_HS	0.0736	0.0131	<0.001			
	tb	3.5382	1.5127	n.r.			

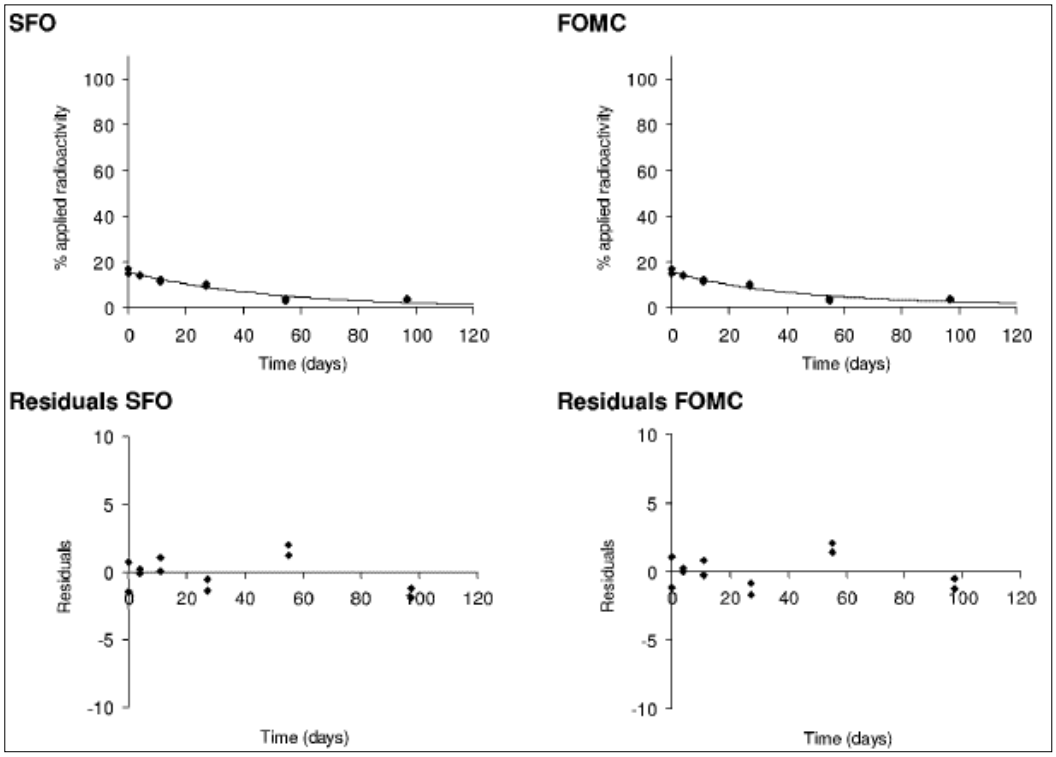


Figure B.8.4-6: Optimised SFO and FOMC kinetic models for dissipation of glyphosate in the sediment phase (Bowler and Johnson (1999, BVL no 2154357), system Cache)

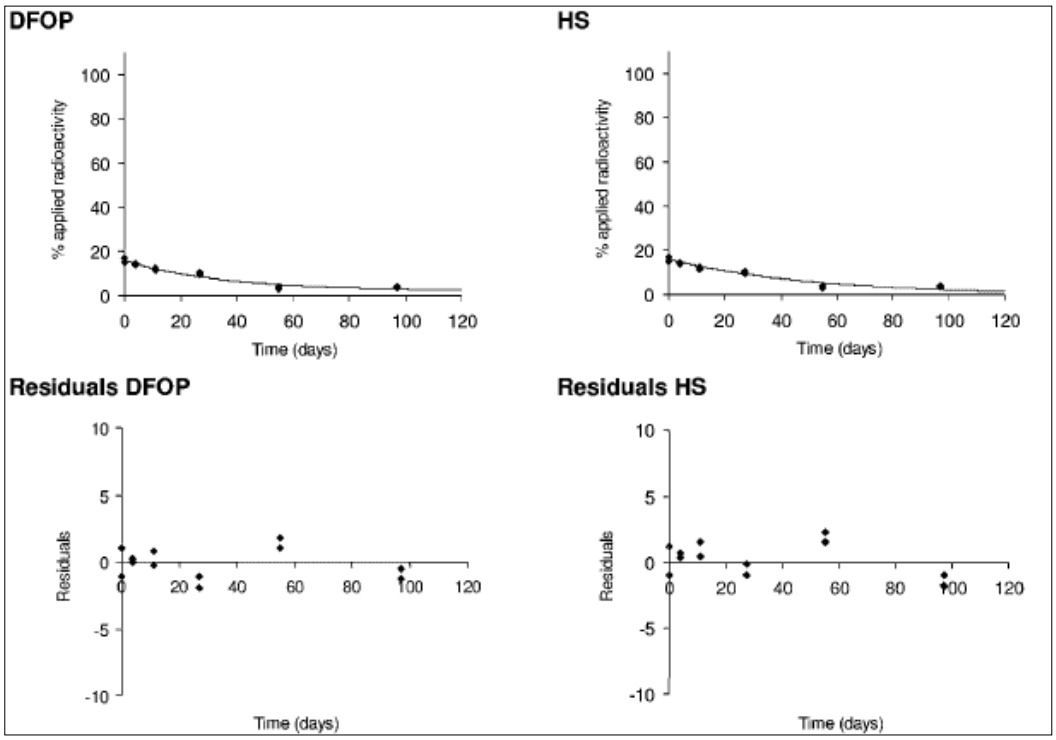


Figure B.8.4-7: Optimised DFOP and HS kinetic models for dissipation of glyphosate in the sediment phase (Bowler and Johnson (1999, BVL no 2154357), system Cache)

Table B.8.4-34: Kinetic evaluation for dissipation of glyphosate in the sediment phase (Bowler and Johnson (1999, BVL no 2154357), system Cache)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	15.5290	0.5850	n.r.	8.4	34.05	113.10
	k_SFO	0.0204	0.0022	<0.001			
FOMC	M_0	15.837	15.4760	n.r.	8.7	31.11	145.04
	α	2.6015	53.9760	n.r.			
	β	101.91	2626.7	n.r.			
DFOP	M_0	15.8700	0.7603	n.r.	9.5	29.56	2353.88
	k1_DFOP	0.0287	0.0335	0.208			
	k2_DFOP	0.0001	0.0602	0.499			
	g	0.8735	1.0273	n.r.			
HS	M0	-[1)]					
	k1_HS	-[1)]					
	k2_HS	-[1)]					
	tb	-[1)]					

[1)] could not be optimised by ModelMaker

Bowler and Johnson (1999, BVL no 2154357), system Putah

Total system:

Though SFO was statistically acceptable, it provided a poor visual fit compared to FOMC. Additionally tested HS and DFOP showed a marginally poorer χ^2-error than FOMC; however, they revealed more realistic degradation endpoints ($DegT_{50}$, $DegT_{90}$) and gave a better fit to later sampling points than FOMC. Since DFOP met M_0 slightly better than HS and also

showed a slightly better χ^2-error, preference was given to DFOP as persistence endpoint. As 10 % of the initially measured substance concentration was not reached within the study period, the modelling endpoint needed to be derived from either HS or DFOP. Based on the slightly better overall fit of DFOP, it was also considered as modelling end point. Consequently, the SFO half-life was calculated from the slow phase of DFOP.

Water phase:
SFO revealed a poor statistical fit, while FOMC was more appropriate, especially at later data point around DT_{90}. Additionally tested HS did not improve χ^2-error though it perfectly met later sampling points. This was probably due to the poor fit around M_0. The additionally tested DFOP provided a better overall fit than FOMC (χ^2 = 7.5). However, FOMC represented later data points around DT_{90} better than DFOP and, hence, preference was given to FOMC as persistence endpoint. Since FOMC was also available for the procedure to estimate modelling endpoints, and since DT_{50} of all bi-phasic models were very similar, FOMC was deemed appropriate to derive a conservative modelling endpoint by back-calculating from FOMC DT_{90}.

Sediment phase:
There were not a sufficient number of data points available after the peak concentration in sediment to run kinetic evaluations.

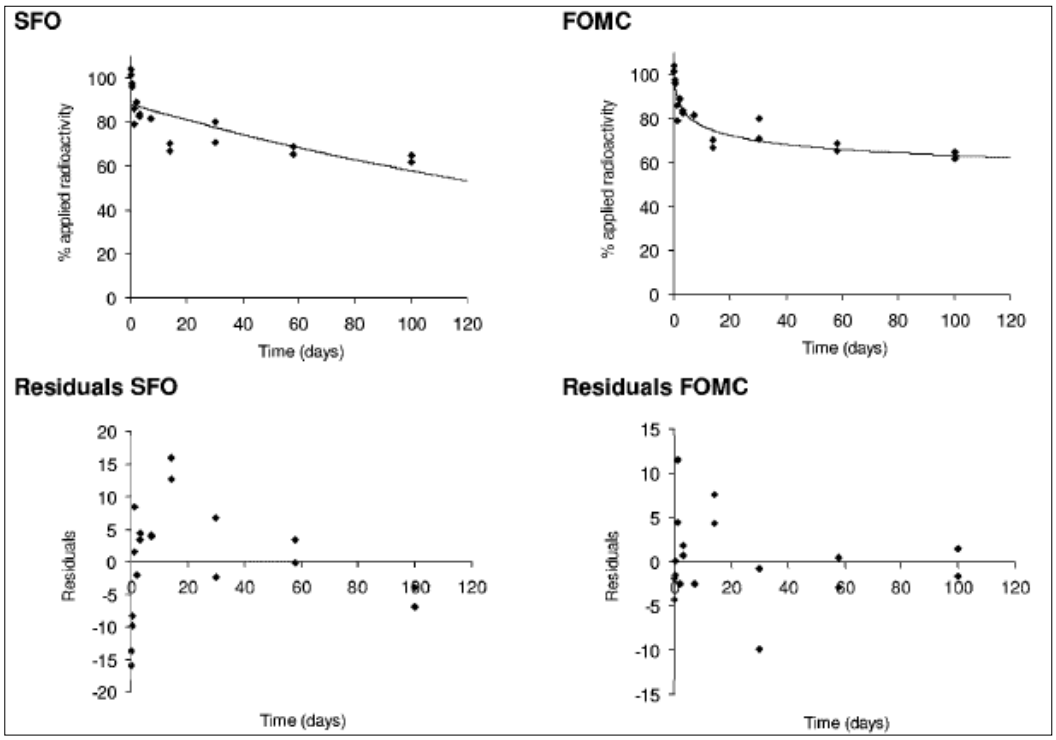


Figure B.8.4-8: Optimised SFO and FOMe kinetic models for degradation of glyphosate in the total system (Bowler and Johnson (1999, BVL no 2154357), system Putah)

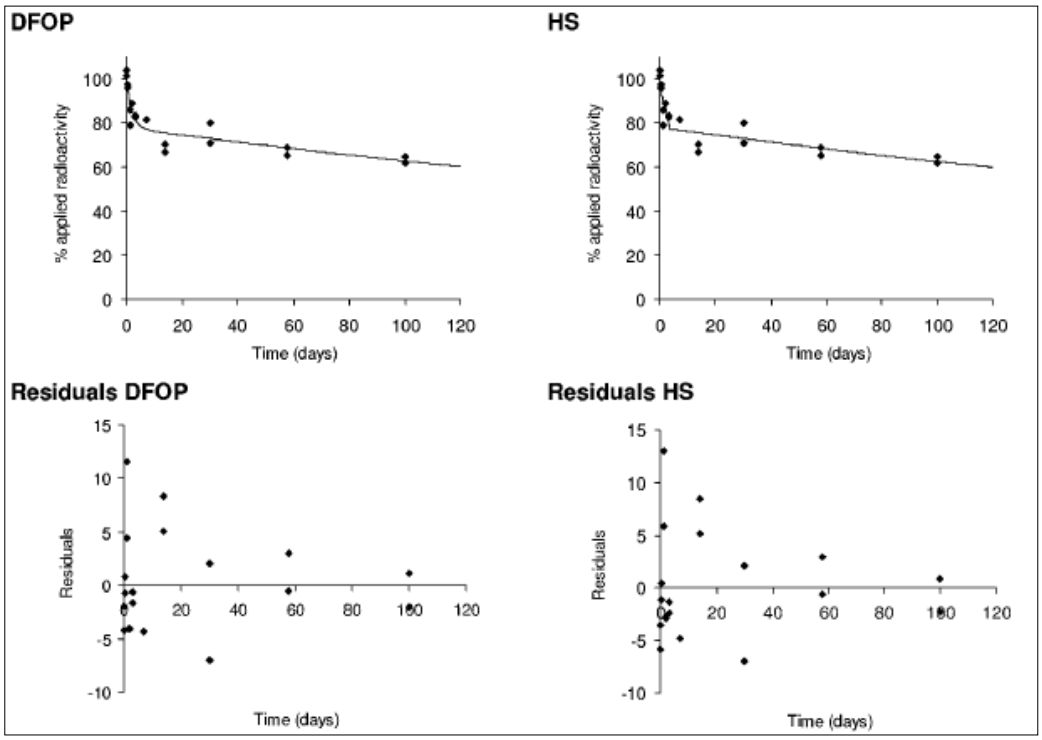


Figure B.8.4-9: Optimised DFOP and HS kinetic models for degradation of glyphosate in the total system (Bowler and Johnson (1999, BVL no 2154357), system Putah)

Table B.8.4-35: Kinetic evaluation for degradation of glyphosate in the total system (Bowler and Johnson (1999, BVL no 2154357), system Putah)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	87.9380	7.8904	n.r.	7.7	166.11	551.82
	k_SFO	0.0042	0.0031	0.095			
FOMC	M_0	99.6720	5.8556	n.r.	4.1	>1000	>1000
	α	0.0849	0.0142	n.r.			
	β	0.4795	0.2190	n.r.			
DFOP	M_0	99.6980	2.8990	n.r.	4.4	210.66	976.54
	k1_DFOP	0.5173	0.0506	<0.001			
	k2_DFOP	0.0021	0.0007	0.006			
	g	0.2216	0.0385	n.r.			
HS	M0	98.0700	2.6840	n.r.	4.9	213.54	957.21
	k1_HS	0.0635	0.0121	<0.001			
	k2_HS	0.0022	0.0008	0.006			
	tb	3.7662	1.2793	n.r.			

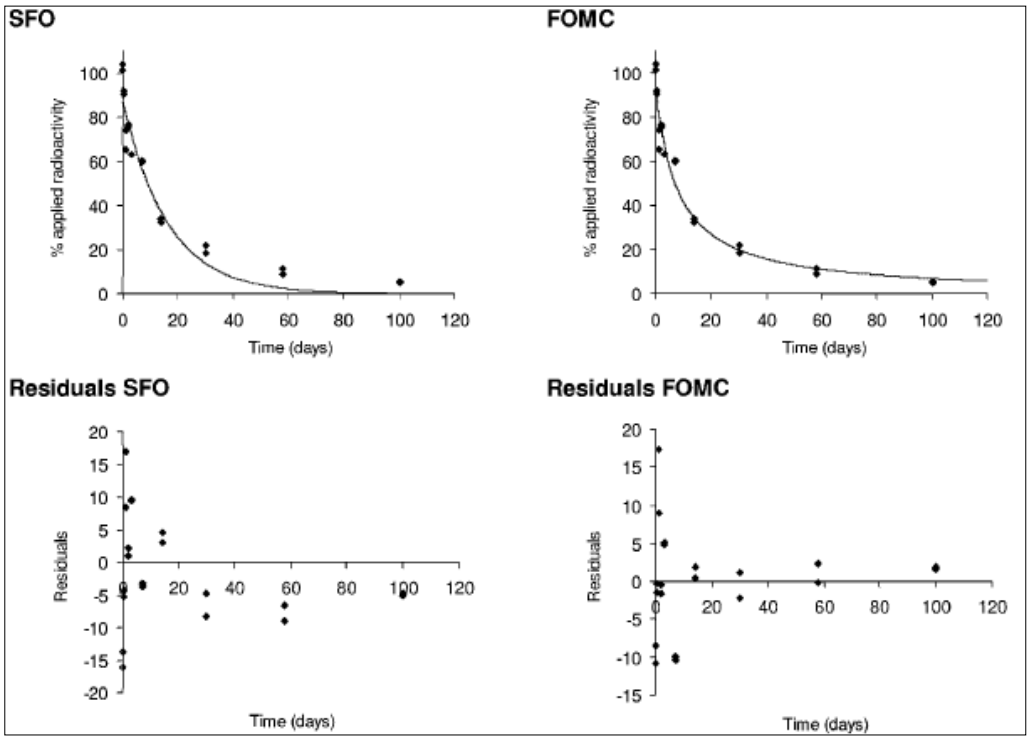


Figure B.8.4-10: Optimised SFO and FOMC kinetic models for dissipation of glyphosate in the water phase (Bowler and Johnson (1999, BVL no 2154357), system Putah)

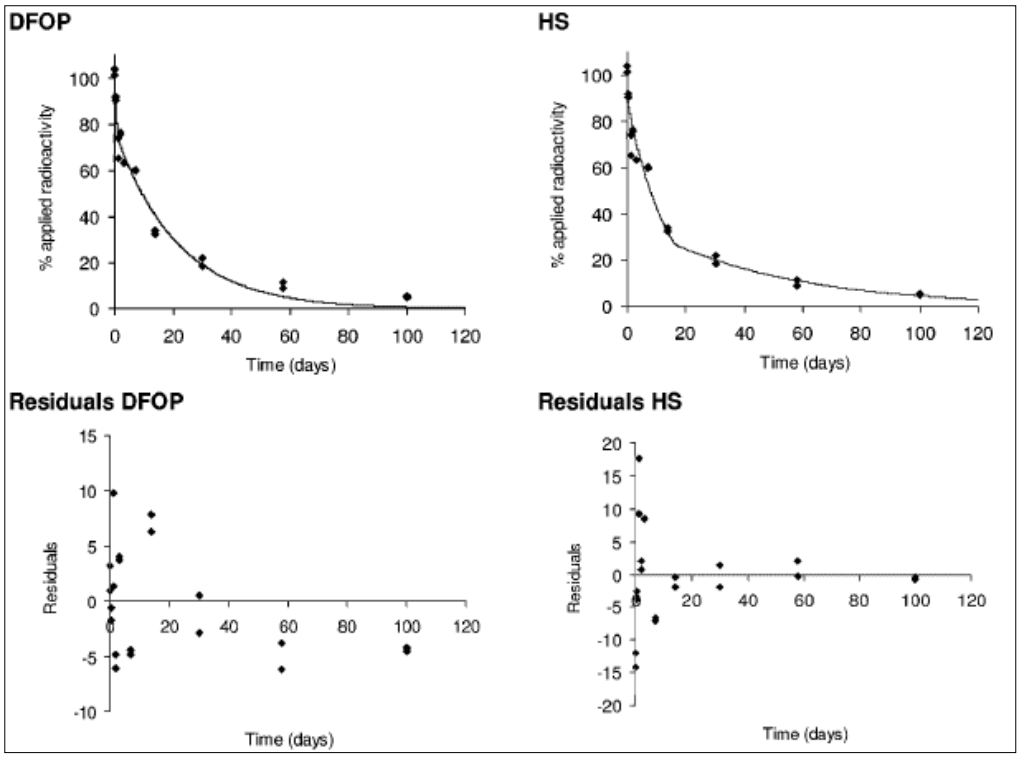


Figure B.8.4-11: Optimised DFOP and HS kinetic models for dissipation of glyphosate in the water phase (Bowler and Johnson (1999, BVL no 2154357), system Putah)

Table B.8.4-36: Kinetic evaluation for dissipation of glyphosate in the water phase (Bowler and Johnson (1999, BVL no 2154357), system Putah)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	87.8060	5.3278	n.r.	12.2	11.27	37.44
	k_SFO	0.0615	0.0142	<0.001			
FOMC	M_0	93.0900	4.5315	n.r.	10.0	8.25	72.40
	α	1.0220	0.3703	n.r.			
	β	8.5009	5.0723	n.r.			
DFOP	M_0	104.8200	5.9728	n.r.	7.5	8.38	43.03
	k1_DFOP	2.8180	1.2560	0.020			
	k2_DFOP	0.0464	0.0100	<0.001			
	g	0.26200	0.0817	n.r.			
HS	M0	89.6990	2.7470	n.r.	11.7	9.45	68.12
	k1_HS	0.0733	0.0092	<0.001			
	k2_HS	0.0212	0.0118	0.045			
	tb	16.4470	3.4297	n.r.			

Möllerfeld and Römbke (1993, BVL no 1934113), system Sandy Sediment

Total system:
In a first comparison, FOMC model was much more appropriate than SFO, both visually and statistically. Consequently, HS and DFOP were tested and revealed even better visual fits than FOMC, especially at later sampling points. On the basis of its almost perfect visual fit, HS was selected as best-fit end point. As neither HS nor DFOP k-rates significantly different from zero, and as, due to degradation of less than 90 % of the initial concentration within the experiment, FOMC was excluded from the recommended procedure to estimate modelling endpoint at level P-I, the selection of the modelling end point was more critical. However, the SFO fit was questionable and, finally, it was deemed appropriate to derive modelling endpoints from the slower k-rate of the HS model.

Water phase:
FOMC showed a more appropriate fit to the data than SFO. Especially, M_0 and χ^2 were better met. Due to extremely high standard errors of the optimised FOMC parameters, HS and DFOP needed to be tested. Both bi-phasic models showed a smaller χ^2-error and highly significant degradation rates. Consequently, both persistence and modelling end points were derived from DFOP fit.

Sediment phase:
Fitting the FOMC model to the sediment data very much improved the visual and statistical acceptance, except that it revealed extremely high half-lives, especially DT_{90}. The additionally tested bi-phasic models HS and DFOP improved the χ^2-error even more and showed similar values <4 %. As the optimisation process of the HS model provided smaller standard errors

and statistically significant t-tests of the degradation rates, preference was given to HS as persistence and modelling end point, although DFOP had a slightly better χ^2-error.

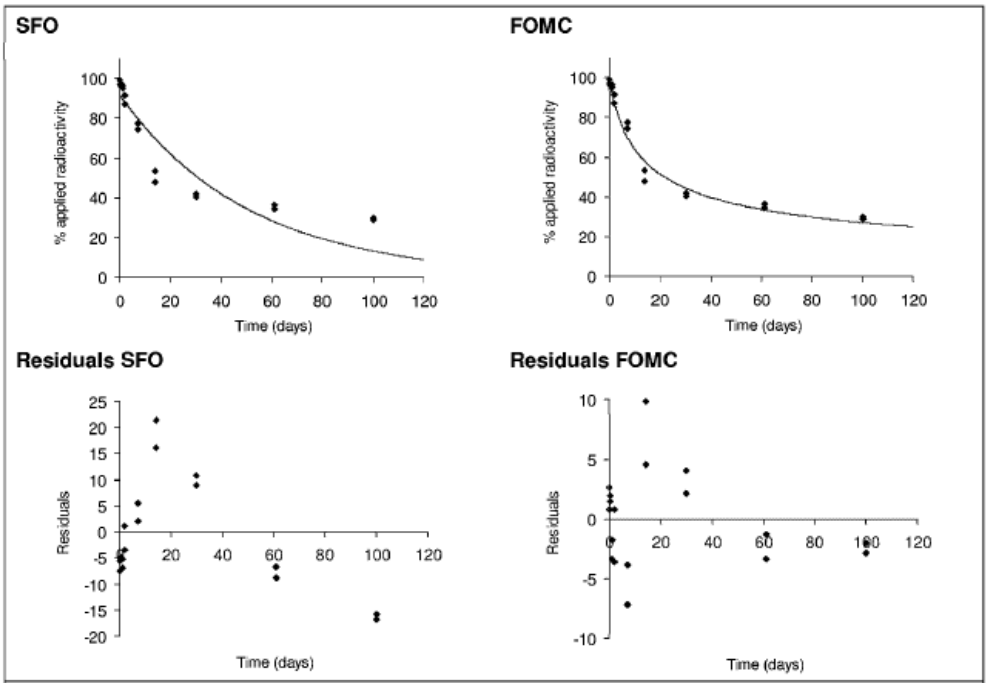


Figure B.8.4-12: Optimised SFO and FOMC kinetic models for degradation of glyphosate in the total system (Möllerfeld and Römbke (1993, BVL no 1934113), system Sandy Sediment)

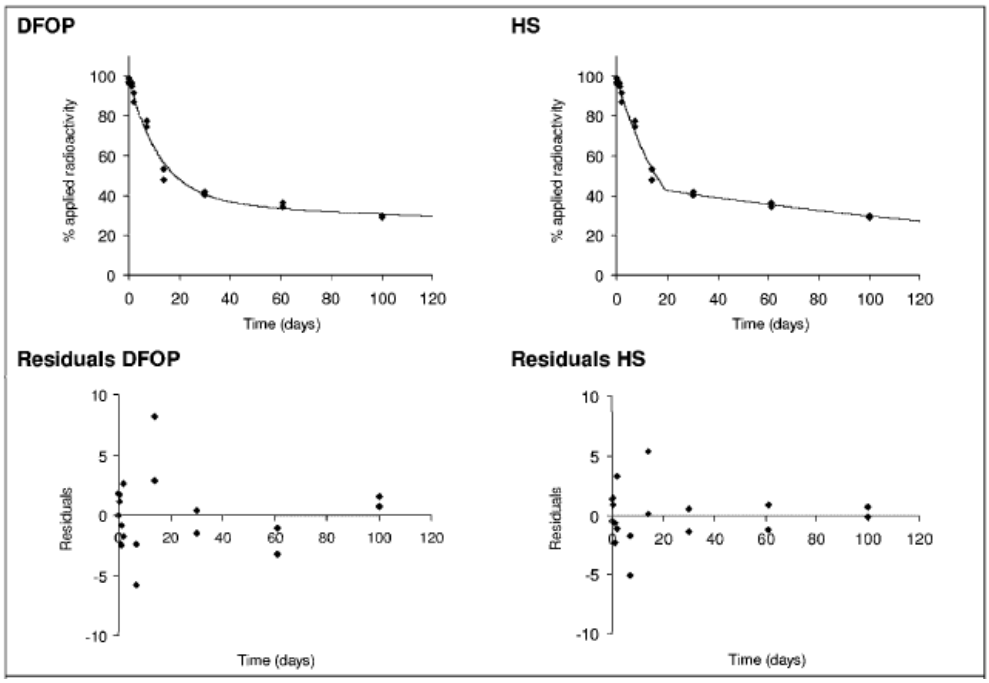


Figure B.8.4-13: Optimised DFOP and HS kinetic models for degradation of glyphosate in the total system (Möllerfeld and Römbke (1993, BVL no 1934113), system Sandy Sediment)

Table B.8.4-37: Kinetic evaluation for degradation of glyphosate in the total system (Möllerfeld and Römbke (1993, BVL no 1934113), system Sandy Sediment)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	91.4020	1.9270	n.r.	11.7	35.81	118.95
	k_SFO	0.0194	0.0016	<0.001			
FOMC	M_0	99.6070	2.7992	n.r.	4.5	22.02	930.35
	α	0.4598	0.0568	n.r.			
	β	6.2632	1.9831	n.r.			
DFOP	M0	98.7260	4.8357	n.r.	3.4	19.05	681.94
	k1_DFOP	0.0797	0.0503	0.068			
	k2_DFOP	0.0019	0.0080	0.405			
	g	0.6227	0.2439	n.r.			
HS	M0	98.3030	4.1920	n.r.	2.2	16.03	346.81
	k1_HS	0.0432	0.0096	<0.001			
	k2_HS	0.0045	0.0044	0.164			
	tb	19.1930	5.6060	n.r.			

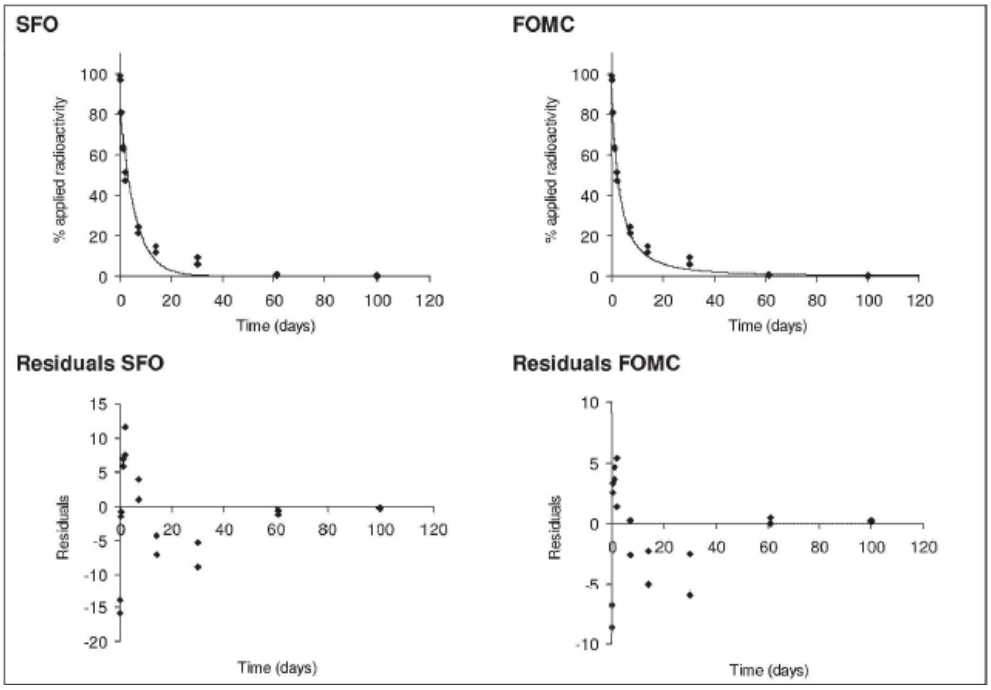


Figure B.8.4-14: Optimised SFO and FOMC kinetic models for dissipation of glyphosate in the water phase (Möllerfeld and Römbke (1993, BVL no 1934113), system Sandy Sediment)

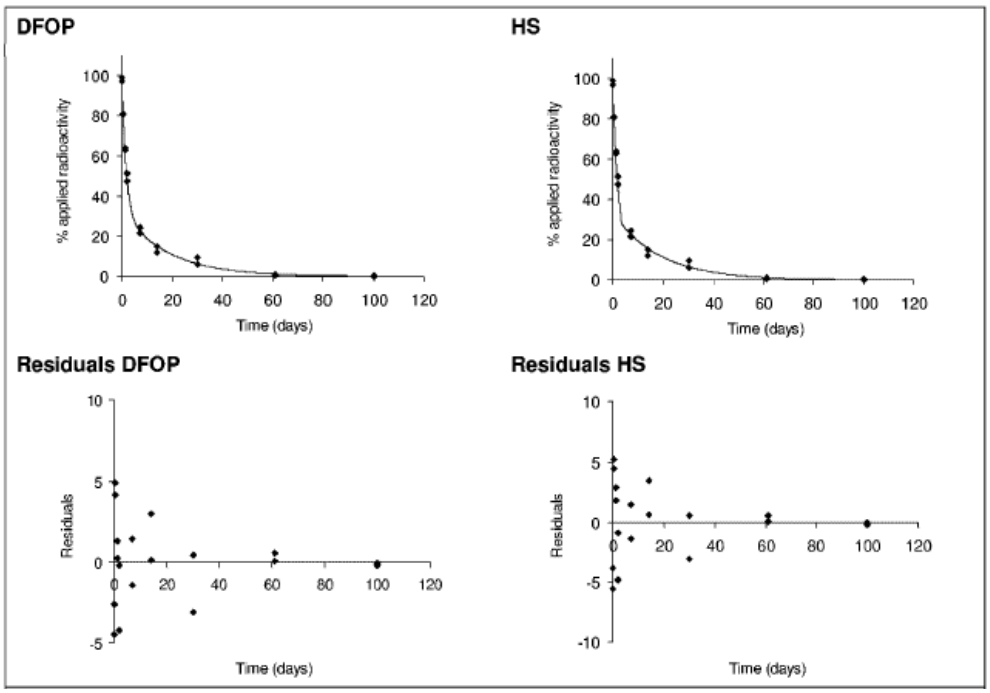


Figure B.8.4-15: Optimised DFOP and HS kinetic models for dissipation of glyphosate in the water phase (Möllerfeld and Römbke (1993, BVL no 1934113), system Sandy Sediment)

Table B.8.4-38: Kinetic evaluation for dissipation of glyphosate in the water phase (Möllerfeld and Römbke (1993, BVL no 1934113), system Sandy Sediment)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M0	82.9830	9.9141	n.r.	15.0	4.11	13.66
	k_SFO	0.1686	0.0627	0.008			
FOMC	M0	90.1150	12.5240	n.r.	8.6	2.75	15.04
	α	1.8310	1.8002	n.r.			
	β	5.9155	8.9327	n.r.			
DFOP	M0	94.3470	2.2924	n.r.	5.2	2.03	22.63
	k1_DFOP	0.6038	0.1251	<0.001			
	k2_DFOP	0.0543	0.0218	0.013			
	g	0.6579	0.0959	n.r.			
HS	M0	93.1640	2.4640	n.r.	6.5	2.02	23.20
	k1_HS	0.3438	0.0286	<0.001			
	k2_HS	0.0557	0.0191	0.006			
	tb	3.5068	2.2458	n.r.			

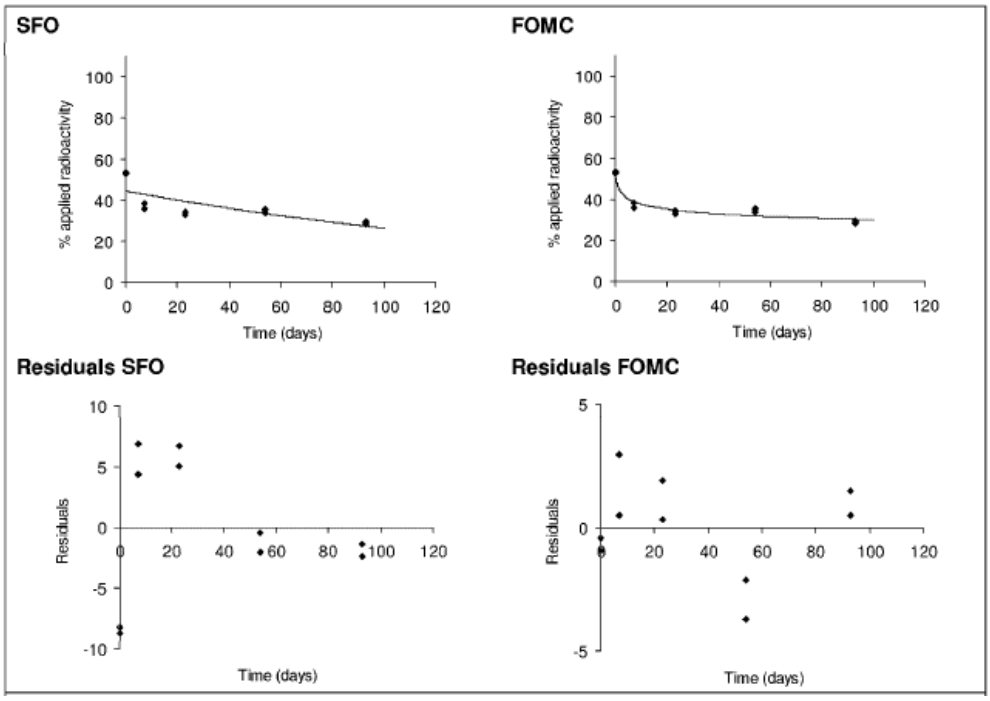


Figure B.8.4-16: Optimised SFO and FOMC kinetic models for dissipation of glyphosate in the sediment phase (Möllerfeld and Römbke (1993, BVL no 1934113), system Sandy Sediment)

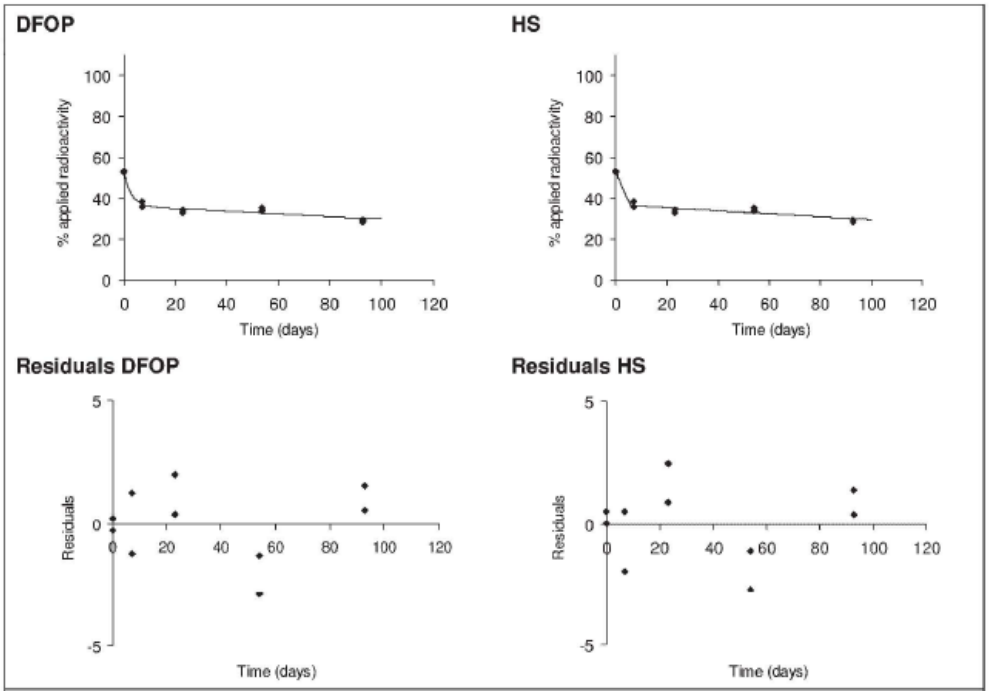


Figure B.8.4-17: Optimised DFOP and HS kinetic models for dissipation of glyphosate in the sediment phase (Möllerfeld and Römbke (1993, BVL no 1934113), system Sandy Sediment)

Table B.8.4-39: Kinetic evaluation for dissipation of glyphosate in the sediment phase (Möllerfeld and Römbke (1993, BVL no 1934113), system Sandy Sediment)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	44.5730	11.6580	n.r.	11.4	132.39	439.79
	k_SFO	0.0052	0.0068	0.2322			
FOMC	M_0	52.4180	2.1286	n.r.	4.1	383.86	>1000
	α	0.1012	0.0115	n.r.			
	β	0.4074	0.0165	n.r.			
DFOP	M_0	53.076	12.6380	n.r.	3.6	159.82	966.67
	k1_DFOP	0.3510	1.8871	0.429			
	k2_DFOP	0.0020	0.0080	0.405			
	g	0.3123	0.3705	n.r.			
HS	M0	53.3520	3.0140	n.r.	3.8	146.26	850.52
	k1_HS	0.0617	0.01328	0.002			
	k2_HS	0.0023	0.0014	0.078			
	tb	6.0443	6.8035	n.r.			

Möllerfeld and Römbke (1993, BVL no 1934113), system Loamy Sediment

Total system:
In a first comparison, both SFO and FOMC showed statistically acceptable optimisation results. Since FOMC provided a much better visual fit than SFO, HS and DFOP were tested additionally; both resulted in non-significant t-tests on the respective k-rates. According to the

χ^2-error, HS provided the best fit to the data, revealing a conservative estimation of $DegT_{50}$. However, as 10 % of the applied material was not reached within the study, and as DFOP met both the last data point and Mo slightly better than HS, preference was given to DFOP as persistence end point. Since SFO showed a very poor and doubtful fit and none of the bi-phasic models revealed significant t-tests, no reliable modelling end point could be determined.

Water phase:
SFO did not adequately represent the fast dissipation process of glyphosate from the water phase. From the bi-phasic models, DFOP showed the best χ^2. However, FOMC was accepted as persistence end point as it provided a good general fit and met M_0 better than HS and DFOP. As 10 % of the initially measured concentration was reached within the experimental period, all three bi-phasic models were tested. Due to non- significant t-tests, neither HS nor DFOP were statistically acceptable. Therefore, the modelling end point was derived from FOMC, resulting in a conservative SFO DT_{50}.

Sediment phase:
Since FOMC could not be optimised by ModelMaker 4.0 in the first comparison with SFO, HS and DFOP were tested immediately. Since DFOP could also not be optimised, SFO and HS were the only remaining kinetic models to derive end points from. Based on the residue data it was doubted if dissipation actually occurs. Therefore, and because of the relatively high standard errors of the optimised parameters, leading to non-significant statistics, none of the models (SFO, HS) were considered reliable. Hence, neither persistence nor modelling end point was derived from the sediment data.

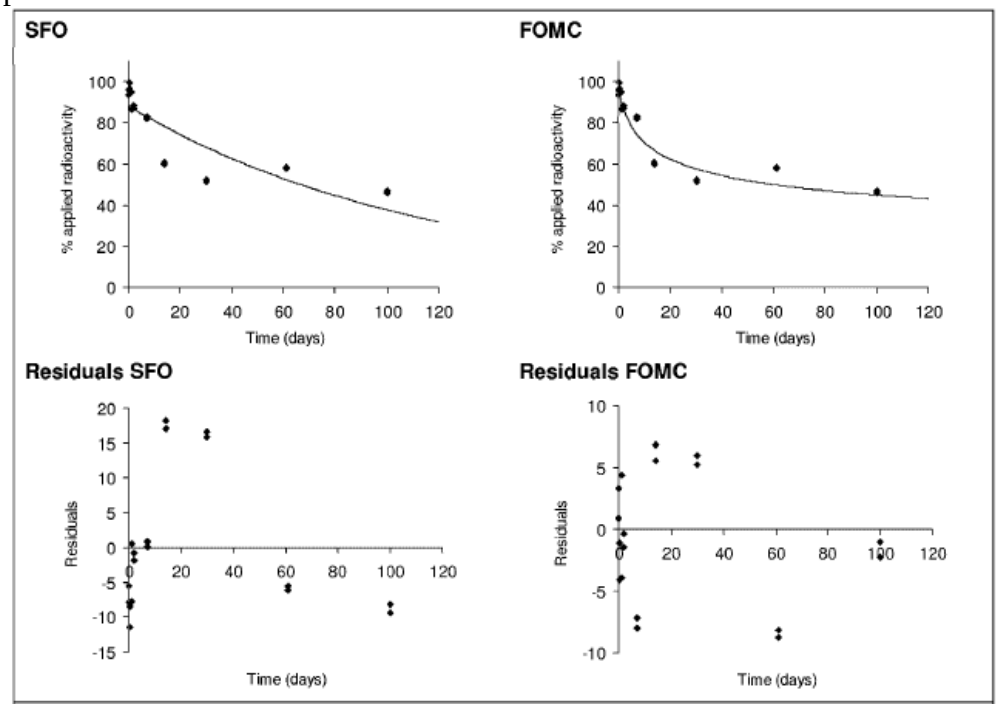


Figure B.8.4-18: Optimised SFO and FOMC kinetic models for degradation of glyphosate in the total system (Möllerfeld and Römbke (1993, BVL no 1934113), system Loamy Sediment)

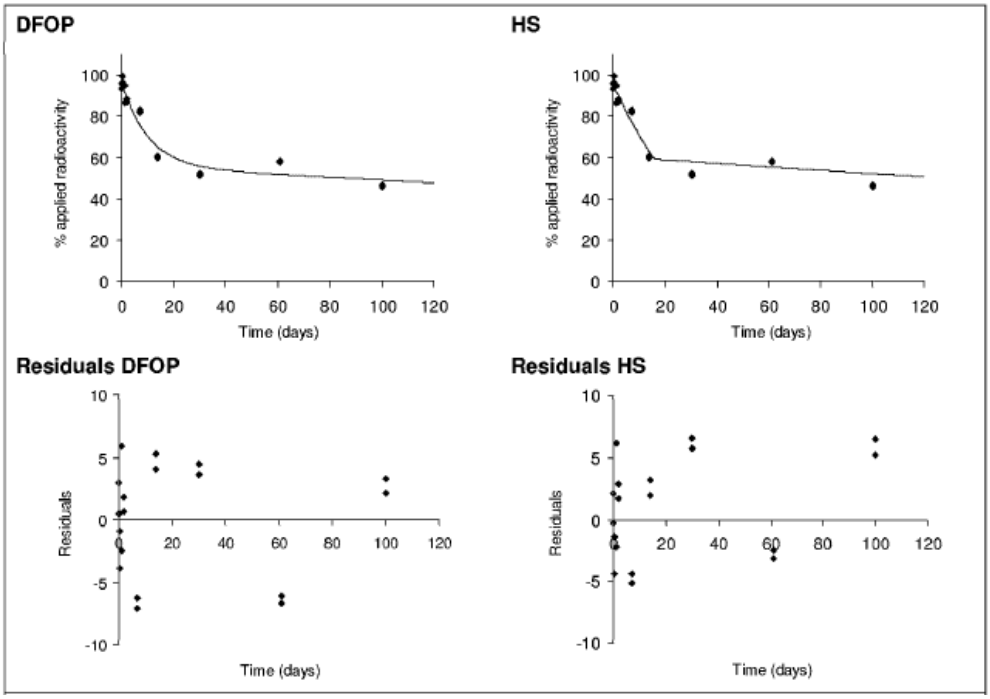


Figure B.8.4-19: Optimised DFOP and HS kinetic models for degradation of glyphosate in the total system (Möllerfeld and Römbke (1993, BVL no 1934113), system Loamy Sediment)

Table B.8.4-40: Kinetic evaluation for degradation of glyphosate in the total system (Möllerfeld and Römbke (1993, BVL no 1934113), system Loamy Sediment)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	88.0100	7.3054	n.r.	10.4	82.01	272.43
	k_SFO	0.0085	0.0034	0.013			
FOMC	M_0	96.8400	2.6653	n.r.	5.5	70.48	∞
	α	0.2200	0.0250	n.r.			
	β	3.1541	1.5202	n.r.			
DFOP	M_0	96.4300	10.5290	n.r.	4.8	116.56	1328.20
	k1_DFOP	0.0997	0.1801	0.294			
	k2_DFOP	0.0013	0.0090	0.443			
	g	0.4163	0.3879	n.r.			
HS	M0	95.6340	11.2230	n.r.	4.6	123.24	1196.55
	k1_HS	0.0297	0.0230	0.109			
	k2_HS	0.0015	0.0076	0.4234			
	tb	18.0220	30.2690	n.r.			

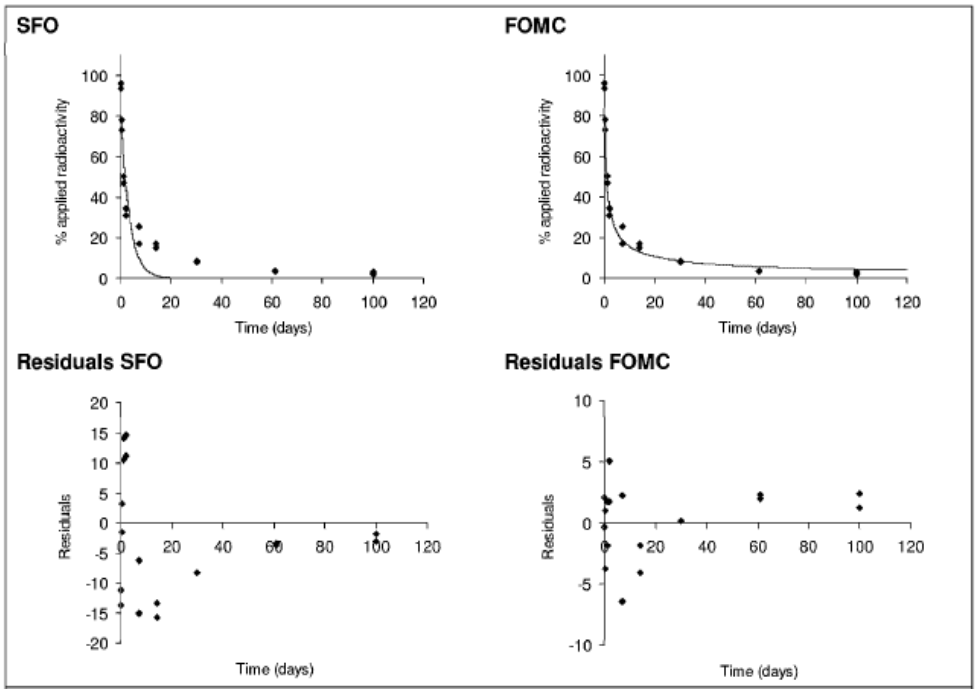


Figure B.8.4-20: Optimised SFO and FOMC kinetic models for dissipation of glyphosate in the water phase (Möllerfeld and Römbke (1993, BVL no 1934113), system Loamy Sediment)

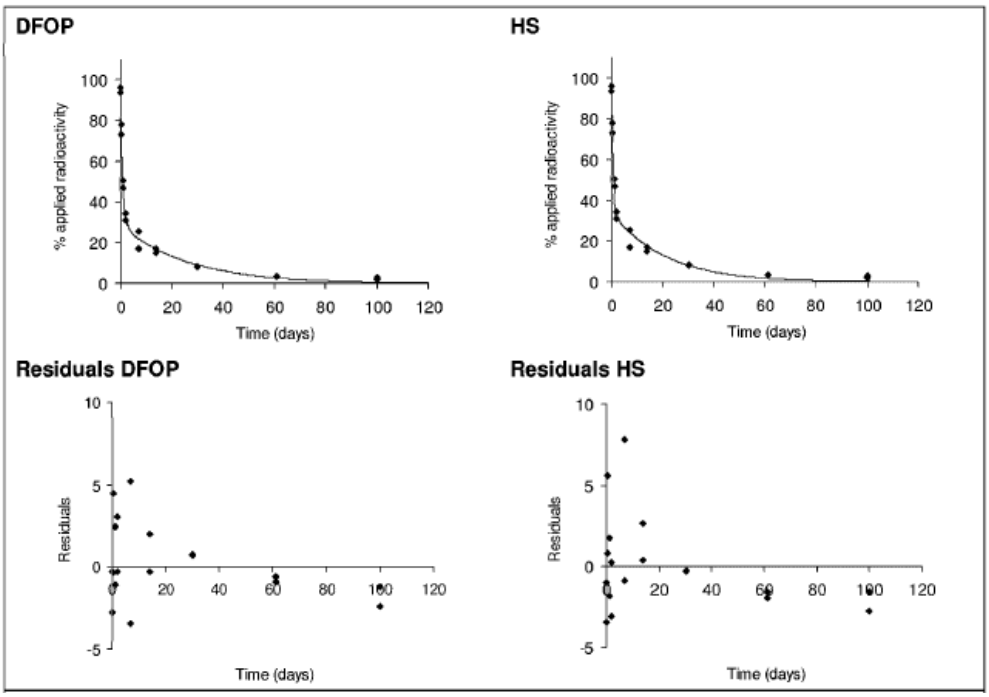
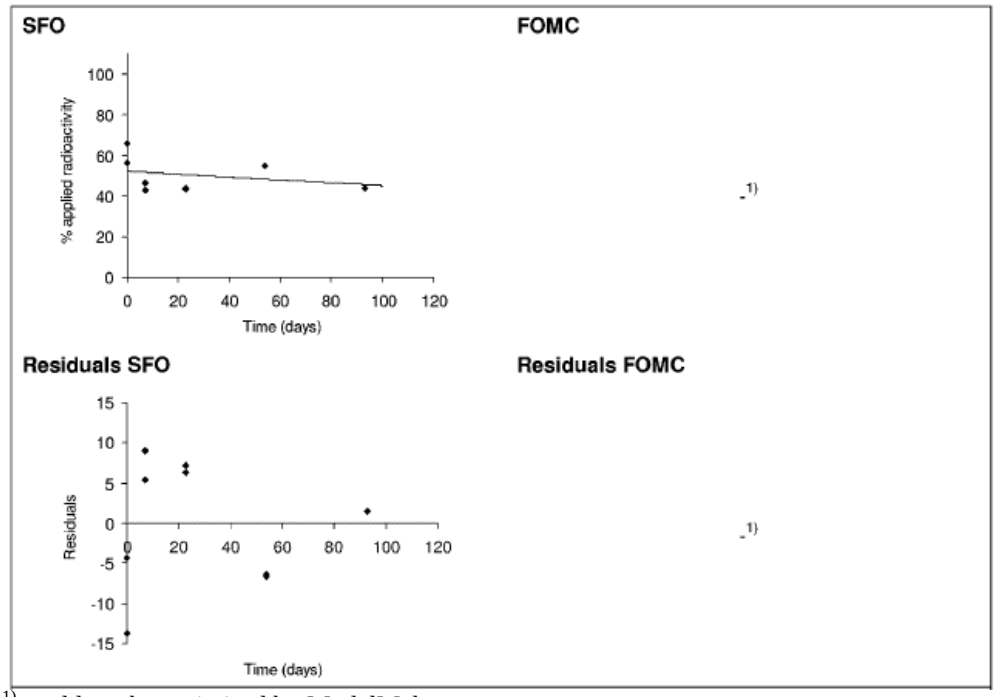


Figure B.8.4-21: Optimised DFOP and HS kinetic models for dissipation of glyphosate in the water phase (Möllerfeld and Römbke (1993, BVL no 1934113), system Loamy Sediment)

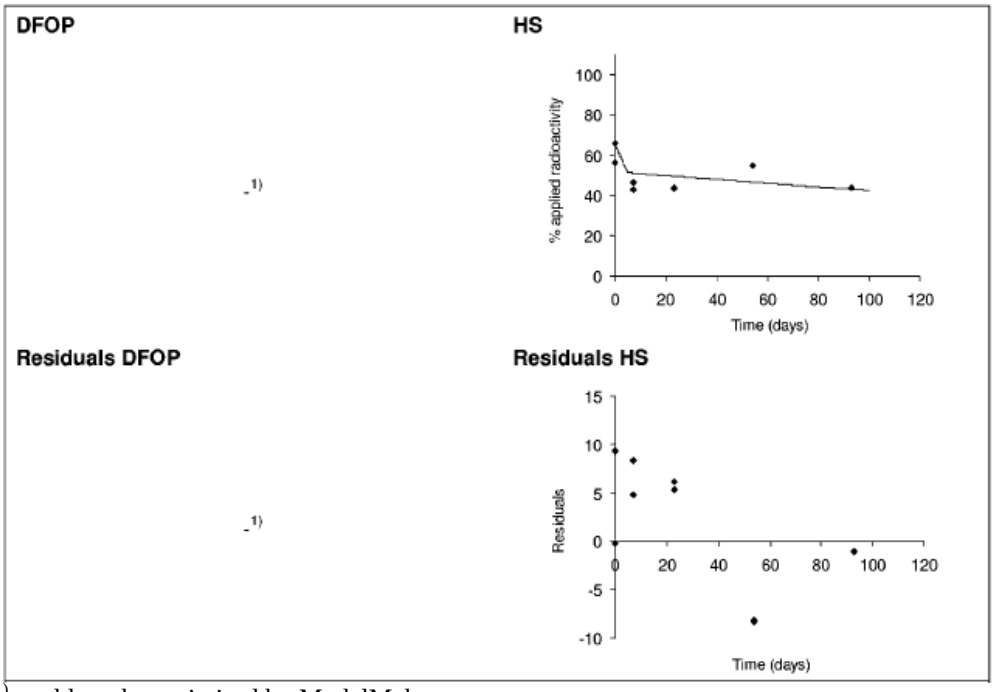
Table B.8.4-41: Kinetic evaluation for dissipation of glyphosate in the water phase (Möllerfeld and Römbke (1993, BVL no 1934113), system Loamy Sediment)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	82.2730	13.7470	n.r.	23.5	2.35	7.80
	k_SFO	0.2951	0.1389	0.025			
FOMC	M_0	95.5650	3.5290	n.r.	5.0	1.06	24.11
	α	0.5783	0.0342	n.r.			
	β	0.4582	0.0351	n.r.			
DFOP	M_0	93.1770	14.9150	n.r.	3.4	1.13	29.20
	k1_DFOP	1.0774	0.8803	0.121			
	k2_DFOP	0.0387	0.0670	0.286			
	g	0.6907	0.2767	n.r.			
HS	M0	92.4680	11.5290	n.r.	5.6	1.09	27.04
	k1_HS	0.6362	0.3203	0.034			
	k2_HS	0.0488	0.0512	0.179			
	tb	1.6747	0.5498	n.r.			



[1)] could not be optimised by ModelMaker

Figure B. 8.4-22: Optimised SFO and FOMC kinetic models for dissipation of glyphosate in the sediment phase (Möllerfeld and Römbke (1993, BVL no 1934113), system Loamy Sediment)



1) could not be optimised by ModelMaker

Figure B.8.4-23: Optimised DFOP and HS kinetic models for dissipation of glyphosate in the sediment phase (Möllerfeld and Römbke (1993, BVL no 1934113), system Loamy Sediment)

Table B.8.4-42: Kinetic evaluation for dissipation of glyphosate in the sediment phase (Möllerfeld and Römbke (1993, BVL no 1934113), system Loamy Sediment)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	52.3400	8.9510	n.r.	10.8	473.56	1573.13
	k_SFO	0.0015	0.0037	0.352			
FOMC	M_0	-1)		n.r.			
	α	-1)		n.r.			
	β	-1)		n.r.			
DFOP	M_0	-1)		n.r.			
	k1_DFOP	-1)					
	k2_DFOP	-1)					
	g	-1)		n.r.			
HS	M0	65.9700	16.1230	n.r.	13.3	228.10	1036.25
	k1_HS	0.0500	0.0594	0.216			
	k2_HS	0.0020	0.0053	0.360			
	tb	4.9749	129.4100	n.r.			

1) could not be optimised by ModelMaker

Heintze (1996, BVL no 1939626), system Pond

Total system:

FOMC provided a better fit compared to SFO, regarding χ^2-error and meeting M_0, and, hence, triggered testing of further bi-phasic models HS and DFOP. Both models revealed very similar χ^2-errors to each other and also similar to FOMC. As the $DegT_{90}$ was not reached within the experimental period, the fact that FOMC met the later data points better than HS and DFOP was considered more important than the high standard errors of α and ß resulting from the optimisation process of the FOMC model. Therefore, FOMC was deemed appropriate as persistence end point. However, as SFO generally represented the degradation behaviour of glyphosate in total system, and as bi-phasic models added only little to the χ^2-error, SFO was accepted as modelling end point revealing a slightly conservative degradation half-life.

Water phase:
The comparison of SFO and FOMC showed that none of the model adequately represents the residue data of the water phase. Though FOMC met Mo slightly better; it provided a χ^2-error that was even worse than that of SFO model, which was probably due to the fact that SFO met the later data points better than FOMC. Testing further bi-phasic models (HS, DFOP) provided much smaller χ^2-errors for both models. HS was identified as the model that fit the residue data best, regarding meeting Mo, χ^2-error, and significance of t-test for degradation rates. Consequently, HS was selected as persistence and modelling end point.

Sediment phase:
Although the data set offered three data points after peak concentration, no reliable fit could be provided by any of the four models. SFO revealed a non-significant dissipation rate and the questions whether there is dissipation at all. FOMC and HS could not be optimised by ModelMaker 4.0, and DFOP provided extremely high standard errors of optimised parameters as well as a non-calculated χ^2-error due to insufficient degrees of freedom. Consequently, this sediment dissipation should be excluded from deriving any end points.

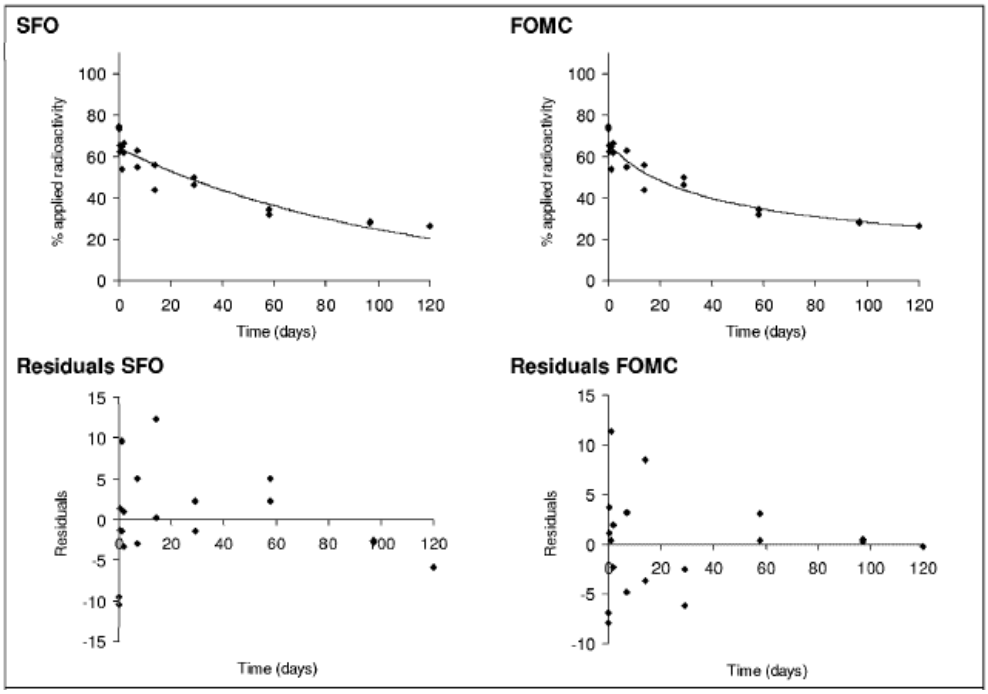


Figure B.8.4-24: Optimised SFO and FOMC kinetic models for degradation of glyphosate in the total system (Heintze (1996, BVL no 1939626), system Pond)

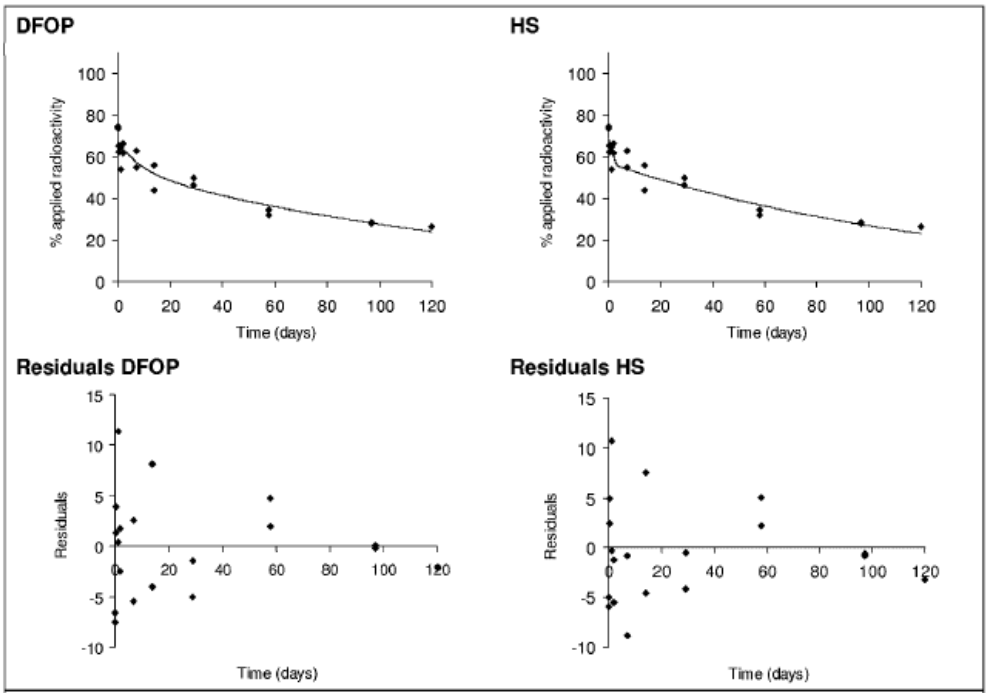


Figure B.8.4-25: Optimised DFOP and HS kinetic models for degradation of glyphosate in the total system (Heintze (1996, BVL no 1939626), system Pond)

Table B.8.4-43: Kinetic evaluation for degradation of glyphosate in the total system (Heintze (1996, BVL no 1939626), system Pond)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	64.1960	3.9637	n.r.	7.2	72.75	241.67
	k_SFO	0.0095	0.0025	<0.001			
FOMC	M_0	66.7690	5.0572	n.r.	5.7	65.86	2053.35
	α	0.5104	0.5545	n.r.			
	β	22.8000	44.9600	n.r.			
DFOP	M_0	67.1290	5.4471	n.r.	6.1	70.93	310.85
	k1_DFOP	0.1112	0.2666	0.341			
	k2_DFOP	0.0067	0.0066	0.163			
	g	0.1955	0.3958	n.r.			
HS	M0	68.7450	1.6320	n.r.	6.3	67.45	281.39
	k1_HS	0.0614	0.0150	<0.001			
	k2_HS	0.0075	0.0012	<0.001			
	tb	3.4458	1.1265	n.r.			

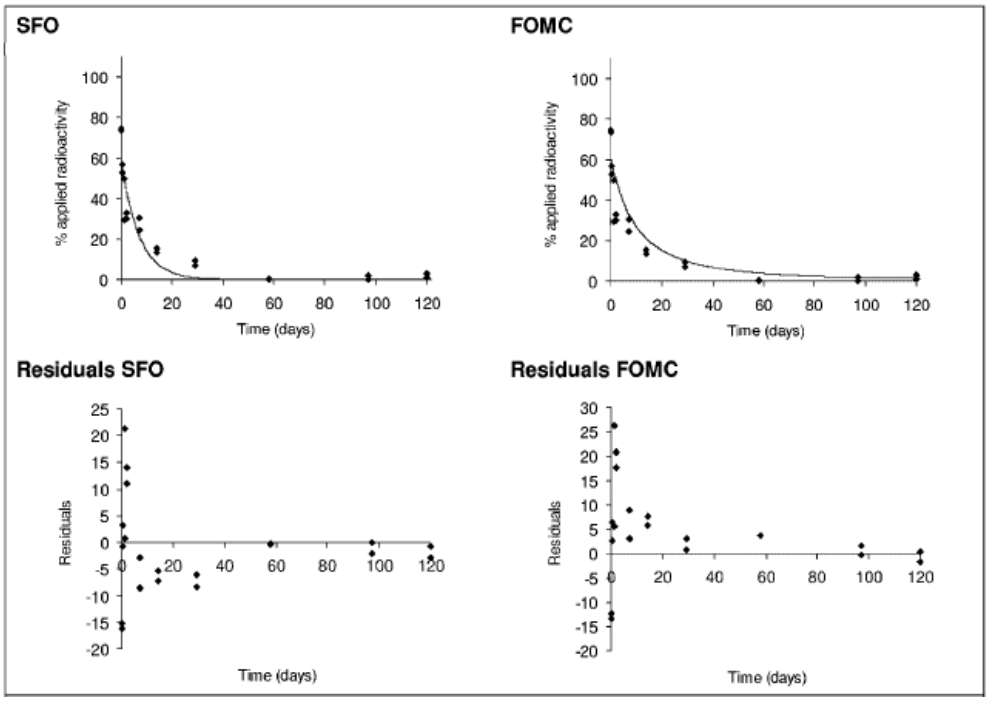


Figure B.8.4-26: Optimised SFO and FOMC kinetic models for dissipation of glyphosate in the water phase (Heintze (1996, BVL no 1939626), system Pond)

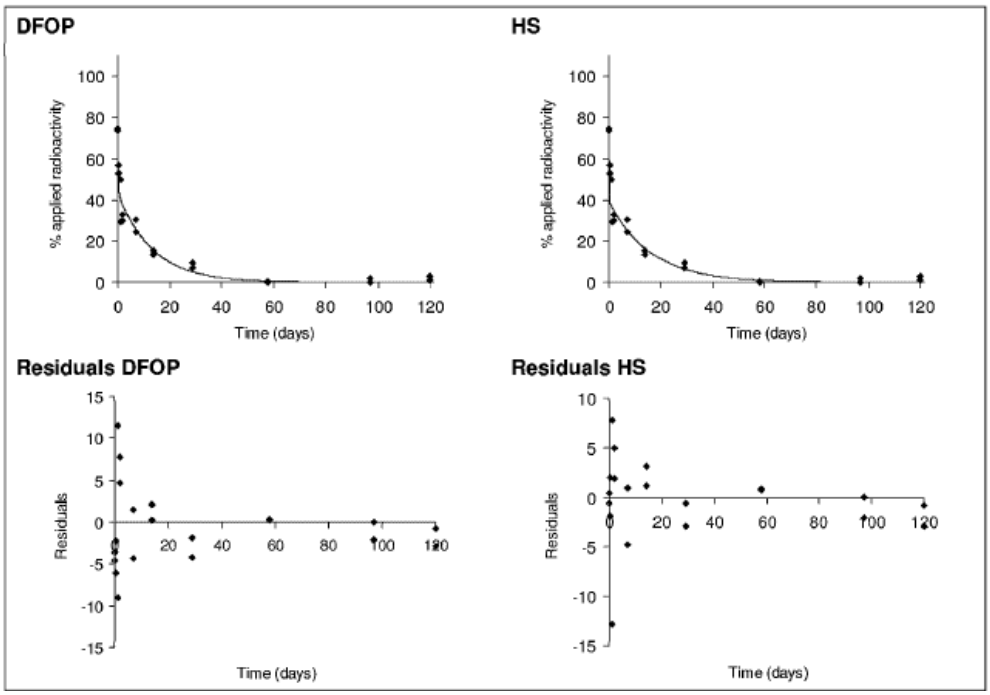
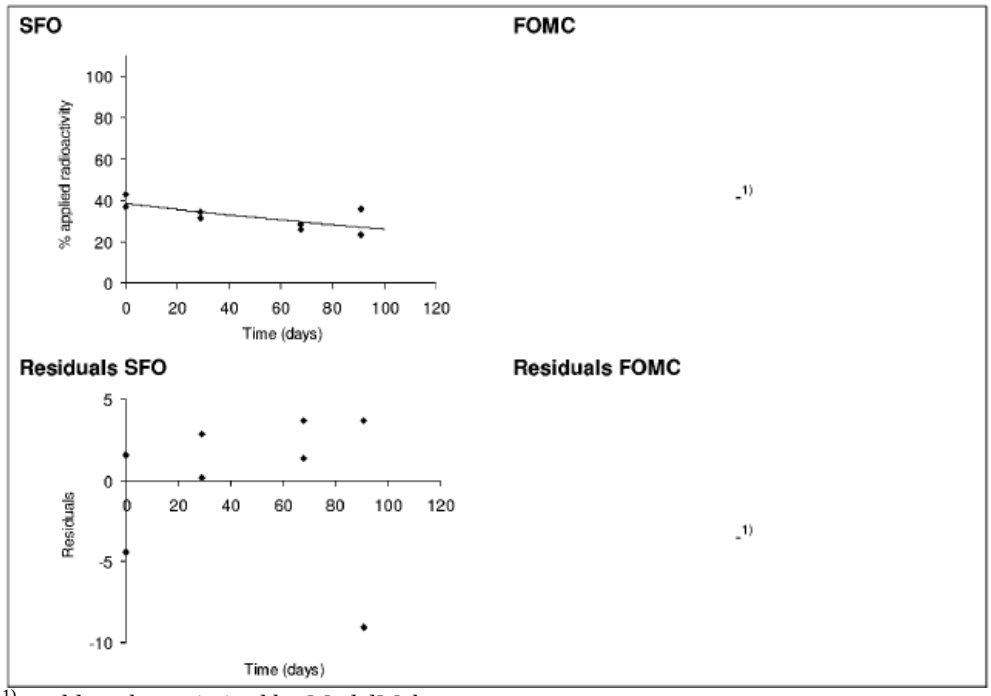


Figure B.8.4-27: Optimised DFOP and HS kinetic models for dissipation of glyphosate in the water phase (Heintze (1996, BVL no 1939626), system Pond)

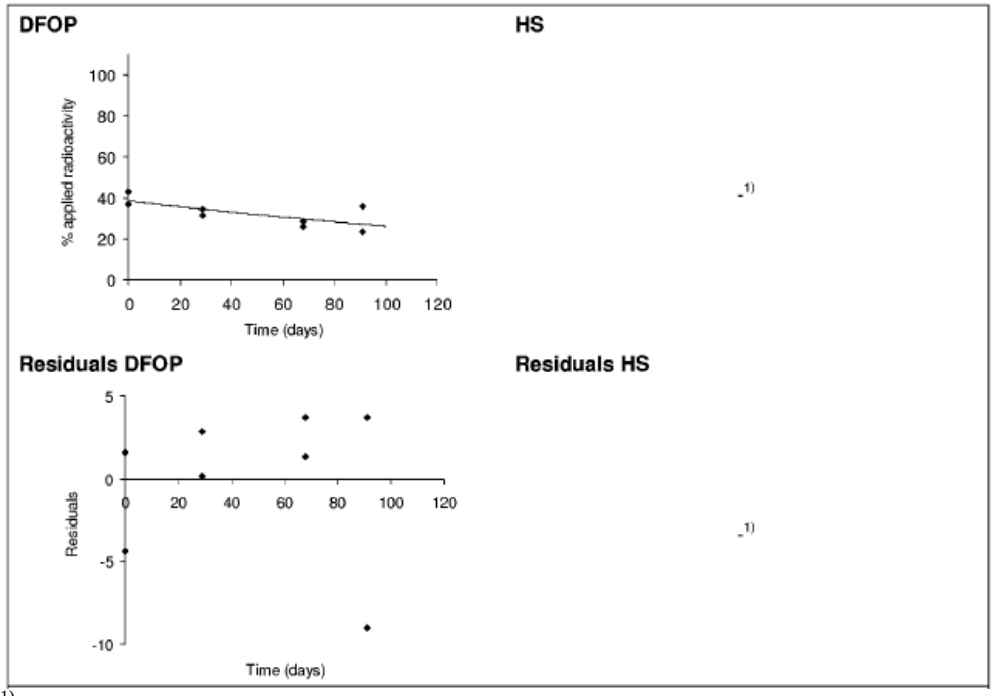
Table B.8.4-44: Kinetic evaluation for dissipation of glyphosate in the water phase (Heintze (1996, BVL no 1939626), system Pond)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	58.4780	13.2560	n.r.	25.8	4.89	16.24
	k_SFO	0.1418	0.0953	0.077			
FOMC	M_0	61.2900	13.502	n.r.	31.8	3.57	14.56
	α	4.1861	9.732	n.r.			
	β	19.8510	53.8920	n.r.			
DFOP	M_0	70.0770	11.2330	n.r.	10.7	2.98	24.75
	k1_DFOP	4.6913	7.9619	0.282			
	k2_DFOP	0.0739	0.0565	0.105			
	g	0.3766	0.1741	n.r.			
HS	M0	74.1130	5.3610	n.r.	6.5	1.00	26.89
	k1_HS	1.1709	0.4716	0.012			
	k2_HS	0.0622	0.0191	0.003			
	tb	0.5693	0.2425	n.r.			



1) could not be optimised by ModelMaker

Figure B.8.4-28: Optimised SFO and FOMC kinetic models for dissipation of glyphosate in the sediment phase (Heintze (1996, BVL no 1939626), system Pond)



1) could not be optimised by ModelMaker

Figure B.8.4-29: Optimised DFOP and HS kinetic models for dissipation of glyphosate in the sediment phase (Heintze (1996, BVL no 1939626), system Pond)

Table B.8.4-45: Kinetic evaluation for dissipation of glyphosate in the sediment phase (Heintze (1996, BVL no 1939626), system Pond)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	38.6320	17.5410	n.r.	5.3	178.45	592.81
	k_SFO	0.0039	0.0089	0.339			
FOMC	M_0	-1)		n.r.			
	α	-1)		n.r.			
	β	-1)		n.r.			
DFOP	M_0	38.6350	17.3750	n.r.	-2)	178.37	592.48
	k1_DFOP	0.0039	1289.6000	0.500			
	k2_DFOP	0.0039	693.9600	0.500			
	g	15.1090	∞	n.r.			
HS	M0	-1)		n.r.			
	k1_HS	-1)					
	k2_HS	-1)					
	tb	-1)		n.r.			

1) could not be optimised by ModelMaker
2) not calculated due to insufficient degrees of freedom

Heintze (1996, BVL no 1939626), system Creek

Total system:
FOMC was not more appropriate than SFO regarding the χ^2-error. However, it gave a slightly better fit of M_0 and around day 60 (= $DegT_{90}$) than SFO. Therefore, testing of further bi-

phasic models (HS, DFOP) was considered relevant. Both HS and DFOP provided the respective advantages of SFO and FOMC: a good fit of M_0 and around $DegT_{90}$ combined with a very good overall fit, expressed as a small χ^2-error. HS was selected as persistence model, while fitting the SFO model was deemed appropriate for derivation of modelling end points.

Water phase:
Again, FOMC was not more appropriate than SFO regarding the χ^2-error; still, further bi-phasic models were tested. Fitting DFOP provided the smallest of all four χ^2-error in combination with the best fit of M_0. Since the overall visual fit was similar to the fit of SFO, end-points were to be selected from these two models: DFOP was selected as persistence end-point, while SFO was selected as modelling end point.

Sediment phase:
Testing SFO model revealed a poor visual fit, supported by a χ^2-error >15 %. Since FOMC could not be optimised by ModelMaker 4.0, additional bi-phasic models were tested immediately, and resulting in the same pattern as the first comparison of SFO and FOMC: HS could not be optimised by ModelMaker 4.0, while DFOP showed an almost identical visual fit to SFO. From the two remaining models, only SFO showed an acceptable χ^2-error and a dissipation rate significantly different from zero. Hence, both persistence and modelling end points were derived from SFO model.

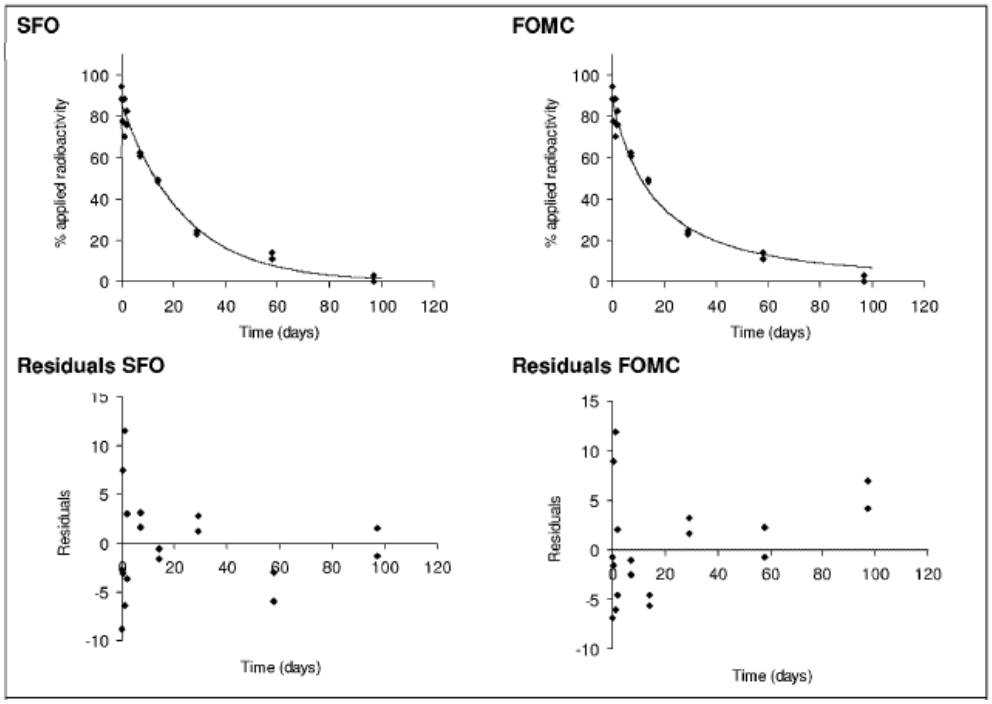


Figure B.8.4-30: Optimised SFO and FOMC kinetic models for degradation of glyphosate in the total system (Heintze (1996, BVL no 1939626), system Creek)

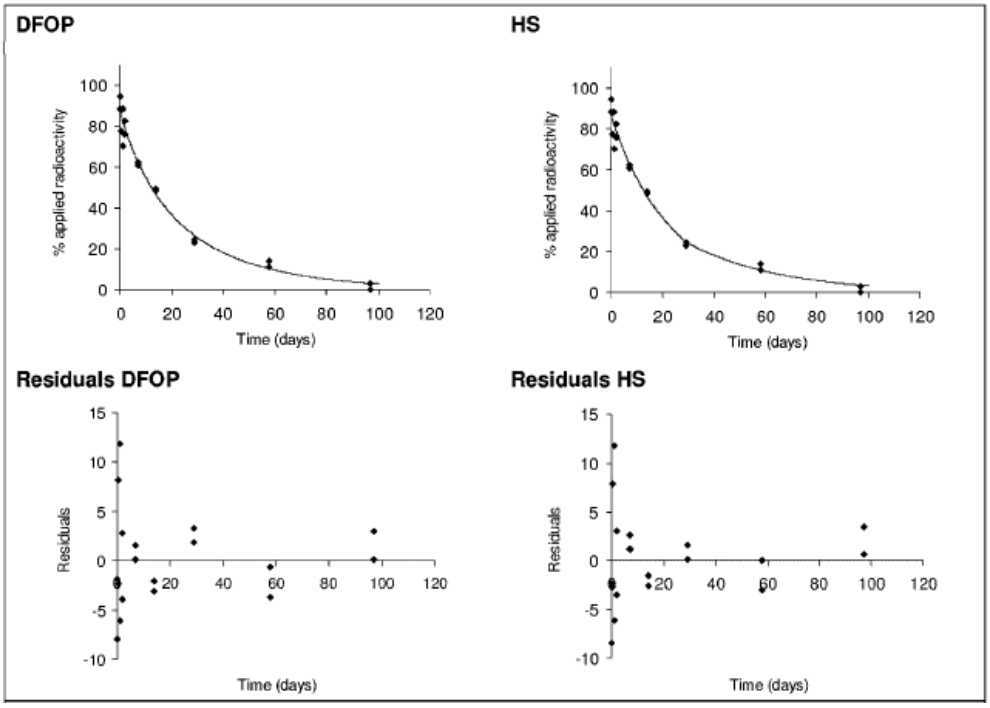


Figure B.8.4-31: Optimised DFOP and HS kinetic models for degradation of glyphosate in the total system (Heintze (1996, BVL no 1939626), system Creek)

Table B.8.4-46: Kinetic evaluation for degradation of glyphosate in the total system (Heintze (1996, BVL no 1939626), system Creek)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	85.7480	10.7620	n.r.	4.3	16.78	55.74
	k_SFO	0.0413	0.0192	0.024			
FOMC	M_0	87.6600	11.4920	n.r.	5.4	14.15	81.21
	α	1.6864	0.6940	n.r.			
	β	27.8370	15.7970	n.r.			
DFOP	M_0	86.6050	3.0242	n.r.	4.4	15.84	63.05
	k1_DFOP	0.0855	0.2071	0.343			
	k2_DFOP	0.0305	0.0315	0.174			
	g	0.3256	1.3315	n.r.			
HS	M0	86.1510	1.9030	n.r.	4.3	16.10	65.93
	k1_HS	0.0431	0.0045	0.001			
	k2_HS	0.0285	0.0122	0.017			
	tb	28.9850	21.9770	n.r.			

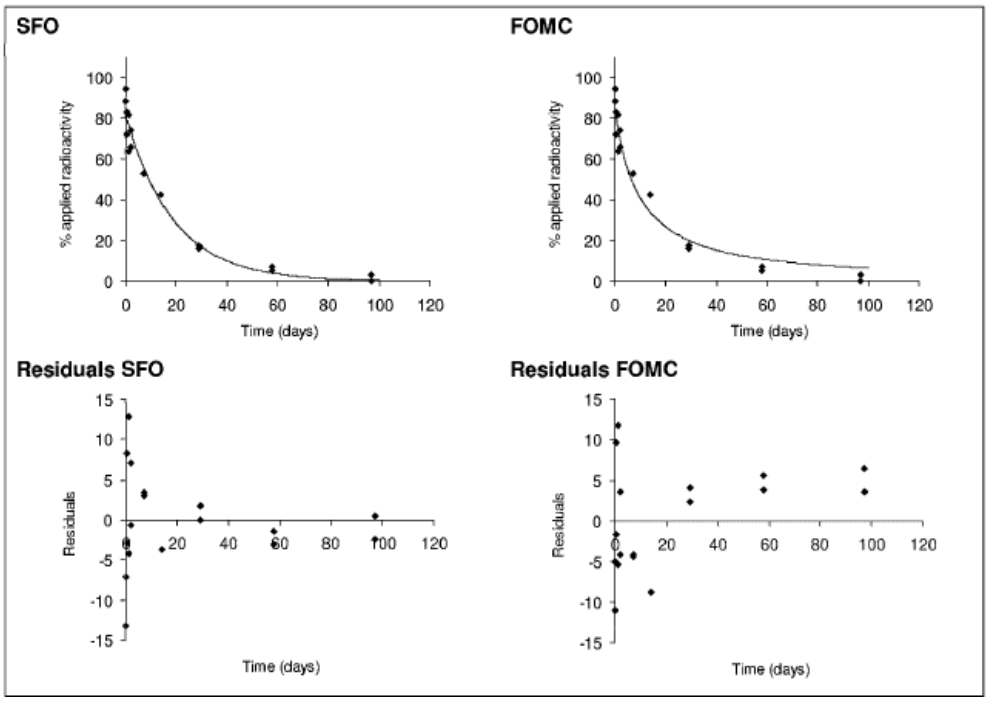


Figure B.8.4-32: Optimised SFO and FOMC kinetic models for dissipation of glyphosate in the water phase (Heintze (1996, BVL no 1939626), system Creek)

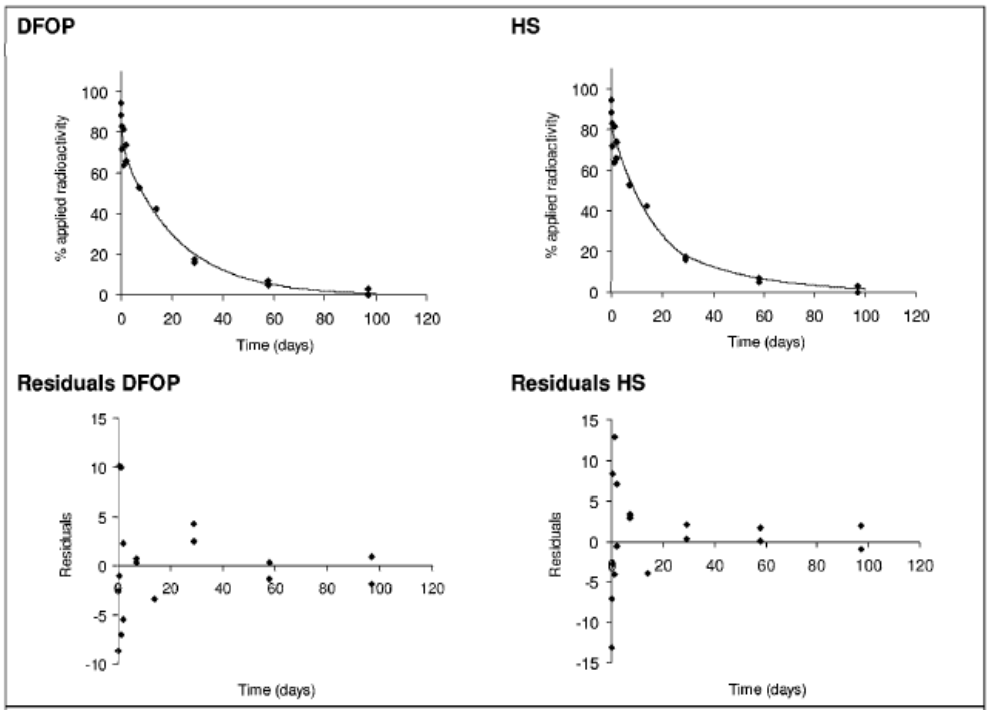
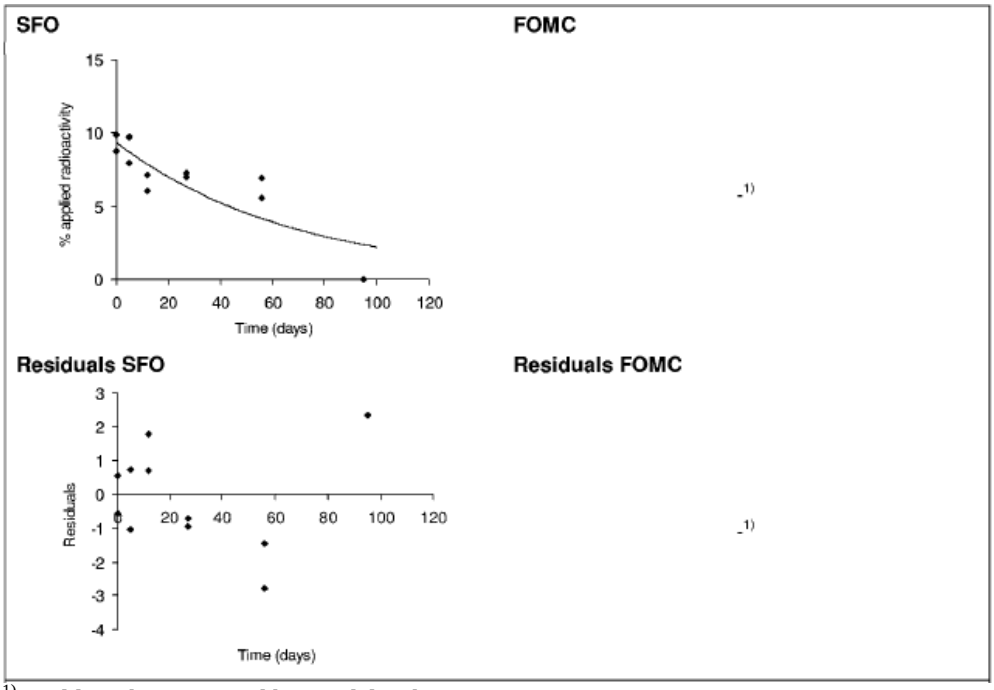


Figure B.8.4-33: Optimised DFOP and HS kinetic models for dissipation of glyphosate in the water phase (Heintze (1996, BVL no 1939626), system Creek)

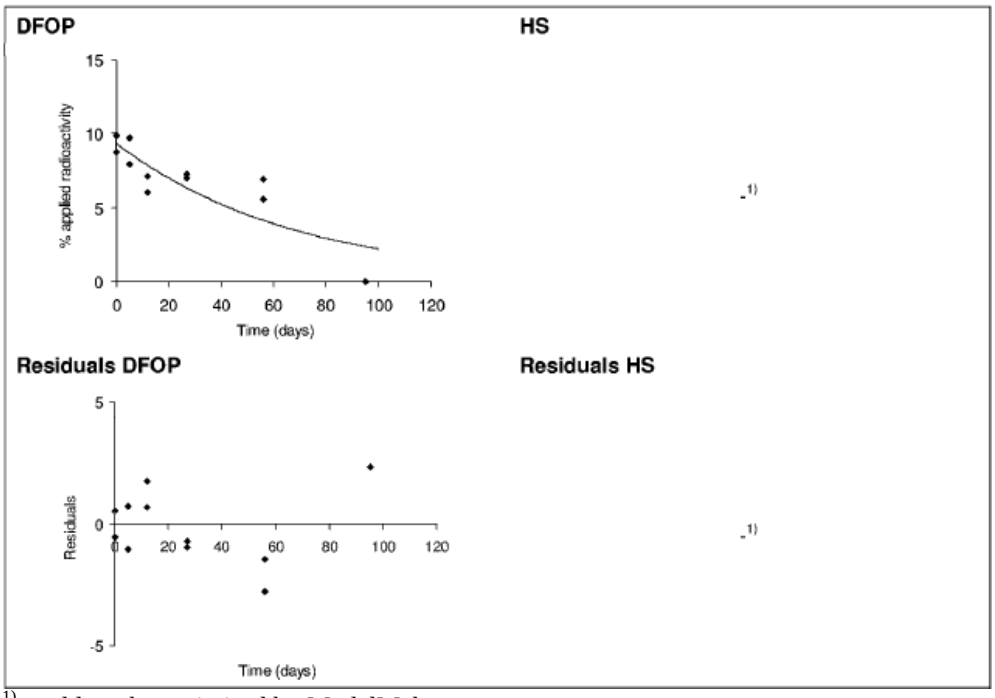
Table B.8.4-47: Kinetic evaluation for dissipation of glyphosate in the water phase (Heintze (1996, BVL no 1939626), system Creek)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	81.2750	12.2000	n.r.	7.3	13.15	43.67
	k_SFO	0.0527	0.0289	0.043			
FOMC	M_0	83.4990	15.6620	n.r.	9.2	9.69	75.22
	α	1.1467	1.2482	n.r.			
	β	11.6650	18.261	n.r.			
DFOP	M_0	85.8530	4.0081	n.r.	5.6	11.95	48.10
	k1_DFOP	1.0591	1.3535	0.2235			
	k2_DFOP	0.0445	0.0113	<0.001			
	g	0.1491	0.0953	n.r.			
HS	M0	81.3820	2.3400	n.r.	8.1	13.02	52.92
	k1_HS	0.0533	0.0071	<0.001			
	k2_HS	0.0330	0.0194	0.056			
	tb	27.5230	96.8820	n.r.			



[1)] could not be optimised by ModelMaker

Figure B.8.4-34: Optimised SFO and FOMC kinetic models for dissipation of glyphosate in the sediment phase (Heintze (1996, BVL no 1939626), system Creek)



[1)] could not be optimised by ModelMaker

Figure B.8.4-35: Optimised DFOP and HS kinetic models for dissipation of glyphosate in the sediment phase (Heintze (1996, BVL no 1939626), system Creek)

Table B.8.4-48: Kinetic evaluation for dissipation of glyphosate in the sediment phase (Heintze (1996, BVL no 1939626), system Creek)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	9.3636	1.5742	n.r.	17.8	47.88	159.06
	k_SFO	0.0145	0.0075	0.041			
FOMC	M_0	-[1)]		n.r.	-[1)]	-[1)]	-[1)]
	α	-[1)]		n.r.			
	β	-[1)]		n.r.			
DFOP	M_0	9.3636	1.3764	n.r.	22.4	47.88	159.06
	k1_DFOP	0.0145	18.9430	0.500			
	k2_DFOP	0.0145	51.9500	0.500			
	g	1.1678	10.700.000	n.r.			
HS	M0	-[1)]		n.r.	-[1)]	-[1)]	-[1)]
	k1_HS	-[1)]					
	k2_HS	-[1)]					
	tb	-[1)]		n.r.			

[1)] could not be optimised by ModelMaker

Feser-Zügner (2002, BVL no 2310270), system Rückhaltebecken

Total system:
FOMC provided a perfect fit (χ^2 = 2.1) to the residue data of AMPA for total system degradation compared to SFO. The SFO model revealed a poor fit, supported by a χ^2-error >15 %:

earlier data points were systematic overestimated while later data points were systematically underestimated. In consequence, HS and DFOP were tested as additional bi-phasic models. Both models showed χ^2-errors larger than that of FOMC, which was probably due the larger residuals of both HS and DFOP, especially around M_0. In consequence, persistence endpoints were derived from FOMC. For derivation of modelling end points, the second best-fit model (DFOP) was selected as it provided statistically significant probabilities that its degradation rates differ from zero.

Water phase:
Again, FOMC provided an almost perfect fit (χ^2 = 2.1) to the residue data of AMPA when compared to SFO; this time for dissipation from the water phase. While SFO showed systematic underestimations of later data points, FOMC tended to overestimate later data points. In consequence, further testing was considered appropriate. Both additional biphasic models (HS, DFOP) revealed poorer visual fits than FOMC, with generally larger residuals and overestimation of earlier data points, while later data points were underestimated in both model fits. Therefore, FOMC was selected as persistence end point. Since SFO showed extremely large residuals around Mo, and since neither HS nor DFOP passed statistical criteria, preference as modelling end point was also given to FOMC.

Sediment phase:
A very good fit of SFO model was even exceeded by an almost perfect FOMC fit and lead to further testing of HS and DFOP models. HS could not be optimised by ModelMaker 4.0, while DFOP provided an even better fit than FOMC (χ^2 = 0.3). Consequently, DFOP was selected as persistence model. Since DFOP did not pass statistical criteria, it was not deemed appropriate as modelling end point. However, since also SFO did not pass statistical criteria and FOMC showed extremely high standard errors for its optimised parameters, none of the model could serve as modelling end point.

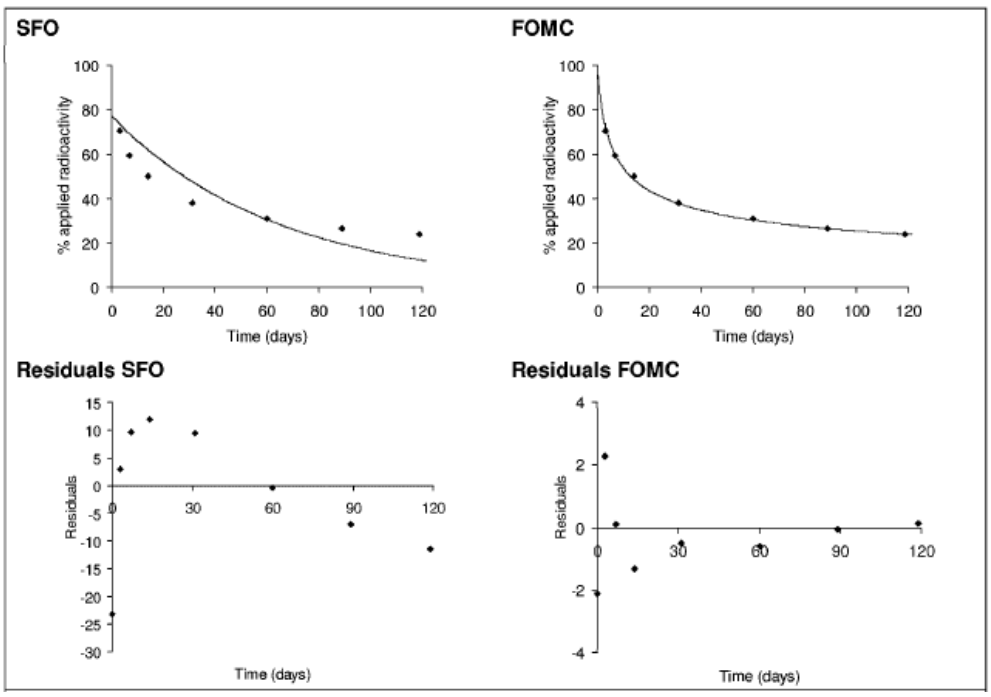


Figure B.8.4-36: Optimised SFO and FOMC kinetic models for degradation of AMPA in the total system (Feser-Zügner (2002, BVL no 2310270), system Rückhaltebecken)

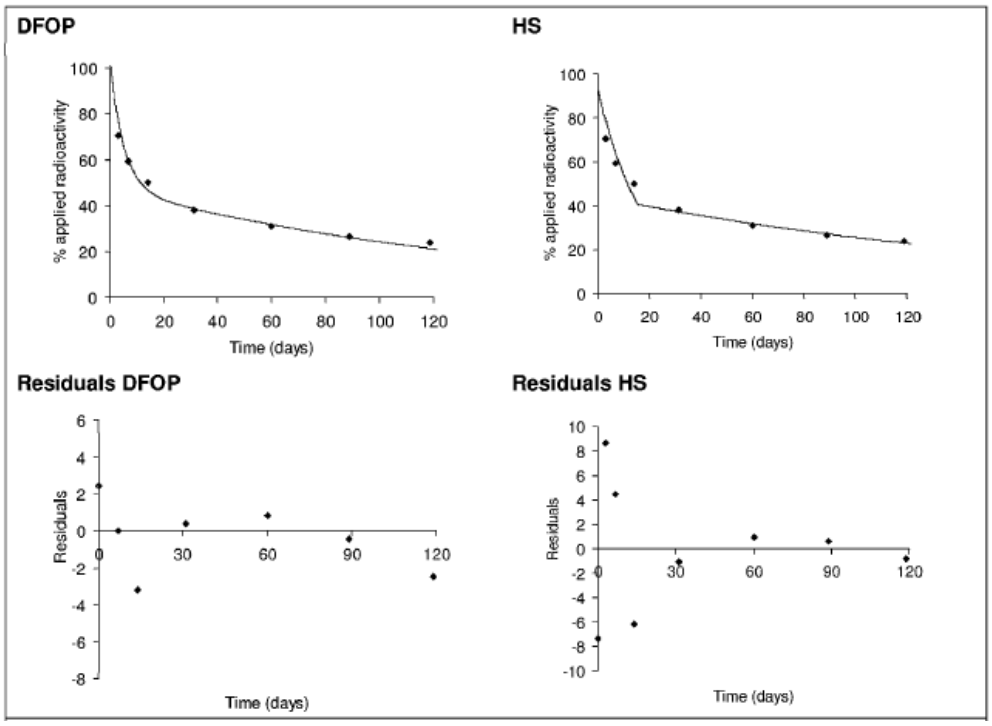


Figure B.8.4-37: Optimised DFOP and HS kinetic models for degradation of AMPA in the total system (Feser-Zügner (2002, BVL no 2310270), system Rückhaltebecken)

Table B.8.4-49: Kinetic evaluation for degradation of AMPA in the total system (Feser-Zügner (2002, BVL no 2310270), system Rückhaltebecken)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	77.0030	17.7780	n.r.	18.3	45.16	150.01
	k_SFO	0.0154	0.0103	0.094			
FOMC	M_0	98.1870	29.1980	n.r.	2.1	13.8	1513.0
	α	0.3539	0.2550	n.r.			
	β	2.2621	5.0921	n.r.			
DFOP	M_0	102.750	8.6433	n.r.	5.5	10.59	227.80
	k1_DFOP	0.1951	0.0637	0.019			
	k2_DFOP	0.0067	0.0038	0.076			
	g	0.5359	0.1119	n.r.			
HS	M0	92.9440	5.7951	n.r.	8.9	12.98	284.70
	k1_HS	0.0534	0.0112	0.004			
	k2_HS	0.0055	0.0033	0.085			
	tb	15.4520	8.1691	n.r.			

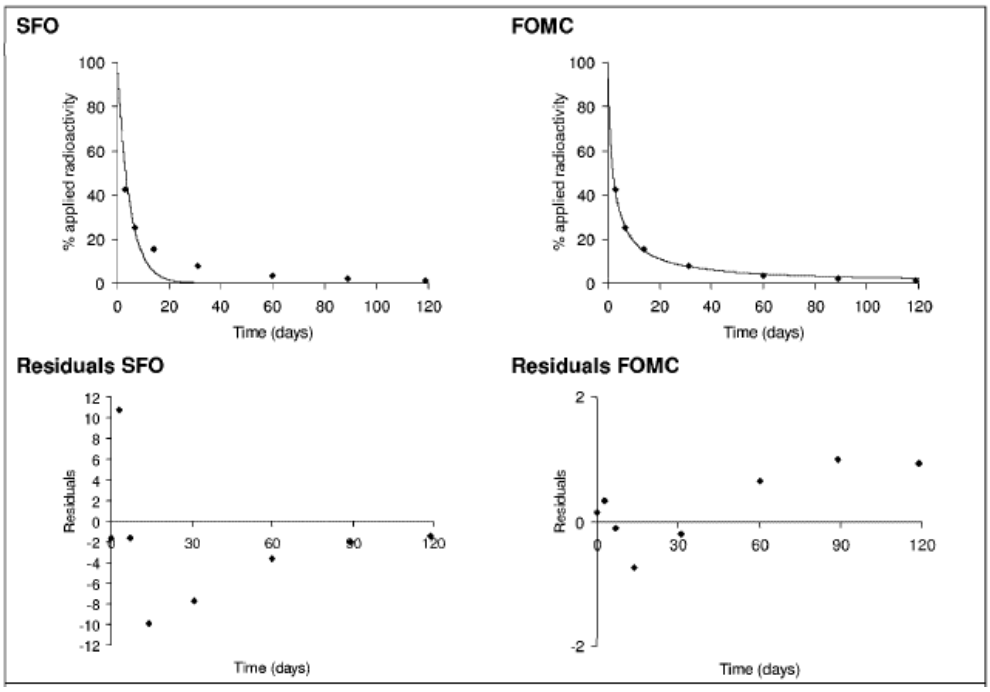


Figure B.8.4-38: Optimised SFO and FOMC kinetic models for dissipation of AMPA in the water phase (Feser-Zügner (2002, BVL no 2310270), system Rückhaltebecken)

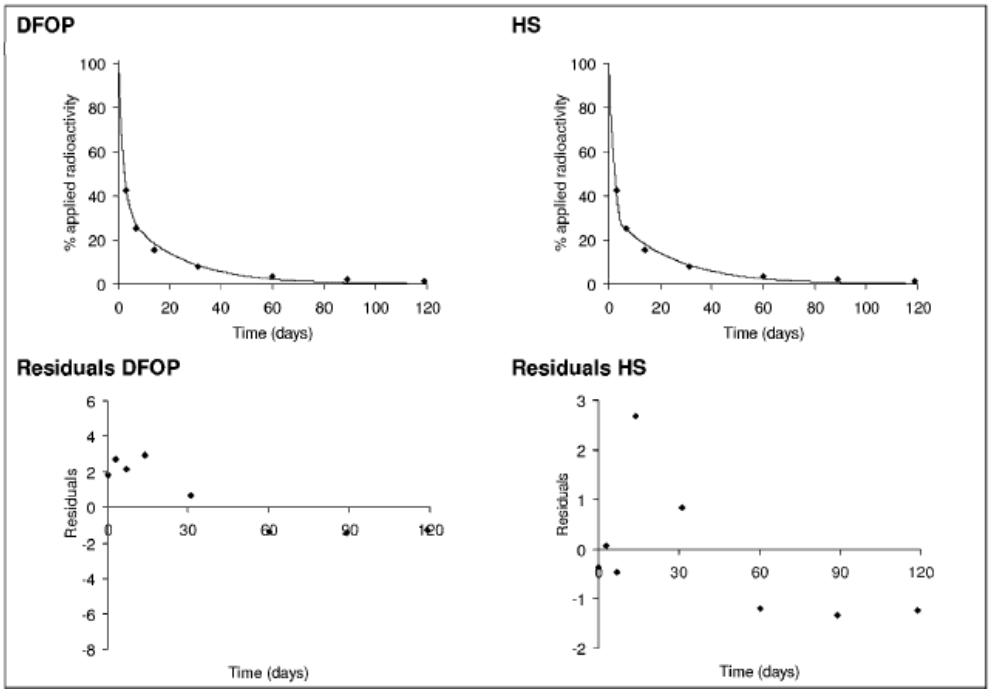


Figure B.8.4-39: Optimised DFOP and HS kinetic models for dissipation of AMPA in the water phase (Feser-Zügner (2002, BVL no 2310270), system Rückhaltebecken)

Table B.8.4-50: Kinetic evaluation for dissipation of AMPA in the water phase (Feser-Zügner (2002, BVL no 2310270), system Rückhaltebecken)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	98.6840	23.0450	n.r.	19.6	3.38	11.24
	k_SFO	0.2048	0.1085	0.054			
FOMC	M_0	100.4600	3.2225	n.r.	2.1	2.2	22.5
	α	0.9102	0.0946	n.r.			
	β	1.9490	0.4266	n.r.			
DFOP	M_0	102.1500	16.8500	n.r.	7.2	2.44	27.08
	k1_DFOP	0.4992	0.5858	0.2211			
	k2_DFOP	0.0452	0.0819	0.305			
	g	0.6603	0.4317	n.r.			
HS	M0	99.9450	9.2485	n.r.	4.7	2.45	27.85
	k1_HS	0.2830	0.0780	0.011			
	k2_HS	0.0435	0.0358	0.146			
	tb	4.5560	1.8599	n.r.			

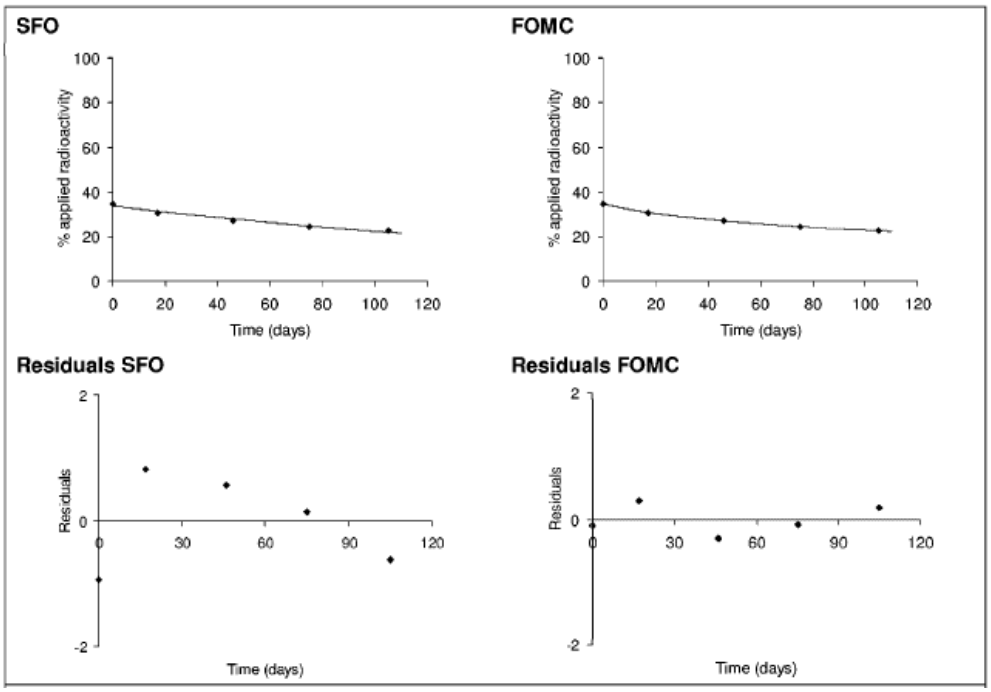
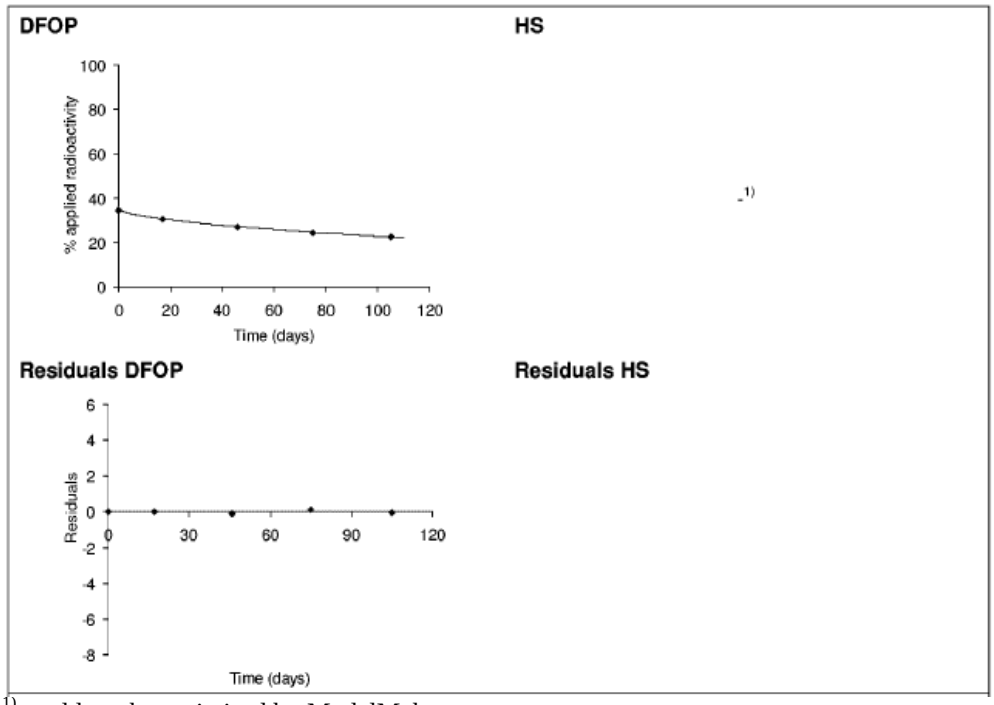


Figure B.8.4-40: Optimised SFO and FOMC kinetic models for dissipation of AMPA in the sediment phase (Feser-Zügner (2002, BVL no 2310270), system Rückhaltebecken)



1) could not be optimised by ModelMaker

Figure B.8.4-41: Optimised DFOP and HS kinetic models for dissipation of AMPA in the sediment phase (Feser-Zügner (2002, BVL no 2310270), system Rückhaltebecken)

Table B.8.4-51: Kinetic evaluation for dissipation of AMPA in the sediment phase (Feser-Zügner (2002, BVL no 2310270), system Rückhaltebecken)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	33.6590	6.2850	n.r.	1.9	168.19	558.72
	k_SFO	0.0041	0.0036	0.168			
FOMC	M_0	34.5020	16.8270	n.r.	0.7	278.88	∞
	α	0.3534	3.4538	n.r.			
	β	45.6540	707.7800	n.r.			
DFOP	M_0	34.5980	17.4250	n.r.	0.3	184.33	678.38
	k1_DFOP	0.0838	2.9757	0.491			
	k2_DFOP	0.0033	0.0220	0.453			
	g	0.0885	1.6978	n.r.			
HS	M0	-1)		n.r.			
	k1_HS	-1)					
	k2_HS	-1)					
	tb	-1)		n.r.			

1) could not be optimised by ModelMaker

Feser-Zügner (2002, BVL no 2310270), system Schäphysen

Total system:
Comparison of SFO and FOMC showed a visually more appropriate bi-phasic model and lead to further testing of HS and DFOP. DFOP was the only model that showed non-systematic

deviations of residuals and that provided a χ^2-error <15 %. DFOP was therefore selected as persistence end point. However, none of the models - not even DFOP - passed statistical criteria (here: t-test for degradation rate) or provided acceptable fits to the data; hence, no reliable modelling end point could be selected.

Water phase:
The very fast dissipation of AMPA from the water phase was more adequate represented by FOMC than by SFO and lead to testing of HS and DFOP. Both models, HS and DFOP, revealed larger χ^2-errors than FOMC, which was probably due to the large residuals at earlier data points. Later data points tended to be underestimated by HS and DFOP, while FOMC overestimated them. In consequence, FOMC was selected as persistence model, and SFO was selected as modelling end point. Since SFO showed a good visual fit around the DT_{50} and provided a more conservative half-live than DFOP and FOMC this decision was deemed to be appropriate.

Sediment phase:
As there was not enough data points after peak concentration in sediment, kinetic evaluations could not be conducted.

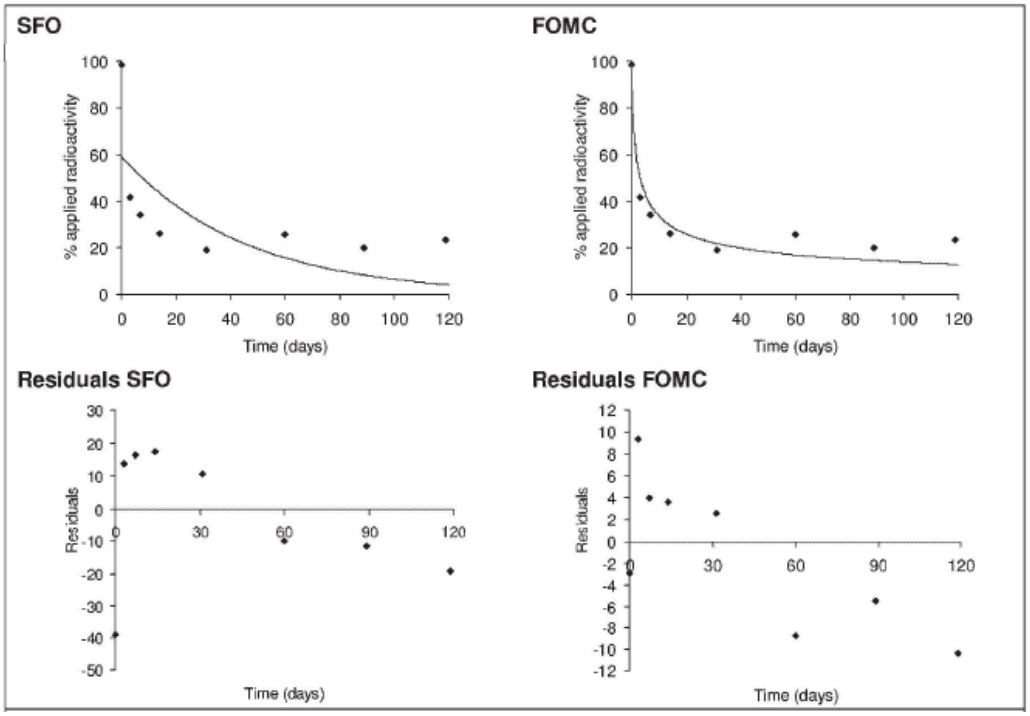


Figure B.8.4-42: Optimised SFO and FOMC kinetic models for degradation of AMPA in the total system (Feser-Zügner (2002, BVL no 2310270), system Schäphysen)

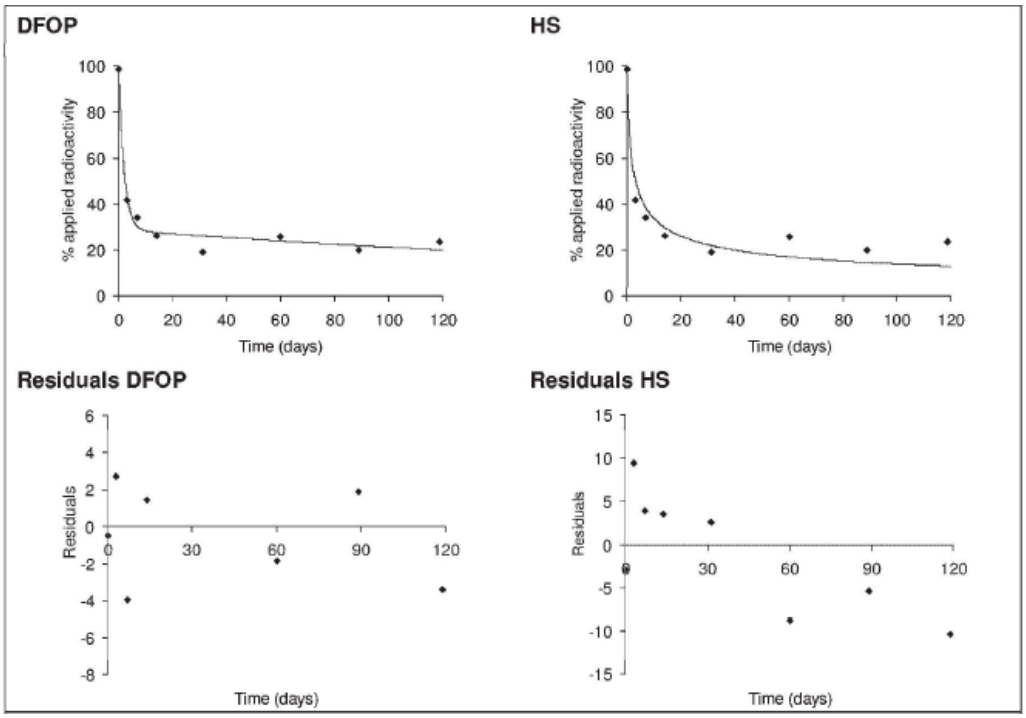


Figure B.8.4-43: Optimised DFOP and HS kinetic models for degradation of AMPA in the total system (Feser-Zügner (2002, BVL no 2310270), system Schäphysen)

Table B.8.4-52: Kinetic evaluation for for degradation of AMPA in the total system (Feser-Zügner (2002, BVL no 2310270), system Schäphysen)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	59.1530	6.8970	n.r.	42.9	31.48	104.59
	k_SFO	0.0220	0.0073	0.012			
FOMC	M_0	95.6180	22.3140	n.r.	15.5	3.7	259.6
	α	0.3952	0.2620	n.r.			
	β	0.7676	1.3655	n.r.			
DFOP	M_0	98.0200	20.7560	n.r.	8.6	2.48	354.99
	k1_DFOP	0.4911	0.5304	0.203			
	k2_DFOP	0.0030	0.0099	0.387			
	g	0.7073	0.1834	n.r.			
HS	M0	97.7080	17.1970	n.r.	16.8	2.41	425.03
	k1_HS	0.2870	0.1420	0.056			
	k2_HS	0.0025	0.0072	0.372			
	tb	4.3230	5.0827	n.r.			

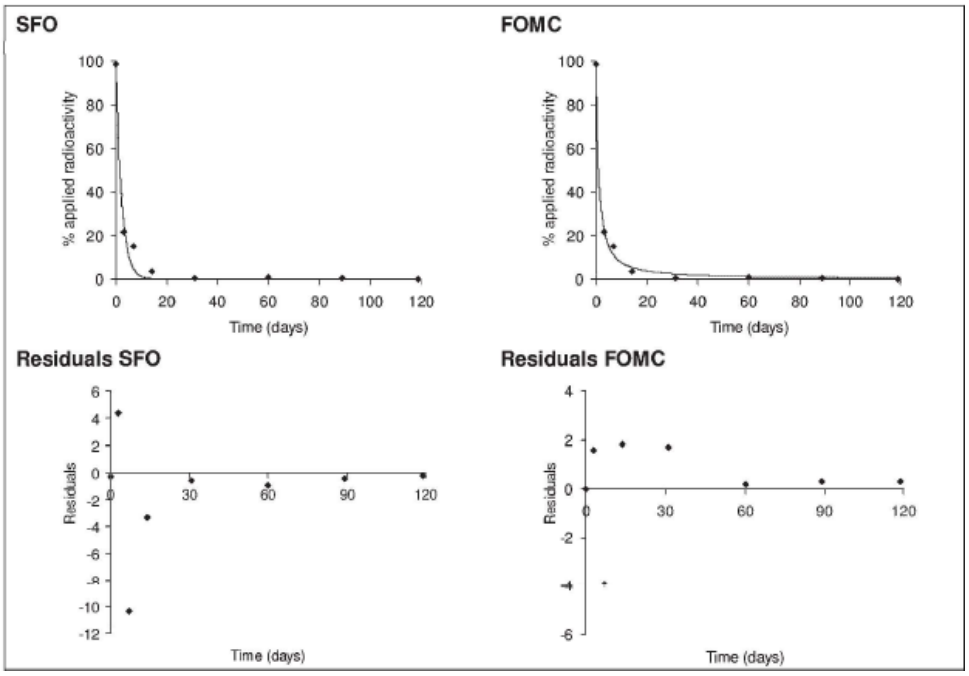


Figure B.8.4-44: Optimised SFO and FOMC kinetic models for dissipation of AMPA in the water phase (Feser-Zügner (2002, BVL no 2310270), system Schäphysen)

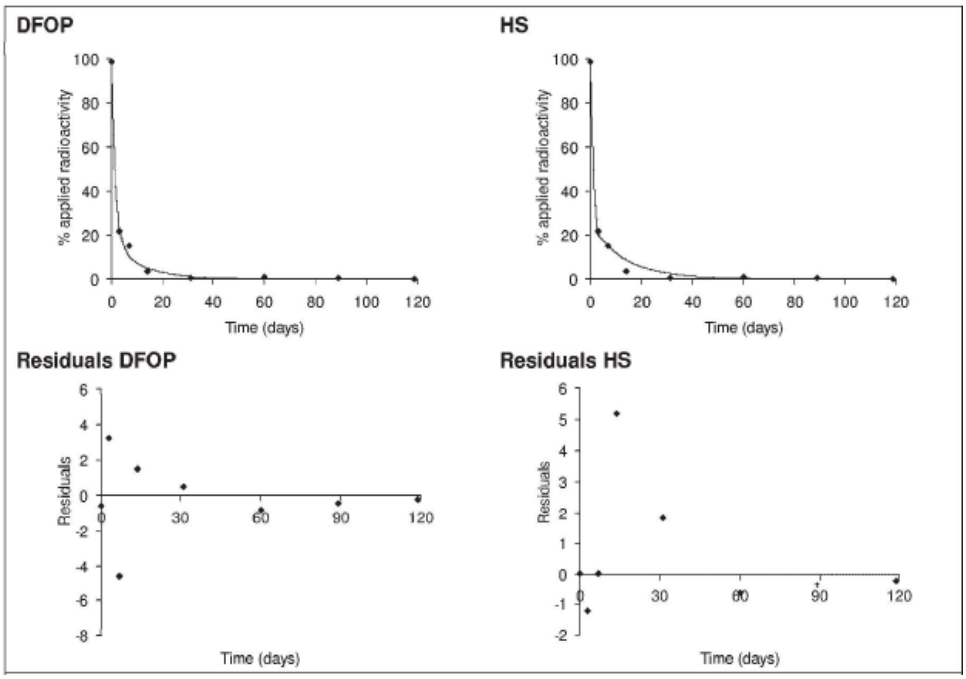


Figure B.8.4-45: Optimised DFOP and HS kinetic models for dissipation of AMPA in the water phase (Feser-Zügner (2002, BVL no 2310270), system Schäphysen)

Table B.8.4-53: Kinetic evaluation for for dissipation of AMPA in the water phase (Feser-Zügner (2002), system Schäphysen, BVL no 2310270)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	98.2080	31.3800	n.r.	18.9	1.58	5.24
	k_SFO	0.4394	0.2980	0.095			
FOMC	M_0	98.4900	2.7854	n.r.	8.5	1.0	7.8
	α	1.1350	2.5E-01	n.r.			
	β	1.1850	5.34E-01	n.r.			
DFOP	M_0	97.9380	13.406	n.r.	10.9	1.37	7.42
	k1_DFOP	0.6430	0.7139	0.209			
	k2_DFOP	0.0887	0.2940	0.389			
	g	0.8203	0.5415	n.r.			
HS	M0	98.5090	13.4700	n.r.	10.5	1.33	12.50
	k1_HS	0.5200	0.2210	0.039			
	k2_HS	0.0755	0.1150	0.2736			
	tb	3.0570	3.8692	n.r.			

Knoch (2003, BVL no 2310273), system Bickenbach

Total system:
SFO gave a poor fit when compared to FOMC, although it passed statistical criteria. FOMC provided a good overall fit (χ^2 = 6.9) with smaller non-systematic residuals than SFO, and a better fit around Mo. However, FOMC overestimated later data points, while SFO extremely underestimated them. Fitting HS and DFOP as further bi-phasic models was a major improvement regarding χ^2-errors and the residuals of the later data points. In addition, both models passed statistical criteria. Therefore, HS was derived as persistence and modelling end point.

Water phase:
SFO model provided a poor visual fit with systematic deviations at later data points. In comparison, FOMC met Mo better and generally provided a better visual fit than SFO although it still showed systematic underestimation at later data points - even if lower than SFO. Fitting the bi-phasic models HS and DFOP showed that still FOMC provided the best fit, according to χ^2-error. However, it underestimated DT_{90}, while DFOP provided a better fit around the DT_{90}. As DFOP also provided a better find around Mo and DT_{50}, it was selected as both persistence and modelling end point.

Sediment phase:
As there was not enough data points after peak concentration in sediment, kinetic evaluations could not be conducted.

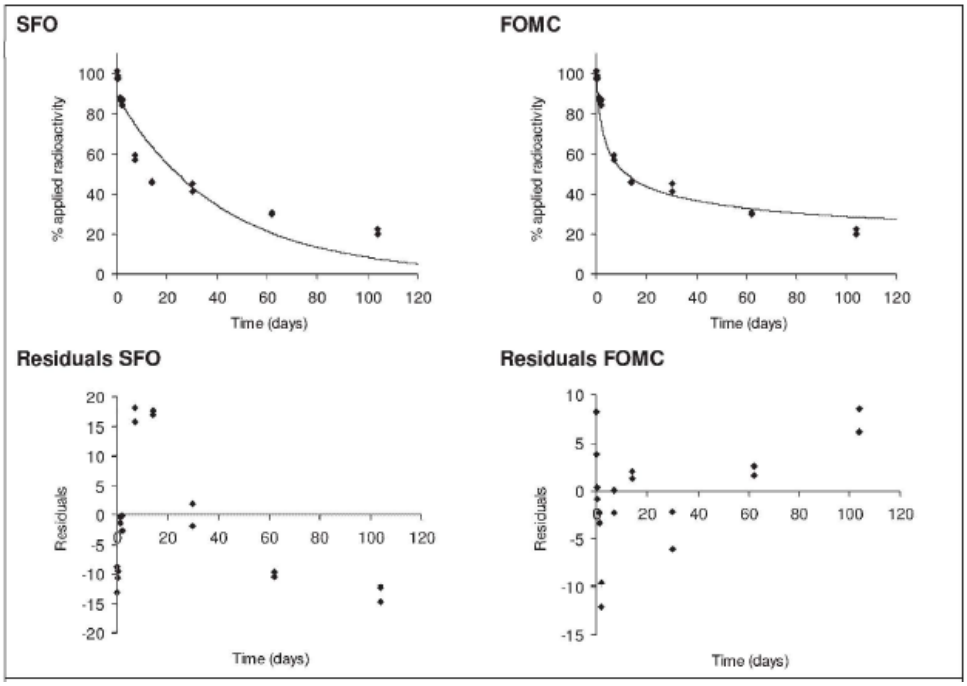


Figure B.8.4-46: Optimised SFO and FOMC kinetic models for degradation of AMPA in the total system (Knoch (2003, BVL no 2310273), system Bickenbach)

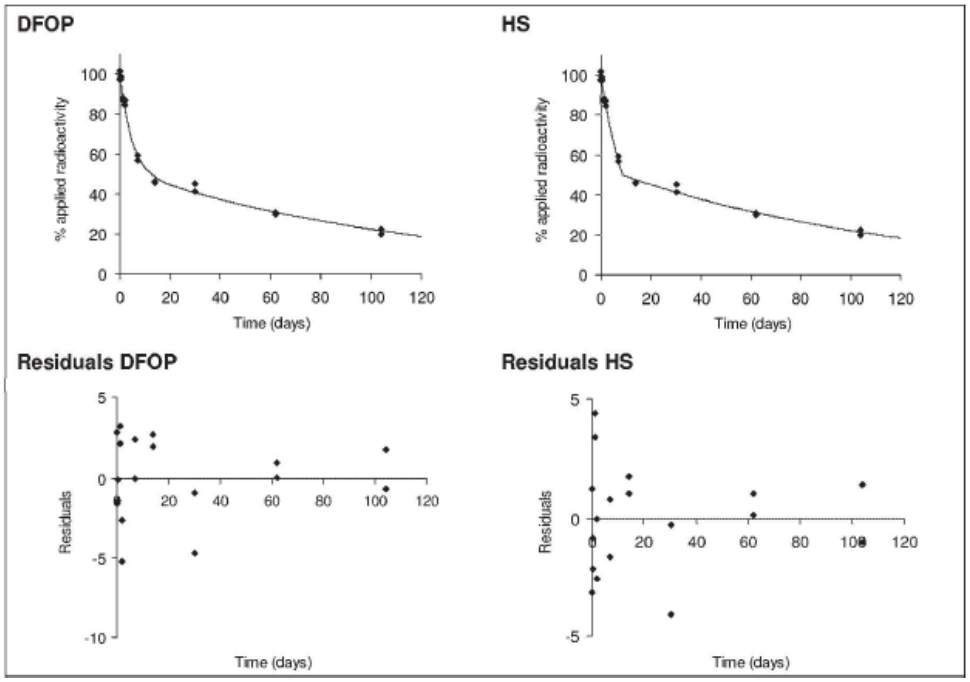


Figure B.8.4-47: Optimised DFOP and HS kinetic models for degradation of AMPA in the total system (Knoch (2003, BVL no 2310273), system Bickenbach)

Table B.8.4-54: Kinetic evaluation for degradation of AMPA in the total system (Knoch (2003, BVL no 2310273), system Bickenbach)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	88.5530	3.6190	n.r.	13.9	29.33	97.45
	k_SFO	0.0236	0.0037	<0.001			
FOMC	M_0	105.5400	4.8549	n.r.	6.9	9.62	4357.96
	α	0.2665	0.0380	n.r.			
	β	0.7714	0.3256	n.r.			
DFOP	M_0	100.1500	3.1529	n.r.	2.9	12.52	195.42
	k1_DFOP	0.2262	0.0778	0.006			
	k2_DFOP	0.0085	0.0026	0.003			
	g	0.4751	0.0676	n.r.			
HS	M0	98.5570	3.4690	n.r.	2.5	10.54	191.25
	k1_HS	0.0765	0.0149	<0.001			
	k2_HS	0.0089	0.0027	0.003			
	tb	8.8697	1.5672	n.r.			

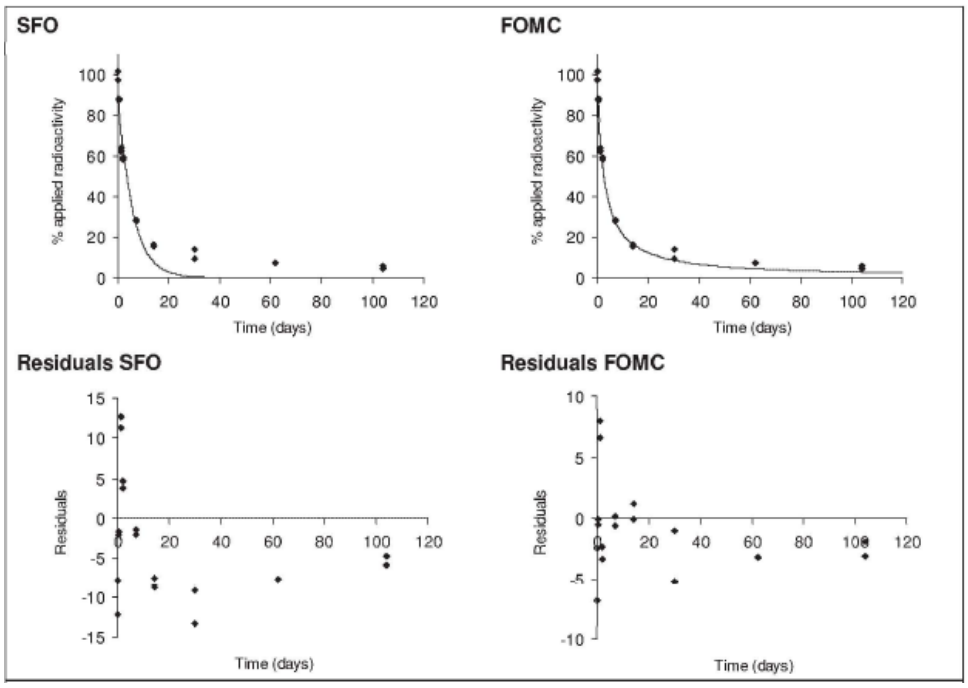


Figure B.8.4-48: Optimised SFO and FOMC kinetic models for dissipation of AMPA in the water phase (Knoch (2003, BVL no 2310273), system Bickenbach)

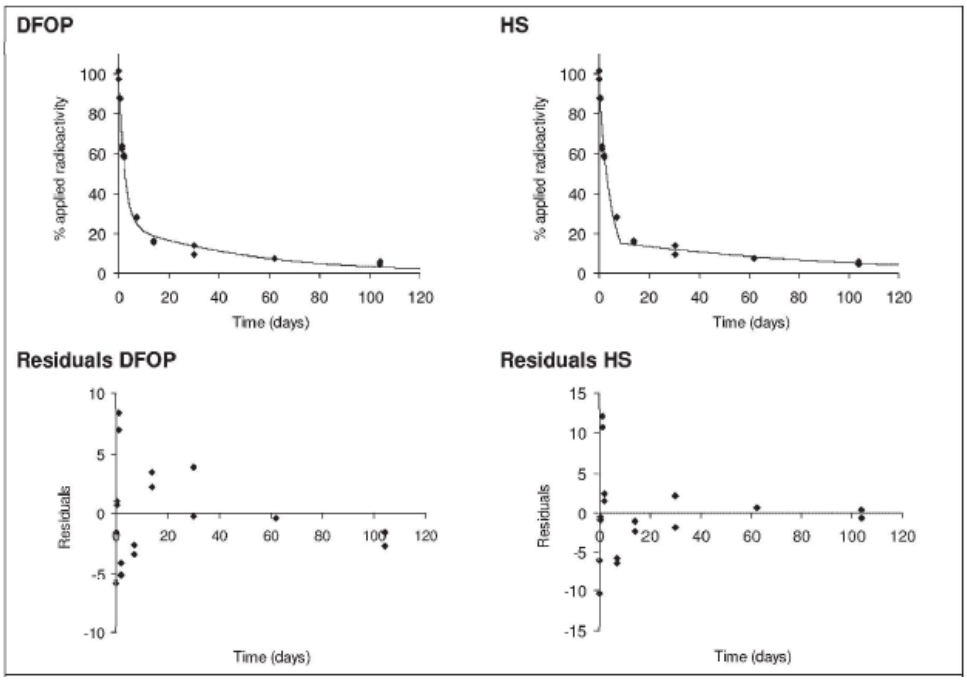


Figure B.8.4-49: Optimised DFOP and HS kinetic models for dissipation of AMPA in the water phase (Knoch (2003, BVL no 2310273), system Bickenbach)

Table B.8.4-55: Kinetic evaluation for dissipation of AMPA in the water phase (Knoch (2003, BVL no 2310273), system Bickenbach)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	89.5090	2.5171	n.r.	14.9	3.99	13.26
	k_SFO	0.173	0.0171	<0.001			
FOMC	M_0	94.9000	11.5990	n.r.	7.0	2.91	27.09
	α	0.9712	0.6402	n.r.			
	β	2.7915	3.3639	n.r.			
DFOP	M_0	95.7290	3.4179	n.r.	7.9	2.54	47.57
	k1_DFOP	0.4208	0.0910	<0.001			
	k2_DFOP	0.0200	0.0097	0.029			
	g	0.7417	0.0660	n.r.			
HS	M0	91.3860	7.9757	n.r.	11.1	3.43	53.70
	k1_HS	0.2020	0.0657	0.004			
	k2_HS	0.0114	0.0228	0.313			
	tb	8.8776	2.6803	n.r.			

Knoch (2003, BVL no 2310273), system Unter Widdersheim

Total system:
The comparison of SFO and FOMC determined FOMC to better fit the residue data, especially as it met M_0 and earlier data points well. The additional tested bi-phasic models HS and DFOP even better fit the residue data; both met M_0 and earlier data points better than FOMC and both adequately represent later data points around $DegT_{50}$. Based on the χ^2-error, persistence endpoints were derived from HS. As HS also passed statistical criteria, with highly sig-

nificant p-values (for t-test on degradation rates), HS was also selected as modelling end point.

Water phase:
Comparison of SFO and FOMC indicated the adequate fit of FOMC model to the residue data of the water phase. FOMC met M_0 better than SFO and provided smaller and randomly distributed residuals at earlier data points, which is important for the very fast dissipation of AMPA from water phase. From the additionally tested HS and DFOP models, only DFOP could be seen as improvement, as fitting HS model revealed a χ^2-error similar to the one of SFO model. DFOP provided a very good visual fit with randomly distributed small residuals up to DT_{90} (ca. day 30). As the slower degradation rate of DFOP also passed the t-test criterion ($p < 0.1$ for water-sediment studies), DFOP was selected as both persistence and modelling end point.

Sediment phase:
Fitting adequate kinetic models to sediment data was again difficult, as obviously there is no dissipation at all during the first 16 to 20 days. FOMC did not add anything to SFO but showed even more unrealistic estimations of optimised values and their respective standard error. Still, DFOP and HS were tested. HS could not be optimised by ModelMaker 4.0 at all, while DFOP showed a very similar fit to SFO. However, due to an insufficient number of degrees of freedom, χ^2-test could not be calculated by FOCUS DegKin tool. And finally, repeating the fitting and optimisation process of SFO to sediment data supported the impression that SFO was not a reliable fit: its dissipation rate passed statistical criteria (here: t-test) only every now and then. Consequently, this sediment dissipation should be excluded from deriving any end points.

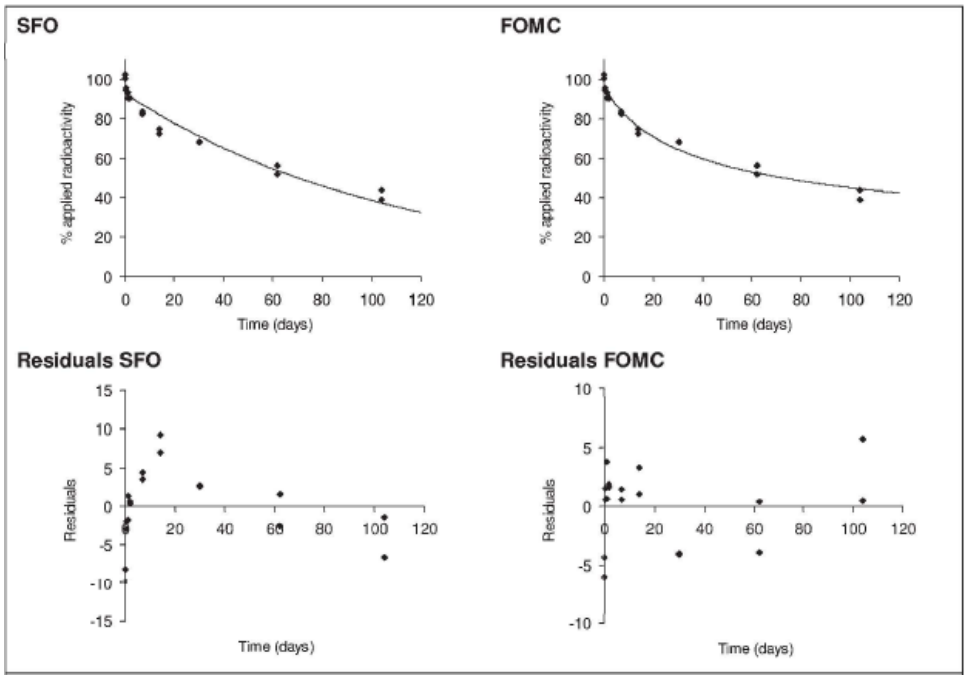


Figure B.8.4-50: Optimised SFO and FOMC kinetic models for degradation of AMPA in the total system (Knoch (2003, BVL no 2310273), system Unter-Widdersheim)

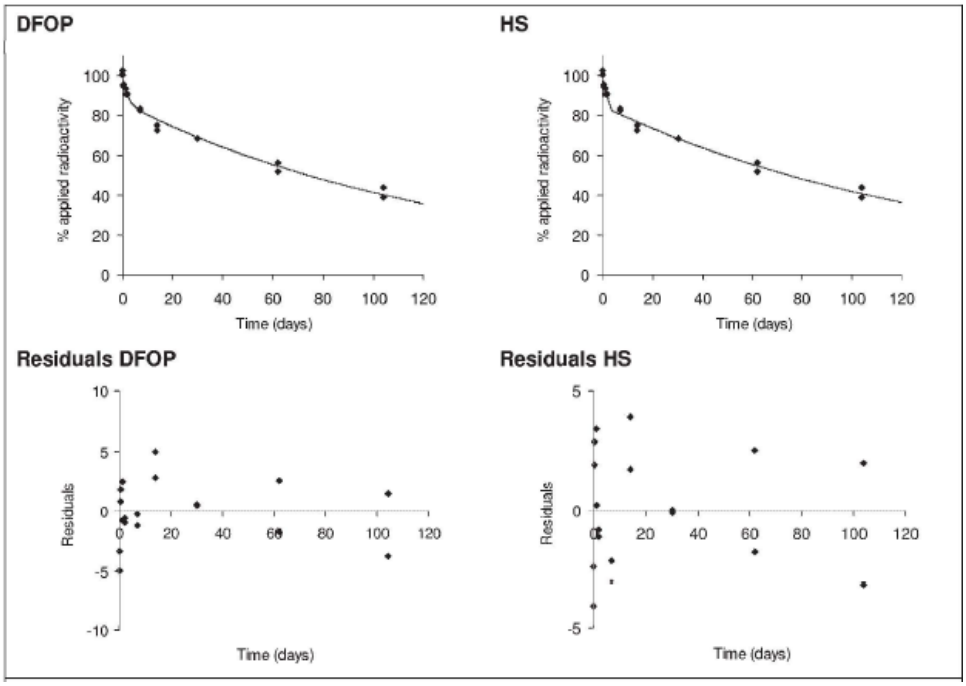


Figure B.8.4-51: Optimised DFOP and HS kinetic models for degradation of AMPA in the total system (Knoch (2003, BVL no 2310273), system Unter-Widdersheim)

Table B.8.4-56: Kinetic evaluation for degradation of AMPA in the total system (Knoch (2003, BVL no 2310273), system Unter-Widdersheim)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	92.5710	1.0524	n.r.	4.8	79.44	263.90
	k_SFO	0.0087	0.0005	<0.001			
FOMC	M_0	96.4080	1.2660	n.r.	3.0	81.82	6064.09
	α	0.3904	0.0355	n.r.			
	β	16.6860	3.1403	n.r.			
DFOP	M_0	97.4180	6.3571	n.r.	2.4	77.97	298.75
	k1_DFOP	0.4190	0.5320	0.222			
	k2_DFOP	0.0073	0.0033	0.022			
	g	0.1173	0.1243	n.r.			
HS	M0	98.3340	2.0640	n.r.	2.3	77.36	307.19
	k1_HS	0.0471	0.0155	0.004			
	k2_HS	0.0070	0.0008	<0.0001			
	tb	3.7798	1.3343	n.r.			

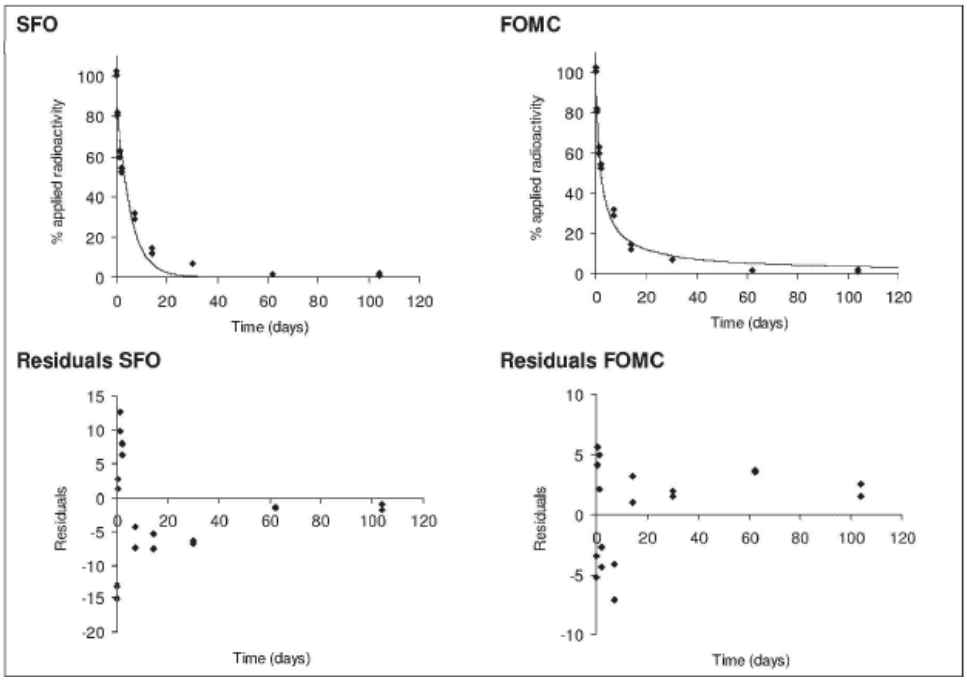


Figure B.8.4-52: Optimised SFO and FOMC kinetic models for dissipation of AMPA in the water phase (Knoch (2003, BVL no 2310273), system Unter-Widdersheim)

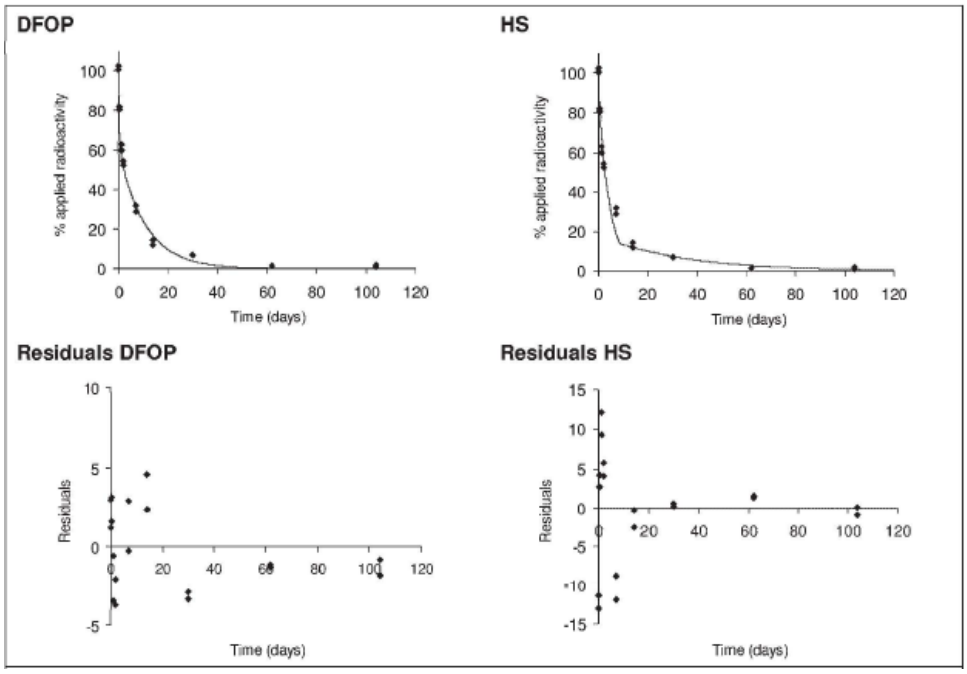


Figure B.8.4-53: Optimised DFOP and HS kinetic models for dissipation of AMPA in the water phase (Knoch (2003, BVL no 2310273), system Unter-Widdersheim)

Table B.8.4-57: Kinetic evaluation for dissipation of AMPA in the water phase (Knoch (2003, BVL no 2310273), system Unter-Widdersheim)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	87.3470	4.7730	n.r.	15.4	3.82	12.70
	k_SFO	0.1813	0.0347	<0.001			
FOMC	M_0	97.2700	3.1524	n.r.	8.0	2.13	26.31
	α	0.7932	0.0699	n.r.			
	β	1.5274	0.2145	n.r.			
DFOP	M_0	103.6700	10.8460	n.r.	5.4	1.81	19.29
	k1_DFOP	2.2291	2.1093	0.154			
	k2_DFOP	0.0912	0.0517	0.050			
	g	0.4187	0.1778	n.r.			
HS	M0	89.3850	5.0860	n.r.	15.5	3.26	23.64
	k1_HS	0.2126	0.0446	<0.001			
	k2_HS	0.0300	0.0391	0.228			
	tb	8.7289	3.0693	n.r.			

Knoch and Spirlet (1999, BVL no 1934122), system Bickenbach

Total system:
FOMC provided a slightly better visual fit compared to SFO, especially at early sampling points, but revealed an extremely high standard error for the optimised parameter ß. Both additionally tested bi-phasic models (HS; DFOP) provided slightly better χ^2-errors than FOMC.

As HS also passed statistical criteria, both persistence and modelling end points were derived from HS.

Water phase:
Compared to SFO, FOMC provided a slightly better fit, especially at earlier data points up to day 30. Still, it overestimated later data points, while SFO underestimated data points between day 30 and day 100. HS and DFOP models were tested additionally: fitting HS model to the water phase residue data did not change the χ^2-error compared to FOMC, but revealed a much more appropriate visual fit, which was probably due to smaller and randomly distributed residuals at later data points. Fitting DFOP model provided the best χ^2-error of all four models and statistically significant p-values for the t-test on both dissipation rates. Consequently, DFOP model was selected as persistence and modelling end point.

Sediment phase:
As there was not enough data points after peak concentration in sediment, kinetic evaluations could not be conducted.

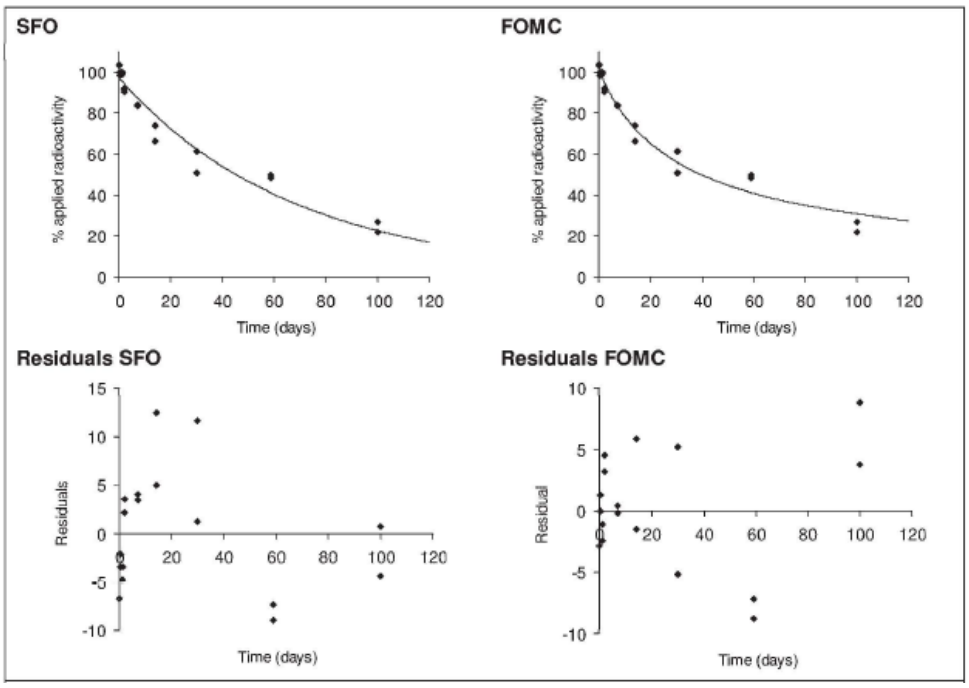


Figure B.8.4-54: Optimised SFO and FOMC kinetic models for degradation of AMPA in the total system (Knoch and Spirlet (1999, BVL no 1934122), system Bickenbach)

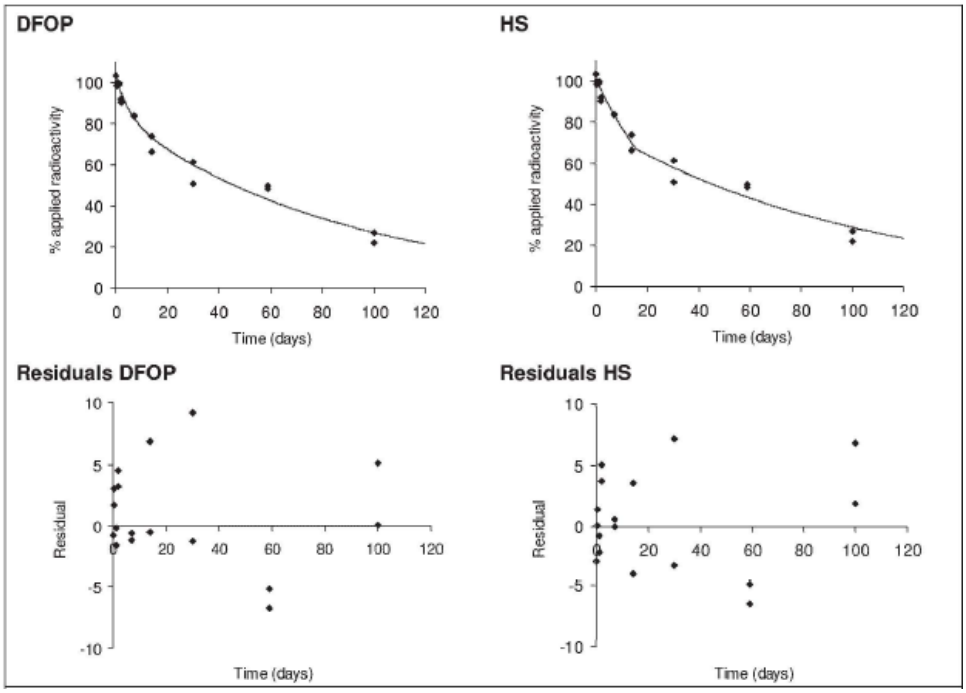


Figure B.8.4-55: Optimised DFOP and HS kinetic models for degradation of AMPA in the total system (Knoch and Spirlet (1999, BVL no 1934122), system Bickenbach)

Table B.8.4-58: Kinetic evaluation for degradation of AMPA in the total system (Knoch and Spirlet (1999, BVL no 1934122), system Bickenbach)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	96.7910	7.6186	n.r.	5.9	47.74	158.58
	k_SFO	0.0145	0.0047	0.003			
FOMC	M_0	100.6300	8.1073	n.r.	4.4	39.01	553.91
	α	0.7289	0.8697	n.r.			
	β	24.5650	48.5500	n.r.			
DFOP	M_0	102.7700	3.5641	n.r.	3.8	43.81	185.08
	k1_DFOP	0.1930	0.1594	0.123			
	k2_DFOP	0.0114	0.0032	0.002			
	g	0.1765	0.1258	n.r.			
HS	M0	100.6000	1.827	n.r.	3.7	44.53	205.21
	k1_HS	0.0259	0.0035	<0.001			
	k2_HS	0.0100	0.0017	<0.001			
	tb	15.5760	6.5271	n.r.			

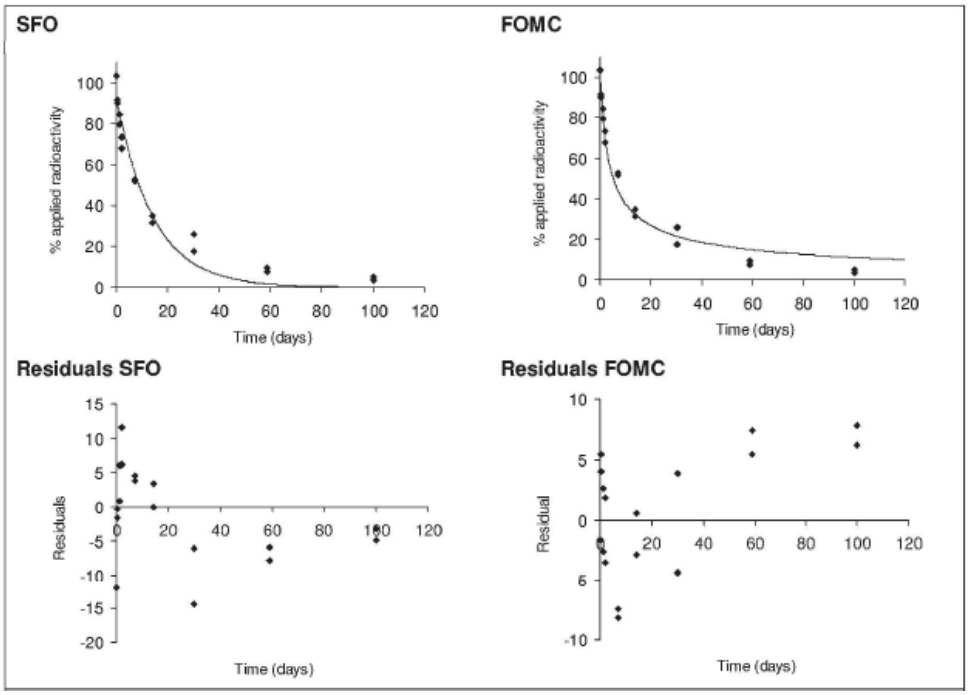


Figure B.8.4-56: Optimised SFO and FOMC kinetic models for dissipation of AMPA in the water phase (Knoch and Spirlet (1999, BVL no 1934122), system Bickenbach)

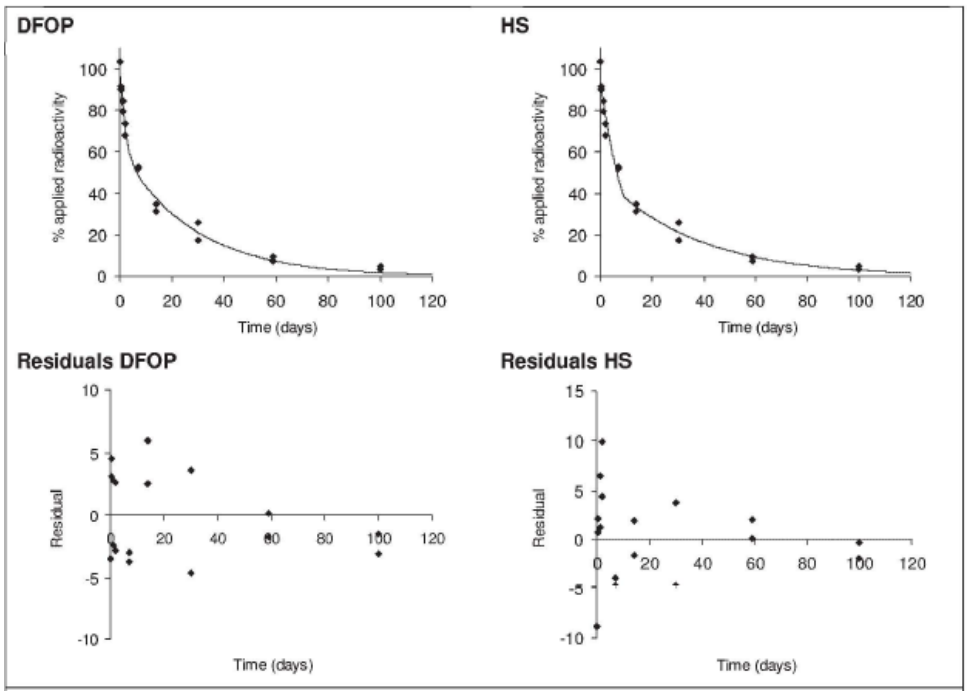


Figure B.8.4-57: Optimised DFOP and HS kinetic models for dissipation of AMPA in the water phase (Knoch and Spirlet (1999, BVL no 1934122), system Bickenbach)

Table B.8.4-59: Kinetic evaluation for dissipation of AMPA in the water phase (Knoch and Spirlet (1999, BVL no 1934122), system Bickenbach)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	91.5180	12.1030	n.r.	10.6	10.11	33.59
	k_SFO	0.0685	0.0330	0.0272			
FOMC	M_0	101.8300	16.691	n.r.	7.3	5.12	116.86
	α	0.5773	0.4349	n.r.			
	β	2.2066	3.7926	n.r.			
DFOP	M_0	100.0300	4.1726	n.r.	4.6	6.59	51.47
	k1_DFOP	0.5252	0.1507	0.002			
	k2_DFOP	0.0353	0.0074	<0.001			
	g	0.3842	0.0826	n.r.			
HS	M0	94.6230	2.2770	n.r.	7.5	7.20	59.41
	k1_HS	0.0963	0.0087	<0.001			
	k2_HS	0.0279	0.0070	<0.001			
	tb	9.4621	39.6560	n.r.			

Knoch and Spirlet (1999, BVL no 1934122), system Unter Widdersheim

Total system:
FOMC (χ^2 = 4.0) was more appropriate than SFO (χ^2 = 11 .7) when fitting both models to total system residue data. Although SFO passed statistical criteria, its visual fit was very poor: it did neither meet M_0 nor later data points (days 30 to 100) very well. According to FOCUS kinetics (FOCUS, 2006, 2011), further bi-phasic models were tested. Both models (HS, DFOP) showed a small χ^2-error and a very good visual fit with non-systematic small residuals at all sampling dates. However, neither HS nor DFOP passed statistical criteria; DFOP even came up with an unrealistic high $DegT_{90}$ of > 11,000 days. In consequence, persistence end-points were derived from HS model. Since none of the biphasic models showed statistically reliable standard errors of the respective optimised values, and SFO gave a poor and questionable visual fit, no reliable modelling end point could be selected.

Water phase:
The comparison of SFO and FOMC models revealed a slightly better χ^2-error for FOMC; probably due to smaller residuals at earlier data points. HS and DFOP were tested additionally and provided a better visual fit for HS only, while DFOP showed a similar pattern to SFO and FOMC. HS model fits very well later data points. For earlier data points, which are more important to the fast dissipation of AMPA from water phase, randomly distributed residuals are smaller than for other models. HS also passed statistical criteria and was therefore deemed appropriate as persistence and modelling end point.

Sediment phase:
All four kinetic models were tested to see whether one of them fit the respective sediment data. For HS and DFOP, again, no χ^2-error could be calculated due to an insufficient number of degrees of freedom as only three data points after peak concentration in sediment were available. None of the four models passed statistical criteria. The visual fit seemed to be acceptable for the bi-phasic models. However, all of them are questionable as obviously there is no dissipation from sediment at all.

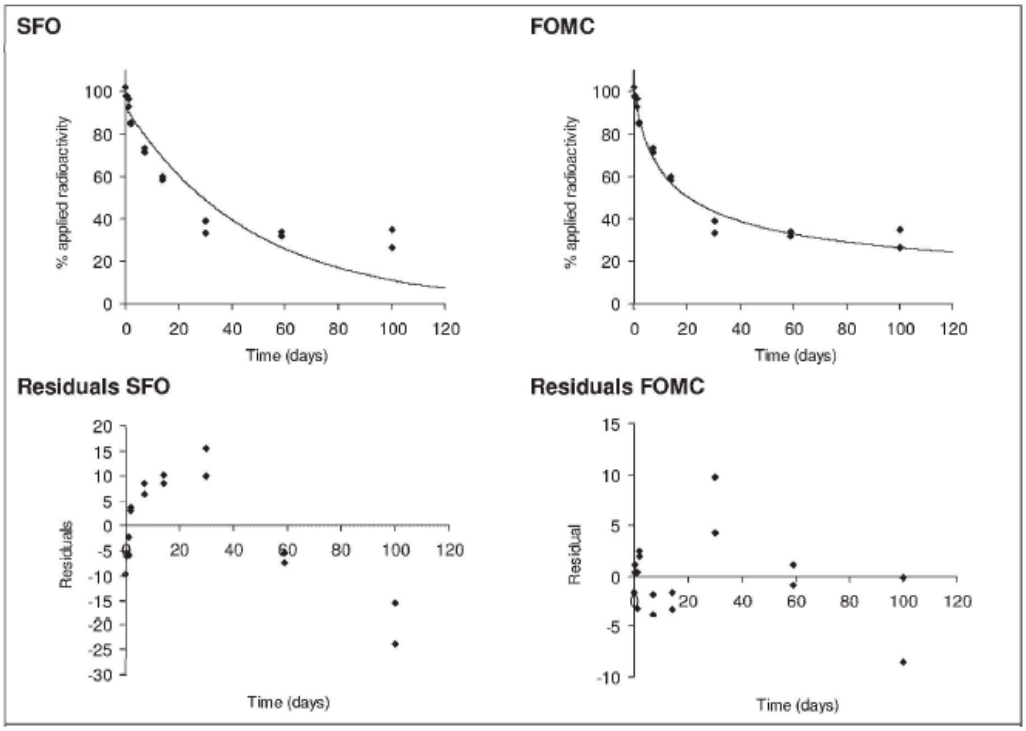


Figure B.8.4-58: Optimised SFO and FOMC kinetic models for degradation of AMPA in the total system (Knoch and Spirlet (1999, BVL no 1934122), system Unter-Widdersheim)

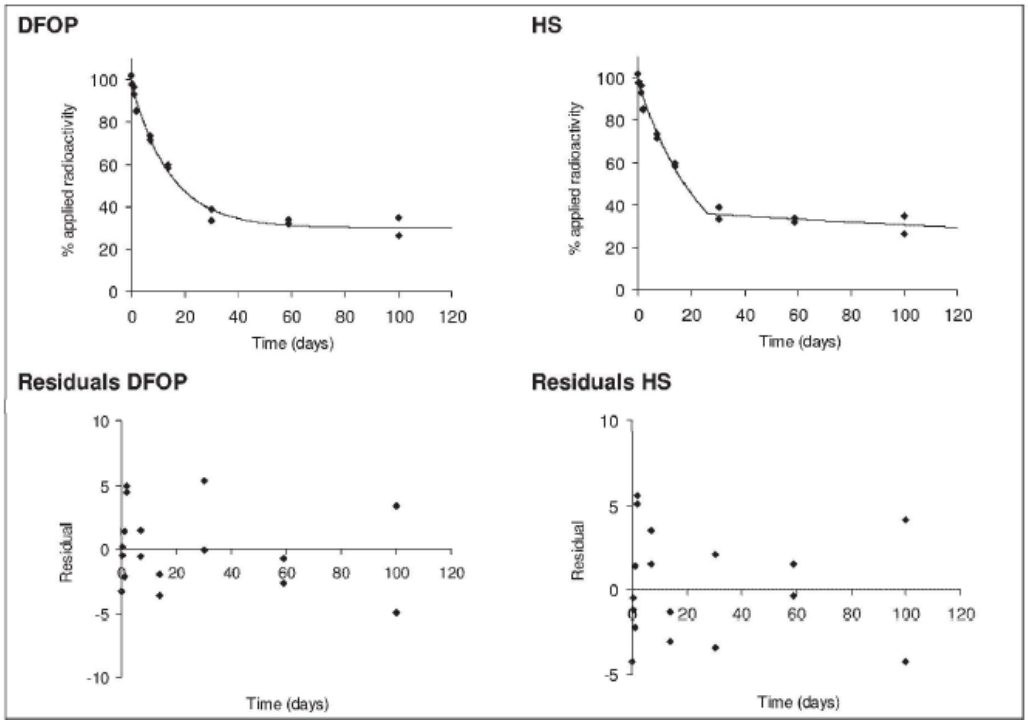


Figure B.8.4-59: Optimised DFOP and HS kinetic models for degradation of AMPA in the total system (Knoch and Spirlet (1999, BVL no 1934122), system Unter-Widdersheim)

Table B.8.4-60: Kinetic evaluation for degradation of AMPA in the total system (Knoch and Spirlet (1999, BVL no 1934122), system Unter-Widdersheim)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	92.5910	7.9732	n.r.	11.7	32.73	108.73
	k_SFO	0.0212	0.0070	0.004			
FOMC	M_0	100.6200	9.5954	n.r.	4.0	20.13	885.03
	α	0.4541	0.1533	n.r.			
	β	5.5891	4.2311	n.r.			
DFOP	M_0	98.8540	3.2509	n.r.	3.2	18.61	∞
	k1_DFOP	0.0683	0.0278	0.014			
	k2_DFOP	0.0001	0.0065	0.494			
	g	0.6941	0.1656	n.r.			
HS	M0	97.9800	2.3280	n.r.	3.4	18.02	640.66
	k1_HS	0.0385	0.0051	<0.001			
	k2_HS	0.0021	0.0026	0.215			
	tb	26.0410	5.1046	n.r.			

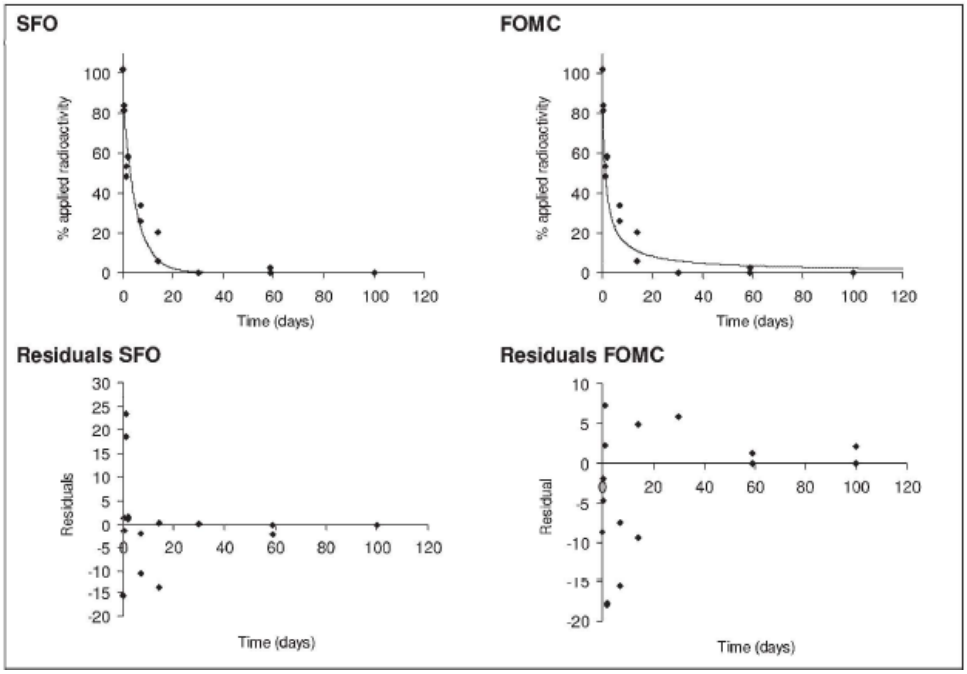


Figure B.8.4-60: Optimised SFO and FOMC kinetic models for dissipation of AMPA in the water phase (Knoch and Spirlet (1999, BVL no 1934122), system Unter-Widdersheim)

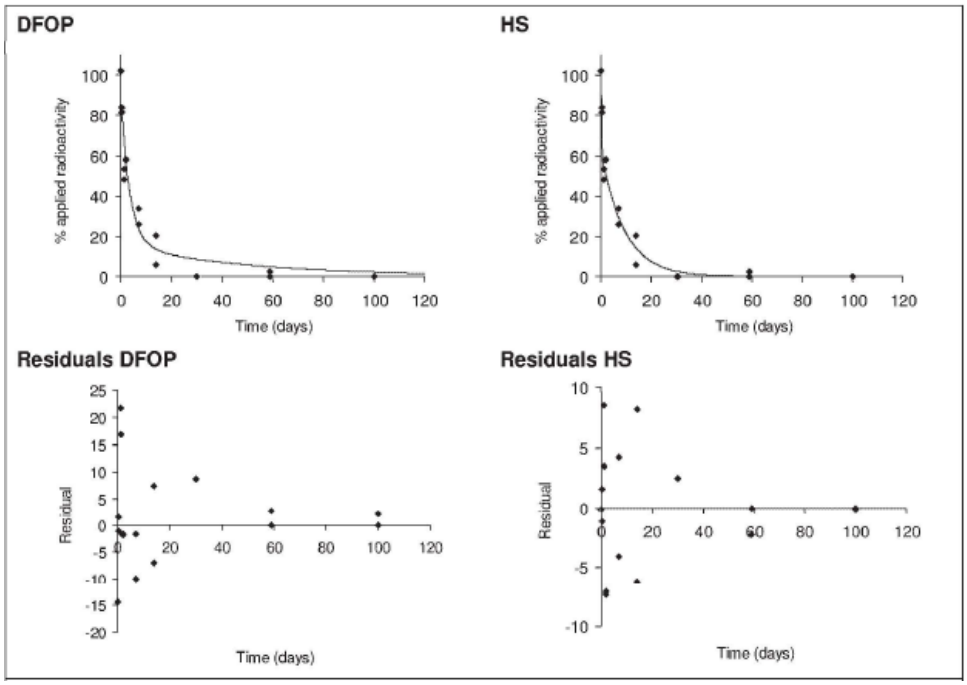


Figure B.8.4-61: Optimised DFOP and HS kinetic models for dissipation of AMPA in the water phase (Knoch and Spirlet (1999, BVL no 1934122), system Unter-Widdersheim)

Table B.8.4-61: Kinetic evaluation for dissipation of AMPA in the water phase (Knoch and Spirlet (1999, BVL no 1934122), system Unter-Widdersheim)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	86.5470	18.0010	n.r.	17.6	3.75	12.45
	k_SFO	0.1849	0.1347	0.094			
FOMC	M_0	93.5610	24.9240	n.r.	16.4	1.50	17.36
	α	0.8272	0.4872	n.r.			
	β	1.1439	0.8580	n.r.			
DFOP	M_0	87.8330	8.1451	n.r.	18.8	3.28	30.03
	k1_DFOP	0.2757	0.1712	0.065			
	k2_DFOP	0.0194	0.0619	0.379			
	g	0.8214	0.3070	n.r.			
HS	M0	102.1100	6.5810	n.r.	7.1	2.02	17.15
	k1_HS	0.8349	0.4018	0.028			
	k2_HS	0.1064	0.0257	<0.001			
	tb	0.6565	0.3835	n.r.			

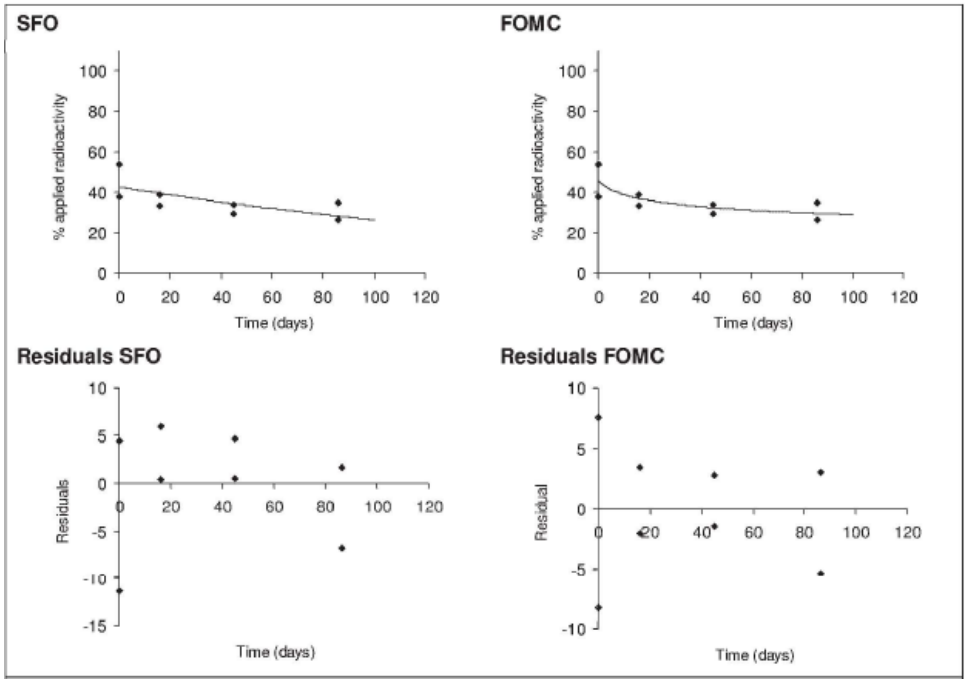


Figure B.8.4-62: Optimised SFO and FOMC kinetic models for dissipation of AMPA in the sediment phase (Knoch and Spirlet (1999, BVL no 1934122), system Unter-Widdersheim)

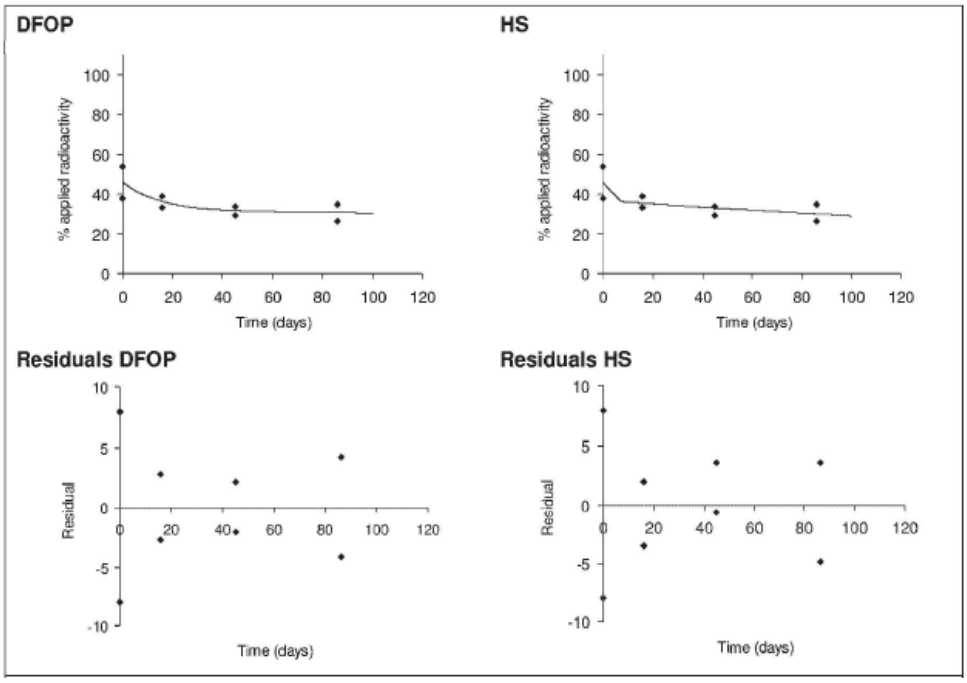


Figure B.8.4-63: Optimised DFOP and HS kinetic models for dissipation of AMPA in the sediment phase (Knoch and Spirlet (1999, BVL no 1934122), system Unter-Widdersheim)

Table B.8.4-62: Kinetic evaluation for dissipation of AMPA in the sediment phase (Knoch and Spirlet (1999, BVL no 1934122), system Unter-Widdersheim)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	42.7140	22.0500	n.r.	6.7	144.76	480.90
	k_SFO	0.0048	0.0128	0.361			
FOMC	M_0	45.8190	24.6920	n.r.	2.2	486.10	∞
	α	0.1537	0.3197	n.r.			
	β	5.4088	7.8718	n.r.			
DFOP	M_0	46.1190	11.8250	n.r.	n.c.[1)]	1093.62	6701.81
	k1_DFOP	0.0670	0.4050	0.438			
	k2_DFOP	0.0003	0.0224	0.495			
	g	0.3157	1.2221	n.r.			
HS	M0	46.1490	3.8370	n.r.	n.c.[1)]	199.76	881.64
	k1_HS	0.0316	0.1857	0.437			
	k2_HS	0.0024	0.0024	0.189			
	tb	7.5846	234.1400	n.r.			

[1)] not calculated due to insufficient degrees of freedom

Conclusions

For FOCUS surface water modelling the geometric mean $DegT_{50,total\ system}$ of 61.2 days for glyphosate and of 83.7 days for AMPA are considered to be acceptable as half-lives for the water phase in combination with a default $DegT_{50}$ of 1000 days for sediment .

RMS Comment

The report of Partsch (2012, BVL no 2316005) presents the new calculations of DT_{50} values following FOCUS Kinetics guidance (2006) for persistence and modelling end points for glyphosate and AMPA from water/sediment studies. The complex kinetic modelling approach from the Notifier to derive reliable end points can be in general accepted by the RMS. However some corrections are necessary, which are described in the following.

Studies taken into account for kinetic evaluation

In Knoch (2003) amounts of AMPA in the sediment reported in the text (p. 30 of study report) differ from amounts reported in the tables (p. 51 of study report). Since it is not clear, which values are correct, the sediment systems Bickenbach and Unter-Widdersheim should be excluded from kinetic evaluation.

It is stated by the Notifier that "*The studies of Muttzall (1993, BVL no 1982136) and McEwen (2004, BVL no 2310275) revealed several deviations from OECD Guidelines for Testing of Chemicals No. 308 (OECD, 2002) such as analytical artefacts and insufficient reporting of important parameters. In consequence, these studies were excluded from kinetic evaluation.*"

There are no further explanations.

In the DAR of 1998, the study of Muttzall (1993, BVL no 1982136) was considered acceptable. A questionable result of this study was the high amount of an unknown substance (44 % or 10 % of radioactivity in the water phase, and 2 % or 1 % in the sediment phase at day 0, afterwards negligible amounts). A possible explanation was that glyphosate might have formed a complex with humic acids what led to a different behaviour on the TLC plates. The RMS proposes to include the study in the assessment and to recalcuate the end points, presuming the unknown fraction is glyphosate.

In McEwen (2004, BVL no 2310275) severe problems were encountered in obtaining chromatography for the extracts in Sediment B. This is believed to be due to the presence of co-extracted endogenous material affecting the ion-exchange chromatography. Therefore, the RMS proposes to exclude Sediment B and the total system B from kinetic evaluation.
The studies of Muttzall (1993, BVL no 1982136) and McEwen (2004, BVL no 2310275) were kinetically evaluated following FOCUS guidance (FOCUS, 2006) to derive persistence and modelling end points. At Level P-I (one-compartment approaches), single first-order and biphasic kinetic models were used to describe the behaviour of glyphosate and AMPA in the water phase, the sediment phase and the total system. No Level P-II was calculated. KinGUII (version 2.2012.202.925) was used as fitting software.

Muttzall (1993, BVL no 1982136), system TNO

Total system:
SFO gave a poor and questionable visual fit. FOMC was more appropriate than SFO with regard to the χ^2-errors and the visual fit. HS model could not be optimised by KinGUII, whereas DFOP showed a visually acceptable fit. Since the standard error and the p-value of k2_DFOP were not sufficiently low, DFOP was not considered reliable. Although the p-value of β (FOMC) was larger than 0.1, persistence end points were derived from FOMC as best-fit model. Due to the fact that 10 % of the initially measured concentration was not reached within the study period, no reliable modelling end point could be selected.

Water phase:
It was not possible to fit kinetics for the dissipation in water.

Sediment phase:
It was not possible to fit kinetics for the dissipation in the sediment for system TNO.

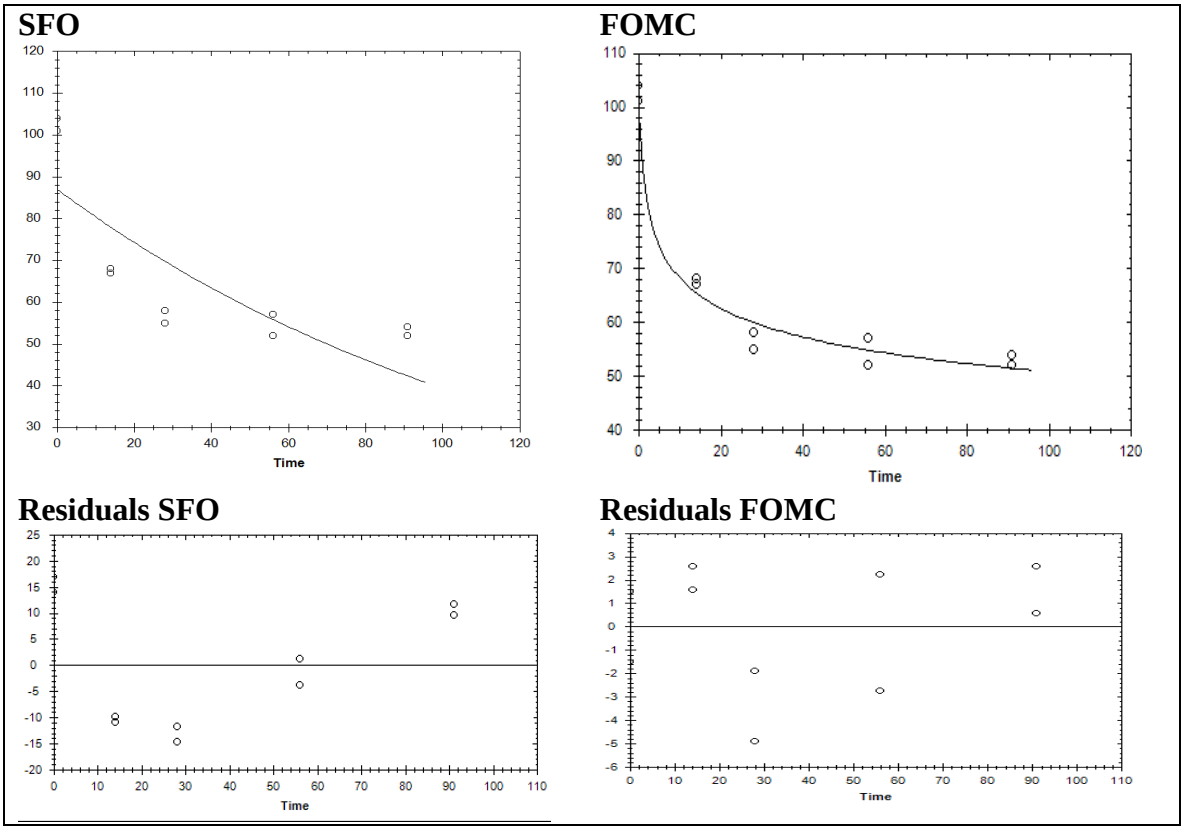


Figure B.8.4-64: Optimised SFO and FOMC kinetic models for dissipation of glyphosate in the total system (Muttzall (1993, BVL no 1982136), system TNO)

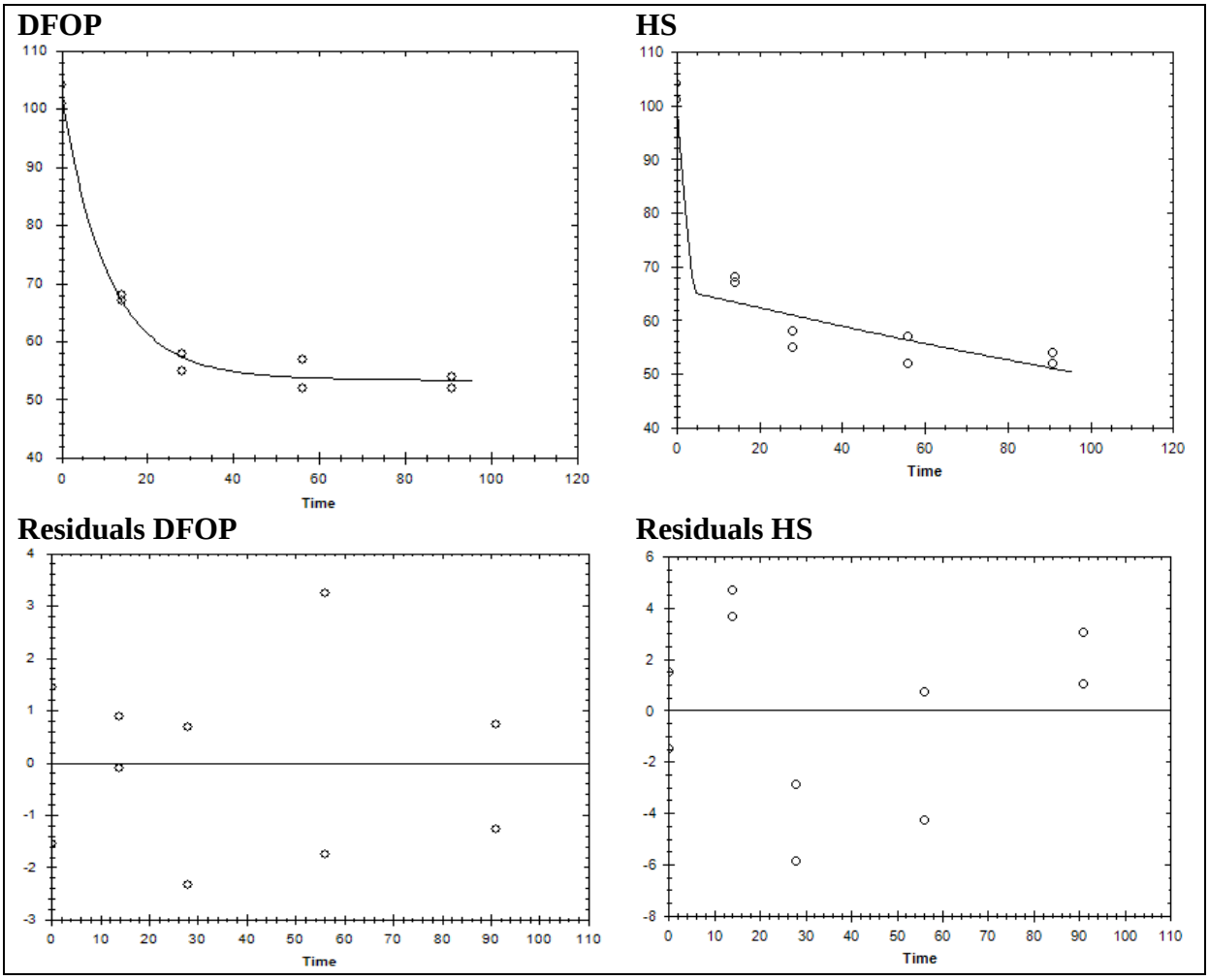


Figure B.8.4-65: Optimised DFOP and HS kinetic models for dissipation of glyphosate in the total system (Muttzall (1993, BVL no 1982136), system TNO)

Table B.8.4-63: Kinetic evaluation for dissipation of glyphosate in the total system (Muttzall (1993, BVL no 1982136), system TNO)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	87.0061	7.2085	1.03e-06	13.6	87.464	290.55
	k_SFO	0.0079	0.0024	0.00588			
FOMC	M_0	102.5133	2.08109	1.86e-10	2.4	93.055	>1000
	α	0.13076	0.02734	0.00100			
	β	0.46642	0.43656	0.16039			
DFOP	M_0	102.5	1.495	3.23e-10	0.7	418.42	>1000
	k1_DFOP	0.09235	0.01595	0.000582			
	k2_DFOP	1.159e-04	9.932e-04	0.455453			

	g	0.4752	3.941e-02	9.88e-06			
HS	M0	102.5	3.049	2.30e-08	3.9	89.152	660.42
	k1_HS	0.1051	n.c.	n.c.			
	k2_HS	2.817e-03	9.478e-04	0.0124			
	tb	4.319	n.c.	n.c.			

n.c.: could not be calculated by KinGUII

Muttzall (1993, BVL no 1982136), system Kromme Rijn

Total system:
FOMC was visually and statistically more appropriate than SFO; hence, HS and DFOP models were additionally tested. HS model could not be optimised by KinGUII, whereas DFOP revealed the best χ^2-error, met M_0 slightly very good, and gave the most appropriate fit for later sampling points. Therefore, persistence and modelling end points were derived from DFOP.

Water phase:
It was not possible to fit kinetics for the dissipation in water.

Sediment phase:
SFO gave an acceptable fit, both visually and statistically. FOMC was not considered reliable, since the p-value and the standard error of β (FOMC) were not sufficiently low. However, biphasic models were additionally tested. Again, HS model could not be optimised by KinGUII, whereas the standard error as well as the p-value for M_0 (DFOP) was not sufficiently low Therefore, persistence and modelling end points were derived from SFO.

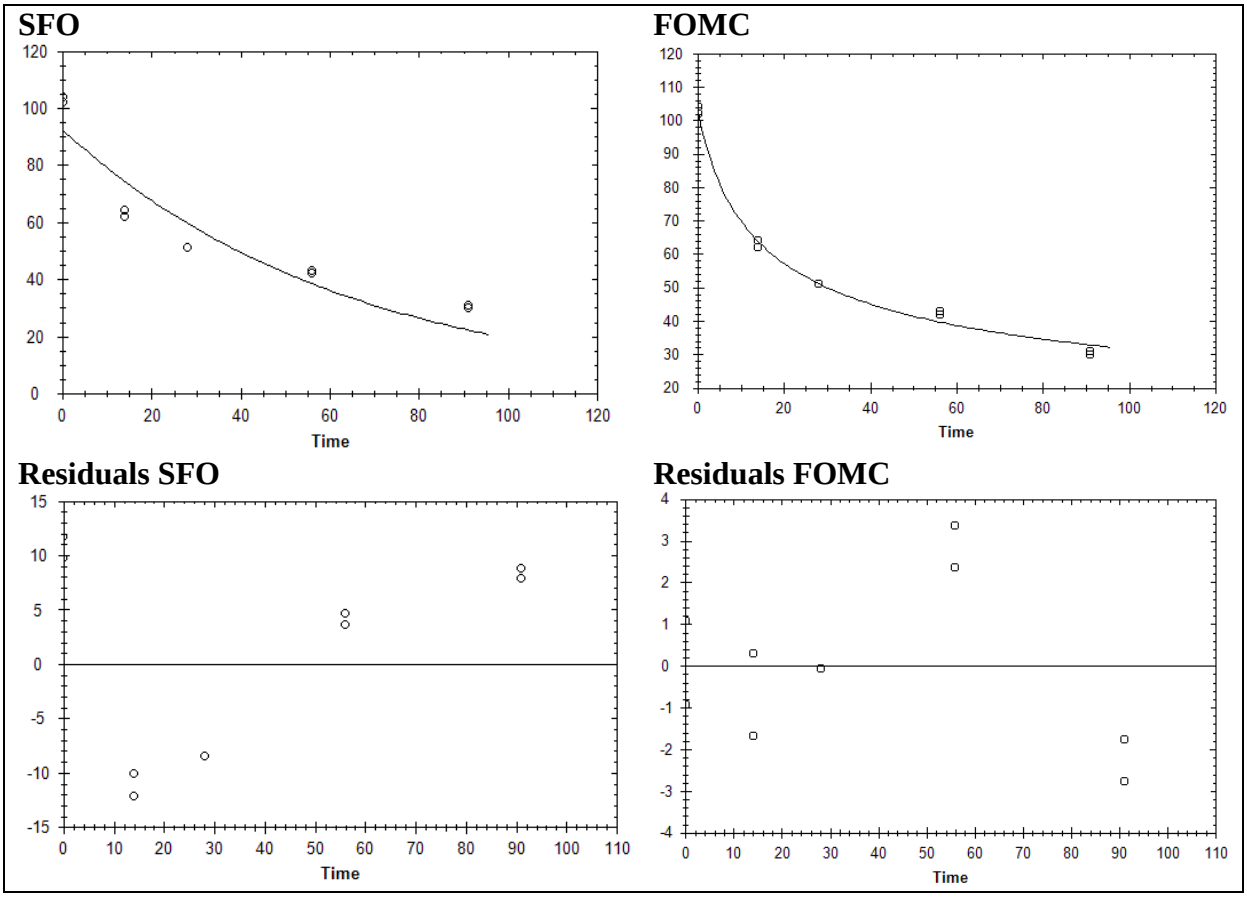


Figure B.8.4-66: Optimised SFO and FOMC kinetic models for dissipation of glyphosate in the total system (Muttzall (1993, BVL no 1982136), system Kromme Rijn)

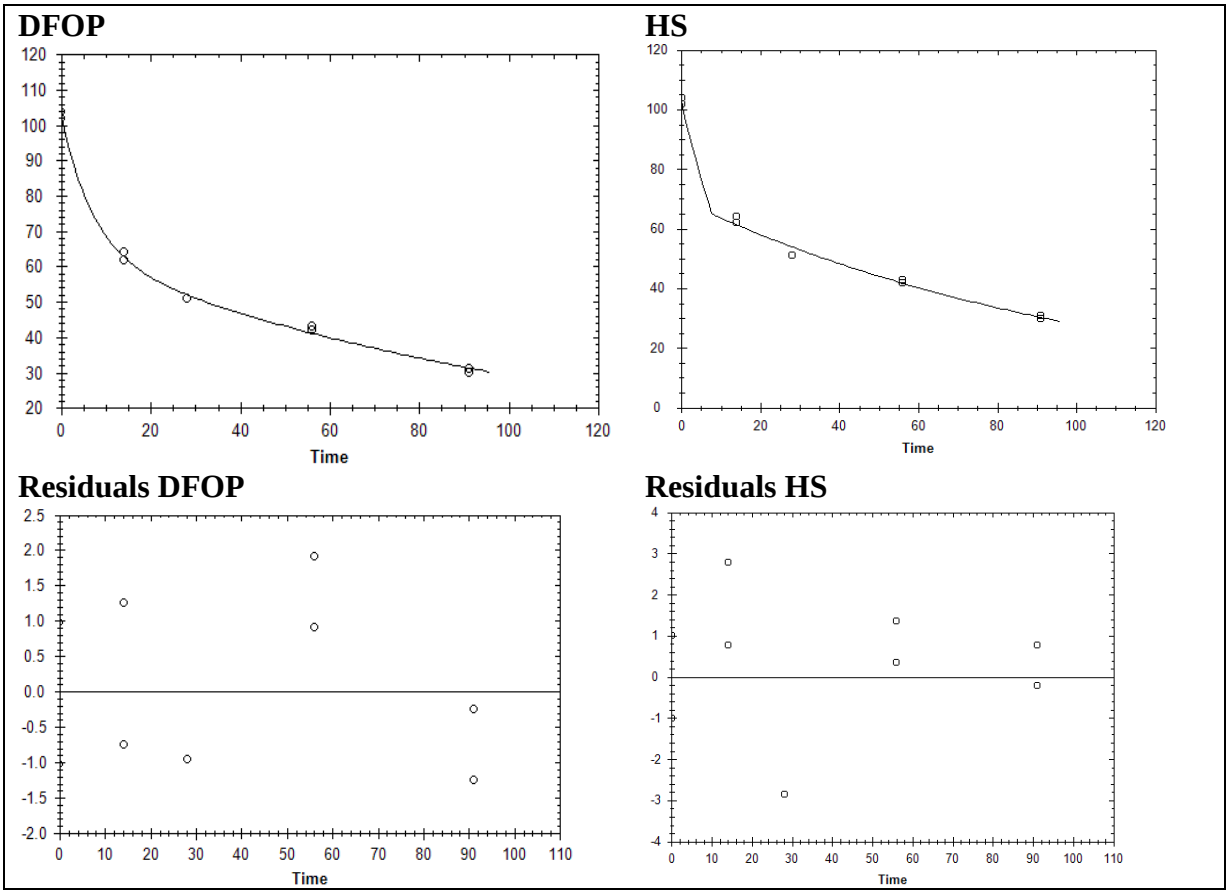


Figure B.8.4-67: Optimised DFOP and HS kinetic models for dissipation of glyphosate in the total system (Muttzall (1993, BVL no 1982136), system Kromme Rijn)

Table B.8.4-64: Kinetic evaluation for dissipation of glyphosate in the total system (Muttzall (1993, BVL no 1982136), system Kromme Rijn)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	92.310827	6.159951	1.94e-07	12.3	44.204	146.84
	k_SFO	0.015681	0.002803	0.000257			
FOMC	M_0	102.92381	1.53090	2.12e-11	2.4	27.376	>1000
	α	0.42925	0.04707	1.96e-05			
	β	6.79814	1.69864	0.00259			
DFOP	M_0	103.0	0.9999	2.82e-11	1.3	28.863	232.92
	k1_DFOP	1.379e-01	2.456e-02	0.00068			
	k2_DFOP	7.817e-03	7.057e-04	1.61e-05			
	g	3.824e-01	2.735e-02	4.17e-06			
HS	M0	103.0	1.558	4.02e-10	2.4	32.849	208.39
	k1_HS	5.909e-02	n.c.	n.c.			

	k2_HS	9.168e-03	6.749e-04	4.94e-06			
	tb	7.852	n.c.	n.c.			

n.c.: could not be calculated by KinGUII

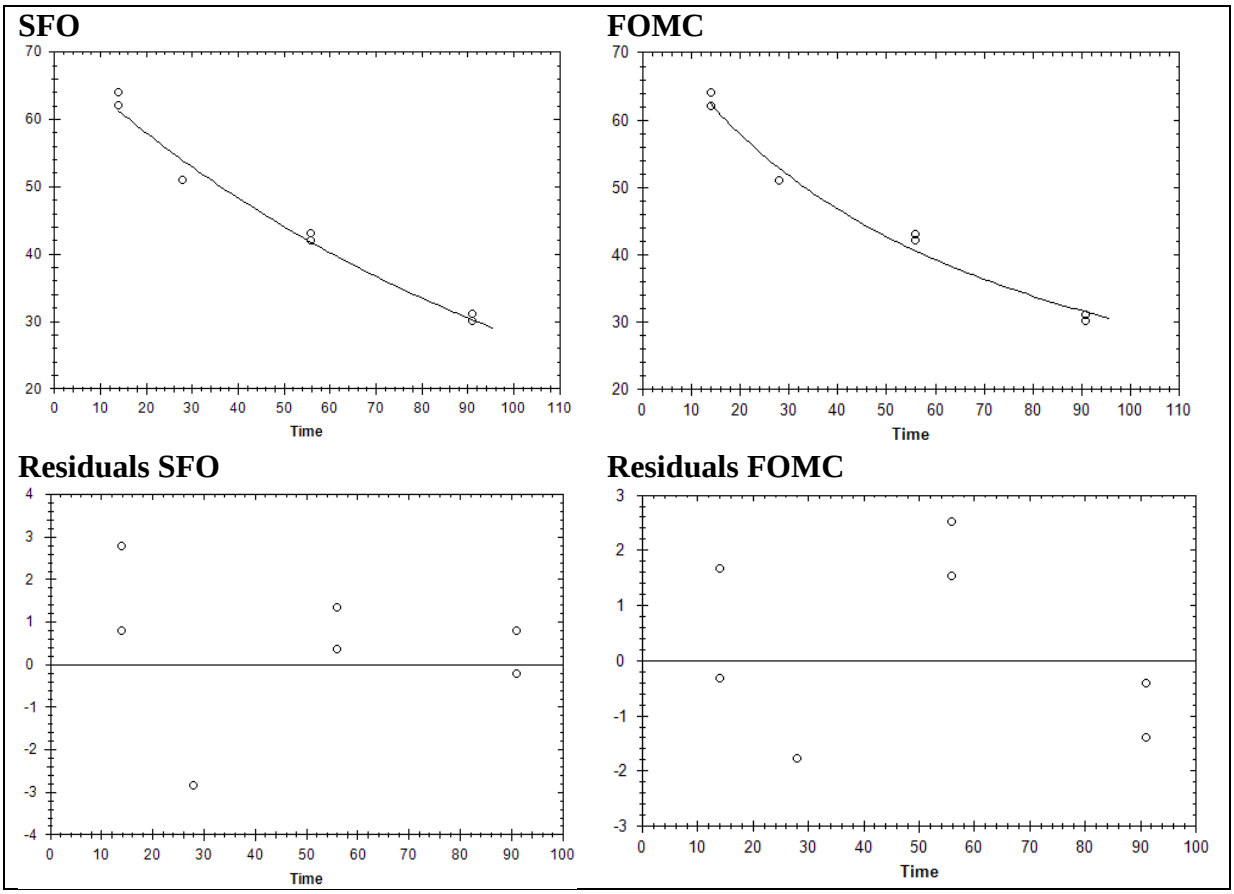


Figure B.8.4-68: Optimised SFO and FOMC kinetic models for dissipation of glyphosate in the sediment (Muttzall (1993, BVL no 1982136), system Kromme Rijn)

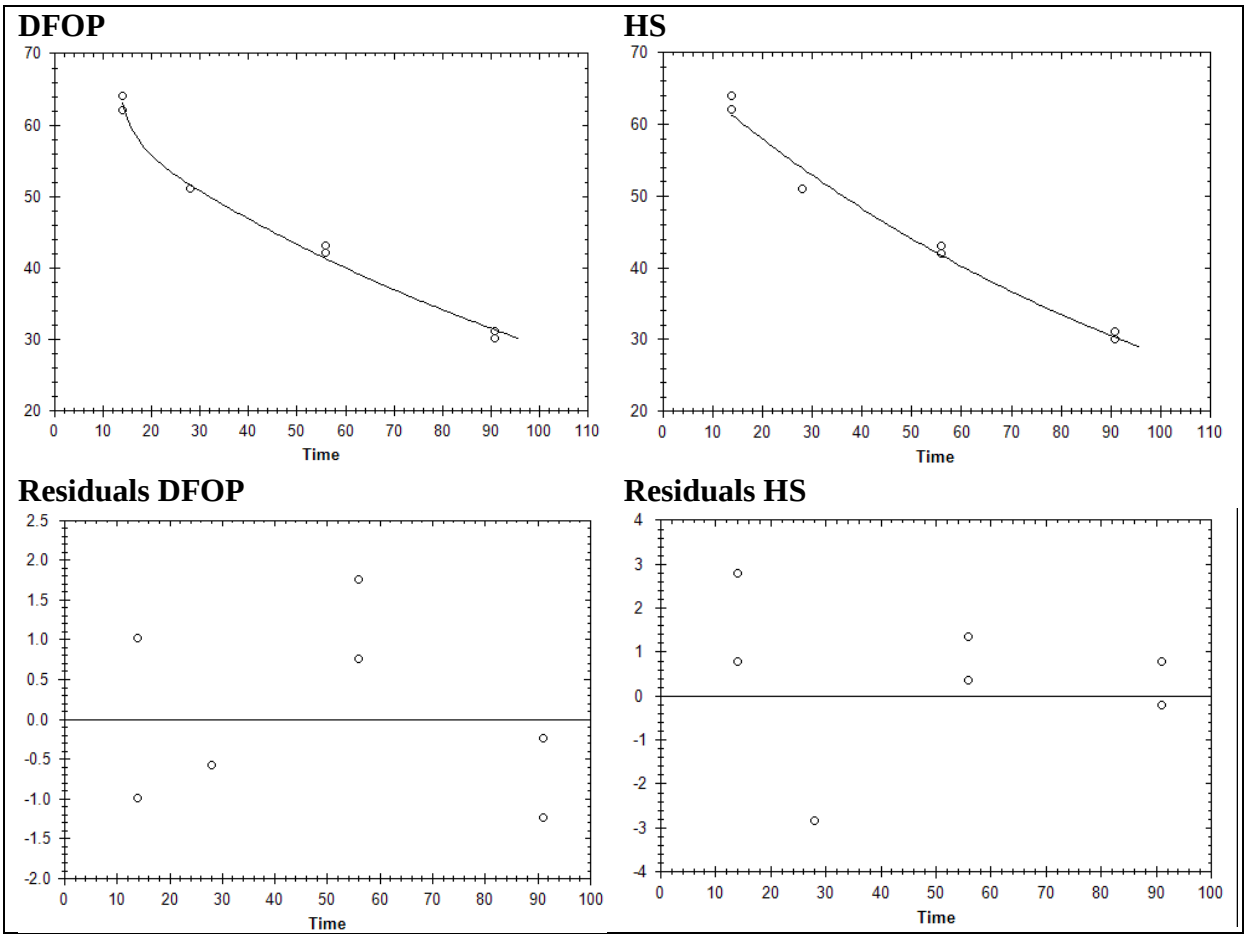


Figure B.8.4-69: Optimised DFOP and HS kinetic models for dissipation of glyphosate in the sediment (Muttzall (1993, BVL no 1982136), system Kromme Rijn)

Table B.8.4-65: Kinetic evaluation for dissipation of glyphosate in the sediment (Muttzall (1993, BVL no 1982136), system Kromme Rijn)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	69.60	1.877	1.28e-08	3.0	75.605	251.16
	k_SFO	9.168e-03	6.513e-04	4.01e-06			
FOMC	M_0	76.1907	7.2558	6.76e-05	2.7	63.567	594.49
	α	0.9684	0.7477	0.126			
	β	60.7877	77.8024	0.235			
DFOP	M_0	49.05	85.18	0.2978	1.5	2.7242	34.194
	k1_DFOP	3.117e-01	1.458e-01	0.0496			
	k2_DFOP	7.940e-03	5.823e-04	8.38e-05			
	g	8.688e-01	2.275e-01	0.0094			
HS	M0	69.58	n.c.	n.c.	3.4	75.641	251.19
	k1_HS	9.168e-03	n.c.	n.c.			
	k2_HS	7.120e-03	7.977e-04	0.000164			
	tb	1.636e-01	n.c.	n.c.			

n.c.: could not be calculated by KinGUII

McEwen (2004, BVL no 2310275), system A

Total system:
Fitting adequate kinetic models to total system data was difficult, as obviously there is no degradation at all during the first 7 to 29 days. Therefore, none of the models tested (SFO, FOMC, DFOP, HS) was considered reliable to describe the degradation of AMPA in the total system.

Water phase:
Testing SFO model revealed a poor visual fit, supported by a χ^2-error >15%. HS model could not be optimised by KinGUII, whereas FOMC and DFOP showed acceptable fits, both visually and statistically. In comparison with DFOP, FOMC provided a smaller χ^2-error. Therefore, persistence and modelling end points were derived from FOMC.

Sediment phase:
As there was not enough data points after peak concentration in sediment, kinetic evaluations could not be conducted.

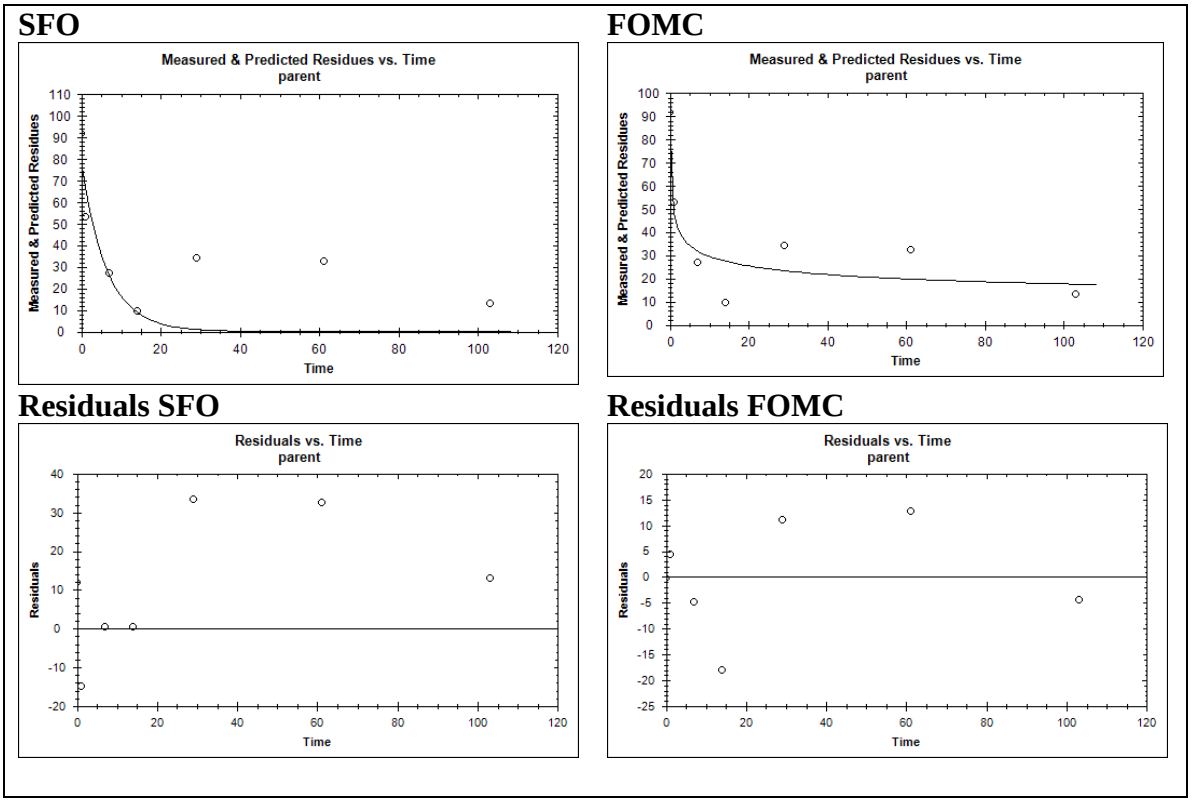


Figure B.8.4-70: Optimised SFO and FOMC kinetic models for degradation of AMPA in the total system (McEwen (2004, BVL no 2310275), system A)

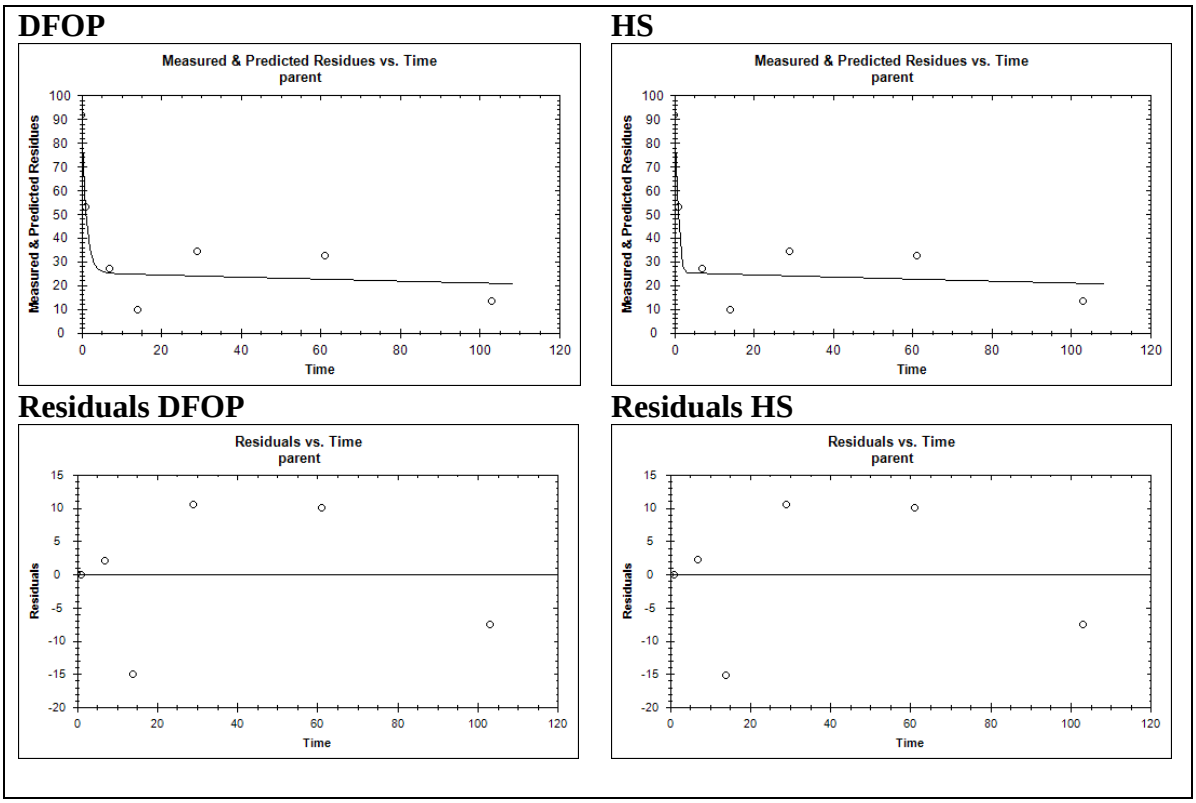


Figure B.8.4-71: Optimised DFOP and HS kinetic models for degradation of AMPA in the total system (McEwen (2004, BVL no 2310275), system A)

Table B.8.4-66: Kinetic evaluation for degradation of AMPA in the total system (McEwen (2004, BVL no 2310275), system A)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	79.5285	20.8116	0.006	42.0	4.41	14.65
	k_SFO	0.1572	0.1642	0.191			
FOMC	M_0	91.70889	12.89805	0.001	22.5	1.32	1860.60
	α	0.22335	0.12339	0.072			
	β	0.06200	0.16919	0.366			
DFOP	M0	91.570456	12.954260	0.003	21.5	1.35	535.62
	k1_DFOP	0.860903	0.542336	0.105			
	k2_DFOP	0.001886	0.006718	0.399			
	g	0.725381	0.106059	0.003			
HS	M0	91.599950	12.943878	0.003	21.5	1.27	524.59
	k1_HS	0.545251	0.281788	0.074			
	k2_HS	0.001932	0.006685	0.396			
	tb	2.372409	1.289723	0.082			

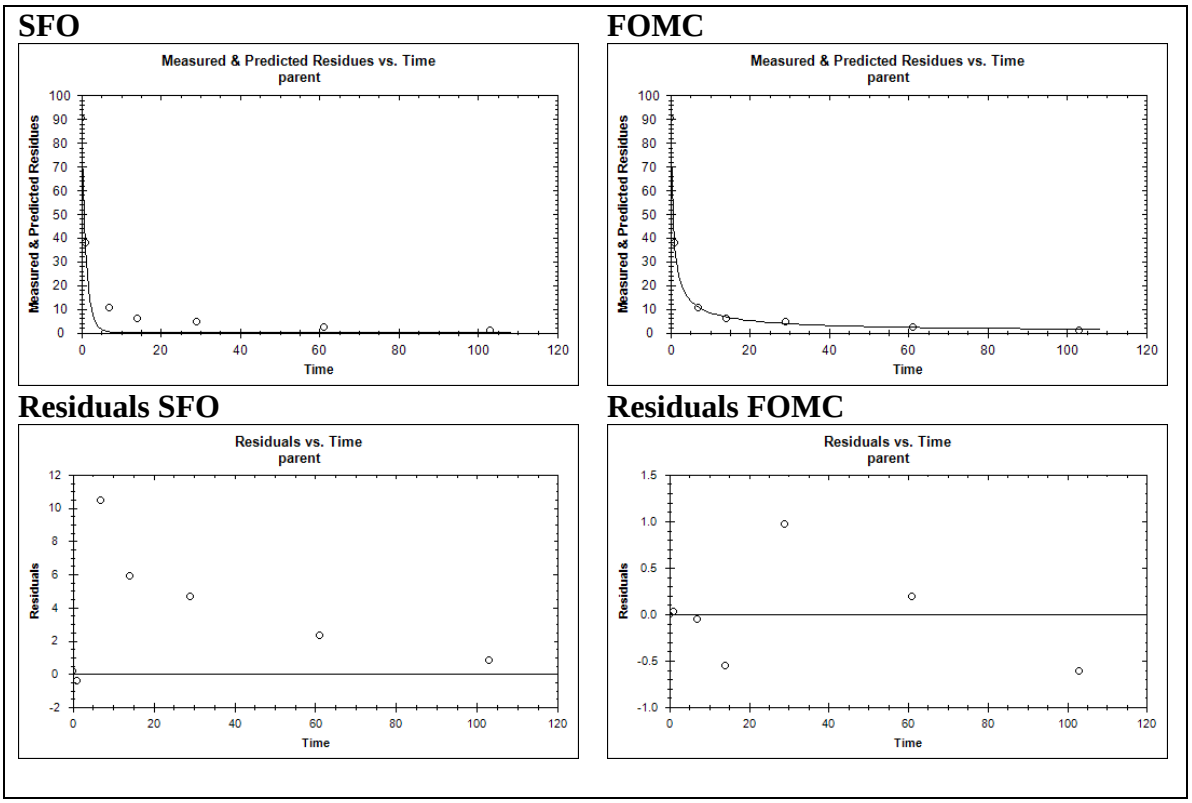


Figure B.8.4-72: Optimised SFO and FOMC kinetic models for dissipation of AMPA in the water phase (McEwen (2004, BVL no 2310275), system A)

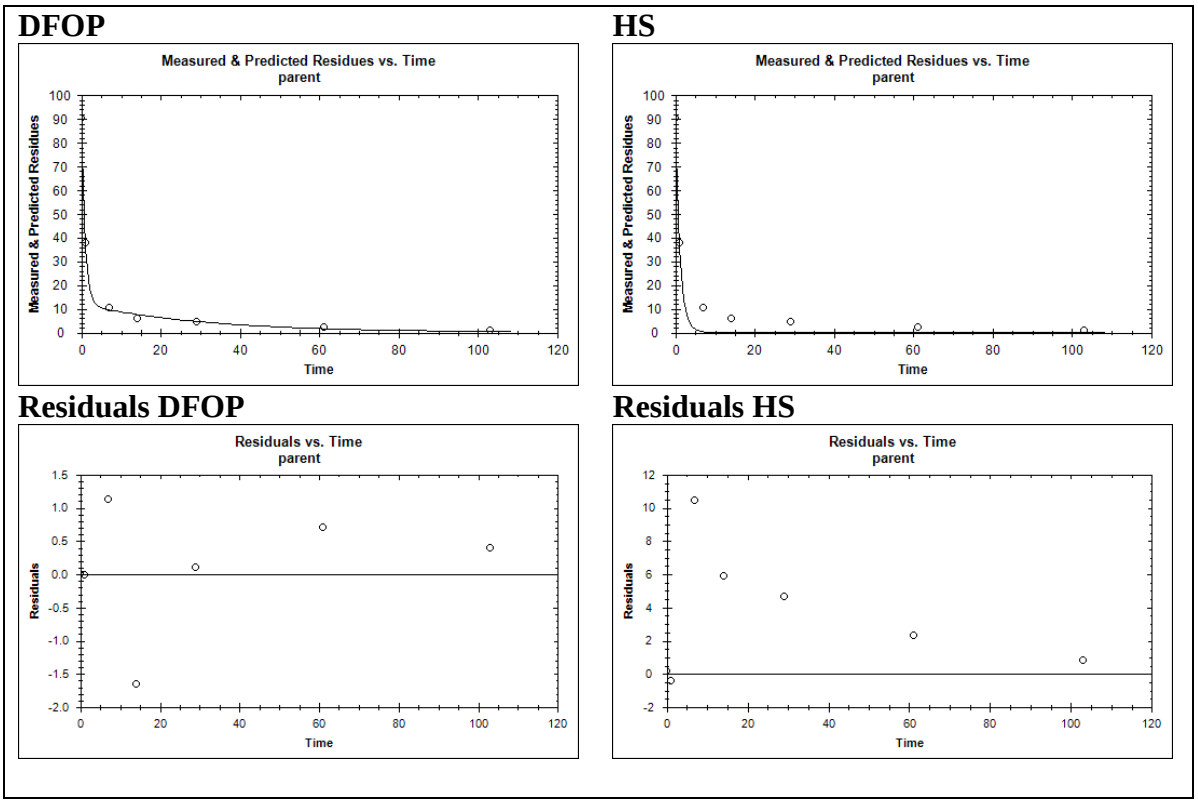


Figure B.8.4-73: Optimised DFOP and HS kinetic models for dissipation of AMPA in the water phase (McEwen (2004, BVL no 2310275), system A)

Table B.8.4-67: Kinetic evaluation for dissipation of AMPA in the water phase (McEwen (2004, BVL no 2310275), system A)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	90.3500	5.9178	< 0.001	18.1	0.80	2.67
	k_SFO	0.8631	0.1742	0.002			
FOMC	M_0	90.50343	0.64577	< 0.001	1.9	0.69	8.87
	α	0.77222	0.03334	< 0.001			
	β	0.47354	0.04366	< 0.001			
DFOP	M0	90.49692	1.25303	< 0.001	3.6	0.77	8.58
	k1_DFOP	1.10153	0.07057	< 0.001			
	k2_DFOP	0.03317	0.01178	0.034			
	g	0.86718	0.02408	< 0.001			
HS	M0	90.349983	n.c.	n.c.	21.6	<0.0001	n.c.
	k1_HS	0.005519	n.c.	n.c.			
	k2_HS	0.863088	n.c.	n.c.			
	tb	0.000000	n.c.	n.c.			

n.c.: could not be calculated by KinGUII

McEwen (2004, BVL no 2310275), system B

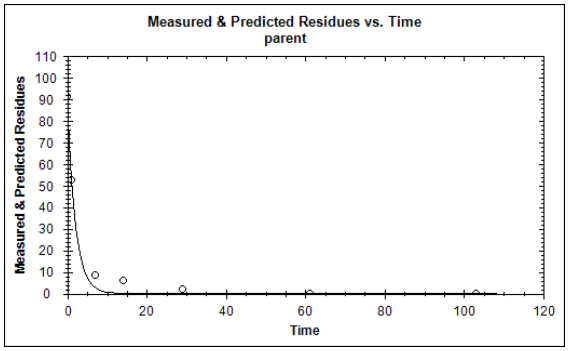
Total system:

Due to problems analysing extracts obtained from Sediment B, believed to be caused by endogenous co-extracted material disrupting the ion-exchange chromatography, this system was excluded from kinetic evaluation.

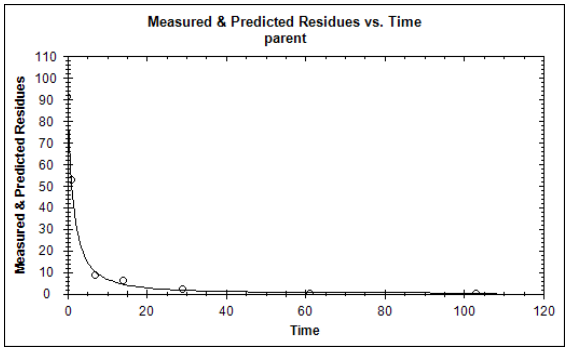
Water phase:
In a first comparison, FOMC model was much more appropriate than SFO, both visually and statistically. HS model could not be optimised by KinGUII, whereas DFOP also showed an acceptable fit. Since DFOP provided a better visual fit and a smaller χ^2-error than FOMC, persistence and modelling end points were derived from DFOP.

Sediment phase:
Due to problems analysing extracts obtained from Sediment B, believed to be caused by endogenous co-extracted material disrupting the ion-exchange chromatography, Sediment B was excluded from kinetic evaluation.

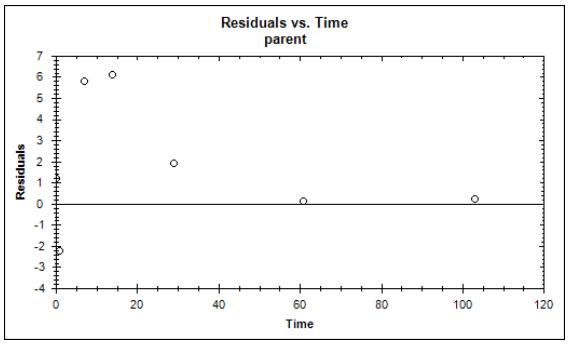
SFO



FOMC



Residuals SFO



Residuals FOMC

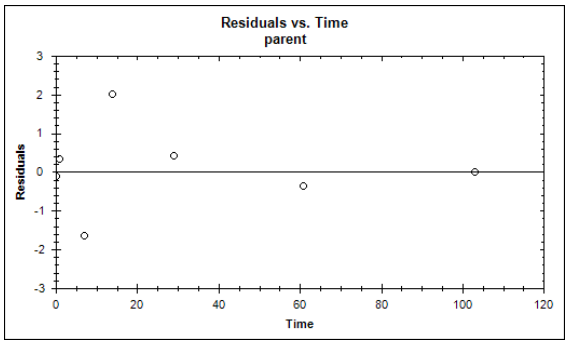


Figure B.8.4-74: Optimised SFO and FOMC kinetic models for dissipation of AMPA in the water phase (McEwen (2004, BVL no 2310275), system B)

DFOP	**HS**

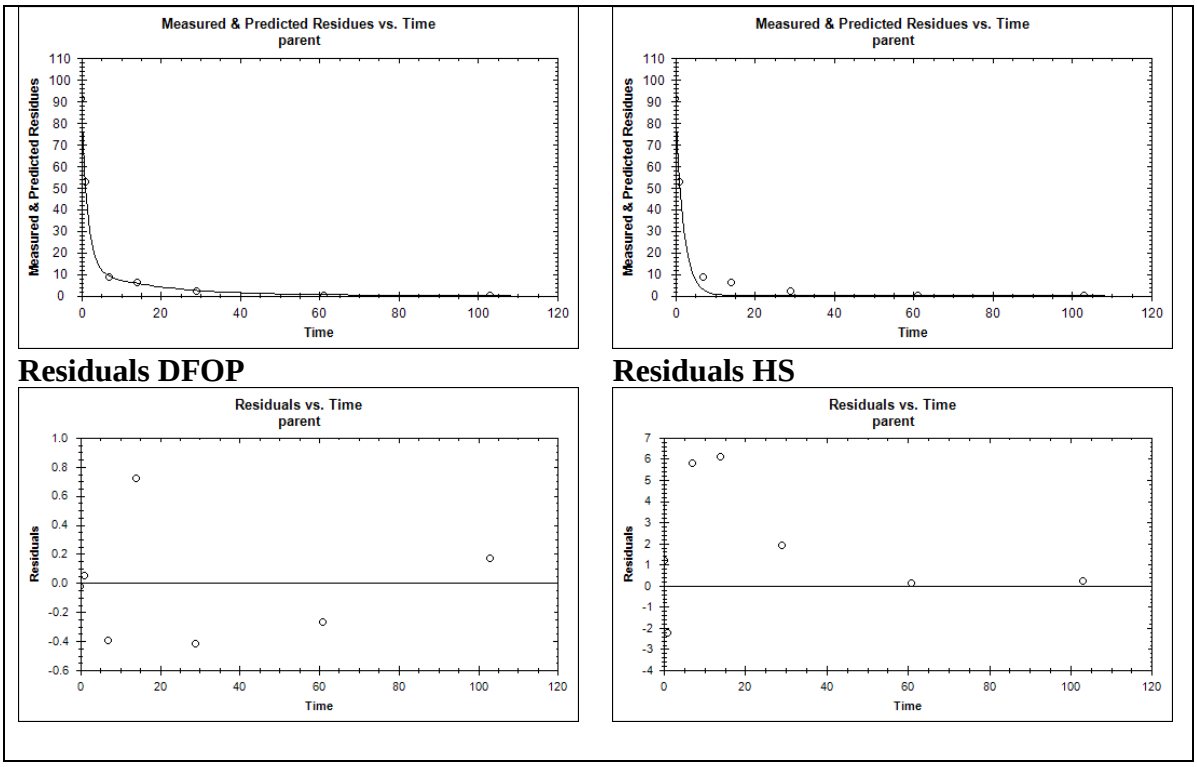


Figure B.8.4-75: Optimised DFOP and HS kinetic models for dissipation of AMPA in the water phase (McEwen (2004, BVL no 2310275), system B)

Table B.8.4-68: Kinetic evaluation for dissipation of AMPA in the water phase (McEwen (2004, BVL no 2310275), system B)

Kinetic model	Parameter	Optimised value	Std. error	p-value (t-test)	% error χ^2 test	DT_{50} (d)	DT_{90} (d)
SFO	M_0	90.03345	4.16427	< 0.001	11.7	1.40	4.66
	k_SFO	0.49431	0.09524	0.002			
FOMC	M_0	91.3006	1.3383	< 0.001	3.8	1.30	7.71
	α	1.6111	0.2356	0.001			
	β	2.4271	0.5072	0.004			
DFOP	M0	91.223294	0.560975	< 0.001	1.5	1.28	6.87
	k1_DFOP	0.057362	0.009511	0.005			
	k2_DFOP	0.653217	0.021203	< 0.001			
	g	0.133904	0.016281	0.002			
HS	M0	90.03	5.376	< 0.001	14.0	< 0.0001	n.c.
	k1_HS	0.02250	n.c.	n.c.			
	k2_HS	0.4943	0.1229	0.014			
	tb	8.120e-09	n.c.	n.c.			

n.c.: could not be calculated by KinGUII

Review of the selected endpoints for persistence and modelling

The goodness of the fits presented in Partsch (2012, BVL no 2316005) was proved by the RMS to derive reliable end points for persistence and modelling according to FOCUS Kinetics guidance (2006). First, a visual inspection of diagrams of estimated and measured values vs. time and of the diagrams of residuals versus time was assessed. Generally, the error at

which χ^2-test is passed of less than 15 % as well as a t-test probability of 0.1 was considered to indicate a good fit for water/sediment systems (FOCUS Kinetics guidance 2006). Additionally, it was checked that the standard errors of the parameters estimated were sufficiently low (at least < 100 %) to assure that these are reliable. However, on a case-by-case basis, the water/sediment endpoints were still be considered acceptable even though one or more of the indices are not met, as long as the end point value can be considered conservative, or can be justified based on weight of evidence from other studies. The following corrections concerning the selected end points for persistence and/or modelling were made by the RMS:

Möllerfeld and Römbke (1993, BVL no 1934113), system Sandy Sediment
Sediment phase:

SFO did not adequately represent the dissipation process of glyphosate from the sediment phase, because of the high p-value and standard error of the rate constant k_SFO as well as the relatively poor visual fit. Due to the fact that the standard error of the parameters estimated with regard to the tested bi-phasic models DFOP and HS were not sufficiently low, preference was given to FOMC as persistence endpoint, but not as modelling endpoint, since 10 % of the initially measured concentration was not reached within the experimental period.

Möllerfeld and Römbke (1993, BVL no 1934113), system Loamy Sediment
Total system:

SFO was considered not acceptable, because the degradation of glyphosate from the total system was overestimated beginning from day 60 of the study (see visual fit). The p-values and standard errors of most of the parameters estimated concerning the bi-phasic models DFOP and HS were not sufficiently low, therefore FOMC was chosen as persistence endpoint, but not as modelling endpoint, since 10 % of the initially measured concentration was not reached within the experimental period.

Heintze (1996, BVL no 1939626), system Pond
Total system:

In view of visuell assessment FOMC showed a more appropriate fit to the data than SFO, but the standard errors of α, β (FOMC) were not considered acceptable. Taking these facts into account, the RMS also recommends testing bi-phasic models. Visual fit of both, DFOP and HS, are considered acceptable, but fitting HS provided better statistical parameters particularly with regard to p-values and standard errors, therefore HS was selected as persistence and modelling end point, even though FOMC provided a much more conservative DT_{90}.

Heintze (1996, BVL no 1939626), system Creek
Total system:

FOMC was not more appropriate than SFO regarding the χ^2-errors, the standard errors of the parameters estimated and the visual fit (in particular, as the degradation of glyphosate was overestimated at the end of the study by using FOMC). Therefore, testing of further bi-phasic models (HS, DFOP) is not considered relevant in order to derive persistence end points. SFO was also selected as persistence end point.

Water phase:

Again, FOMC was not more appropriate than SFO regarding the χ^2-errors, the standard errors of the parameters estimated and the visual fit (in particular, as the degradation of glyphosate was overestimated at the end of the study by using FOMC). Therefore, testing of further bi-phasic models (HS, DFOP) is not considered relevant in order to derive persistence endpoints. SFO was also selected as persistence end point. Besides, the p-value and the standard error of k1_DFOP were not sufficiently low.

Sediment phase:

Regarding the visual fits and the statistical parameters χ^2-error, p-value and standard error none of the models tested (SFO, HS) was considered reliable to describe the dissipation of glyphosate in the sediment phase.

Feser-Zügner (2002, BVL no 2310270), system Rückhaltebecken
Sediment phase:

The statistical parameters of the models tested (SFO, FOMC, HS) did not indicate a good fit. Therefore, none of the models was selected for persistence or modelling.

Feser-Zügner (2002, BVL no 2310270), system Schäphysen
Total system:

No reliable fit could be achieved for the degradation of AMPA in the total system according to the criteria named above.

Water phase:

The dissipation of AMPA from the water phase was not adequate represented by SFO. Both models, HS and DFOP, revealed larger χ^2-errors than FOMC, as well as partly high p-values and standard errors of the parameters estimated. Therefore, FOMC was also selected as modelling end point.

Knoch (2003, BVL no 2310273), system Unter-Widdersheim
Water phase:
Comparison of SFO and FOMC indicated the adequate fit of FOMC model to the residue data of the water phase. The additionally testes models HS and DFOP could not be seen as improvement, as HS revealed a χ^2-error similar to the one of SFO model and a high p-value for k2_HS and DFOP revealed a high p-value for k1_DFOP. Furthermore, the dissipation of AMPA from the water phase was overestimated at the end of the study by DFOP model. Therefore, FOMC was chosen as persistence and modelling end point.

Knoch and Spirlet (1999, BVL no 1934122), system Bickenbach
Total system:

Since 10 % of the initially measured concentration was not reached within the experimental period, SFO DT_{50} for modelling was recalculated from slower k-rate of HS kinetics according to FOCUS Kinetics guidance (2006).

Knoch and Spirlet (1999, BVL no 1934122), system Unter-Widdersheim

Total system:

FOMC was more appropriate than SFO when fitting both models to total system residue data. Although SFO passed statistical criteria, its visual fit was very poor: especially, the degradation of AMPA from the water phase was overestimated at the end of the study. HS and DFOP showed a small χ^2-error and a good visual fit with non-systematic small residuals at all sampling dates. However, neither HS nor DFOP passed statistical criteria (p-value and standard error). In consequence, persistence end points were derived from FOMC model.

End points with regard to the P criterion of potential POP, vPvB or PBT substances
Persistence end points were derived by the Notifier selecting best fit kinetics. Apart from the fact that the RMS has corrected some end points (see above), the RMS recommends using the endpoints from FOMC, HS or DFOP kinetics only to determine whether various aquatic ecotoxicology studies are triggered, but not to decide whether the P criterion is fulfilled. To maintain a conservative approach with regard to the P criterion, the RMS proposes to use recalculated SFO DT_{50} values in cases where FOMC, DFOP or HS kinetics gave the best fit. Thereby, SFO DT_{50} values should be recalculated from the overall DT_{90} of biphasic kinetics (DT_{90}/3.32) according to EFSA DG SANCO working document on evidence needed to identify POP, PBT and vPvB properties for pesticides from 25.09.2012- rev.3.

Summary of persistence and modelling end points of glyphosate and AMPA selected by the RMS

All DT_{50} values for glyphosate and AMPA in water/sediment systems selected by the RMS for persistence and modelling are summarised in Table B.8.4-69 and Table B.8.4-70.

Table B.8.4-69: Persistence and modelling end points of glyphosate in water/sediment systems selected by the RMS

Study	**System**	**Persistence endpoints at Level P-I**				**Modelling endpoints at Level P-I**	
		Model	**DT_{50} 4) (days)**	**DT_{90} 4) (days)**	**SFO DT_{50} 4) (days)**	**Model**	**SFO DT_{50} 4) (days)**
Glyphosate (total system)							
Bowler & Johnson (1999), BVL no 2154357	Cache	FOMC	8.47	45.89	13.82 5)	FOMC	13.82 1)
	Putah	DFOP	210.66	976.54	294.14 5)	DFOP	329.85 2)
Möllerfeld & Römbke (1993), BVL no 1934113	Loamy Sediment	FOMC	70.48	∞	- 6)	- 3)	- 3)
	Sandy Sediment	HS	16.03	346.81	104.46 5)	HS	154.19 2)
Heintze (1996), BVL no 1939626	Creek	SFO	16.78	55.74	16.78	SFO	16.78
	Pond	HS	67.45	281.39	84.76 5)	HS	92.42 2)
Muttzall (1993), BVL no 1982136	TNO	FOMC	93.06	>1000	>301.20 5)	- 3)	- 3)
	Kromme Rijn	DFOP	28.86	232.92	70.16 5)	DFOP	88.67 2)
Minimum			-	-	13.82		13.82
Maximum			-	-	301.20		329.85
Geometric mean (n = 7/6 8))			**-**	**-**	**74.52**		**67.74**
Glyphosate (water phase)							
Bowler & Johnson (1999), BVL no 2154357	Cache	HS	4.98	26.84	8.08 5)	SFO	6.94
	Putah	FOMC	8.25	72.40	21.81 5)	FOMC	21.81 1)

Möllerfeld & Römbke (1993), BVL no 1934113	Loamy Sediment	FOMC	1.06	24.11	7.26[5)]	FOMC	7.26[1)]
	Sandy Sediment	DFOP	2.03	22.63	6.82[5)]	DFOP	6.82[1)]
Heintze (1996), BVL no 1939626	Creek	SFO	13.15	43.67	13.15	SFO	13.15
	Pond	HS	1.00	26.89	8.10[5)]	HS	8.10[1)]
Muttzall (1993), BVL no 1982136	TNO	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]
	Kromme Rijn	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]
Minimum			-	-	6.82		6.82
Maximum			-	-	21.81		21.81
Geometric mean (n = 6)			**-**	**-**	**9.88**		**9.63**
Glyphosate (sediment phase)							
Bowler & Johnson (1999), BVL no 2154357	Cache	SFO	34.05	113.10	34.05	SFO	34.05
	Putah	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]
Möllerfeld & Römbke (1993), BVL no 1934113	Loamy Sediment	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]
	Sandy Sediment	FOMC	383.86	∞	-[6)]	-[3)]	-[3)]
Heintze (1996), BVL no 1939626	Creek	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]
	Pond	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]
Muttzall (1993), BVL no 1982136	TNO	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]
	Kromme Rijn	SFO	75.61	251.16	75.61	SFO	75.61
Minimum			-	-	34.05		34.05
Maximum			-	-	75.61		75.61
Geometric mean (n = 2)			**-**	**-**	-[7)]		-[7)]

1) Back-calculated from DT_{90} of bi-phasic model (DT_{90}/3.32)
2) Calculated from slower k-rate
3) no reliable fit achieved
4) DT_{50} = degradation DT_{50} for total system, Dissipation DT_{50} for water and sediment phase
5) Back-calculated SFO to derive end points for P criteria (SFO DT_{50} = DT_{90}/3.32)
6) Back-calculation of SFO DT_{50} not possible
7) Not calculated, since a sufficient number of DT_{50} values were not available
8) Number of values for deriving persistence end point (SFO DT_{50}) and the modelling end point

Table B.8.4-70: Persistence and modelling end points of AMPA in water/sediment systems selected by the RMS

Study	**System**	**Persistence end points at Level P-I**				**Modelling end points at Level P-I**	
		Model	**DT_{50}[4)] (days)**	**DT_{90}[4)] (days)**	**SFO DT_{50}[4)] (days)**	**Model**	**SFO DT_{50}[4)] (days)**
AMPA (total system)							
Feser-Zügner (2002), BVL no 2310270	Rückhaltebecken	FOMC	13.80	1513.00	455.72[5)]	DFOP	102.87[2)]
	Schäphysen	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]
Knoch (2003), BVL no 2310273	Bickenbach	HS	10.54	191.25	57.61[5)]	HS	77.83[2)]
	Unter-Widdersheim	HS	77.36	307.19	92.53[5)]	HS	98.98[2)]
Knoch & Spirlet (1999) BVL no 1934122	Bickenbach	HS	44.53	205.21	61.81[5)]	HS	69.31[2)]
	Unter-Widdersheim	FOMC	20.13	885.03	266.58[5)]	-[3)]	-[3)]
McEwen (2004), BVL no 2310275	A	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]
	B	-[6)]	-[6)]	-[6)]	-[6)]	-[6)]	-[6)]
Minimum			-	-	57.61		69.31
Maximum			-	-	455.72		102.87
Geometric mean (n = 5/4[7)])			**-**	**-**	**131.97**		**86.09**
AMPA (water phase)							
Feser-Zügner (2002), BVL no 2310270	Rückhaltebecken	FOMC	2.20	22.50	6.78[5)]	FOMC	6.78[1)]
	Schäphysen	FOMC	1.00	7.80	2.35[5)]	FOMC	2.35[1)]

Knoch (2003), BVL no 2310273	Bickenbach	DFOP	2.54	47.57	14.33[5)]	DFOP	14.33[1)]
	Unter-Widdersheim	FOMC	2.13	26.31	7.92[5)]	FOMC	7.92[1)]
Knoch & Spirlet (1999) BVL no 1934122	Bickenbach	DFOP	6.59	51.47	15.50[5)]	DFOP	15.50[1)]
	Unter-Widdersheim	HS	2.02	17.15	5.17[5)]	HS	5.17[1)]
McEwen (2004), BVL no 2310275	A	FOMC	0.69	8.87	2.67[5)]	FOMC	2.67[1)]
	B	DFOP	1.28	6.87	2.07[5)]	DFOP	2.07[1)]
Minimum			-	-	2.07		2.07
Maximum			-	-	15.50		15.50
Geometric mean (n = 8)			**-**	**-**	**5.47**		**5.47**
AMPA (sediment phase)							
Feser-Zügner (2002), BVL no 2310270	Rückhaltebecken	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]
	Schäphysen	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]
Knoch (2003), BVL no 2310273	Bickenbach	-[8)]	-[8)]	-[8)]	-[8)]	-[8)]	-[8)]
	Unter-Widdersheim	-[8)]	-[8)]	-[8)]	-[8)]	-[8)]	-[8)]
Knoch & Spirlet (1999) BVL no 1934122	Bickenbach	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]
	Unter-Widdersheim	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]
McEwen (2004), BVL no 2310275	A	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]	-[3)]
	B	-[6)]	-[6)]	-[6)]	-[6)]	-[6)]	-[6)]

1) Back-calculated from DT_{90} of bi-phasic model (DT_{90}/3.32)
2) Calculated from slower k-rate
3) no reliable fit achieved
4) DT_{50} = degradation DT_{50} for total system, Dissipation DT_{50} for water and sediment phase
5) Back-calculated SFO to derive end points for P criteria (SFO DT_{50} = DT_{90}/3.32)
6) excluded from kinetic evaluation due to analytical problems
7) Number of values for deriving persistence end point (SFO DT_{50}) and the modelling end point
8) excluded from kinetic evaluation due to different amounts of AMPA in the sediment reported in the study

B.8.4.5 Other studies on fate and beaviour in water (Review of open literature)

B.8.4.5.1 Photochemical degradation (open literature)

One article is available on the behaviour of glyphosate when subjected to irradiation in the presence of H_2O_2: Manassero et al. (2010) reported that the reaction rate depends on the initial herbicide concentration and has an optimum plateau of a hydrogen peroxide to glyphosate molar concentration ratio between 7 and 19. The expected non linear dependence on the irradiation rate was observed. Test results are of minor relevance as they are not obtained by a standard test procedure. They can be assessed as additional information only.

Two additional studies reported on the degradation of glyphosate in aqeous medium by photocatalytic mineralization (Chen *et al.*, 2012 and Zhang *et al.*, 2012). The authors analyzed the effect of the presence of UV/TiO_2 or titania nanotubes on degradation, respectively.

For a more detailed descriptions and the evaluation of the open literature studies, please refer to B.8.10. The respective references are listed there as well.

B.8.4.5.2 Water/sediment study (open literature)

Two studies (Degenhardt *et al.*, 2012 and Tsui and Chu, 2008) are available on outdoor freshwater investigations. The results are of no relevance for the glyphosate assessment since the experimental sites were in wetland outside the EU. This information might be used as additional information only.

Another article (Mercurio et al., 2014) was available regarding the degradation of glyphosate in seawater. The results are less relevant for the glyphosate assessment since the authors investigated degradation of glyphosate in natural seawater without any sediment, which does not reflect the conditions of the presence of glyphosate in the aquatic environment.

For a more detailed descriptions and the evaluation of the open literature studies, please refer to B.8.10. The respective references are listed there as well.

B.8.5 Impact on water treatment procedures (Annex IIIA 9.6.4)

B.8.5.1 Information on drinking water treatment procedures

The 2001 EU glyphosate evaluation concludes "Glyphosate and its metabolite AMPA may be classified as low mobile in soil. Therefore, it is not necessary to consider the impact on water treatment procedures." However, the impact of glyphosate and its removal from water sources by treatment processes commonly used for production of drinking water have been extensively studied: There are two literature reviews (Jönsson, 2012, BVL no 2316001, Jönsson and Camm, 2010, BVL no 2316003) on the removal of glyphosate and AMPA during standard water treatment processes available, which are described in detail below.

In summary, the literature review of Jönsson (2012, BVL no 2316001) showed that glyphosate and AMPA can be removed by bank filtration and slow sand filtration to a varying extent. More specifically the elimination of glyphosate by these water treatment processes was between 17 % and > 75 % of the initial amounts. In comparison to glyphosate, AMPA was eliminated more effective; the removal efficiency was between 46 % and 95 %. Biological activated carbon is not expected to effectively remove glyphosate and AMPA from water. Jönsson and Camm (2010, BVL no 2316003) showed that chlorination, ozonation as well as the combination of O_3 and H_2O_2 can provide a high degree of removal for glyphosate and AMPA under typical conditions used in water treatment, whereas bankside or dune infiltration, coagulation and clarification would each contribute some removal, but alone would not provide a secure barrier in relation to meeting a 0.1 µg l^{-1} standard. Furthermore, adsorption using Granular Activated Carbon (GAC) and Powdered Activated Charcoal (PAC) does not provide an effective barrier to glyphosate or AMPA. AOP, more precisely, UV and H_2O_2 or ozone and H_2O_2 showed good removal of glyphosate, but not of AMPA. Almost complete removal of AMPA (but not of glyphosate) was achieved by ClO_2.

KIIA 9.6.4/1 (Jönsson, 2012, BVL no 2316001)

Annex point:	KIIA 9.6.4/01
Author(s):	Jönsson, J.
Title:	Review of sustainable water treatment
Date:	March, 2012
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Yes

Materials and Methods
This is a desk study, based on literature review.

Results and discussion

The results are of the literature study are summarised in Table B.8.5-1.

Table B.8.5-1: Overview on different treatments and results

Compound	Redox conditions	Process	C_0 (µg/l)	Residence time (days)	Removal (%)	Reference
Glyphosate	Anaerobic	BF	0.07	30-300	>30	Post et al., 2000
Glyphosate	Anaerobic	BF	0.12	Unknown	17	Post et al., 2000
Glyphosate	Aerobic & anaerobic	BF and SSF	<0.05-0.09	Unknown	~50	Schlett et al., 2005
Glyphosate	Aerobic	SSF	<0.05-0.19	Unknown	>75	Schlett et al., 2005
Glyphosate	Aerobic	Soil column[2)]	10	25	>95	Lindner et al., 2000
Glyphosate	Aerobic	Batch river water[2)]	150000	72	40-72	Zaranyika and Nyandoro, 1993
Glyphosate	Aerobic	Batch soil samples[2)]	100 µg/g	50	95	Getenga and Kengara, 2004
Glyphosate	Initially aerobic	Batch river water[2)]	100	56	54-89	Wang et al., 1994
Glyphosate	Initially aerobic	BF	3.5, 11.6	Half life 7-10 days	80[1)]	Krause et al. (2009)
AMPA	Anaerobic	BF	0.46	30-300	46-87	Post et al., 2000
AMPA	Anaerobic	BF	0.54	450-2000	85-94	Stuyfzand et al., 2004
AMPA	Anaerobic	BF	1.8	Unknown	90	Post et al., 2000
AMPA	Aerobic & anaerobic	BF and SSF	0.23-1.1	Unknown	≤ 95	Schlett et al., 2005
AMPA	Aerobic	SSF	0.08-0.7	Unknown	>89	Schlett et al., 2005
AMPA	Aerobic	SSF	0.04-0.48	Unknown	≤ 94	Hopman et al., 1995

BF=Bank Filtration, SSF=Slow Sand Filter, C_0=initial concentration
80 % removal under test conditions, but removal to < 0.1 µg/L identified from modelling for high initial concentrations with half life shown
Degradation in batches of spiked river water or removal by soil samples in batches or flow-through columns

This table shows that bank filtration (BF) and slow sand filtration (SSF) can remove glyphosate and AMPA. The general trend seems to be that the concentration of AMPA is higher than glyphosate but that AMPA is more readily degraded or removed. The degradation of glyphosate seems to benefit from aerobic conditions whereas AMPA is readily degraded both under aerobic and anaerobic conditions. Although no information was found for biological activated carbon (BAC), previous tests by WRc using powdered activated carbon (PAC) suggest that granulated activated carbon (GAC) alone is not effective for adsorption of either glyphosate or AMPA. The mechanism of biological removal of organic contaminants by BAC could rely on adsorption of the organics before biodegradation and BAC may therefore be less effective for glyphosate and AMPA.

Conclusions

Glyphosate and AMPA can be removed by sustainable water treatments like BF and SSF. Although no information is available for BAC, this treatment is not expected to effectively remove glyphosate and AMPA from raw water.

RMS Comment
The results of the literature study are not required for the purposes of the environmental fate assessment; they do only provide additional information.

KIIA 9.6.4/2 (Jönsson and Camm, 2010, BVL no 2316003)

Annex point:	KIIA 9.6.4/2
Author(s):	Jönsson, J., Camm, R.
Title:	Removal of glyphosate and AMPA by water treatment
Date:	October, 2010
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Yes

Materials and Methods
The first part of the study was based on a literature review.

Laboratory Batch tests were carried out to investigate the removal of glyphosate and AMPA by oxidation using ozone, O_3 in combination with hydrogen peroxide (H_2O_2), Cl_2 and ClO_2, and by adsorption using PAC.

The stock solutions of glyphosate and AMPA were prepared by dissolving high purity solids in deionised water. For the AMPA tests using PAC and for all glyphosate tests, a 10 litre sample of Swindon tap water was spiked with 3 µg l^{-1} of either glyphosate or AMPA. Samples of the spiked water were taken for analysis to establish the initial concentration of pesticides, and the remainder of the spiked water was used in the tests. This concentration was agreed as the maximum concentration likely to be found in raw waters.

Ozonation alone: A one litre sub-sample of spiked water was ozonated using a pilot-scale O_3 generator and a bubble diffuser stone. Following ozonation for 10 s, the O_3 residual was measured immediately, and at 5 minute intervals, during a 15 minute contact time. At the end of the contact period, the residual ozone was quenched with sodium thiosulphate ($Na_2S_2O_3$).

Ozonation with hydrogen peroxide: A further set of tests were carried out with simultaneous use of O_3 and H_2O_2, at 0.5 and 1.0 mg l^{-1}. The ozonation conditions were identical to the test with O_3 alone with the temperature kept constant at 15 ±0.6 °C. The O_3 residual was measured immediately after ozonation, and then at 5 minute intervals, during a 15 minute contact time. At the end of the contact period, the residual O_3 was quenched with sodium thiosulphate.

Chlorine: One-litre samples of the spiked water were dosed with sodium hypochlorite (NaClO) at 1.5 mg Cl_2 l^{-1}. The dosed water was left for 30 minutes at the desired temperature. At the end of the contact period, the residual Cl_2 was measured and then quenched with sodium thiosulphate.

Chlorine dioxide: The tests with Cl_2 was repeated but with ClO_2 as the oxidant. The ClO_2 was added as crushed tablets, supplied by Accepta. The initial target concentration of ClO_2 was 1 mg l^{-1}.

Powdered Activated Charcoal (PAC): Tests were carried out to investigate the performance of 3 different types of coal based PAC. One litre samples of the spiked water were dosed with the three different PAC at 5, 15 and 25 mg l^{-1}. The dosed water was left stirring for 1 hour, to keep the PAC in suspension. The samples were then filtered through GF/C grade filter paper to remove the carbon, prior to analysis.

UV and hydrogen peroxide: After a literature review, an advanced oxidation process (AOP) pilot plant test was performed. The AOP pilot rig, consists of in-line hydrogen peroxide dosing, ozone dosing and a UV reactor, which can be used individually or in combination. The retention time in the unit is around 30-60 s, most of which is in the UV reactor. Two tests were performed, each with the same matrix of operating conditions. For the first test, the feed tap water was spiked with glyphosate to the same target concentration as previous tests, 3 µg/L. For the second test, the feed water was spiked with AMPA to a target concentration of 3 µg/L. The matrix of operating conditions was:

UV, dose 740 mJ/cm^{-2}
UV, 1240 mJ cm^{-2}
UV, 740 mJ cm^{-2}, + H_2O_2, 5 mg l^{-1}
UV, 1240 mJ cm^{-2}, + H_2O_2, 5 mg l^{-1}
O_3, 2 mg l^{-1} + H_2O_2, 2 mg l^{-1}
O_3, 2 mg l^{-1}
O_3, 2 mg l^{-1}, with sample left standing for 9 minutes to provide ozone contact time

In the oxidation tests with glyphosate spiking, the treated water samples were also analysed for AMPA, to investigate whether any of the glyphosate was degraded only to AMPA by oxidation.

All samples were analysed for glyphosate and AMPA using the following method. Water samples are treated with fmoc (9-fluorenylmethyl chloroformate) derivatising reagent prior to concentration by solid phase extraction. The extracts are then analysed by high performance liquid chromatography (HPLC) using primary mass spectroscopic (MS) detection in negative ion electrospray with selective ion monitoring. The reported limit of detection (LOD) for the method is 0.006 µg l^{-1} for glyphosate and 0.016 µg l^{-1} for AMPA.

Results and discussion
The results are of the literature study are summarised in Table B.8.5-2.

Table B.8.5-2: Removal of glyphosate and AMPA by treatment processes

Treatment Process	Glyphosate removal (%)	AMPA removal (%)
Bank and dune filtration	20 to 50	25 to 95
Aluminium coagulant and clarification	15 to 40	20 to 25
	Not a reliable barrier for glyphosate and AMPA	
Iron coagulant and clari-	40 to 70	20 to 85

fication	Not a reliable barrier for glyphosate and AMPA	
Slow sand filtration	Insufficient information but likely to be less effective than bank or dune filtration and therefore of little practical benefit	
Chlorination	74 to > 99	40 to >95
	Likely to provide the main barrier to glyphosate and AMPA at most water treatment works	
Chlorine dioxide	Insufficient information but not expected to be effective	
Ozonation	60 to >99	25 to 95
	Provides an additional barrier at works where already installed for other pesticides and micropollutants	
UV irradiation	No information found. Highly unlikely to be effective alone at doses used in water treatment. May be effective at very high doses not currently used for water treatment.	
UV/hydrogen peroxide	Little direct information available, but indications that a combination of UV with hydrogen peroxide would be effective	
Advanced oxidation	No information found, but would be expected to be effective through free radical mechanisms. Little used for water treatment at the present time.	
Activated carbon adsorption	10 to 90	20 to 70
	Higher removals relate to virgin GAC and are unlikely to be achieved under practical conditions. Not a reliable barrier for glyphosate and AMPA.	
Membrane filtration	>90 (NF/RO) >50 (UF)* *depending on membrane type	>95 (NF/RO) No information found for UF
	Membrane processes not widely used in water treatment, and unlikely to be installed solely as a barrier to pesticides and other organic micropollutants.	
Air stripping	No information found, not expected to be effective based on chemical characteristics.	

Chlorine, which is one of the most common disinfectants (oxidants) used in water treatment in Europe, can provide a high degree of removal (>95 %) for glyphosate and AMPA under typical conditions used in water treatment. Ozonation, another oxidant commonly used for pesticide removal, can also provide more than 95 % removal of glyphosate and AMPA. Bankside or dune infiltration, coagulation/ clarification/ filtration and slow sand filtration, commonly used in water treatment, would each contribute some removal, but alone would not provide a secure barrier in relation to meeting a 0.1 µg l^{-1} standard. Depending on the treatment processes used, waterworks which include chlorine could deal with between 1 and 4 µg l^{-1} (glyphosate + AMPA) in the raw water to maintain less than 0.1 µg l^{-1} in the treated water, but if the works also includes ozonation total concentrations of above 30 µg l^{-1} could be treated. The most common water treatment process installed for removal of pesticides worldwide is adsorption using granular activated carbon (GAC). However, this does not provide an effective barrier to glyphosate or AMPA.

The results of the laboratory batch tests are summarised in Table B.8.5-3.

Table B.8.5-3: Removal of glyphosate and AMPA during laboratory batch tests

Treatment Process	Glyphosate	AMPA

	Conditions	**Removal (%)**	**Conditions**	**Removal (%)**
Ozonation	T° ~ 7, 11, 15 °C Residual O_3 : 0.41, 0.76 mg/L Conc.: 2.6 , 2.7 µg/L	>99	T° ~ 5, 10, 13 °C Residual O_3 : 0.5 mg/L Conc.: 3.65 µg/L	>99
Ozonation + hydrogen peroxide	H_2O_2 : 0.5, 1.0 mg/L Residual O_3 : 0.09, 0.18, 0.24, 0.46 mg/L Conc.: 2.6, 2.7 µg/L	98 - >99	H_2O_2 : 0.5, 1.0 mg/L Residual O3 : 0.16, 0.04 mg/L Conc.: 3.65 µg/L	85 – 97
Chlorine	pH : 6, 7.5, 8.5 T° : 5, 10, 20°C Residual Cl_2 : 1.4 mg/L Conc.: 2.17, 3.17 µg/L	71 - >99 (removal decrease with T°)	pH : 6, 7., 8.5 T° : 6, 10, 20 °C Residual Cl_2: 1.4 mg/L Conc.: 3.65 µg/L	>99
Chlorine dioxide	pH : 6-8.6 T° : 4-23 °C Residual ClO_2 : 0.4-1.35 mg/L Conc.: 2.17, 2.47 µg/L	17-93 % (removal decrease with T°)	pH : 6.2 - 8.4 T° : 6, 10, 20 °C Residual Cl_2: 1 - 1.4 mg/L Conc.: 3.65 µg/L	>99
Powdered Activated Charcoal	PAC conc : 5, 15, 25 mg/L Conc.: 3.13 µg/L	0-22	PAC conc : 5, 15, 25 mg/L Conc.: 3.13 µg/L	0-31

Ozone was highly effective in removing both glyphosate and AMPA and virtually complete removal was achieved under all conditions tested. The combination of O_3/H_2O_2 was as effective as O_3 alone in removing glyphosate and complete removal was achieved under all conditions tested. The Cl_2 results indicate that changes in pH had little influence on the removal of glyphosate by chlorine; but that the temperature had a larger influence on the glyphosate removal with 71 % being removed at 5 °C compared to 96 % at 20 °C. The removal of glyphosate by ClO_2 was less effective than that for other oxidants, ranging from 17 % to 93 %. The highest removal was seen for the low pH samples (pH ~6) with high temperature (~22 °C) and high ClO_2 concentrations. However, complete removal of AMPA was seen for all conditions tested, suggesting AMPA is readily removed by ClO_2. Although the results are somewhat scattered, it is clear the investigated PACs would not provide adequate removal of glyphosate and AMPA.

The results of the Advanced Oxidation Processes (AOP) pilot plant tests are summarised in Table B.8.5-4.

Table B.8.5-4: Removal of glyphosate and AMPA during AOP pilot plant tests

Treatment Process	**Glyphosate**		**AMPA**	
	Conditions	**Removal (%)**	**Conditions**	**Removal (%)**
UV (740 mJ/cm^2)	1 min contact time Conc.: 1.72 µg/L	25	1 min contact time Conc.: 2.31 µg/L	6
UV (1240 mJ/cm^2)	1 min contact time Conc.: 1.72 µg/L	36	1 min contact time Conc.: 2.31 µg/L	32
UV (740 mJ/cm^2) H_2O_2 5 mg/L	1 min contact time Conc.: 1.72 µg/L Residual H_2O_2 : 5.5 mg/L	88	1 min contact time Conc.: 2.31 µg/L Residual H_2O_2: 4.98 mg/L	8
UV (1240 mJ/cm^2)	1 min contact time	91	1 min contact time	6

H_2O_2 5 mg/L	Conc.: 1.72 µg/L Residual H_2O_2 : 5.16 mg/L		Conc.: 2.31 µg/L Residual H_2O_2: 4.65 mg/L	
O_3 2 mg/L **H_2O_2 2 mg/L**	1 min contact time Conc.: 1.72 µg/L	96->99 (duplicates)	1 min contact time Conc.: 2.31 µg/L	35
O_3 2 mg/L	1 min contact time Conc.: 1.72 µg/L Residual O_3 : 0.83 mg/L	96	1 min contact time Conc.: 2.31 µg/L Residual O_3 : 0.90 mg/L	63
O_3 2 mg/L	10 min contact time Conc.: 1.72 µg/L Residual O_3 : 0.36 mg/L	97	10 min contact time Conc.: 2.31 µg/L Residual O_3 :0.52 mg/L	>99

UV alone did not remove significant amounts of glyphosate or AMPA even at relatively high doses (1240 mJ cm^{-2}). UV in conjunction with H_2O_2 showed good removal of glyphosate (approximately 90 %) but significant amounts of AMPA was also generated and AMPA was poorly removed by this treatment (<10 %).

An applied dose of 2 mg l^{-1} ozone removed greater than 95 % of the glyphosate, this removal being essentially achieved within 1 minute contact time after the eductor. This indicates a very high rate of reaction with molecular ozone. This is consistent with the previous laboratory tests with ozone, but the earlier laboratory tests showed better removal of AMPA (literature search) by ozone alone. Near complete removal of glyphosate was also seen for the combination of ozone and H_2O_2, >95 % was removed after 1 minute. Again, the removal of AMPA was not as good as in previous tests, but this is probably an effect of the short contact time (1 minute).

Conclusions

The majority of water treatment works worldwide use chlorine for disinfection, and therefore has an effective barrier for glyphosate and AMPA. Exceptions to this would be works in mainland Europe which use chlorine dioxide for disinfection and protection of the water in distribution, instead of chlorine. In this situation, the removal of glyphosate would be more variable, but complete removal of AMPA (>99 %) could be expected.

The most common water treatment process installed for removal of pesticides worldwide is adsorption using granular activated carbon. This system does not to provide an effective barrier to glyphosate and AMPA. However, at many treatment works ozone is also installed for removal of pesticides or other organic micropollutants, and would be highly effective for glyphosate and AMPA removal under the dose and contact time conditions typically used. As expected, UV disinfection processes are not very effective in removing glyphosate and AMPA, but in combination with hydrogen peroxide could provide an efficient barrier for glyphosate (but not AMPA).

Other processes commonly used in water treatment (bankside or dune infiltration, coagulation/ clarification/ filtration and slow sand filtration) would each contribute some removal, but each process in isolation is unlikely to provide a secure barrier in relation to meeting a 0.1 µg l^{-1} standard.

RMS Comment

The results of the study are not required for the purposes of the environmental fate assessment; they do only provide additional information.

B.8.5.2 Effects on biological methods for sewage treatment

The evaluation of open literature shows that glyphosate and AMPA can reach sewage water treatment plants (Ghanem et al., 2007; Augustin, 2003), but there are no information available about effects on biological methods for sewage treatment. Furthermore, it remains unclear if the findings originate from a technically correct and regulation compliant use of the respective plant protection products in agricultural areas.

B.8.5.3 Other studies on impact on water treatment procedures (Review of open literature)

Several publications also confirm that glyphosate and AMPA can be removed from raw water by treatment processes. In particular, it was shown that glyphosate is readily removed from water by ozonation, chlorination, the modified electro-Fenton-like (EF-like) with Mn^{2+}, UV/TiO_2, the combination of hydrogen peroxide and UV radiation as well as reverse osmosis and subsequent activated carbon filtration. Cl_2 and O_3, are highly effective in degrading glyphosate and AMPA. Removal by coagulation is ineffective as a barrier against contamination in drinking water. Removal or degradation by bank filtration, slow sand filtration and ClO_2 is variable. In addition, there is a potential of ~~bank filtration~~ rapid sand filtration to eliminate glyphosate from water. Other treatment processes, e.g. reverse osmosis exclusively or Granular Activated Carbon (GAC) filtration, are less effective. Ozonation is also a suitable method for the removal of AMPA from water, whereas GAC filtration is not efficient in removing AMPA. The chlorination products of glyphosate are similar to those expected from chlorination of amino acids, proteins, peptides, and many other natural organic matters present in drinking water. Furthermore, it seems that glyphosate degradation by the combination of hydrogen peroxide and UV radiation does not lead to stable toxic end products.

For a more detailed descriptions and the evaluation of the open literature studies, please refer to B.8.10. The respective references are listed there as well.

B.8.6 Predicted environmental concentrations in surface water and in groundwater (PEC_{GW}; PEC_{SW}) (Annex IIIA1 9.6; 9.7)

B.8.6.1 Predicted environmental concentrations (PEC_{GW}) in groundwater

KIIIA 9.6/01 (Anyusheva, 2012, BVL no 2315999)

Annex point:	IIIA 9.6/01
Author(s):	Anyusheva, M.
Title:	Predicted environmental concentrations of glyphosate and its metabolite AMPA in groundwater (PEC_{GW}) using FOCUS PEARL 4.4.4 and FOCUS PELMO 4.4.3 following application to various crops in the EU
Date:	April 25, 2012
Guideline(s):	FOCUS (2002): FOCUS groundwater scenarios in the EU review of active substances. Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000, version 2002. FOCUS (2009): Assessing potential for movement of active substances and their metabolites to ground water in the EU. Report of the FOCUS Ground Water Work Group, EC Document Reference San-

	co/13144/2010 version 1, 604 pp. FOCUS (2011b): Generic guidance for Tier 1 FOCUS ground water assessments, version 2.0. FOCUS groundwater scenarios working group.
Deviations:	No
GLP:	No (modelling study: does not contain laboratory work)
Acceptability:	Due to the fact that RMS has corrected the end points for modelling provided by the applicant, the study is not acceptable. Therefore, new PECgw calculations were performed by the RMS.

Material and methods

Not applicable, no materials were used as this study is a computer simulation.

The groundwater leaching models FOCUS PEARL 4.4.4 and FOCUS PELMO 4.4.3 (FOCUS, 2002, 2009, 2011b) were used for calculation of PEC_{GW}.

The exposure assessment was based on a representative use pattern derived from the representative GAP. The application scenarios selected for the leaching assessment are summarised in Table B.8.6-1.

Table B.8.6-1: Application scenarios considered for simulations

Crop	**FOCUSgwcrop**	**Application rate (g glyphosate acid/ha)**	**No. of appl.**	**Maximum yearly application rate (g glyphosate acid/ha)**	**Min. interval between applications (d)**	**Application period**
Various crops (autumn application)	Winter cereals	2160	2	4320	21	Pre-planting /pre-emergence
Various crops (spring + autumn application)	Spring cereals	2160	2	4320	21	Pre-planting /pre-emergence + post-harvest
Various crops (spring application)	Potatoes	2160	2	4320	21	Pre-planting /pre-emergence
Orchards, citrus, vines, tree nuts	Apples	2880 + 720 + 720	1 + 1 + 1	4320	28	Post-emergence of weeds

For winter cereals and potato crops, the applications dates (representing pre-planting applications as defined in the representative GAP) were chosen using the planting dates defined for the respective crop (FOCUS, 2002, 2011b). The second application was set to the day of planting; and the first application to 21 days before planting according to the minimum application interval. For spring cereals, the first application was assumed to take place 14 days before seeding and the second application 14 days after harvest in order to account for a real-

istic worst case pre-emergence + pre- or post-harvest use in summer. The first application in apples was based on a realistic estimate of the first possible date of weed emergence and the following two applications were arranged with respect to the minimum interval of 28 days. A worst-case zero interception was assumed for all applications. The detailed application dates are summarised in Table B.8.6-2.

Table B.8.6-2: Application dates used for modelling

Crop	Scenario	1st application	2nd application	3rd application
Winter cereals	Châteaudun	29-Sep	20-Oct	n/a
	Hamburg	21-Sep	12-Oct	n/a
	Jokioinen	20-Aug	10-Sep	n/a
	Kremsmünster	04-Oct	25-Oct	n/a
	Okehampton	16-Sep	07-Oct	n/a
	Piacenza	04-Nov	25-Nov	n/a
	Porto	25-Oct	15-Nov	n/a
	Sevilla	25-Oct	15-Nov	n/a
	Thiva	25-Oct	15-Nov	n/a
Spring cereals	Châteaudun	6-Feb	3-Aug	n/a
	Hamburg	24-Feb	3-Sep	n/a
	Jokioinen	23-Apr	8-Sep	n/a
	Kremsmünster	24-Feb	3-Sep	n/a
	Okehampton	11-Mar	3-Sep	n/a
	Porto	6-Feb	3-Aug	n/a
Potatoes	Châteaudun	25-Mar	15-Apr	n/a
	Hamburg	10-Apr	1-May	n/a
	Jokioinen	24-Apr	15-May	n/a
	Kremsmünster	10-Apr	1-May	n/a
	Okehampton	25-Mar	15-Apr	n/a
	Piacenza	11-Mar	1-Apr	n/a
	Porto	7-Feb	28-Feb	n/a
	Sevilla	25-Dec	15-Jan	n/a
	Thiva	25-Jan	15-Feb	n/a
Apples	Châteaudun	15-Feb	15-Mar	12-Apr
	Hamburg	01-Mar	29-Mar	26-Apr
	Jokioinen	15-Mar	12-Apr	10-May
	Kremsmünster	01-Mar	29-Mar	26-Apr
	Okehampton	01-Mar	29-Mar	26-Apr
	Piacenza	15-Feb	15-Mar	12-Apr
	Porto	15-Feb	15-Mar	12-Apr

	Sevilla	15-Feb	15-Mar	12-Apr
	Thiva	15-Feb	15-Mar	12-Apr

n/a = not analysed

Parameters used for estimating the predicted environmental concentration of glyphosate acid and its metabolite AMPA in groundwater (PEC_{GW}) are presented in Table B.8.6-3. The normalised geometric mean laboratory half-lives of glyphosate and AMPA of 12.8 days (n = 13) and 55.7 days (n = 7) were used for modelling purposes. The arithmetic mean formation fraction of 0.34 (n = 12) was used for formation of AMPA. Regarding sorption, the arithmetic mean K_{foc} and 1/n value of glyphosate (16810 mL/g and 0.80, n = 15) and AMPA (9749 mL/g and 0.80, n = 16) were selected for modelling purposes. Since FOCUS PEARL requires input of K_{fom}, the K_{fom} value was derived by dividing the K_{foc} by 1.724. In order to obtain a conservative simulation, plant uptake factors for both glyphosate and AMPA were set to zero. Apart from the input parameters explicitly discussed, all variables in the models were left at their default values.

Table B.8.6-3: Summary of input parameters for glyphosate acid and its metabolite AMPA for the leaching simulation models FOCUS PEARL 4.4.4 and FOCUS PELMO 4.4.3

Parameter	**Unit**	**Glyphosate acid**	**AMPA**
Molecular Mass	g/mol	169	111
Water solubility (20 °C)	mg/L	10500	10500 [1)]
Vapour pressure (25 °C)	Pa	1.31×10^{-5}	1.31×10^{-5} [1)]
$DegT_{50}$ soil[2)]	days	12.8	55.7
K_{foc}	mL/g	16810	9749
K_{fom}	mL/g	9750.6	5654.9
Freundlich exponent (1/n)	-	0.80	0.80
Formation fraction	-	-	0.34
Plant uptake factor	-	0 [2)]	0 [2)]

1) parent data
2) worst-case assumption

Results and discussion

In all simulations the PEC_{GW} values of glyphosate acid and AMPA at 1 m soil depth were below the groundwater threshold value of 0.1 µg/L. The results of the calculations with FOCUS PEARL 4.4.4 and FOCUS PELMO 4.4.3 predicting the leaching of glyphosate acid and AMPA are presented in Table B.8.6-4 and Table B.8.6-5 respectively.

Table B.8.6-4: PEC_{GW} of glyphosate acid and AMPA at 1 m soil depth (simulations with PEARL 4.4.4)

Crop	**Scenario**	**PEC_{GW} (µg/L)**	
		Glyphosate acid	**AMPA**
Winter cereals	Châteaudun	<0.001	<0.001
	Hamburg	<0.001	<0.001
	Jokioinen	<0.001	<0.001
	Kremsmünster	<0.001	<0.001
	Okehampton	<0.001	<0.001
	Piacenza	<0.001	<0.001
	Porto	<0.001	<0.001
	Sevilla	<0.001	<0.001
	Châteaudun	<0.001	<0.001
Spring cereals	Châteaudun	<0.001	<0.001
	Hamburg	<0.001	<0.001
	Jokioinen	<0.001	<0.001
	Kremsmünster	<0.001	<0.001
	Okehampton	<0.001	<0.001
	Porto	<0.001	<0.001
Potatoes	Châteaudun	<0.001	<0.001
	Hamburg	<0.001	<0.001
	Jokioinen	<0.001	<0.001
	Kremsmünster	<0.001	<0.001
	Okehampton	<0.001	<0.001
	Piacenza	<0.001	<0.001
	Porto	<0.001	<0.001
	Sevilla	<0.001	<0.001
	Châteaudun	<0.001	<0.001
Apples	Châteaudun	<0.001	<0.001
	Hamburg	<0.001	<0.001
	Jokioinen	<0.001	<0.001
	Kremsmünster	<0.001	<0.001
	Okehampton	<0.001	<0.001
	Piacenza	<0.001	<0.001
	Porto	<0.001	<0.001
	Sevilla	<0.001	<0.001
	Châteaudun	<0.001	<0.001

Table B.8.6-5: PEC_{GW} of glyphosate acid and AMPA at 1 m soil depth (simulations with PELMO 4.4.3)

Crop	**Scenario**	**PEC_{GW} (µg/L)**	
		Glyphosate acid	**AMPA**
Winter cereals	Châteaudun	<0.001	<0.001
	Hamburg	<0.001	<0.001
	Jokioinen	<0.001	<0.001
	Kremsmünster	<0.001	<0.001
	Okehampton	<0.001	<0.001
	Piacenza	<0.001	<0.001
	Porto	<0.001	<0.001
	Sevilla	<0.001	<0.001
	Châteaudun	<0.001	<0.001
Spring cereals	Châteaudun	<0.001	<0.001
	Hamburg	<0.001	<0.001
	Jokioinen	<0.001	<0.001
	Kremsmünster	<0.001	<0.001
	Okehampton	<0.001	<0.001
	Porto	<0.001	<0.001
Potatoes	Châteaudun	<0.001	<0.001
	Hamburg	<0.001	<0.001
	Jokioinen	<0.001	<0.001
	Kremsmünster	<0.001	<0.001
	Okehampton	<0.001	<0.001
	Piacenza	<0.001	<0.001
	Porto	<0.001	<0.001
	Sevilla	<0.001	<0.001
	Châteaudun	<0.001	<0.001
Apples	Châteaudun	<0.001	<0.001
	Hamburg	<0.001	<0.001
	Jokioinen	<0.001	<0.001
	Kremsmünster	<0.001	<0.001
	Okehampton	<0.001	<0.001
	Piacenza	<0.001	<0.001
	Porto	<0.001	<0.001
	Sevilla	<0.001	<0.001
	Châteaudun	<0.001	<0.001

Conclusions

Predicted environmental concentrations for glyphosate acid and its metabolite AMPA in groundwater (PEC_{GW}) were calculated for use on various crops in Europe according to guidance provided by FOCUS using two models, FOCUS PEARL 4.4.4 and FOCUS PELMO 4.4.3 (FOCUS, 2002, 2009, 2011b).

In all simulations the 80th percentile PEC_{GW} values of glyphosate acid and AMPA at 1 m soil depth were below the groundwater threshold value of 0.1µg/L. Therefore, it can be concluded that the use of glyphosate is not likely to pose an unacceptable risk to groundwater if the active substance is used in compliance with the label recommendations.

Additional field testing is not required based on results showed above.

RMS Comment

Due to the fact that RMS has corrected the end points for modelling provided by the applicant, the study is not acceptable. Therefore, new PEC_{GW} calculations were performed by the RMS.

PEC_{GW} calculations by the RMS

In order to calculate PEC_{GW}, the RMS used the FOCUS groundwater model FOCUS PELMO 4.4.3. Detailed information on substance properties and input parameters, application scenarios and the modelling results are presented in the following.

Substance properties and input parameters

Parameters used for estimating the predicted environmental concentration of glyphosate and its metabolite AMPA in groundwater (PEC_{GW}) are presented in Table B.8.6-6 and Table B.8.6-7.

Table B.8.6-6: Input parameters for glyphosate for the leaching simulation with FOCUS PELMO 4.4.3

Parameter	Unit	Glyphosate	Remarks/Reference
Molecular Mass	g/mol	169	-
Water solubility (20°C)	mg/L	10500 (20 °C)	Vol. 3 B2 (Physical and chemical properties)
Vapour pressure (25°C)	Pa	1.31×10^{-5} (25 °C)	Vol. 3 B2 (Physical and chemical properties)
$DegT_{50}$ soil	days	~~21.03~~ 20.51	Geometric mean of the DT_{50} values of all soils
K_{oc}	mL/g	15844 [2)]	Arithmetic mean of the K_{oc} values of all soils
Freundlich exponent (1/n)	-	0.914 [2)]	Arithmetic mean of the 1/n values of all soils
Plant uptake factor	-	0 [1)]	

[1)] worst-case assumption

[2)] As an outcome of the discussions in the Pesticides Peer Review Meeting 126 the arithmetic mean Kfoc and 1/n values for glyphosate have been amended. The experts agreed that for the EU approval no additional exposure calculations were

necessary, due to the limited effect on the mean endpoints. The correct values to be used in future PEC simulations are Kfoc:15388 and 1/n: 0.93

Table B.8.6-7: Input parameters for AMPA for the leaching simulation with FOCUS PELMO 4.4.3

Parameter	Unit	AMPA	Remarks/Reference
Molecular Mass	g/mol	111	-
Water solubility (20°C)	mg/L	10500 (20 °C) [1)]	Vol. 3 B2 (Physical and chemical properties)
Vapour pressure (25°C)	Pa	1.31×10^{-5} (25 °C) [1)]	Vol. 3 B2 (Physical and chemical properties)
$DegT_{50}$ soil	days	88.84	Geometric mean of the DT_{50} values of all soils
K_{oc}	mL/g	9749	Arithmetic mean of the K_{oc} values of all soils
Freundlich exponent (1/n)	-	0.853 [3)]	Arithmetic mean of the 1/n values of all soils
Formation fraction	-	~~0.37~~ 0.36	Arithmetic mean of all formation fractions
Plant uptake factor	-	0 [2)]	

1) parent data
2) worst-case assumption
3) As an outcome of the discussions in the Pesticides Peer Review Meeting 126 the arithmetic mean 1/n value for AMPA has been amended. The experts agreed that for the EU approval no additional exposure calculations were necessary, due to the limited effect on the mean endpoints. The correct arithmetic mean 1/n value to be used in future PEC simulations is 0.81

Application scenarios

The exposure assessment was based on a representative use pattern derived from the representative GAP as described by Anyusheva (2012, BVL no 2315999). The application scenarios selected for the leaching assessment are summarised in Table B.8.6-8.

Table B.8.6-8: Application scenarios considered for simulations

Crop	$FOCUS_{gw}$crop	Application rate (g glyphosate acid/ha)	No. of appl.	Maximum yearly application rate (g glyphosate acid/ha)	Min. interval between applications (d)	Application period
Various crops (autumn application)	Winter cereals	2160	2	4320	21	Pre-planting /pre-emergence
Various crops (spring + autumn applica-	Spring cereals	2160	2	4320	21	Pre-planting /pre-emergence + post-harvest

tion)						
Various crops (spring application)	Potatoes	2160	2	4320	21	Pre-planting /pre-emergence
Orchards, citrus, vines, tree nuts	Pome fruit (Apples)	2880 + 720 + 720	1 + 1 + 1	4320	28	Post-emergence of weeds

For winter cereals and potato crops, the applications dates were chosen using the planting dates defined for the respective crop. The second application was set to the day of planting; and the first application to 21 days before planting according to the minimum application interval. For spring cereals, the first application was assumed to take place 14 days before seeding and the second application 14 days after harvest in order to account for a realistic worst case pre-emergence and pre- or post-harvest use in summer. The first application in apples was based on a realistic estimate of the first possible date of weed emergence and the following two applications were arranged with respect to the minimum interval of 28 days.

A worst-case zero interception was assumed for all applications.

The application dates were selected according to the software AppDate and are summarised in Table B.8.6-9.

Table B.8.6-9: Application dates used for modelling

Crop	Scenario	1st application	2nd application	3rd application
Winter cereals	Châteaudun	29-Sep	20-Oct	-
	Hamburg	21-Sep	12-Oct	-
	Jokioinen	20-Aug	10-Sep	-
	Kremsmünster	21-Sep	12-Oct	-
	Okehampton	16-Sep	07-Oct	-
	Piacenza	04-Nov	25-Nov	-
	Porto	04-Nov	25-Nov	-
	Sevilla	25-Oct	15-Nov	-
	Thiva	25-Oct	15-Nov	-
Spring cereals	Châteaudun	6-Feb	3-Aug	-
	Hamburg	24-Feb	3-Sep	-
	Jokioinen	23-Apr	8-Sep	-
	Kremsmünster	24-Feb	3-Sep	-
	Okehampton	11-Mar	3-Sep	-
	Porto	6-Feb	3-Aug	-
Potatoes	Châteaudun	25-Mar	15-Apr	-
	Hamburg	10-Apr	1-May	-
	Jokioinen	24-Apr	15-May	-
	Kremsmünster	10-Apr	1-May	-
	Okehampton	25-Mar	15-Apr	-
	Piacenza	20-Mar	10-Apr	-
	Porto	7-Feb	28-Feb	-

	Sevilla	25-Dec	15-Jan	-
	Thiva	25-Jan	15-Feb	-
Apples	Châteaudun	15-Feb	15-Mar	12-Apr
	Hamburg	01-Mar	29-Mar	26-Apr
	Jokioinen	15-Mar	12-Apr	10-May
	Kremsmünster	01-Mar	29-Mar	26-Apr
	Okehampton	01-Mar	29-Mar	26-Apr
	Piacenza	15-Feb	15-Mar	12-Apr
	Porto	15-Feb	15-Mar	12-Apr
	Sevilla	15-Feb	15-Mar	12-Apr
	Thiva	15-Feb	15-Mar	12-Apr

PEC_{GW} values of glyphosate and AMPA

The results of PEC_{GW} calculation, i.e. the 80th percentile PEC_{GW} at 1 m soil depth (mg L^{-1}), for glyphosate and AMPA are given in Table B.8.6-10.

Table B.8.6-10: PEC_{GW} of glyphosate and AMPA at 1 m soil depth (simulations with FOCUS PELMO 4.4.3)

Crop	**Scenario**	**80th percentile PEC_{GW} at 1 m soil depth (mg L-1)**	
		Glyphosate	**AMPA**
Winter cereals	Châteaudun	<0.001	<0.001
	Hamburg	<0.001	<0.001
	Jokioinen	<0.001	<0.001
	Kremsmünster	<0.001	<0.001
	Okehampton	<0.001	<0.001
	Piacenza	<0.001	<0.001
	Porto	<0.001	<0.001
	Sevilla	<0.001	<0.001
	Thiva	<0.001	<0.001
Spring cereals	Châteaudun	<0.001	<0.001
	Hamburg	<0.001	<0.001
	Jokioinen	<0.001	<0.001
	Kremsmünster	<0.001	<0.001
	Okehampton	<0.001	<0.001
	Porto	<0.001	<0.001
Potatoes	Châteaudun	<0.001	<0.001
	Hamburg	<0.001	<0.001
	Jokioinen	<0.001	<0.001
	Kremsmünster	<0.001	<0.001
	Okehampton	<0.001	<0.001
	Piacenza	<0.001	<0.001
	Porto	<0.001	<0.001
	Sevilla	<0.001	<0.001
	Thiva	<0.001	<0.001

Apples	Châteaudun	<0.001	<0.001
	Hamburg	<0.001	<0.001
	Jokioinen	<0.001	<0.001
	Kremsmünster	<0.001	<0.001
	Okehampton	<0.001	<0.001
	Piacenza	<0.001	<0.001
	Porto	<0.001	<0.001
	Sevilla	<0.001	<0.001
	Thiva	<0.001	<0.001

In all simulations the 80th percentile PEC_{GW} values of glyphosate acid and AMPA at 1 m soil depth were below the groundwater threshold value of 0.1 µg/L indicating that the use of glyphosate as intended is not likely to pose an unacceptable risk to groundwater via direct leaching.

One has to admit that monitoring results in Europe (see B.8.6.3.1) point out glyphosate occurence in water resources. Regarding the pathways of glyphosate into groundwater when used for agricultural purposes as intended, RMS considers that groundwater contamination > 0.1 µg/L via direct leaching is generally not expected as the substance is strongly adsorbed to soil particles. Exceptions may be made, e.g. for preferential flow as described in point B.8.2.4.2 and B.8.2.4.3. Sanchís et al. (2012, BVL no 2537361, see B.8.6.3.1) describe that surface waters exist in 10 out of 11 sampling sites where glyphosate was (at least partly) detected. Due to this fact, surface run-off and/or drainage into these waters with subsequent bank filtration into groundwater cannot be excluded as pathway.

B.8.6.2 Predicted environmental concentrations in surface water (PEC_{SW}) and sediment (PEC_{Sed})

KIIIA 9.7/01& KIIIA 9.8/01 (Anyusheva, 2012, BVL no 2316007)

Annex point:	IIIA 9.7/01 IIIA 9.8/01
Author(s):	Anyusheva, M.
Title:	Predicted environmental concentrations of glyphosate and its metabolites AMPA and HMPA in surface water (PEC_{SW}) and sediment (PEC_{Sed}) following application to various crops in the EU Report no. 303605-3
Date:	27/04/2012
Guideline(s):	FOCUS (2001): FOCUS Surface Water Scenarios in the EU Evaluation Process under 91 /414/EEC. Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001 rev. 2, 245 pp FOCUS (2011 b): Generic guidance for FOCUS surface water Scenarios, version 1.0.
Deviations:	None
GLP:	No- not subject to GLP
Acceptability:	Only acceptable for HMPA, since RMS derived different degradation and adsorption endpoints for glyphosate and AMPA; besides additional FOCUSsw crops were simulated by RMS

Materials and Methods

Step 1 and Step 2 PEC_{SW} and PEC_{Sed} values for glyphosate and its metabolites AMPA, HMPA were derived using the model FOCUS Steps1-2 (version 2.1). Additionally, Step 3 values were derived for glyphosate using the model FOCUS SWASH (version 3.1). Step 3 simulations for glyphosate were carried out using FOCUS SWASH (version 3.1) with the Chemical Application Method (CAM) 1 (soil linear) including a standard application depth of 4 cm.

The substance related input parameter for glyphosate and its metabolites AMPA and HMPA used for FOCUS SW simulations are presented in Table B.8.6-11.

Table B.8.6-11: Substance related input data of glyphosate and its metabolites AMPA and HMPA used for the simulations

Parameter	Glyphosate	AMPA	HMPA
Molecular Mass (g/mol)	169	111	112
Water solubility (mg/L)	10500 (pH 2, 20°C)	10500 (pH 2, 20°C)*	-**
DT_{50} soil (days)	12.8	55.7	-
DT_{50} water (days)	61.2	83.7	-**
DT_{50} sediment (days)	61.2/ 1000***	83.7	-**
DT_{50} total system (days)	61.2	83.7	-**
K_{foc} (mL/g)	16810	9749	-**
Max. occurrence soil (%)	n/a	50.1	0
Max occurrence w/sed (%)	n/a	27.26	10
Freundlich exponent 1/n (-)	0.80	-	-**
Plant uptake factor (-)	0	-	-**

* parent data
** Not relevant (calculations based on PEC_{SW} and PEC_{Sed} of glyphosate and only maximum values were determined)
*** values used in Step 1-2/ Step 3 calculations
n/a not applicable

The exposure assessment was based on a representative use pattern derived from the representative GAP.

The application scenarios selected for simulations at Step 1 and 2 are summarised in Table B.8.6-12. The FOCUSsw crops winter and spring cereals were used to represent the use of glyphosate in all transplanted or seeded field crops. Since, glyphosate is not intended to be sprayed onto the perennials itself but onto the weeds below, the $FOCUS_{sw}$ crop option 'application, hand (crop <50 cm)' was used to represent the use of glyphosate in orchard crops, vines including citrus & nut trees (= perennial crops). A single application of 4320 g as/ha was assumed as worst case for the application of glyphosate in perennial crops.

Table B.8.6-12: Application scenarios for simulations at Step 1-2

Crop	FOCUSsw crop	Application rate (g a.s./ha)	No. of applications	Min. intervall (d)	FOCUSsw scenarios	Interception
Various crops (field)	Winter and spring cereals	2160	2	21	North & South Europe	No interception
Various crops (perennial)	Appln., hand (crop<50cm)	4320	1	-	North & South Europe	No interception

The application scenarios selected for simulations at Step 3 are summarised in Table B.8.1-19. Simulations were performed for both single and multiple applications according to FOCUS (2011).

Table B.8.6-13: Application scenarios considered for simulations at Step 3

Crop	FOCUSsw crop	Application rate (g as/ha)	No. of applications	Min. intervall (d)	Application period
Various crops (autumn application)	Winter cereals	2160	2	21	pre-planting/ pre-emergence
		2160	1**	-	
Various crops (autumn application)	Spring cereals	2160	2	21***	pre-planting/ pre-emergence + post-harvest
		2160	1*	-	
		2160	1**	-	
Various crops (spring application)	Potatoes	2160	2		pre-planting/ pre-emergence
		2160	1		
Orchards, citrus, vines, tree nuts	Pome/ stone fruit	2880/ 720/ 720	3	28	Post-emergence of weeds
		2880	1*	-	

* Single application in spring (according to FOCUS, 2001, 2011 b, a single application should be evaluated additionally)

** Single application in autumn (according to FOCUS, 2001, 2011 b, a single application should be evaluated additionally)

*** For the determination of the application window, an application interval of 160 days was assumed in order to reflect preemergence application in spring and the following application in autumn

The 'ground spray' application method was chosen for winter and spring cereals as well as potatoes.

For pome/stone fruit, the 'granular' application method was chosen to exclude simulation with the high spray drift rates which are assigned for application to pome/stone fruit trees itself but are unrealistic for the application around the base of trunks as intended for glyphosate. Since the 'granular' application method implies a drift rate of 0, the realistic drift rates had to be set manually. Therefore, a special drift assessment was performed (FOCUS, 2001, 2011b) when running the TOXSWA program. The spray drift rates were amended manually in the respective *.twa files of the model. The spray drift rates used were based on the FOCUS spray drift calculator for the FOCUSsw crop 'appl. hand (crop <50 cm)' and are shown in Table B.8.6-14.

Table B.8.6-14: Drift rates for application around the base of the pome/stone fruit tree trunks

FOCUSsw crop	Application rate	Amended drift rate from $FOCUS_{sw}$ crop 'appl. Hand (crop <50 cm)' (%)		
		Pond	Ditch	Stream**
Pome/ stone fruit	1 x 2880	0.219	1.927	1.716
Pome/stone fruit	2880/ 720/ 720*	0.154	1.405	1.245

* Since the model Steps 1-2 can only handle one application rate within a multiple application scenario, the highest single dose rates were used for modelling as a worst-case approach.

** Including a factor of 1.2 to account for pesticide input from the upstream catchment (FOCUS 2001, 2011b).

Appropriate application windows for winter cereals as well as for potatoes assuming pre-planting/ pre-emergence application of glyphosate were chosen based on recommended emergence dates of the crops specified in FOCUS (2001, 2011b). For spring cereals application

windows were chosen assuming one pre-planting/ pre-emergence application and one post harvest application of glyphosate using emergence and harvest dates as specified in FOCUS (2001, 2011b). The beginning of the application window for pome/stone fruit was assigned to each individual scenario by expert judgement. The application windows for winter and spring cereals, potatoes and pome/stone fruit trees and the actual application dates within the application windows as determined by the Pesticide Application Timer (PAT) are presented in Table B.8.6-15, Table B.8.6-16, Table B.8.6-17 and Table B.8.6-18.

Table B.8.6-15: Application windows and application dates for single and multiple application of glyphosate to winter cereals

Scenario	1 x 2.16 kg as/ha		2 x 2.16 kg as/ha	
	Possible window of application	**Application date set by PAT**	**Possible window of application**	**Application date set by PAT**
D1	26-Aug (238) – 25-Sep (268)	28-Aug	05-Aug (217) – 25-Sep (268)	05-Aug/28-Aug
D2	25-Sep (268)– 25-Okt (298)	09-Oct	04-Sep (247) – 25-Okt (298)	04-Sep/09-Oct
D3	22-Okt (295) – 21-Nov (325)	05-Nov	01-Okt (274) – 21-Nov (325)	30-Sep/05-Nov
D4	23-Aug (235) – 22-Sep (265)	27-Aug	02-Aug (214) – 22-Sep (265)	27-Aug/18-Sep
D5	11-Okt (284) – 10-Nov (314)	11-Oct	20-Sep (263) – 10-Nov (314)	14-Oct/04-Nov
D6	31-Okt (304) – 30-Nov (334)	31-Oct	10-Okt (283) – 30-Nov (334)	10-Oct/31-Oct
R1	13-Okt (286) – 12-Nov (316)	13-Oct	22-Sep (265) – 12-Nov (316)	22-Sep/13-Oct
R3	01-Nov (305) – 01-Dez (335)	15-Nov	11-Oct (284) – 01-Dez(335)	27-Oct/17-Nov
R4	11-Okt (284) – 10-Nov (314)	18-Oct	20-Sep (263) – 10-Nov (314)	23-Sep/15-Oct

Table B.8.6-16: Application windows and application dates for single and multiple application of glyphosate to spring cereals

Scenario	1 x 2.16 kg as/ha (spring)		1 x 2.16 kg as/ha (autumn)		2 x 2.16 kg as/ha (1 spring + 1 autumn)	
	Possible window of application	**Application date set by PAT**	**Possible window of application**	**Application date set by PAT**	**Possible window of application**	**Application date set by PAT**
D1	05-Apr (95) – 05-May (125)	25-Apr	18-Sep (261) - 18-Oct (291)	03-Oct	05-Apr (95) – 12-Oct (285)	25-Apr/03-Oct
D3	02-Mrz (61)– 01-Apr (91)	01-Mar	03-Sep (246) - 03-Oct (276)	26-Sep	02-Mrz (61)– 08-Sep (251)	01-Mar/18-Aug
D4	27-Mrz (86) – 26-Apr (116)	18-Apr	09-Sep (252) - 09-Oct (282)	10-Sep	27-Mrz (86) – 03-Oct (285)	18-Apr/28-Sep

D5	13-Feb (44) – 15-Mrz (74)	21-Feb	03-Aug (215) - 02-Sep (245)	04-Aug	13-Feb (44) – 22-Aug (234)	21-Feb/04-Aug
R4	13-Feb (44)– 15-Mrz (74)	02-Mar	29-Jul (210) - 28-Aug (240)	31-Jul	13-Feb (44)– 22-Aug (234)	02-Mar/09-Aug

Table B.8.6-17: Application windows and application dates for single and multiple application of glyphosate to potatoes

Crop	Scenario	1 x 2.16 kg as/ha		2 x 2.16 kg as/ha	
		Possible window of application	Application date set by PAT	Possible window of application	Application date set by PAT
potatoes, pre-planting	D3	10-Apr (100) – 10-May (130)	09-Apr	20-Mar (79) – 10-May (130)	04-Apr/04-May
	D4	22-Apr (112) – 22-May (142)	17-May	01-Apr (91) – 22-May (142)	18-Apr/17-May
	D6, early	11-Mar (70)– 10-Apr (100)	14-Mar	15-Feb (46) – 10-Apr (100)	27-Feb/02-Apr
	D6, late	06-Jul (187)– 05-Aug (217)	25-Jul	15-Jun (166) – 05-Aug (217)	23-Jun/25-Jul
	R1	05-Apr (95)– 05-May (125)	26-Apr	15-Mrz (74) – 05-May (125)	17-Mar/26-Apr
	R2	13-Feb (44) – 15-May (74)	06-Mar	23-Jan (23) – 15-May (74)	04-Feb/06-Mar
	R3	11-Mar (70) – 10-Apr (100)	28-Mar	18-Feb (49) – 10-Apr (100)	19-Feb/20-Mar

Table B.8.6-18: Application windows and application dates for single and multiple application of glyphosate to pome/ stone fruits

Crop	Scenario	1 x 2.88 kg as/ha		1 x 2.88 kg as/ha + 2 x0.72 kg as/ha	
		Possible window of application	Application date set by PAT	Possible window of application	Application date set by PAT
Pome/ stone fruit, pre-planting	D3	01-Mar (60) - 31-Mar (90)	29-Feb	01-Mar (60) - 26-May (146)	29-Feb/04-Apr/04-May
	D4	15-Mar (74) - 14-Apr (104)	19-Mar	15-Mar (74) - 09-Jun (160)	19-Mar /18-Apr/30-May
	D5	15-Feb (46) - 17-Mar (76)	21-Feb	15-Feb (46) - 12-May (132)	21-Feb/08-Apr/11-May
	R1	01-Mar (60)- 31-Mar (90)	17-Mar	01-Mar (60) - 26-May (146)	17-Mar/26-Apr /24-May
	R2	15-Feb (46)- 17-Mar (76)	06-Mar	15-Feb (46) - 12-May (132)	06-Mar/10-Apr/11-May
	R3	15-Feb (46)- 17-Mar (76)	19-Feb	15-Feb (46) - 12-May (132)	19-Feb/20-Mar/ 17 -Apr
	R4	15-Feb (46)- 17-Mar (76)	02-Mar	15-Feb (46) - 12-May (132)	02-Mar/04-Apr/08-May

Results and discussion

The global maximum maximum Step 1 and 2 PEC_{SW} and PEC_{Sed} of glyphosate and its metabolites AMPA and HMPA are summarised in Table B.8.6-19.

Table B.8.6-19: Global maximum PEC_{SW} and PEC_{Sed} of glyphosate and its metabolites AMPA and HMPA at Step 1 -2

FOCUS Step/Scenario	Crop	Application rate	Glyphosate		AMPA		HMPA	
			PEC_{SW} (µg/L)	PEC_{Sed} (µg/kg)	PEC_{SW} (µg/L)	PEC_{Sed} (µg/kg)	PEC_{SW} (µg/L)	PEC_{Sed} (µg/kg)
Step 1	Not crop specific	1 x4320 g/ha	101	10500	41.0	3320	6.71	696
Step 2 (North Europe, Oct – Feb)	Spring and winter cereals	2 x 2160 g/ha	18.4	2660	14.9	1430	1.22	196
	Appl. Hand (crop > 50 cm)	1 x4320 g/ha	39.7	4430	16.9	1620	2.63	294
Step 2 (North Europe, Mar – May)*	Spring and winter cereals	2 x 2160 g/ha	18.4	1310	6.31	593	1.22	86.8
	Appl. Hand (crop > 50 cm)	1 x4320 g/ha	39.7	1930	7.21	674	2.63	128
Step 2 (North Europe, Jun-Sep)	Spring and winter cereals	2 x 2160 g/ha	18.4	1310	6.31	593	1.22	86.8
	Appl. Hand (crop > 50 cm)	1 x4320 g/ha	39.7	1930	7.21	674	2.63	128
Step 2 (South Europe, Oct – Feb)	Spring and winter cereals	2 x 2160 g/ha	18.4	2410	12.0	1150	1.22	160
	Appl. Hand (crop > 50 cm)	1 x4320 g/ha	39.7	3590	13.7	1300	2.63	238
Step 2 (South Europe, Mar - May)	Spring and winter cereals	2 x 2160 g/ha	18.4	2410	12.0	1150	1.22	160
	Appl. Hand (crop > 50 cm)	1 x4320 g/ha	39.7	3590	13.7	1300	2.63	238
Step 2 (South Europe, Jun – Seb)	Spring and winter cereals	2 x 2160 g/ha	18.4	1860	9.16	871	1.22	123
	Appl. Hand (crop > 50 cm)	1 x4320 g/ha	39.7	2760	10.4	988	2.63	183

The global maximum maximum Step 3 PEC_{SW} and PEC_{Sed} of glyphosate after pre-planting/ pre-emergence application to winter cereals are given in Table B.8.6-20. The global maximum maximum Step 3 PEC_{SW} and PEC_{Sed} of glyphosate after pre-planting/ pre-emergence and post-harvest application to spring cereals are given in Table B.8.6-21. The global maximum maximum Step 3 PEC_{SW} and PEC_{Sed} of glyphosate after pre-planting/ pre-emergence application to potatoes are given in Table B.8.6-22. The global maximum maximum Step 3 PEC_{SW} and PEC_{Sed} of glyphosate after weed emergence application to pome/stone fruit are given in Table B.8.6-23.

Table B.8.6-20: Global maximum PEC_{SW} and PEC_{Sed} of glyphosate following pre-planting/ pre-emergence application to winter cereals

FOCUS STEP 3 Scenario	Water Body	1 × 2.16 kg/ha		2 × 2.16 kg/ha	
		PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)	PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)
D1	ditch	13.4	81.2	13.2	138
D1	stream	11.7	7.82	10.1	12.6
D2	ditch	13.5	60.8	12.2	98.0
D2	stream	12.0	53.9	10.7	85.7
D3	ditch	13.2	7.05	11.6	16.1
D4	pond	0.445	7.21	0.528	11.8
D4	stream	11.5	2.55	9.91	4.18
D5	pond	12.4	7.60	10.7	12.4
D5	stream	12.4	3.62	10.7	5.93
D6	ditch	13.4	48.9	11.9	80.8
R1	pond	0.445	8.79	0.531	14.5
R1	stream	8.72	28.0	7.57	50.1
R3	stream	12.1	809	10.7	1703
R4	stream	8.23	488	7.57	238

Table B.8.6-21: Global maximum PEC_{SW} and PEC_{Sed} of glyphosate following pre-planting/ pre-emergence and post-harvest application to spring cereals

FOCUS STEP 3 Scenario	Water Body	1 × 2.16 kg/ha (spring)		1 × 2.16 kg/ha (autumn)		2 × 2.16 kg/ha (autumn + spring)	
		PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)	PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)	PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)
D1	ditch	13.4	29.7	13.4	81.1	11.8	86.4
D1	stream	11.0	0.963	11.7	7.84	10.1	7.27
D3	ditch	13.2	7.63	13.3	10.9	11.7	22.5
D4	pond	0.445	7.05	0.445	7.19	0.386	10.8
D4	stream	10.3	0.428	11.5	2.47	9.56	1.14
D5	pond	0.444	6.78	0.445	6.86	0.385	10.2
D5	stream	8.47	0.105	12.4	3.61	10.7	3.19
R4	stream	8.68	57.8	8.76	31.7	7.57	178

Table B.8.6-22: Global maximum PEC_{SW} and PEC_{Sed} of glyphosate following pre-planting/pre-emergence application to potatoes

FOCUS STEP 3 Scenario	Water Body	1 × 2.16 kg/ha		2 × 2.16 kg/ha	
		PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)	PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)
D3	ditch	11.0	7.70	9.51	12.6
D4	pond	0.431	6.32	0.477	10.5
D4	stream	9.17	0.479	7.88	0.704
D6, early app.	ditch	11.0	8.15	9.38	5.19
D6, late app.	ditch	11.1	36.4	9.60	38.9
R1	pond	0.431	8.05	0.472	16.5
R1	stream	7.57	34.3	6.53	123
R2	stream	9.98	35.0	8.60	1690
R3	stream	10.7	25.3	9.23	63.4

Table B.8.6-23: Global maximum PEC_{SW} and PEC_{Sed} of glyphosate following post weed emergence application to the trunks of and the soil below pome/stone fruit trees

FOCUS STEP 3 Scenario	Water Body	1 × 0.288 kg a.s./ha		1 × 0.288 kg as/ha + 2 × 0.72 kg as/ha	
		PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)	PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)
D3	ditch	17.7	12.2	12.9	10.8
D4	pond	0.595	9.19	0.417	9.45
D4	stream	13.1	0.39	9.47	0.555
D5	pond	0.594	8.98	0.417	9.29
D5	stream	11.3	0.141	8.18	0.268
R1	pond	0.595	9.32	0.417	9.43
R1	stream	11.7	2.18	8.46	3.47
R2	stream	14.4	2.74	10.4	4.29
R3	stream	16.5	4.46	11.9	3.79
R4	stream	11.6	16.6	8.37	23.8

Conclusions

Predicted environmental concentrations in surface water (PEC_{SW}) and sediment (PEC_{Sed}) were calculated for the active substance glyphosate and its metabolites AMPA and HMPA. The simulations were performed for pre-emergence/pre-planting glyphosate use on winter cereals and potatoes, for pre-emergence/pre-planting and post-harvest glyphosate use on spring cereals and for post-weed emergence glyphosate use on pome/stone fruit. Step 1 and Step 2 simu-

lations were performed for glyphosate and its metabolites AMPA and HMPA using the simulation model FOCUS STEPS 1-2 (version 2.1). For glyphosate, additional calculations at Step 3 were performed with FOCUS SWASH (version 3.1).

The overall maximum PEC_{SW} value of glyphosate at Step 1, 2 and 3 was 101, 39.7 and 17.7 µg/L, respectively. The overall maximum PEC_{SW} value of AMPA at Step 1 and 2 was 41.0 and 16.9 µg/L. The overall maximum PEC_{SW} value of HMPA at Step 1 and 2 was 6.71 and 2.63 µg/L.

RMS Comment
The approach used by the study author to derive PEC_{SW} and PEC_{Sed} values for glyphosate and its metabolites AMPA and HMPA for the application of glyphosate to winter and spring cereals, potatoes and pome/ stone fruit is considered acceptable by the RMS. However, different endpoints were derived by the RMS for the degradation of glyphosate and AMPA in soil and water/sediment systems and for the adsorption of both compounds to soil. Thus, the derived PEC_{SW} and PEC_{Sed} values are only acceptable for HMPA. The resulting PEC_{SW} and PEC_{Sed} for HMPA will be used for risk assessment.

The re-calculations of PEC_{SW} and PEC_{Sed} values for glyphosate and AMPA are described below.

PEC_{SW} and PEC_{Sed} calculations by the RMS

Materials and Methods
New Step 1 and Step 2 PEC_{SW} and PEC_{Sed} values for glyphosate and its metabolite AMPA were derived using the model FOCUS Steps 1-2 (version 2.1).

The substance related input parameter for glyphosate and its metabolites AMPA used for FOCUS SW simulations are presented in Table B.8.6-24.

Table B.8.6-24: Substance related input data of glyphosate and its metabolites AMPA used by the RMS for the simulations

Parameter	Glyphosate	AMPA
Molecular Mass (g/mol)	169.07	111
Water solubility (mg/L)	10500 (pH 2, 20°C)	10500 (pH 2, 20°C)*
DT_{50} soil (days)	~~21.03~~ 20.51	88.84
DT_{50} water (days)	67.74	86.09
DT_{50} sediment (days)	67.74 / 1000**	86.09
DT_{50} total system (days)	67.74	86.09
K_{oc} (mL/g)	15844 ***	9749
1/n	0.91 ***	-
Max. occurrence soil (%)	n/a	50.1
Max occurrence w/sed (%)	n/a	27.1

* parent data
** values used in Step 1-2/ Step 3 calculations
**** As an outcome of the discussions in the Pesticides Peer Review Meeting 126 the arithmetic mean Kfoc and 1/n values for glyphosate have been amended. The experts agreed that for the EU approval no additional exposure calculations were necessary, due to the limited effect on the mean endpoints. The correct values to be used in future PEC simulations are Kfoc:15388 and 1/n: 0.93
n/a not applicable

The same application scenario as selected by the notfier was used for simulations at Step 1 and Step 2. The application scenario is summarised in Table B.8.6-12. The additional FOCUSsw crops field beans, maize, spring & winter oil-seed rape, sugar beets, vegetables (bulb, fruiting, leafy), grass & alfalfa & legumes were modelled toegther with the crops winter and spring cereals to represent the use of glyphosate in all transplanted or seeded field crops.

The same application scenarios as selected by the notfier and summarised in Table B.8.1-19 were used for simulations at Step 3 for winter cereals, potatoes and pome/stone fruit. However, for spring cereals two pre-planting, pre-emergence applications in spring were simulated according to the representive GAP list for glyphosate. Additionally, Step 3 simulations were performed for winter oilseed rape, spring oilseed rape, maize and legumes. The application scenarios used for modelling by the RMS are presented in Table B. 8.6-25. Simulations were performed for both single and multiple applications according to FOCUS (2011).

Table B. 8.6-25 Application scenarios considered by the RMS for simulations at Step 3

Crop	FOCUSsw crop	Application rate (g a.s./ha)	No. of applications	Min. intervall (d)	Application period
Various crops (autumn application)	Winter cereals, winter oil seed rape	2160	2	21	pre-planting/ pre-emergence
		2160	1**	-	
Various crops (spring application)	Spring cereals, maize, legumes, potatoes	2160	2	21	pre-planting/ pre-emergence
		2160	1*	-	
Orchards, citrus, vines, tree nuts	Pome/ stone fruit	2880/ 720/ 720	3	28	Post-emergence of weeds
		2880	1*	-	

* Single application In spring (according to FOCUS, 2001, 2011 b, a single application should be evaluated additionally)

** Single application in autumn (according to FOCUS, 2001, 2011 b, a single application should be evaluated additionally)

The 'ground spray' application method was chosen for winter and spring cereals, winter and spring oil seed rape, maize, legumes and potatoes. Regarding the application method to the trunks of and the soil below pome/stone fruit trees, the RMS followed the approach also used by the Notifier.

For pre-planting/ pre-emergence application of glyphosate to winter cereals and potatoes and for post-weed application of glyphosate to pome/ stone fruit trees, the application windows as chosen by the Notifier were used for modelling. For spring cereals, winter and spring oil seed rape, maize and legumes, appropriate application windows assuming pre-planting/ pre-emergence application of glyphosate were chosen based on recommended on emergence dates of the crops specified in FOCUS (2001, 2011b). The application windows for spring cereals, potatoes and pome/stone fruit trees and the actual application dates within the application windows as determined by the Pesticide Application Timer (PAT) are presented in Table B.8.6-15, Table B.8.6-17 and Table B.8.6-18. The application windows for spring cereals, winter and spring oil seed rape, maize and legumes and the actual application dates within the application windows as determined by PAT are presented in Table B. 8.6-26, Table B. 8.6-27, Table B. 8.6-28, Table B. 8.6-29 and Table B. 8.6-30.

Table B. 8.6-26 Application windows and application dates for single and multiple application of glyphosate to spring cereals cereals

Scenario	1 x 2.16 kg a.s./ha		2 x 2.16 kg a.s./ha	
	Possible window of application	Application date set by PAT	Possible window of application	Application date set by PAT
D1	05-Apr (95) – 05-May (125)	25-Apr	15-Mar (74)– 05-May (125)	29-Mar & 25-Apr
D3	02-Mar (61)– 01-Apr (91)	01-Mar	09-Feb (40) – 01-Apr (91)	29-Feb & 24-Mar
D4	27-Mar (86) – 26-Apr (116)	18-Apr	06-Mar (65)– 26-Apr (116)	19-Mar & 18-Apr
D5	13-Feb (44) – 15-Mrz (74)	21-Feb	23-Jan (23) – 15-Mar (74)	10-Feb & 07-Mar
R4	13-Feb (44)– 15-Mar (74)	02-Mar	23-Jan (23) – 15-Mar (74)	04-Feb & 02-Mar

Table B. 8.6-27 Application windows and application dates for single and multiple application of glyphosate to winter oilseed rape

Scenario	1 x 2.16 kg a.s./ha		2 x 2.16 kg a.s./ha	
	Possible window of application	Application date set by PAT	Possible window of application	Application date set by PAT
D2	16 Aug (228) - 15-Sep (258)	03-Sep	26-Jul (207) - 15-Sep (258)	06-Aug/03-Sep
D3	03-Aug (215) - 02-Sep (245)	02-Aug	13-Jul (194) - 02-Sep (245)	30-Jul/21-Aug
D4	04-Aug (216) - 03-Sep (246)	27-Aug	14-Jul (195) - 03-Sep (246)	18-Jul/27-Aug
D5	21-Aug (233) - 20-Sep (263)	27-Aug	31-Jul (212) - 20-Sep (263)	04-Aug/27-Aug
R1	05-Aug (217) - 04-Sep (247)	20-Aug	15-Jul (196) - 04-Sep (247)	28-Jul/20-Aug
R3	05-Sep (248) - 21-Oct (294)	23-Sep	15-Aug (227) - 21-Okt (294)	28-Aug/23-Sep

Table B. 8.6-28 Application windows and application dates for single and multiple application of glyphosate to spring oilseed rape

Scenario	1 x 2.16 kg a.s./ha		2 x 2.16 kg a.s./ha	
	Possible window of	Application date	Possible window of	Application date set

	application	**set by PAT**	**application**	**by PAT**
D1	05-Apr (95) – 05-May (125)	25-Apr	15-Mar (74)– 05-May (125)	29-Mar/25-Apr
D3	02-Mar (61)– 01-Apr (91)	01-Mar	09-Feb (40) – 01-Apr (91)	29-Feb/24-Mar
D4	27-Mar (86) – 26-Apr (116)	18-Apr	06-Mar (65)– 26-Apr (116)	19-Mar/18-Apr
D5	13-Feb (44) – 15-Mrz (74)	21-Feb	23-Jan (23) – 15-Mar (74)	10-Feb/07-Mar
R4	13-Feb (44)– 15-Mar (74)	02-Mar	23-Jan (23) – 15-Mar (74)	04-Feb/02-Mar

Table B. 8.6-29 Application windows and application dates for single and multiple application of glyphosate to maize

Scenario	**1 x 2.16 kg a.s./ha**		**2 x 2.16 kg a.s./ha**	
	Possible window of application	**Application date set by PAT**	**Possible window of application**	**Application date set by PAT**
D3	05-Apr (95) –05-May (125)	04-Apr	15-Mar (74) – 05-May (125)	16-Mar/06-Apr
D4	10-Apr (100) –10-May (130)	18-Apr	20-Mar (79)– 10-May (130)	20-Mar/ 18-Apr
D5	10-Apr (100) –10-May (130)	14-Apr	20-Mar (79)– 10-May (130)	08-Apr/ 01-May
D6	21-Mar (80) – 20-Apr (110)	09-Apr	28-Feb (59) - 20_Apr (110)	05-Mar/ 09-Apr
R1	03-Apr (93) – 03-May (123)	26-Apr	13-Mar (72) - 03-May (123)	17-Mar/ 26-Apr
R2	01-Apr (91) – 01-May (121)	22-Apr	11-Mar (70) – 31-Jul (212)	22-Mar/ 22-Apr
R3	16-Mar (75) – 01-Mai (121)	28-Mar	11-Mar (70) - 01-May (121)	28-Mar/ 22-Apr
R4	11-Mar (70) – 10-Apr (100)	11-Mar	18-Feb (49) - 10-Apr (100)	02-Mar/ 31-Mar

Table B. 8.6-30 Application windows and application dates for single and multiple application of glyphosate to legumes

Scenario	**1 x 2.16 kg a.s./ha**		**2 x 2.16 kg a.s./ha**	
	Possible window of application	**Application date set by PAT**	**Possible window of application**	**Application date set by PAT**
D3	10-Mar (69)	16-Mar	23-Feb (54) -	29-Feb/ 04-Apr

	– 15-Apr (105)		15-Apr (105)	
D4	24-Mar (83) – 23-Apr (113)	18-Apr	03-Mar (62) - 23-Apr (113)	03-Mar/ 18-Apr
D5	13-Feb (44) – 15-Mar (74)	21-Feb	23-Jan (23) - 15-Mar (74)	10-Feb/ 07-Mar
D6	21-Mar (80) – 20-Apr (110)	09-Apr	28-Feb (59)- 20-Apr (110)	05-Mar/ 09-Apr
R1	16-Mar (75)2.16 – 15-Apr (105)	17-Mar	23-Feb (54) - 15-Apr (105)	24-Feb/ 17-Mar
R2	21-Mar (80) – 20-Apr (110)	22-Mar	28-Feb (59) - 20-Apr (110)	06-Mar/ 10-Apr
R3	22-Mar (81) – 21-Apr (111)	28-Mar	01-Mar (60) - 21-Apr (111)	01-Mar/ 28-Mar
R4	22-Mar (81) – 07-May (127)	08-Apr	01-Mar (60) - 21-Apr (111)	05-Mar/ 08-Apr

Results and discussion

The global maximum maximum Step 1 and Step 2 PEC_{SW} and PEC_{Sed} of glyphosate and its metabolites AMPA calculated by the RMS are summarised in Table B.8.6-31.

Table B.8.6-31: Global maximum PEC_{SW} and PEC_{Sed} of glyphosate and its metabolites AMPA and HMPA at Step 1 -2

FOCUS Step/Scenario	Crop	Application rate	Glyphosate		AMPA	
			PEC_{SW} (µg/L)	PEC_{Sed} (µg/kg)	PEC_{SW} (µg/L)	PEC_{Sed} (µg/kg)
Step 1	Not crop specific	1 ×4320 g/ha	104.81	10300	40.90	3300
Step 2 (North Europe, Oct – Feb)	Field crops*	2 × 2160 g/ha	~~23.58~~ 23.38	~~3600~~ 3570	15.76	1520
	Appl. Hand (crop < 50 cm)**	1 ×4320 g/ha	39.73	~~4780~~ 4770	17.16	1640
Step 2 (North Europe, Mar – May)*	Field crops*	2 × 2160 g/ha	18.49	~~1570~~ 1560	6.67	628.4
	Appl. Hand (crop < 50 cm)**	1 ×4320 g/ha	39.73	2070	7.32	685.1
Step 2 (North Europe, Jun-Sep)	Field crops*	2 × 2160 g/ha	18.49	~~1570~~ 1560	6.67	628.4
	Appl. Hand (crop < 50 cm)**	1 ×4320 g/ha	39.73	2070	7.32	685.1
Step 2 (South Europe, Oct – Feb)	Field crops*	2 × 2160 g/ha	~~19.30~~ 19.14	~~2920~~ 2900	12.73	1220
	Appl. Hand (crop < 50 cm)**	1 ×4320 g/ha	39.73	~~3880~~ 3870	13.88	1320
Step 2 (South Europe, Mar - May)	Field crops*	2 × 2160 g/ha	~~19.30~~ 19.14	~~2920~~ 2900	12.73	1220
	Appl. Hand (crop < 50 cm)**	1 ×4320 g/ha	39.73	~~3880~~ 3870	13.88	1320
Step 2 (South Europe, Jun – Seb)	Field crops*	2 × 2160 g/ha	18.49	~~2250~~ 2230	9.70	924.0
	Appl. Hand (crop < 50 cm)**	1 ×4320 g/ha	39.73	~~2980~~ 2970	10.60	1000

* Spring & winter cereals, field beans, maize, spring & winter oil-seed rape, sugar beets, vegetables (bulb, fruiting, leafy), grass & alfalfa & legumes

** Application to the trunks of and the soil under perennials

The global maximum maximum Step 3 PEC_{SW} and PEC_{Sed} of glyphosate after pre-planting/ pre-emergence application to winter cereals, to spring cereals, to winter oil seed rape, spring oil seed rape, potatoes, maize and legumes are given in Table B. 8.6-32, Table B. 8.6-33, Table B. 8.6-34, Table B. 8.6-35, Table B. 8.6-36, Table B. 8.6-37, Table B. 8.6-38 and Table B. 8.6-39, , respectively.

Table B. 8.6-32 Global maximum PEC_{SW} and PEC_{Sed} of glyphosate following pre-planting/pre-emergence application to winter cereals (calculation of RMS)

FOCUS STEP 3 Scenario	Water Body	1 × 2.16 kg/ha		2 × 2.16 kg/ha	
		PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)	PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)
D1	ditch	13.608	71.425	14.170	117.576
D1	stream	11.899	7.722	10.293	10.531
D2	ditch	13.622	57.576	12.765	85.108
D2	stream	12.116	51.082	11.182	73.995
D3	ditch	13.394	6.991	11.777	12.344
D4	pond	0.461	5.694	0.582	9.389
D4	stream	11.627	2.557	10.054	3.582
D5	pond	0.461	6.024	0.591	9.878
D5	stream	12.546	4.798	10.849	5.128
D6	ditch	13.566	45.680	12.184	67.199
R1	pond	0.461	7.989	0.592	13.831
R1	stream	8.850	25.962	7.687	47.807
R3	stream	12.277	815.228	10.841	1696.174
R4	stream	8.355	468.878	7.694	214.027

Table B. 8.6-33 Global maximum PEC_{SW} and PEC_{Sed} of glyphosate following pre-planting/pre-emergence application to spring cereals (calculation of RMS)

FOCUS STEP 3 Scenario	Water Body	1 × 2.16 kg/ha		2 × 2.16 kg/ha	
		PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)	PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)
D1	ditch	13.546	28.478	11.857	31.442
D1	stream	11.161	0.975	9.650	1.039
D3	ditch	13.404	7.557	11.751	12.097

D4	pond	0.461	5.319	0.531	8.505
D4	stream	10.447	0.434	9.033	0.535
D5	pond	0.460	5.224	0.541	8.360
D5	stream	8.591	0.107	8.977	0.316
R4	stream	8.809	63.360	7.686	105.090

Table B. 8.6-34 Global maximum PEC_{SW} and PEC_{Sed} of glyphosate following pre-planting/pre-emergence application to winter oil seed rape (calculation of RMS)

FOCUS STEP 3 Scenario	Water Body	1 × 2.16 kg/ha		2 × 2.16 kg/ha	
		PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)	PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)
D2	ditch	13.622	57.427	12.345	78.794
D2	stream	12.116	50.942	10.660	58.093
D3	ditch	13.538	28.639	11.940	40.701
D4	pond	0.461	5.694	0.522	8.657
D4	stream	11.627	2.557	10.054	3.134
D5	pond	0.461	5.541	0.581	8.693
D5	stream	12.546	3.617	10.849	4.919
R1	pond	0.462	5.193	0.568	8.198
R1	stream	8.887	7.750	7.684	11.546
R3	stream	12.490	160.896	10.801	227.865

Table B. 8.6-35 Global maximum PECSW and PECSed of glyphosate following pre-planting/pre-emergence application to spring oil seed rape (calculation of RMS)

FOCUS STEP 3 Scenario	Water Body	1 × 2.16 kg/ha		2 × 2.16 kg/ha	
		PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)	PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)
D1	ditch	13.546	28.478	11.857	31.442
D1	stream	11.161	0.975	9.650	1.039
D3	ditch	13.427	9.793	11.738	12.996
D4	pond	0.461	5.323	0.531	8.509
D4	stream	10.447	0.434	9.033	0.535
D5	pond	0.460	5.225	0.541	8.362
D5	stream	8.591	0.107	8.977	0.316
R1	pond	0.463	9.748	0.777	28.795

R1	stream	8.616	76.161	7.591	366.862

Table B. 8.6-36 Global maximum PECSW and PECSed of glyphosate following pre-planting/pre-emergence application to potatoes (calculation of RMS)

FOCUS STEP 3 Scenario	Water Body	1 × 2.16 kg/ha		2 × 2.16 kg/ha	
		PEC_{SW} global max (µg/L)	PEC_{Sed}global max (µg/kg)	PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)
D3	ditch	11.115	7.604	9.649	10.454
D4	pond	0.446	4.828	0.526	7.871
D4	stream	9.298	0.485	8.001	0.615
D6, early app.	ditch	11.205	32.899	9.518	4.286
D6, late app.	ditch	11.205	32.899	9.743	31.731
R1	pond	0.447	6.964	0.569	14.265
R1	stream	7.685	35.792	6.634	110.556
R2	stream	10.115	46.144	8.742	1730.618
R3	stream	10.824	26.095	9.360	54.887

Table B. 8.6-37 Global maximum PECSW and PECSed of glyphosate following pre-planting/pre-emergence application to maize (calculation of RMS)

FOCUS STEP 3 Scenario	Water Body	1 × 2.16 kg/ha		2 × 2.16 kg/ha	
		PEC_{SW} global max (µg/L)	PEC_{Sed}global max (µg/kg)	PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)
D3	ditch	11.102	7.605	9.644	10.945
D4	pond	0.446	5.156	0.517	8.237
D4	stream	9.064	0.376	7.800	0.469
D5	pond	0.446	5.022	0.551	7.891
D5	stream	9.802	0.423	8.443	0.507
D6	ditch	11.110	8.379	9.646	10.476
R1	pond	0.447	6.931	0.569	14.217
R1	stream	7.685	35.102	6.634	109.876
R2	stream	10.223	24.159	8.810	678.650
R3	stream	10.825	244.954	9.392	244.742
R4	stream	7.682	60.609	6.621	393.570

Table B. 8.6-38 Global maximum PECSW and PECSed of glyphosate following pre-planting/pre-emergence application to legumes (calculation of RMS)

FOCUS STEP 3 Scenario	Water Body	1 × 2.16 kg/ha		2 × 2.16 kg/ha	
		PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)	PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)
D3	ditch	11.103	7.575	9.640	9.281
D4	pond	0.446	5.149	0.479	8.234
D4	stream	9.064	0.376	8.154	0.585
D5	pond	0.446	5.062	0.523	8.088
D5	stream	7.453	0.0929	7.751	0.273
D6	ditch	11.110	8379	9.646	10.476
R1	pond	0.446	8.786	0.648	14.159
R1	stream	7.710	73.485	6.502	100.506
R2	stream	10.198	678.046	8.765	196.543
R3	stream	10.828	244.935	9.330	505.314
R4	stream	7.678	208.671	6.611	344.072

Table B. 8.6-39 Global maximum PEC_{SW} and PEC_{Sed} of glyphosate following post weed emergence application to the trunks of and the soil below pome/stone fruit trees (calculation of RMS)

FOCUS STEP 3 Scenario	Water Body	2.88 kg/ha		2.88 + 0.72 + 0.72 kg/ha	
		PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)	PEC_{SW} global max (µg/L)	PEC_{Sed} global max (µg/kg)
D3	ditch	6.209	4.161	4.537	6.484
D4	pond	0.213	2.500	0.238	4.802
D4	stream	4.594	0.137	3.748	0.446
D5	pond	0.213	2.459	0.245	4.764
D5	stream	3.971	0.0495	3.811	0.242
R1	pond	0.213	2.531	0.252	4.820
R1	stream	6.505	1.605	2.978	3.179
R2	stream	5.358	3.725	3.937	5.612
R3	stream	5.794	2.117	4.203	4.378
R4	stream	4.063	17.616	2981	25.323

Conclusions

Predicted environmental concentrations in surface water (PEC_{SW}) and sediment (PEC_{Sed}) were calculated by the RMS for the active substance glyphosate and its metabolites AMPA for the application of glyphosate to various field crops and to the trunks of and the soil below pome

fruit trees. The derived PEC_{SW} and PEC_{Sed} of glyphosate and AMPA will be used for environmental risk assessment.

B.8.6.3 Monitoring data

B.8.6.3.1 Groundwater and surface water

Several studies (Calliera et al., 2011, BVL no 2310280; Schmidt and Reichert, 2006, BVL no 2310282; Franke et al., 2010, BVL no 2310284; Carter and Pepper, 2005, BVL no 2310285; Anonymous, 2012, BVL no 2310289; Horth, 2012, BVL no 2310291) on groundwater monitoring were submitted by the Notifier. In one of these studies (Horth, 2012, BVL no 2310291) results from surface water monitoring are described as well. The main results of these studies are summarized in the following.

Calliera et al. (2011, BVL no 2310280) reported that glyphosate has been measured in groundwater aquifers at 5 locations in Italy exceeding the limit concentration 0.1 µg/L. A detailed investigation has allowed plausible explanations of the origin of the glyphosate findings at 4 of the 5 locations. Thereby, the origin of the glyphosate concentrations could be allocated to surface inflow or to point source contamination. In one location the investigation is still ongoing. Until now, the findings could not be correlated with the normal and proper use of glyphosate in the field.

Schmidt and Reichert (2006, BVL no 2310282) reported that the Federal Office of Consumer Protection and Food Safety (BVL) has requested registration owners of glyphosate containing plant protection products to investigate the causes of findings ≥ 0.1 µg/L of glyphosate and its metabolite AMPA in the groundwater, which were reported from monitoring points in Germany since 2003. Thereby, glyphosate was found at 5 wells, while the metabolite AMPA appeared at 21 locations. The further detailed investigation during this study has furnished a plausible explanation of the origin of glyphosate or AMPA findings for all 24 locations of finding. The findings can be classified in groups of causes: Five wells showed inflow of surface water or bank filtrate and one well was affected by a waste deposit. In one case the well was located inside a sewage plant and showed the influence of the waste water. In another case the sample was contaminated at the well which serves as a processing water well for a tank filling place. 16 findings were due to an analysis which was obviously deficient. Furthermore, it was shown that there is not a single case in which there is a direct connection with the normal and proper use of the active ingredient in the field and the findings of glyphosate and AMPA in groundwater.

Groundwater monitoring in the Netherlands is discussed by Franke et al. (2010, BVL no 2310284). At 6 out of 189 sampling locations glyphosate was measured above the detection limit and drinking water threshold of 0.1 µg/L carried out in one report, and at 4 out of 691 measurements carried out in a second report. The results show that some wells were not fully protected and it is conceivable that contact with surface water may occur. Uncertainty related to the data processing was evidenced as well. For 6 sites where glyphosate was detected, no explanation could be found during this investigation. In general, it remains unclear if the findings can be correlated with the normal and proper use of glyphosate in the field.

The situation in the Vemmenhög catchment in Southern Sweden is described by Carter and Pepper (2005, BVL no 2310285). In this catchment glyphosate was detected in two pairs of boreholes between August 2004 and February 2005. In the first well the concentration

reached 0.045 µg/L whilst in the second well located glyphosate was detected at 0.18 and 0.035 µg/L. A review of the regional characteristics showed that extensive drainage systems are in place in the catchment. About 23 % of the catchment was treated with glyphosate and this included application to the immediate or near vicinity of the boreholes. Historical data reviews as well as observations at the site demonstrate that there can be potential for direct hydrological connectivity between surface water and shallow groundwater via artificial drainage systems.

Anonymous (2012, BVL no 2310289) described that the annual reports of the French Environmental Institute (Institut Français de l'Environnement, Ifen) monitoring the plant protection products in the French waters mentioned the detection of glyphosate and its degradation product, AMPA, above 0.1 µg/L in several groundwater sampling sites. A selection of 27 sites for further investigation was performed. Thereby, two sites were rejected early in the process due to their low vulnerability and no further investigation was performed. Out of the 25 remaining sites, 16 showed a single detect of glyphosate (without AMPA) and three showed a single detect for glyphosate and AMPA. With the exception of three sites, all other sites had samples analyzed within the months/years after the detection showing glyphosate/AMPA results < 0.1 µg/L, demonstrating that the contamination was not widespread in the aquifer and not long-term. Multiple detects were observed in six sites, two of which were used for drinking water supply. At those sites, the detections occurred the same year, and the analyses performed the following years showed no further contamination by glyphosate and AMPA. The four other sites with multiple detects had no well protection area, and were not suited for drinking water supply (private or fire well, piezometer, qualitometer, spring). It has to be noted that information on glyphosate use (agricultural and non-agricultural uses) is not provided in the study.

Horth (2012, BVL no 2310291) provided a review that covers glyphosate and AMPA monitoring results for surface (fresh) waters and groundwater across Europe, i.e. all 27 Member States of the European Union, as well as Norway and Switzerland, where information was available. Glyphosate has been analysed in almost 75000 surface water samples from about 4000 sites (from 1993-2011) and was detected in 33 % of samples, with 23 % above 0.1 µg/L. The maximum concentrations of glyphosate acid found in surface water reached from 1.3 to 370 µg/L. The highest glyphosate values in surface water were detected in Sweden (370 µg/L), Ireland (186 µg/L) and Belgium (139 µg/L). The main metabolite AMPA has been analysed in about 56700 samples from nearly 3000 sites (1997-2011) and was detected in 54 % of samples, with 46 % above 0.1 µg/L and maximum concentrations reaching from 0.22 to > 200 µg/L. It has to be noted that glyphosate and AMPA monitored in this study exceed the predicted environmental concentrations for glyphosate acid and AMPA in surface water (PEC_{SW}) calculated using the FOCUS (2000) surface water models, even though worst case applications was assumed. Nevertheless, the calculated TER values referring to the monitored concentrations in the study by Horth (2012, BVL no 2310291) with the respective acceptability criteria show that the risk for aquatic organisms is acceptable. Furthermore, glyphosate and AMPA have been increasingly analysed and occasionally detected in groundwater. Glyphosate has been analysed in 66662 samples from about 675 sites (1993-2010) and detected in 1 % of samples, with 0.64 % above 0.1 µg/L; AMPA has been analysed in 51652 samples from 1345 sites (1993 - 2011) and detected in 2.6 % of samples, with 0.77 % above 0.1 µg/L. The highest numbers of glyphosate detections have been reported from Denmark (4.7 µg/L) and France (24 µg/L). Findings exceeding the limit concentration 0.1 µg/L have also been measured in groundwater aquifers in Austria, Ireland, the Netherlands and the UK.

Clarification of glyphosate groundwater findings > 0.1 µg/L is presented and discussed partly. The following main causes have been identified in the study:

- In Austria, Glyphosate was detected in isolated cases. AMPA was detected somewhat more frequently and at higher concentrations. There is no information about the type of groundwater, although the results, as presented, related to pore groundwater only, not the springs from fractured aquifers. Traces of AMPA were also found in two spring water samples and it was considered unclear at the time whether these findings were related to glyphosate or to aminophosphonates from detergents.
- Recent reported findings in Denmark all relate to groundwater at less than 15 meter depth. Investigations into earlier detections have shown that these occurred in shallow groundwater wells only.~~, and appeared to be due to rapid transfer of surface water from nearby surfaces. The author of a recent investigation into four sites concluded that the occurrence of glyphosate and AMPA was unlikely to be caused by agricultural and other approved usages. Work is ongoing in Denmark to establish leaching of glyphosate through soils.~~
- Earlier investigations in France relating to a small number of detections of glyphosate in drinking waters, most of which were derived from groundwater, revealed that the detections were most likely due to sample contamination or analytical problems. However, these related to 2001-2003 and more recent findings in groundwater may warrant further investigation. From the findings of a recent study to analyse the potential contamination of groundwater with glyphosate (and AMPA) at 27 sites from 2007-2010, it is clear that none of the glyphosate detections could be attributed to long-term contamination of typical groundwater. The majority of detections occurred once only, which is a clear indication that there is no real groundwater contamination, and the small number of multiple detections occurred in shallow groundwater (spring water) or wells unsuitable for groundwater monitoring, suggesting superficial short-term contamination.
- For the glyphosate groundwater findings > 0.1 µg/L in Ireland no clarification is given in the study.
- According to the present study the detection of glyphosate in groundwater in Switzerland may be attributable to short-term contamination of shallow groundwater or spring water.
- In the Netherlands, both glyphosate and AMPA have been detected in a small number of groundwater samples (once each in 10 different wells). These were investigated in detail and it was concluded that 5 of the results (all at levels below 0.15 µg/L were uncertain (high margins of error) whilst overall, all sampling points with positive detections were in cultivation areas with sandy or highly sandy soils, and samples were taken mainly from shallow groundwater.
- In the UK a number of positive samples and high maximum concentrations were found in Wales in the investigations of pollution incidents. There seems to be a particular problem in Wales, which may warrant further investigation.

In addition to the groundwater monitoring studies submitted by the Notifier the paper ”Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry” (Sanchís et al., 2012, BVL no 2537361) was submitted to the German Federal Environment Agency (UBA) by the Federal Office of Consumer Protection and Food Safety (BVL) for evaluation in February 2012. The article reports that a total of 129

groundwater samples from wells located in 11 different sampling sites in Catalonia, Spain, were collected by the Catalan Water Agency between May and September in 2007, 2008, 2009 and 2010. With the exception of one, all the areas studied presented a high impact from intensive agriculture. The samples were analysed by an immunoassay in the first step and to some extent by a LC-MS/MS measurement in the second step. The concentrations of glyphosate range from method limit of quantification (MLQQ) to 2.6 µg/L, the average was 202 ng/L (for the average calculation, samples under limit of quantification were computed as half the MLQQ). By the examination of the study, the presence of glyphosate in groundwater could not exclusively be related to the use as herbicide for agricultural applications. Other sources of glyphosate, e.g. the control of weeds on streams and drains, around railways, roads, sports fields and industrial areas have to be considered as well. Furthermore, the pathways of glyphosate into groundwater are not investigated by the authors. Due to the fact that surface waters exist in 10 out of 11 sampling sites, surface run-off and/or drainage into these waters with subsequent bank filtration into groundwater cannot be excluded.

In the peer review process (evaluation table, open point 4.18) the RMS was asked to summarise information from the VEWIN (Board for the Dutch drinking water companies) provided by the Netherlands (reporting table, no. 4(69)), which is presented in the following: The VEWIN maintains a database in which for 9 abstraction points substances are monitored (most recent data 2008-2012). Glyphosate exceeds the 0.1 µg/L on the basis of 90th percentile values for 2 of the 8 locations. The 90th percentile concentration for AMPA exceeds the limits at 7 out of 8 drinking water abstraction points.

With reference to Germany, groundwater findings of plant protection products, which exceed the authorisation limit concentration, are typically identified by regular monitoring programs conducted by the federal states in Germany (LAWA dataset). In general it has to be noted that between 1997 and 2007 the number of samples taken per year was between 89 and 430, whereas a larger number of samples (> 1500) were obtained since 2008, possibly due to the large sale volume of respective plant protection products. Therefore, the new data are much more representative. To sum the results, glyphosate was not detected in groundwater in concentrations > 0.1 µg/L for many years (1997-2001, 2003, 2006-2007). In 1996 2 samples (1.4 %), in 2002 1 sample (0.4 %), in 2004 1 sample (0.5 %) and in 2005 5 samples (2.1 %) contained glyphosate in concentrations > 0.1 µg/L. In 2008 glyphosate concentrations > 0.1 µg/L were detected in 7 samples (0.5 %), in 2009 in 6 samples (0.4 %) and in 2011 in 7 samples (0.4 %).

To summarise the groundwater monitoring data, it has to be pointed out that glyphosate has been detected in Europe with 0.64 % above the limit concentration 0.1 µg/L; and AMPA with 0.77 % above 0.1 µg/L (Horth, 2012, BVL no 2310291). Detailed groundwater monitoring studies demonstrating that glyphosate (at least partly) exceeded 0.1 µg/l are available from Italy (Calliera et al., 2011, BVL no 2310280), Germany (Schmidt and Reichert, 2006, BVL no 2310282), The Netherlands (Franke et al., 2010, BVL no 2310284), Sweden (Carter and Pepper, 2005, BVL no 2310285), France (Anonymous, 2012, BVL no 2310289) and Spain (Sanchís et al., 2012, BVL no 2537361). In some cases, clarification could be presented by the authors; e.g. causes for glyphosate findings in groundwater aquifers > 0.1 µg/L were point source contamination, affection by waste deposit, deficient analysis, no fully protected wells, potential for direct hydrological connectivity between surface water and shallow groundwater via artificial drainage systems and short-term contamination of shallow groundwater or spring water. However, it remains often unclear if findings above the authorisation limit originate

from a technically correct and regulation compliant use of the respective plant protection products in agricultural areas, or misuses or if construction defects on the groundwater abstraction points are reasonable for the limit exceedances etc. Another emerging issue is that other sources of glyphosate than agricultural applications, e.g. the control of weeds on streams and drains, around railways, roads, sports fields and industrial areas have to be considered as well. Regarding the pathways of glyphosate into groundwater when used for agricultural purposes as intended, RMS considers that groundwater contamination > 0.1 µg/L via direct leaching is generally not expected as the substance is strongly adsorbed to soil particles. Exceptions may be made, e.g. for preferential flow as described in point B.8.2.4.2 and B.8.2.4.3. Sanchís et al. (2012, BVL no 2537361) describe that surface waters exist in 10 out of 11 sampling sites where glyphosate was (at least partly) detected. Due to this fact, surface run-off and/or drainage into these waters with subsequent bank filtration into groundwater cannot be excluded as pathway. During the EU evaluation of the active substances the pathway surface run-off and drainage into an adjacent ditch with subsequent bank filtration into groundwater is not considered until now.

In addition, control and inspection programs at local authority level in Germany show high rates (35 % in 2009 and 36 % in 2010 during controls that were not event-related) of non-compliance regarding the application of plant protection products on walks and places in housing areas, foot-walks, traffic islands and paved surfaces in private properties (Anonymous, 2011, BVL no 2537364 and Anonymous, 2012, BVL no 2537365). This indicates that misuses of plant protection products on paved surfaces, which often contain glyphosate as active ingredient, by non-professional users occur to a relatively large extent. Therefore, current discussions in Germany are focusing on whether the findings of glyphosate and its metabolite AMPA in surface waters originate to some extent from these misuses of plant protection products containing glyphosate on paved surfaces by non-professional users. However, a monitoring study performed in UK (Ramwell et al., 2014, see point B.8.11.6.2) indicates otherwise: Glyphosate losses from urban areas that arise solely from amateur usage have been quantified. Thereby, it was shown that in spite of overdosing occurring, glyphosate concentrations in drain flow were lower than concentrations reported elsewhere from professional use in urban areas. Due to the fact that the representativeness of the study for other EU Member States conditions is called into question and as a precaution, the other EU Member States should look at this issue and, if necessary, take appropriate risk management measures at the national level. ~~In any event, the other EU Member States should be aware of this problem and, if necessary, take appropriate risk management measures at the national level.~~

Next to the use of glyphosate based products by non-professional users, we point to the environmental risks associated with the intended uses of glyphosate as desiccant. An herbicide application to time harvest poses additional ecological risks for surface water, ground water and for non-target species. We refer to Directive 2009/128/EC of the European Parliament and of the Council that establishes a framework for Community actions to achieve a more sustainable use of pesticides (Plant Protection Framework Directive). Directive 2009/128/EC commit Member States to adopt national action plans (NAP) for sustainable use of plant protection products in Article 4. The aims are to further reduce the risks and impacts to human health and the natural environment associated with the use of pesticides and to limit the application of pesticides to the necessary degree. Member States might consider the appropriateness of additional herbicide uses, especially when addressing sustainable use of plant protection products according to Directive 2009/128/EC.

In the peer review process (evaluation table, data requirement 4.5) the applicant was asked to provide information on the possible runoff of glyphosate and consequent concentrations of glyphosate and AMPA in surface water, that might result when application occurs indirectly on hard man made surfaces, consequent to the representative uses of 'all seeded or transplanted crops' and 'all seeded crops' (first two rows of the GAP table).
GTF argumented that direct (spot) application onto hard surfaces are not part of the representative GAP supporting the glyphosate Annex I renewal submission and that indirect applications on hard man made surfaces are outside the scope the current EU risk assessment paradigms and no guidance is available on what such an assessment should look like or which assumptions such assessment should underpin.
Therefore, regarding any hard and man made surface risk assessments, the GTF refers to the re-authorization process of individual company's PPPs containing glyphosate under Article 43 to defend this use at zonal/national level.
RMS considers that additional specific information in order to assess the potential contamination of surface water by runoff as well as the potential contamination of groundwater via runoff in surface water with subsequent bank filtration are only required, if applications of glyphosate on hard man made surfaces, like railway facilities are intended at the national level.

KIIA 7.12/1 (Calliera et al., 2011, BVL no 2310280)

Annex point:	KIIA 7.12/1
Author(s):	Calliera M., Ferrari F., Lamastra L.
Title:	Investigation of the potential glyphosate groundwater contamination in Lomabardia region (North Italy)
Date:	October 20, 2011
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Yes

Materials and Methods
The clarification of the reported glyphosate findings was done in stepwise procedure. In the first part of the study, all accessible information (including information on methods and tools implemented or used by ARPA Lombardia for planning monitoring programs and also information on the analytical method) was requested from the responsible authorities (ARPA-Lombardia) and checked. As this information was insufficient to clarify the findings, detailed information on soil characteristics, technical and hydrogeological information was gathered, and a site inspection carried out to verify the well status and inspect the well surrounding. Local authorities or the owners of the wells were also contacted.

As the available information did not allow to assess the quality of the analyses and the water sampling method, parallel samples from contaminated sites, and from surrounding areas where piezometers were available, were collected to assess residues levels, characterise the water, and to investigate the impact of a different analytical method: the analytical method used by ARPA Lombardia (FMOCCl derivatisation with HPLC:fluorescence detector) was implemented in the laboratories of the University of Piacenza and compared with method using FMOCCl derivatisation followed by LC/MS/MS (method LOD of 0.02 µg/L).

Results and discussion

Table B.8.6-40 provides an overview of the findings and the result of the assessment.

Table B.8.6-40: Overview on findings of glyphosate/AMPA and results of the assessments

Site	Date of findings	Glyphosate level (µg/L)	Cause of the findings	Date of resampling	Glyphosate level (µg/L)
Pandiono (CR)	10 May 2007	0.9	Inflow of surface water	28 Nov 2010	< LOQ
Trigolo (CR)	22 May 2007	0.2	Point source contamination	28 Nov 2010	0.252
Caselle Lurani (LO)	08 May 2007	0.2	Point source contamination	30 Nov 2010	0.163
Asola (MN)	05 June 2007	0.7	Investigation still ongoing	16 Oct 2010	0.525
San Benedetto (MN)	06 June 2007	1.2	Point source contamination	12 Jan 2011	1.375

Pandino: The inspection of the monitoring well showed that the piezometer was not sealed and that surface water and mud from the adjacent areas were standing between the cast iron manhole and the piezometer, and could thus flow into the well.

Trigolo: The owner of the site explained that during the investigation period he weeded the stretch of land around the piezometer with a glyphosate containing herbicide to facilitate access. A careful inspection of the site showed that the base of the piezometer was not well isolated and could thus lead to preferential flows.

Asola: Investigations are ongoing to confirm hypotheses of possible contamination such as infiltration of rainwater from paved surfaces and drainages

Casselle Lurani: The old well (more than one century) is located in a private courtyard of a farm, and is not completely sealed. Surface water from the courtyard can enter directly into the well from the manhole. The farmer uses the well to wash the spray equipment and tractor next to the well.

San Benedetto: the well is located in the courtyard in front of the product and sprayer storage of a company applying herbicides on railways. The area next to the well is used to clean and maintain the trucks used for herbicide application.

The results of the analysis of the samples taken more than 3 years after the reported detect did confirm the levels observed in 2007 in the wells from Trigolo, Caselle Lurani, Asola and San Benedetto, demonstrating the persistence of the contamination.

Conclusions

The detailed investigation has allowed plausible explanations of the origin of the glyphosate findings at 4 of the 5 locations. In one location the investigation is still ongoing. In all cases the origin of the glyphosate concentrations could be allocated to surface inflow or to point source contamination. Up until now, there was not a single case for which the findings could be correlated with the normal and proper use of the active ingredient in the field.

RMS Comment

It has to be noted that glyphosate findings exceeding the limit concentration 0.1 µg/L have been measured in groundwater aquifers at 5 locations in Italy. A detailed investigation has allowed plausible explanations of the origin of the glyphosate findings at 4 of the 5 locations. Thereby, the origin of the glyphosate concentrations could be allocated to surface inflow or to point source contamination. In one location the investigation is still ongoing. Until now, the findings could not be correlated with the normal and proper use of glyphosate in the field.

KIIA 7.12/2 (Schmidt and Reichert, 2006, BVL no 2310282)

Annex point:	KIIA 7.12/2
Author(s):	Schmidt B., Reichert N.
Title:	Clarification of well-related findings of glyphosate and AMPA in groundwater
Date:	December 14, 2006
Guideline(s):	Aden et al, 2002
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Yes

Materials and Methods

During groundwater monitoring in Germany, glyphosate was found only at 5 wells, while the metabolite AMPA appeared at 21 locations ≥ 0.1 µg/L since 2003. Therefore, the Federal Office of Consumer Protection and Food Safety (BVL) has requested registration owners of glyphosate containing plant protection products to investigate the causes of findings ≥ 0.1 µg/L of glyphosate and its metabolite AMPA in the groundwater.

The clarification of the reported glyphosate and AMPA findings was done in stepwise procedure. In a first step all easily accessible information was requested from the responsible authorities. Then it was checked within a pre-evaluation, if on this basis definite conclusions regarding the validity and origin of the finding are possible. Provided other causes than the normal and proper use of the active ingredient or the origins of the findings were obvious, the investigations were terminated. For findings, where this was not the case, a detailed investigation was conducted. For this purpose relevant data with regard to technical, hydrogeological information were gathered and local authorities or the owners of the wells were contacted. Then a site inspection was conducted and if possible an interview with persons who are able to contribute to the clarification, as farmers, well operators etc. was performed. In an additional step information on the analytical details were queried from the laboratories.

Results and discussion

Table B.8.6-41 provides an overview of the findings and the result of the assessment.

Table B.8.6-41: Overview on findings of glyphosate/AMPA and results of the assessments

Well	Finding glyphosate [µg/L] (year)	Finding AMPA [µg/L] (year)	Cause of the finding
Bavaria			
Sulzbach	0.25 (2003) 0.25 (2004)	–	well contamination by surface water
Woelsbach	B18: 0.16 (2004) B26: 0.12 (2004)	–	influence from a waste deposit
Escherndorf	0.06 (2003)	0.20 (2003)	sample contamination
Bamberg (Luisenhain FB1/FB2 and Gereuth FB9)	FB1: 0.16, FB2 0.12 FB9 0.32 (2001)	–	bank filtrate
Hessen			
Meineringhausen	–	0.11 (2004)	analysis not valid
Muehlenberg	–	0.16 (2004)	analysis not valid
Battenberg B2	–	0.11 (2004)	analysis not valid
Ronshausen	–	0.11 (2004)	analysis not valid
Schoenberg	–	0.14 (2004) 0.16 (2005)	analysis not valid
Bicken	–	0.10 (2004)	analysis not valid
Kleinlueder	–	0.18 (2005)	analysis not valid
Spring Weiher (Ober-Hoegern)	–	0.16 (2004)	analysis not valid
B5 (Ober-Hoegern)	–	0.13 (2004)	analysis not valid
BUGA (Praunheim III)	–	0.18 (2004)	analysis not valid
Geisenheim	–	0.14 (2004)	analysis not valid
Niederrad I	–	0.40 (2004)	analysis not valid
Walldorf	–	0.12 (2004)	analysis not valid
Messenhausen	–	0.11 (2004)	analysis not valid
Seeheim-Jugenheim	–	0.10 (2004	analysis not valid
Viernheim	–	0.10 (2004)	analysis not valid
Baden-Wuerttemberg			
Riesbuerg-Pflaumloch	0.17 (2002) 0.08 (2003)	0.5 (2002) 0.27 (2003)	wastewater influence from a sewage plant
Laufenburg	–	0.15 (2002) 0.12 (2003)	bank filtrate
Laudenbach	–	0.18 (2002) 0.06 (2003)	surface water inflow
Weinheim	–	0.11 (2002) 0.15 (2003)	deficient monitoring well quality/contamination by surface or sewage water

Conclusions
The detailed investigation has allowed providing plausible explanations of the origin of the glyphosate and AMPA findings at the 24 locations. In all cases, if the analysis was not false positive, the origin of the glyphosate and/or AMPA concentrations could be allocated to surface or waste water influences. There was not a single case for which the findings could be correlated with the normal and proper use of the active ingredient in the field.

RMS Comment
The Federal Office of Consumer Protection and Food Safety (BVL) has requested registration owners of glyphosate containing plant protection products to investigate the causes of findings ≥ 0.1 µg/L of glyphosate and its metabolite AMPA in the groundwater, which were reported from monitoring points in Germany, more specifically in Bavaria, Baden-Wuerttemberg and Hessen since 2003. Thereby, glyphosate was found at 5 wells, while the metabolite AMPA appeared at 21 locations.

The further detailed investigation during this study has furnished a plausible explanation of the origin of glyphosate or AMPA findings for all 24 locations of finding. The findings can be classified in groups of causes: Five wells showed inflow of surface water or bank filtrate (Sulzbach, Bamberg, Laufenburg, Laudenbach and Weinheim) and one well was affected by a waste deposit (Woelsau). In one case the well was located inside a sewage plant and showed the influence of the waste water (Riesbuerg-Pflaumloch). In Escherndorf the sample was contaminated at the well which serves as a processing water well for a tank filling place. 16 findings reported from Hessen were due to an analysis which was obviously deficient.

Furthermore, it was shown that there is not a single case in which there is a direct connection with the normal and proper use of the active ingredient in the field and the findings of glyphosate and AMPA in groundwater.

KIIA 7.12/3 (Franke et al., 2010, BVL no 2310284)

Annex point:	KIIA 7.12/3
Author(s):	Franke, A.C., Groeneveld, R.M.W., Kempenaar, C.
Title:	Evaluatie van metingen van glyfosaat en AMPA in grondwater in Nederland (Evaluation of glyphosate and AMPA measurements in groundwater in the Netherlands)
Date:	October, 2010
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Yes

Materials and Methods
The clarification of the reported glyphosate and AMPA findings was done in stepwise procedure. In a first step information on the analytical method, sampling and data processing and on the characteristics well surroundings were gathered and evaluated. The second step involved an on-site investigation with a special focus on the protection of the well.

Results and discussion
Glyphosate was found in 6 out of the 189 measurements (3.71 % of all measurements) carried out in one report (2008, Royal Haskoning), and 4 out of 691 measurements (0.58 % of all

measurements) carried out in the second report (2007, RIVM). Table B.8.6-42 summarises available information about the different locations investigated. The locations 1-10 related to the 2008 report, locations 11 to 14 to the 2007 report.

Table B.8.6-42: Overview on findings of glyphosate/AMPA and results of the assessments

Location	Concentration (µg/L)	Date	Description	Groundwater body	Land use in surrounding area	Well protection[1)]	Sample processing[2)]
1. South Limburg, Vaals	0.16 glyphosate	8th October 2007	Spring near Sinselbeek	Chalk South Limburg	Agriculture/Nature (forest)	+/-	+
2. South Limburg, Valkenburg	0.13 glyphosate	11th October 2007	Spring on plateau near Geul	Chalk South Limburg	Agriculture/Nature (forest)	+/-	+
3. Central Limburg, Maasbracht	0.20 glyphosate 0.12 AMPA	11th July 2007	Semi-deep groundwater (6-8 m)	Maas Deep Channel	Agriculture	-	+
4. North Limburg, Tegelen,	0.12 glyphosate	19th Sept. 2007	Semi-deep groundwater (13-15 m)	Maas Sand	Agriculture	+/-	+
5. North Limburg, Nuland,	0.62 glyphosate 0.23 AMPA	1st October 2007	Shallow groundwater (2 m)	Maas Deep Channel	Agriculture/ Groundwater protection area	+	+
6. North Limburg, Laarbeek,	0.13 glyphosate	23rd October 2007	Shallow (phreatic) groundwater (2-3 m)	Maas Sand	Agriculture	+	+
7 Central Limburg, Grathem,	0.17 AMPA	17th October 2007	Semi-deep groundwater (3 m)	Maas Deep Channel	Recreation (bungalow park)	+/-	+
8. North Limburg, Broekhuizn	0.17 AMPA	24th Sept. 2007	Semi-deep groundwater (8-10 m)	Maas Sand	Agriculture	+/-	+
9. North Limburg, Gennep,	0.17 AMPA	15th August 2007	Semi-deep groundwater (8-10 m)	Maas Sand	Urban area	-	-
	0.13 AMPA		Deep groundwater (18-20 m)				
10 North Brabant, Eindhoven,	0.29 AMPA	3rd October 2007	Semi-deep groundwater (3 m)	Maas Sand	Car park/sports grounds	+	Uncertain bottle code
11. South Holland, Noordwijkerhout	0.99 glyphosate	2003-04	Shallow (< 7 m)		Agricultural (bulbs), residential district	+	
12. North Holland, Texel	4.74 glyphosate	2006	Shallow (< 7 m)		Agricultural	+	
13. Groningen, Hoogezand	0.47 glyphosate	2006	10m deep		Agricultural	+	
14. Groningen, Winschoten	0.32 glyphosate	2006	10m deep		Agricultural	+	

1) On the basis of inspection on location, + signifies good protection of the well, +- signifies that the appearance of surface translocation or drift is unlikely but cannot be excluded, - signifies a likely chance of drift or surface translocation.

2) On the basis of insights into possible errors which occurred during the processing of the samples and the analysis of the data, + signifies no indication that errors have occurred, - signifies that possible errors have occurred.

The results show that at least 2 sites (Maasbracht and Gennep) showed a poor well protection that surface water influx cannot be excluded. At five other sites, the well wasn't fully protected either (2 springs, and 3 sites where the covers did not fully close) and it is conceivable that contact with surface water my occur. Uncertainty related to the data processing was evidenced at 2 sites. For 6 of the 10 sites at which glyphosate was detected, no explanation could be found during this investigation.

It should be noted that numerous AMPA detects were reported from the 2007 studies, but not investigated in his report. AMPA is considered as a non-relevant metabolite in The Netherlands and the 0.1 µg/L trigger does thus not apply.

Conclusions
This has evidenced a potential contact between ground and surface water due to a bad sealing of the well cover for 7 wells, and uncertainty related to data processing in 2 sites. No explanation for 6 out of the 10 glyphosate detects could be found within the remit of this study.

RMS Comment
At 6 out of 189 sampling locations glyphosate was measured above the detection limit and drinking water threshold of 0.1 µg/L carried out in one report (Royal Haskoning, 2008), and at 4 out of 691 measurements carried out in a second report (RIVM, 2007). The results show that some wells were not fully protected and it is conceivable that contact with surface water may occur. Uncertainty related to the data processing was evidenced as well. For 6 sites at which glyphosate was detected, no explanation could be found during this investigation. Irrespective of the pathways of glyphosate into groundwater and the uncertainty related to the data processing, it remains unclear if the findings can be correlated with the normal and proper use of glyphosate in the field.

KIIA 7.12/4 (Carter and Pepper, 2005, BVL no 2310285)

Annex point:	KIIA 7.12/4
Author(s):	Carter A., Pepper, T.
Title:	An investigation of reported borehole contamination in the Vemmenhög Catchment, Sweden
Date:	December, 2005
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Yes

Materials and Methods
The clarification of the reported glyphosate and AMPA findings was done in stepwise procedure. In a first step all information on the characteristics of the watershed, water management, land and glyphosate use were gathered and evaluated. The second step involved an on-site investigation of the watershed, including inspection of the boreholes and evaluation of the farm management practices.

Results and discussion

Table B.8.6-43 shows the glyphosate concentrations in water samples taken from 4 boreholes of the Vemmenhög catchment.

Table B.8.6-43: Overview on findings of glyphosate in the Vemmenhög catchment

Sample date/location	Glyphosate residue in µg/L (LOD)	GW depth (m)	Date of adjacent glyphosate application
19/08/2004			
North/East 1	0.045 (0.03)	-3.27	29/10/2003
North/East 2	ND (0.03)	-3.03	
Center West 1	ND (0.03)	-2.14	05/07/2003
Center West 2	ND (0.03)	-2.12	
16/11/2004			
North/East 1	ND (0.02)	-3.24	No
North/East 2	ND (0.02)	-3.13	
Center West 1	0.18 (0.02)	-1.42	20/10/2004
Center West 2	ND (0.02)	-1.13	
09/02/2005			
North/East 1	ND (0.02)	-3.24	No
North/East 2	ND (0.02)	-2.89	
Center West 1	0.035 (0.02)	-1.51	20/10/2004
Center West 2	ND (0.02)	-1.23	

The investigation showed that groundwater often remains close or above field drain depth over the winter period, responding to recharge from excess rainfall. The field drainage in the catchment has been designed to rapidly remove excess water from the surface and also from rooting layers. Although the drains are generally installed at approximately 1-1.5 m depth, they may be as deep as 4 m to accommodate the undulated topography and maintain gradient requirements. There is thus a potential for direct hydrological connectivity between surface water and shallow groundwater.

Conclusions

The detailed investigation has evidenced a potential contact between ground and surface water through the drainage system in place at this watershed.

RMS Comment

Glyphosate was detected in two pairs of boreholes between August 2004 and February 2005 in the Vemmenhög catchment in Southern Sweden. In the first well the concentration reached 0.045 µg/L whilst in the second well located glyphosate was detected at 0.18 and 0.035 µg/L. A review of the regional characteristics showed that extensive drainage systems are in place in the catchment. About 23 % of the catchment was treated with glyphosate and this included application to the immediate or near vicinity of the boreholes. Historical data reviews as well as observations at the site demonstrate that there can be potential for direct hydrological connectivity between surface water and shallow groundwater via artificial drainage systems.

KIIA 7.12/5 (Anonymous, 2012, BVL no 2310289)

Annex point:	KIIA 7.12/05
Author(s):	Anonymous
Title:	Analysis of groundwater contamination with glyphosate/AMPA
Date:	February, 2012

Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Yes

Materials and Methods

The annual reports of the French Environmental Institute (Institut Français de l'Environnement, Ifen) monitoring the plant protection products in the French waters mentioned the detection of glyphosate and its degradation product, AMPA, above 0.1 µg/L in several groundwater sampling sites. A selection of 27 sites for further investigation was performed based on the information available in the ADES database (Accès au Données sur les Eaux Souterraines – access to groundwater data) at the start of the project (April 2010).

The investigation of the groundwater contamination with glyphosate/AMPA included three phases:

- Phase 1: Survey on the sampling and analysis conditions: first, the parties organizing the analyses were contacted with a view of identifying the laboratories that performed the analyses with glyphosate/AMPA detections. After identification, those laboratories were contacted in order to identify the various elements of the sampling and analysis process.

- Phase 2: Investigation of the wells and their environment:
  - The hydrogeological characteristics of each well are described mainly on the basis of available information from existing databases. The collected elements were later corroborated during site visits. The various researched data included the use of the well, the existence of a well protection (water supply well), water yield, depth of the works and, if possible, groundwater level, the geology at the well and in its proximity. The analysis of these elements enabled the assessment of the "hydrogeological vulnerability of the well".
  - The analysis of the soil characteristics was performed based on soil cores taken within 1 km around the well within the boundaries of the catchment area. Therefore, three soil cores were taken at each site. When the topographic features allowed it (slopes, break of slope, deep ditch), a soil profile study was performed, in order to describe the existing soils in a more detailed manner. The collected soil information included the soil texture, colour of the different horizons, soil depth, depth of rock weathering layer occurrence and depth of occurrence for the parent rock itself, load of coarse elements, hydromorphy, organic matter content, and characteristics of the underlying geological layer.
  - Within 1 km around the well and depending on the well catchment area, the land use was surveyed for the following elements:
    - agricultural area: crop type for each plot (when the plot is ploughed and where possible, the previous crop was identified on the basis of crop residues);
    - non-agricultural area: residential areas, industrial and commercial areas, road infrastructure. Any development likely to contribute to groundwater contamination through glyphosate use.

    For each well, a cartographic representation on an orthophoto base was prepared, comprising the above elements.

- Phase 3: Summary of data and definition of a confidence (or reliability) index related to groundwater glyphosate/AMPA contamination. This index is a crossing between the risk of groundwater contamination by glyphosate (based on land (glyphosate potential) use and site/aquifer vulnerability) and the characteristics of the analyses performed (laboratory, method, detection frequency, presence of other plant protection products, nitrates, coliforms). It does not take into account the inherent physico-chemical properties of glyphosate and its metabolite AMPA. A reliability index from 1 to 10 (low to high confidence) has been estimated for each site.

Results and discussion

Table B.8.6-44 provides information on the method used by laboratories that performed the glyphosate and AMPA analyses, as well as the number of sites covered by the lab.

Table B.8.6-44: Methods of analyses

Laboratory	Clean-up step	Derivatization	Detection	Quantification	Number of sites
IPL (Maxeville)	Concentration and clean-up on FPC cartridge	FMOC-Cl	LC/MS/MS	Internal standard (cysteic acid)	13
SGS laboratory	Acidification and sample concentration	O-Phthalaldehyde	HPLC - Fluorescence	External standard	1
CAR (Illkirch)	Sample concentration and acidification	O-Phthalaldehyde	HPLC - Fluorescence	External standard	1
IPL (Lille)	No	FMOC-Cl	LC/MS/MS	Internal standard ($^{13}C^{15}N$ glyphosate)	2
LASAT (La Rochelle)	No	FMOC-Cl	HPLC - Fluorescence	External standard	1
ASPOSAN (Montbonnot)	No	FMOC-Cl	HPLC - Fluorescence	External standard	2
LD26 (Valence)	No	FMOC-Cl	HPLC - Fluorescence	External standard	4
Labo des Pyrénées (Lagor)	Concentration after derivatisation	FMOC-Cl	HPLC - Fluorescence	External standard	1

The most reliable methods are the ones involving LC/MS/MS with an internal standard, which is the case for the IPL laboratories (Lille and Maxeville). However, the laboratory from Maxeville has only been acquired by IPL in 2008, and the analytical method previously used may not have been the one described in the table above.

The quantification limit of most of the analytical methods was at 0.1 µg/L; suggesting that a relatively high margin of error will be associated to detects close to this value (below 0.15 µg/L).

Table B.8.6-45 summarises the results of the investigation for the 27 wells.

Table B.8.6-45: Investigation results

Site (use of the well)	Well water table (m)	Well depth (m)	Findings	Results investigation	Confidence index[1)]
Sites rejected (not investigated)					
Lureuil	81	-	0.14 µg/L glyphosate (04/2008)	Confined aquifer- low vulnerability	low
Sarreinseming	256	-	0.23 µg/L glyphosate/ 2 µg/L AMPA (04/2008)	Low vulnerability	low
Sites with single glyphosate detects(no AMPA) (2007-2008)					
Nort sur Erdre (DW supply)	63	up to 8 m	0.17 µg/L glyphosate (06/2007) 15 subsequent analyses <0.1 µg/L (2007-2010)	Highly vulnerable water body – permeable soils - Numerous pesticides>0.1 µg/L + nitrates >50 mg/L Glyphosate use likely	high
Avant-les-Ramerupt (DW supply)	50	Up to 10.7 m	0.15 µg/L glyphosate (10/2007) 2 subsequent analyses <0.1 µg/L (2010)	Highly vulnerable water body – permeable soils Some pesticides >0.1 µg/L Glyphosate use likely	high
Boissy le Repos (Petroleum research)	64	-	0.3 µg/L glyphosate (04/2007) Single analysis	Water body of medium vulnerability - No well protection Glyphosate use unlikely	low
Bouy (DW supply)	28	up to 8.3 m	0.13 µg/L glyphosate (10/2007) 4 subsequent analyses <0.1 µg/L (2009-2010)	Water body of medium to high vulnerability – permeable soils Glyphosate use likely	medium
Vernoy (DW supply)	6.6	up to 0.9 m	0.27 µg/L glyphosate (10/2007) Analysis 2 days later : <0.1 µg/L 21 subsequent analyses <0.1 µg/L (2008-2010)	Highly vulnerable water body Numerous pesticides>0.1 µg/L –regular detection of coliforms Glyphosate use likely	high
Tonnay Charente (DW supply)	9	up to 6.2 m	0.19 µg/L glyphosate (11/2007) No subsequent analysis	Highly vulnerable water body and permeable soils – nitrates > 50 mg/L Glyphosate use likely	high
Castagnède (DW supply)	-	-	1.19 µg/L glyphosate (06/2007) One subsequent analysis <0.1 µg/L (2008)	Highly vulnerable water body (possible contact with surface water) – no other pesticide detects – permeable soils Glyphosate use unlikely	low
Sites with single glyphosate detects(no AMPA) (2007-2008)					
La Chapelle Agnon (DW supply)	-	-	0.21 µg/L glyphosate (10/2008) Four subsequent analyses <0.1 µg/L (2009-2010)	Water body of average to high vulnerability – permeable soils -no other pesticides detected Glyphosate use unlikely	low

Table B.8.6-31: continued

Site (use of the well)	Well water table (m)	Well depth (m)	Findings	Results investigation	Confidence index[1)]
La Roche Noire (DW supply)	-	-	0.12 µg/L glyphosate (10/2008) Three subsequent analyses <0.1 µg/L	Highly vulnerable water body (possible contact with surface water) – permeable soils - one other pesticide detect Glyphosate use possible	low
Saint Cyr sous Dourdan (DW supply)	88	up to 3 m	2.06 µg/L glyphosate (09/2007) One subsequent analysis (2009) <0.1 µg/L	Highly vulnerable water body – no other pesticide detected >0.1 µg/L Glyphosate use possible	medium
Houvin-Houvigneul (DW supply)	54.5	up to 35.1 m	0.3 µg/L glyphosate (11/2007) One subsequent analysis (2010) <0.1 µg/L	Highly vulnerable water body – no other pesticide detected >0.1 µg/L Glyphosate use likely	high
Aubignan (Private well - Qualitometer)	7	-	0.2 µg/L glyphosate (10/2007) No subsequent analysis	Highly vulnerable water body – permeable soils - few other pesticide detected >0.1 µg/L Glyphosate use likely No well protection	high
Grosne (DW supply)	5.5	-	0.12 µg/L glyphosate (12/2007) One subsequent analysis <0.1 µg/L (2009)	Water body of medium vulnerability – no other pesticide detected Glyphosate use possible	low
Issans (DW supply)	5.2 m (spring)		0.2 µg/L glyphosate (08/2007) 3 subsequent analyses <0.1 µg/L	Highly vulnerable water body – many other pesticides detected – permeable soils Glyphosate use likely	very high
Villers-Farlay (DW supply)	-	-	0.31 µg/L glyphosate (07/2007) Four subsequent analyses <0.1 µg/L (2010)	Highly vulnerable water body – many other pesticides detected –permeable soils Glyphosate use likely	high
Machecoul (DW supply)	-	-	0.13 µg/L glyphosate (06/2007) 20 subsequent analyses <0.1 µg/L	Highly vulnerable water body – many other pesticides detected – very permeable soils Glyphosate use likely	high

Table B.8.6-31: continued

Site (use of the well)	Well water table (m)	Well depth (m)	Findings	Results investigation	Confidence index[1)]
Sites with simultaneous glyphosate and AMPA single detects (2007-2008)					
Saint Georges d'Esperanche (DW supply)	56	up to 34.7 m	0.22 µg/L glyphosate (01/2008) 0.22 µg/L AMPA (01/2008) 14 subsequent analyses <0.1 µg/L (2008-2010)	Water body of medium to high vulnerability – few other pesticides detected – very permeable soils Glyphosate use likely	high
Monteynard (DW supply)	-	-	0.3 µg/L glyphosate (01/2008) 0.14 µg/L AMPA (01/2008) Two subsequent analyses <0.1 µg/L (2009-2010)	Highly vulnerable water body – no other pesticide detected >0.1 µg/L Glyphosate use unlikely	low
La Flotte (private well)	20	up to 8m	0.21 µg/L glyphosate (09/2008) 0.19 µg/L AMPA (09/2008) Four subsequent analyses <0.1 µg/L (2009-2010)	Water body of medium to high vulnerability – very permeable soils Glyphosate use very likely No well protection area	very high
Sites with multiple detects					
Chepy (fire well)	4	-	0.56 µg/L glyphosate (09/2007) 3.4 µg/L AMPA (09/2007) 2.36 µg/L AMPA (10/2008) 0.63 µg/L glyphosate (04/2009) 0.21 µg/l AMPA (0.4/2009) 0.63 µg/L glyphosate (10/2010) 1.04 µg/L AMPA (10/2010)	Very highly vulnerable water body – many other pesticides detected – nitrates >50 mg/L Glyphosate use very likely No well protection area	very high
Corbeilles (piezometer)	19	up to 9 m	0.254 µg/L glyphosate (05/2007) 0.14 µg/L glyphosate (10/2010) 0.19 µg/L glyphosate (12/2010) Regular AMPA detects 2007-2010 (average 0.2 µg/L)	Very highly vulnerable water body – many other pesticides detected – nitrates >50 mg/L Glyphosate use	very high
Blanzay (DW supply)	60	up to 9.5 m	0.2 µg/L glyphosate (10/2008) 0.5 µg/L glyphosate (12/2008) Nine subsequent analysis <0.1 µg/L (2009-2010)	Highly vulnerable water body – few other pesticides detected – nitrates >50 mg/L Glyphosate use likely	very high

Table B.8.6-31: continued

Site (use of the well)	**Well water table (m)**	**Well depth (m)**	**Findings**	**Results investigation**	**Confidence index[1)]**
Fontenay le Pesnel (DW supply)	3.8	up to 2 m	0.137 µg/L glyphosate 04/2007) 12.9 µg/L glyphosate (10/2007) 0.92 µg/L AMPA (10/2007) Six subsequent analyses <0.1 µg/L (2008-2010)	Highly vulnerable water body – few other pesticides detected Glyphosate use likely	high
Evans (Qualitometer)	spring	-	0.16 µg/L glyphosate (07/2008) 0.95 µg/L AMPA (07/2008) 0.1 µg/L glyphsoate (07/2010) 1.1 µg/L AMPA (07/2010) 0.24 µg/L glyphosate (05/2010) 0.73 µg/L AMPA (05/2010)	Highly vulnerable water body – many other pesticides detected Glyphosate use likely	very high
Avrille (private use)	6.5	up to 1.65 m	0.68 µg/L glyphosate (05/2007) 0.3 µg/L glyphosate (03/2008) AMPA regularly detected – average 0.41 µg/L (1007-2010)	Highly vulnerable water body – many other pesticides detected – nitrates >50 mg/L permeable soils Glyphosate use very likely	very high

Two sites were rejected early in the process due to their low vulnerability and no further investigation was performed. Out of the 25 remaining sites, 16 showed a single detect of glyphosate (without AMPA) and three showed a single detect for both glyphosate and AMPA. With the exception of three sites at which no subsequent glyphosate analysis were performed after the detect, all other sites had samples analysed within the months/years after the detect showing glyphosate/AMPA results <0.1 µg/L, demonstrating that the contamination was not widespread in the aquifer and not long-term.

Multiple detects were observed in six sites, two of which were used for drinking water supply. At those sites, the detects occurred the same year, and the analyses performed the following years showed no further contamination by glyphosate and AMPA. The four other sites with multiple detects had no well protection area, and were not suited for drinking water supply (private or fire well, piezometer, qualitometer, spring).

The estimated confidence index was very high for seven of the 25: five of those are sites with multiple detects, one is a private well with no well protection area, and one is a site used for DW supply (spring in a karstic soil, shallow water which showed many detects of plant protection products including one isolated detects of glyphosate).

The estimated confidence index was low for six of the 25 sites: in those areas the use of glyphosate was questionable.

Conclusions

In an attempt to investigate the presence of glyphosate/AMPA in groundwater, a selection of 27 groundwater abstraction sites was evaluated. Two sites were rejected early in the process due to their low vulnerability, suggesting that the reported detect was not accurate.

An in-depth investigation was performed on the 25 remaining sites. The results show that in 76 % of the cases (19 wells), the detections were sporadic (one sample of several analysis), demonstrating that the contamination was not widespread in the aquifer. Multiple detects over one year were observed in 2 sites, used for drinking water supply, but they did not occur the following years. Multiple detections over several years were observed in four sites which are not used for drinking water supply and none had a well protection area.

In summary, none of the glyphosate detects could be attributed to long-term contamination of typical groundwater.

RMS Comment

The annual reports of the French Environmental Institute (Institut Français de l'Environnement, Ifen) monitoring the plant protection products in the French waters mentioned the detection of glyphosate and its degradation product, AMPA, above 0.1 µg/L in several groundwater sampling sites. A selection of 27 sites for further investigation was performed.

Thereby, two sites were rejected early in the process due to their low vulnerability and no further investigation was performed. Out of the 25 remaining sites, 16 showed a single detect of glyphosate (without AMPA) and three showed a single detect for glyphosate and AMPA. With the exception of three sites, all other sites had samples analysed within the months/years after the detection showing glyphosate/AMPA results <0.1 µg/L, demonstrating that the contamination was not widespread in the aquifer and not long-term. Multiple detects were observed in six sites, two of which were used for drinking water supply. At those sites, the detections occurred the same year, and the analyses performed the following years showed no further contamination by glyphosate and AMPA. The four other sites with multiple detects had no well protection area, and were not suited for drinking water supply (private or fire well, piezometer, qualitometer, spring).

It has to be noted that this groundwater glyphosate/AMPA contamination constitutes a limited approach for the following reasons:

- Heterogeneity of analytical data and sometimes absence of data
- Only one site visit focused on the analysis of the physical setting within a 1 km range
- Difficulty concerning the confidence index related to glyphosate/AMPA groundwater detection
- Absence of information on glyphosate use (agricultural and non-agricultural uses)

KIIA 7.12/6 (Horth, 2012, BVL no 2310291)

Annex point:	KIIA 7.12/06
Author(s):	Horth, H.
Title:	Survey of glyphosate and AMPA in groundwaters and surface waters in Europe
Date:	April, 2012

Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Yes

Materials and Methods

This investigation is a desk study, and the information was obtained from professional contacts across Europe (government departments and research organisations in each of the countries), and including some data provided by Monsanto Europe, as well as from web and literature searches, and by querying on line databases.

Results and discussion

Table B.8.6-46: and Table B.8.6-47: provide an overview of the main data for surface water and groundwater, respectively. The summarised data is not precise but presents a best estimate, mainly because of the various forms in which the data were obtained, e.g. some results in terms of samples, others in terms of sites, and other gaps in information.

In total, there is data for 17 countries, 14 each for surface water and groundwater, with most countries including both. However, the Czech and Slovak Republics monitor only surface water, and data for Spain was available for surface water only; for Malta and Switzerland only groundwater data was obtained. Data were mainly collated at national level, but in some cases regional, as for Belgium (two regions), Italy (one region), and Germany (surface water data for several Länder (i.e. Federal States)). Seven countries have confirmed that there is no monitoring of glyphosate and AMPA (Bulgaria, Cyprus, Hungary, Latvia, Lithuania, Luxembourg and Romania), no information was obtained from the remaining five countries (Estonia, Greece, Poland, Portugal and Slovenia).

Table B.8.6-46: Summary of glyphosate and AMPA data in surface water in Europe

Country/ Substance	Date	No. sites	No. samples	Detected (samples)		Samples ≥ 0.1 µg/L		Max. Conc.	LoQ (LoD)
				No.	%	No.	%	µg/L	µg/L
Austria									
AMPA	2001-02	-[1)]	345	≥90	≥26	90	26	3.4	-[1)]
Belgium (Flanders - F and Wallonia - W)									
Glyphosate F	2007-11	198	5350	4450	83.2	1387	25.9	139	0.05-0.4
AMPA F	2007-11	198	5351	4967	92.8	3215	60.1	47	0.05-0.4
Glyphosate W	2001-06	26	531	≥ 429	≥ 81	429	81	1.3	≤ 0.1
Czech Republic									
Glyphosate	2010-11	41	359	168	47.8	96	28.7	5.3	0.025-0.05
AMPA	2010-11	9	165	165	100	138	83.6	1.37	0.05
Finland									
Glyphosate	2002-09	3	26	3	11.5	2	7.7	0.46	0.1
AMPA	2002-09	3	26	3	11.5	1	3.8	0.22	0.05
France									
Glyphosate	97-2009	≥2493	57171	17251	30.2	13655	23.9	50	0.03-0.2
AMPA	98-2009	≥2217	46969	24325	51.8	22062	47.0	48.9	0.02-0.5
Germany (Baden-Württemberg, Hessen, Rheinland-Pfalz, Thüringen & River Rhine combined)									
Glyphosate	97-2011	105	1298	386	29.7	96	7.4	4.7	0.02-1.5
AMPA	97-2011	66	782	571	57.5	514	65.7	3.6	0.05-0.5
Ireland									
Glyphosate	2005-11	256	2483	139	5.6	≥42	≥1.7	186	0.08-0.1/ 20
AMPA	2010-11	-[1)]	496	1	0.2	1	0.2	>200	20
Italy (Lombardia Region)									
Glyphosate	2005-08	150	919	224	24.3	224	24.3	37.6	0.1
AMPA	2008	59	239	208	87.0	208	87.0	37	0.1
Norway									
Glyphosate	97-06	11	80	74	92.5	≤ 57	≤ 71	0.93	(0.01)
AMPA	97-06	11	80	74	92.5	≤ 48	≤ 60	0.54	(0.01)
Slovak Republic									
Glyphosate	2006-10	142	2092	321	15.3	261	12.6	3.6	(0.05)
Spain 2)									
Glyphosate	2006-08	115	748	96	7.4	80	11	15.3	0.003-0.1
Sweden									
Glyphosate	2000-10	≥ 21	1306	360	27.6	≥15	≥1.1	370	<0.1
AMPA	2000-10	≥ 21	1285	244	19.0	≥14	≥1.1	4.0	<0.1
The Netherlands									
Glyphosate	2010	293	1349	254 (s)	87 (s)	198 (s)	68 (s)	>1.0	<0.1
AMPA	2010	293	1374	293 (s)	100 (s)	≥ 40 (s)	≥14 (s)	>8.0	-[1)]
UK									
Glyphosate	93-2011	≥105	3730	759	20.3	759	20.3	8.2	0.1
Total									
Glyphosate	93-2011	≥3959	75350	≥24914	≥33	≥17301	≥23	1.3-370	0.003-1.5 (20)
AMPA	97-2011	≥2879	57112	≥30941	≥54	≥26331	≥46	0.22->200	0.02-0.5 (20)

LoQ = Limit of Quantification, LoD = Limit of Detection

1) No information

2) Data from sites with known quality problems
(s) sites (number of samples not known)

Glyphosate has been analysed in almost 75 000 surface water samples from about 4 000 sites (from 1993-2011) and detected in 33 % of samples, with 23 % above 0.1 µg/L; AMPA has been analysed in about 56 700 samples from nearly 3 000 sites (1997-2011) and detected in 54 % of samples, with 46 % above 0.1 µg/L.

Glyphosate has a high usage rate and has been rated among the most frequently detected herbicides in some countries, notably in the Netherlands. It has been suggested that urban run-off can be a significant source of glyphosate in surface waters (France and the Netherlands). There have been some indications of an upward trend detections and concentrations found in recent years, e.g. in Belgium – Flanders and the Netherlands (not confirmed in recent years, and the opposite in NL - Flevoland), but the data may not be adequate to conclude on trends and co-incides with higher numbers of sites and samples analysed.

Some countries have proposed various environmental quality standards (EQS) or objectives for glyphosate in surface water, ranging from a proposed EQS of 28 µg/L in Mecklenburg-Vorpommern (Germany) and 60 µg/L in Ireland (now accepted), to a proposed no effect concentration (PNEC) of 10 µg/L and a maximum admissible concentration (MAC) of 100 µg/L in Belgium - Flanders, and a Maximum Tolerable Risk (MTR) standard at 77 µg/L, and a pesticide authorisation standard of 64 µg/L in the Netherlands, whilst some professionals (Belgium – Wallonia and Rheinland-Pfalz (Germany) suggested that an EQS should be set. However, none of these standards have been exceeded on a regular basis. Perhaps more importantly, the Netherlands apply the drinking water standard of 0.1 µg/L for pesticides to surface water intakes at waterworks, and LAWA in Germany has set a target value of 0.1 µg/L for the same purpose.

Where data allowed interpretation, glyphosate has been linked to application periods (from spring through to autumn) and run-off events and does not seem to persist. The more persistent presence of AMPA in surface waters throughout the year may be mainly derived from aminophosphonate containing complexing agents in detergents and cooling waters, entering surface waters via wastewater treatment effluents, rather than from the degradation of glyphosate. This seems to be a fairly widely accepted view now, with more evidence having become available, and might explain why AMPA is not always found at higher concentrations, as for example in Sweden, where the population density is low.

Table B.8.6-47: Summary of glyphosate and AMPA data in groundwater in Europe

Country/Substance	Date	No. sites	No. samples	Detected (samples)		Samples ≥ 0.1 µg l^{-1}		Max. Conc.	LoQ (LoD)
				No.	%	No.	%	µg/L	µg/L
Austria									
Glyphosate	2004	~950	3633	7	0.19	2	0.06	>0.1	<0.1
AMPA	2004	~950	3636	44	1.2	11	0.3	0.75	<0.1
Belgium (Flanders & Wallonia)									
Glyphosate (Flanders)	2007-08	450	1088	1	0.1	0	nr	0.011	0.01
AMPA (Flanders)	2007-11	504	3933	707	18	-[1)]	nr	1.85	0.01
Glyphosate (Wallonia)	2000-06	450	≥450	0	nr	0	nr	<0.025	<0.025

Country/Substance	Date	No. sites	No. samples	Detected (samples)		Samples ≥ 0.1 µg l^{-1}		Max. Conc.	LoQ (LoD)
				No.	%	No.	%	µg/L	µg/L
AMPA (Wallonia)	2000-06	450	≥450	13 (s)	3 (s)	0	nr	< 0.05	<0.025
Denmark									
Glyphosate	1993-10	1825	9908	117	1.2	21	0.21	4.7	(0.01-<0.1)
AMPA	1993-10	1840	9906	84	0.84	18	0.18	4.2	(0.01-<0.1)
Finland									
Glyphosate	2002-08	80	80	0	nr	0	nr	nr	0.1
AMPA	2002-08	80	80	0	nr	0	nr	nr	0.05
France									
Glyphosate	99-09	≥7403	45960	515	1.1	390	0.8	24	0.01-0.1
AMPA	99-09	≥7184	30529	442	1.4	321	1.1	19	0.01-0.1
Germany									
Glyphosate	2007	196	≥ 196	7 (s)	3.6 (s)	0	nr	≤ 0.1	<0.1
AMPA	2007	326	≥ 326	10 (s)	3.1 (s)	5	1.5	≥ 1	-[1)]
Ireland									
Glyphosate	2007-09	92	679	6	0.8	1	0.1	0.19	<0.1
Italy (Lombardia Region)									
Glyphosate	2005-08	359	961	0	nr	0	nr	<0.1	0.1
AMPA	2007-08	359	≥619	3	≤0.5	3	≤0.5	0.9	0.1
Malta									
Glyphosate	2009	18	≥ 18	0	nr	0	nr	nr	(0.01)
Norway									
Glyphosate	99-00	7	8	0	nr	0	nr	nr	(0.01)
AMPA	99-00	7	8	1	12.5	0	nr	0.02	(0.01)
Sweden									
Glyphosate	2009-10	≥ 21	1247	1	0.08	0	nr	0.04	(<0.03)
AMPA	2009-10	≥ 21	1242	3	0.24	1	0.08	0.72	(<0.03)
Switzerland									
Glyphosate	2005-06	117	≥ 234	4 (s)	3.4	3 (s)	2.6 (s)	0.21	(0.05)
AMPA	2005-06	117	≥ 232	≥ 10 ≤ 17 (s)	≥ 9 ≤ 14 (s)	≥ 6 ≤ 11 (s)	≥ 5 ≤ 9 (s)	0.46	(0.05)
The Netherlands									
Glyphosate	2003-06	<691	691	4	0.58	4	0.58	4.7	(<0.1)
AMPA	2003-06	<691	691	21	3.0	21	3.0	5.1	(<0.1)
UK									
Glyphosate	95-07	≥ 217	1509	13	0.9	≤ 3	≤ 0.2	0.47	(0.014-0.4)
Total									
Glyphosate	93-2010	12876	≥66662	675	1.0	424	0.64	0.01-24	0.01-0.4
AMPA	93-2011	12525	≥51652	1345	2.6	398	0.77	0.02-19	0.01-0.1

LoQ = Limit of Quantification, LoD = Limit of Detection
1) No information
(s) sites (number of samples not known)
nr = not relevant

Glyphosate and AMPA have been increasingly analysed and occasionally detected in groundwater. Glyphosate has been analysed in almost 67 000 samples from about 675 sites (1993-2010) and detected in 1 % of samples, with 0.6 % above 0.1 µg/L; AMPA has been analysed in 52 000 samples from 1 345 sites (1993-2011) and detected in 2.6 % of samples, with 0.8 % above 0.1 µg/L. These seem to occur in shallow water or spring water, which is often included in groundwater surveys, sometimes associated with contamination incidents (where the information is available), and even unsuitable sampling sites and analytical techniques (investigations in France and Germany).

To date, there seems to be no evidence of any persistent and confirmed groundwater contamination with glyphosate or AMPA. In many cases detections occur in isolated samples rather than consistently at the same sampling site. Where the necessary information is available, it is frequently shown that glyphosate detections are only observed in shallow groundwater (e.g. Denmark and the Netherlands) or wells with surface water influence, for example contamination.

Reports from some countries stated that groundwater contamination with glyphosate and AMPA was not of concern, e.g. Belgium – Wallonia, Finland, Norway, the Czech and Slovak Republics. Some countries have reduced or abandoned glyphosate monitoring in groundwater as a result of special investigations or routine monitoring, where it was rarely found, e.g. Austria, Belgium - Flanders, Baden-Württemberg (Germany), Italy, Sweden and the UK.

Whereas an increase in glyphosate detection and/or concentrations before 2009 in Denmark was indicated, a decrease was reported in 2010. It would need further data and statistical analyses before any conclusions can be drawn.

Conclusions

Ground and surface water monitoring data were gathered from 17 European countries, 14 each for surface and groundwater, with most countries including both. Glyphosate and AMPA have been extensively monitored and frequently detected in surface water above the 0.1 µg/L drinking water standard (23 % of the samples for glyphosate and 46 % for AMPA), but typically below the proposed environmental quality standards or objectives (ecotoxicologically relevant concentration). In groundwater, glyphosate and AMPA have been increasingly monitored and occasionally detected above the 0.1 µg/L limit (0.6 % of the analysed samples for glyphosate and 0.8 % for AMPA). To date, there seems to be no evidence of any persistent and confirmed groundwater contamination with glyphosate or AMPA.

RMS Comment

Horth (2012, BVL no 2310291) provided a review that covers glyphosate and AMPA monitoring results for surface (fresh) waters and groundwater across Europe, i.e. all 27 Member States of the EU.

Glyphosate has been analysed in almost 75000 surface water samples from about 4000 sites (from 1993-2011) and was detected in 33 % of samples, with 23 % above 0.1 µg/L. The maximum concentrations of glyphosate acid found in surface water reached from 1.3 to 370 µg/L. The highest glyphosate values in surface water were detected in Sweden (370 µg/L), Ireland (186 µg/L) and Belgium (139 µg/L). The main metabolite AMPA has been analysed in about 56700 samples from nearly 3000 sites (1997-2011) and was detected in 54 % of samples, with 46 % above 0.1 µg/L and maximum concentrations reaching from 0.22 to > 200 µg/L. It has

to be noted that glyphosate and AMPA monitored in this study exceed the predicted environmental concentrations for glyphosate acid and AMPA in surface water (PEC_{SW}) calculated using the FOCUS (2000) surface water models, even though worst case applications was assumed. Nevertheless, the calculated TER values referring to the monitored concentrations in the study by Horth (2012, BVL no 2310291) with the respective acceptability criteria show that the risk for aquatic organisms is acceptable.

Furthermore, glyphosate and AMPA have been increasingly analysed and occasionally detected in groundwater. Glyphosate has been analysed in 66662 samples from about 675 sites (1993-2010) and detected in 1 % of samples, with 0.64 % above 0.1 µg/L; AMPA has been analysed in 51652 samples from 1345 sites (1993-2011) and detected in 2.6 % of samples, with 0.77 % above 0.1 µg/L. The highest numbers of glyphosate detections have been reported from Denmark (4.7 µg/L) and France (24 µg/L). Findings exceeding the limit concentration 0.1 µg/L have also been measured in groundwater aquifers in Austria, Ireland, the Netherlands and the UK.

Clarification of glyphosate groundwater findings > 0.1 µg/L is presented and discussed partly. The following main causes have been identified in the study:

- In Austria, Glyphosate was detected in isolated cases. AMPA was detected somewhat more frequently and at higher concentrations. There is no information about the type of groundwater, although the results, as presented, related to pore groundwater only, not the springs from fractured aquifers. Traces of AMPA were also found in two spring water samples and it was considered unclear at the time whether these findings were related to glyphosate or to aminophosphonates from detergents.
- Recent reported findings in Denmark all relate to groundwater at less than 15 meter depth. Investigations into earlier detections have shown that these occurred in shallow groundwater wells only.~~, and appeared to be due to rapid transfer of surface water from nearby surfaces. The author of a recent investigation into four sites concluded that the occurrence of glyphosate and AMPA was unlikely to be caused by agricultural and other approved usages. Work is ongoing in Denmark to establish leaching of glyphosate through soils.~~
- Earlier investigations in France relating to a small number of detections of glyphosate in drinking waters, most of which were derived from groundwater, revealed that the detections were most likely due to sample contamination or analytical problems. However, these related to 2001-2003 and more recent findings in groundwater may warrant further investigation. From the findings of a recent study to analyse the potential contamination of groundwater with glyphosate (and AMPA) at 27 sites from 2007-2010, it is clear that none of the glyphosate detections could be attributed to long-term contamination of typical groundwater. The majority of detections occurred once only, which is a clear indication that there is no real groundwater contamination, and the small number of multiple detections occurred in shallow groundwater (spring water) or wells unsuitable for groundwater monitoring, suggesting superficial short-term contamination.
- For the glyphosate groundwater findings > 0.1 µg/L in Ireland no clarification is given in the study.
- According to the present study the detection of glyphosate in groundwater in Switzerland may be attributable to short-term contamination of shallow groundwater or spring water.

- In The Netherlands, both glyphosate and AMPA have been detected in a small number of groundwater samples (once each in 10 different wells). These were investigated in detail and it was concluded that 5 of the results (all at levels below 0.15 µg/L were uncertain (high margins of error) whilst overall, all sampling points with positive detections were in cultivation areas with sandy or highly sandy soils, and samples were taken mainly from shallow groundwater.
- In the UK a number of positive samples and high maximum concentrations were found in Wales in the investigations of pollution incidents. There seems to be a particular problem in Wales, which may warrant further investigation.

Additional study (Sanchís et al., 2012, BVL no 2537361)

In addition to the groundwater monitoring studies submitted by the applicant the paper ”Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry” by Josep Sanchís, Lina Kantiani, Marta Llorca, Fernando Rubio, Antoni Ginebreda, Josep Fraile, Teresa Garrido and Marinella Farré has been online published in Anal Bioanal Chem in February 2012. This publication was submitted to the German Federal Environment Agency (UBA) by the Federal Office of Consumer Protection and Food Safety (BVL) for evaluation in February 2012.

The article reports that a total of 129 groundwater samples from wells located in 11 different sampling sites in Catalonia, Spain, were collected by the Catalan Water Agency between May and September in 2007, 2008, 2009 and 2010. With the exception of one, all the areas studied presented a high impact from intensive agriculture. The samples were analysed by an immunoassay in the first step and to some extent by a LC-MS/MS measurement in the second step. The concentrations of glyphosate range from method limit of quantification (MLQQ) to 2.6 µg/L, the average was 202 ng/L (for the average calculation, samples under limit of quantification were computed as half the MLQQ).

In the publication, it is pointed out that more contaminated areas were found in regions of thriving agriculture activity. However, the highest concentration of glyphosate was measured in sampling site no. 1, which corresponds to an area with only moderate agricultural activity. Therefore, the presence of glyphosate in groundwater cannot exclusively be related to the use as herbicide for agricultural applications. Other sources of glyphosate, e.g. the control of weeds on streams and drains, around railways, roads, sports fields and industrial areas have to be considered as well.

The pathways of glyphosate into groundwater are not investigated by the authors. With the aid of a literature review it is shown that glyphosate can be moved by leaching through uniform gravelly soils and structured soils with macro-pores. Furthermore, glyphosate can be accumulated in soil by precipitation. Spring rains after years of heavy drought could then have favoured the dissolution of glyphosate retained in soil. Due to the fact that surface waters exist in 10 out of 11 sampling sites, surface run-off and/or drainage into these waters with subsequent bank filtration into groundwater cannot be excluded.

According to Regulation (EC) No 1107/2009 it is the immediate task of each Member State to authorise the placing on the market of a plant protection product in its territory. This can imply risk mitigation measures, e.g. buffer zones for application, drift-reduction techniques, a

combination of buffer zones for application and drift-reduction techniques or grassed buffer strips. For authorisation in Germany, exposure assessment of groundwater considers two pathways: (i) direct leaching into groundwater after soil passage and (ii) surface run-off and drainage into an adjacent ditch with subsequent bank filtration into groundwater. During the EU evaluation of the active substances the second pathway is not considered. As stated above, this pathway cannot be excluded as a source of glyphosate in groundwater samples in Catalonia, Spain. At the present, Germany has no knowledge whether this pathway is considered during the authorisation of plant protection products in Spain.

B.8.6.3.2 Drinking water

The Notifier submitted one study (Horth and Gendebien, 2008, BVL no 2310278) reviewing the findings of glyphosate and AMPA in drinking water in selected European countries, which is described in detail below.

To sum up the results of Horth and Gendebien (2008, BVL no 2310278), no glyphosate exceedances of the individual pesticide standard for drinking water of 0.1 µg/L were reported from Belgium, Germany and Ireland, whereas concentrations > 0.1 µg/L have been reported from France, The Netherlands, Sweden and the UK. For AMPA, concentrations > 0.1 µg/L were reported from France and Sweden. Some glyphosate exceedances were attributed to probable problems with the analysis. One of the glyphosate detections turned out to be for raw water rather than finished drinking water. Generally, it remains unclear if the findings can be correlated with the normal and proper use of glyphosate in the field. Nevertheless, the RMS considers that the findings of glyphosate and AMPA exceeding the pesticide standard for drinking water of 0.1 µg/L are giving cause for concern, especially since these compounds can be removed from raw water by drinking water treatment processes to a varying extent (see B.8.5.1). Therefore, the RMS recommends further investigation to seek clarification, such as more details of the sampling sites, type of groundwater and geological conditions, pathway into drinking water and removal during drinking water treatment.

KIIA 7.12/7 (Horth and Gendebien, 2008, BVL no 2310278)

Annex point:	KIIA 7.12/7
Author(s):	Horth, H., Gendebien A.
Title:	Review of glyphosate and AMPA in drinking water in selected European countries
Date:	September, 2008
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Yes

Materials and Methods

Reporting by EU Member States to the Commission under the Drinking Water Directive 98/83/EC (1998) will be incorporated into WISE (Water Information System for Europe) in the near future. However, at present, there are no clear indications of the details of reporting, and data are available in various forms for some Member States.

Available information was sought for Belgium, Denmark, France, Germany, Ireland, Spain, Sweden, the Netherlands, Czech Republic, Greece, Italy and the UK. Information was obtained from web searches and professional contacts.

Drinking water quality reports issued by the responsible national authorities were reviewed for Belgium, Denmark, France, Germany, Ireland, Spain, the Netherlands and the UK. For Sweden data were accessed from a database.

No relevant information was obtained from Greece and Italy. The Czech Republic confirmed that glyphosate was not among the substances monitored.

Results and discussion
The results are summarised in Table B.8.6-48.

Table B.8.6-48: Summary of glyphosate and AMPA monitoring and detection in drinking water in eight EU countries

Country	**Year(s)**	**Monitoring**		**Detection (number)**		**Concentration ≥ 0.1 µg/L (number)**	
		Glyphosate	**AMPA**	**Glyphosate**	**AMPA**	**Glyphosate**	**AMPA**
Belgium	2002-04	not known	not known	?	?	0	0
Denmark	2002-04 [1)]	probably	probably	?	?	0	0
	2001-05 [2)]	yes	yes	54 [3)]		21 [3)]	
France	1993-98	not known	not known	?	?	0	0
	2001-03	yes	yes	26	22	18	15
	2004-06	probably	probably	?	?	0	0
Germany	2002-04	probably	probably	?	?	0	0
	2005	yes	yes	0	0	0	0
Ireland	2005-06	not known	not known	?	?	0	0
Sweden	2000-07	yes	yes	7	14	4	≥ 4
Spain	2002-04	nm	nm	nr	nr	nr	nr
The Netherlands	2000-06	yes	probably	14	?	2	?
UK							
- England	2000-06	yes	not known	?	?	4	?
- Northern Ireland	2002-06	yes	not known	?	?	6 [4)]	?
- Scotland	2005	not known	not known	?	?	≤ 2 [5)]	?

1) large public supplies
2) small/private wells of shallow groundwater, probably untreated
3) glyphosate and AMPA presented as combined amounts
4) 2 of these in private supplies
5) only 2 exceedances of the pesticide standard but substance(s) not specified
nm = not monitored, nr = not relevant, ? = no information

Conclusions

No glyphosate exceedances of the individual pesticide standard for drinking water of 0.1 µg/L were reported from Belgium, Germany and Ireland. A small number of sporadic results > 0.1 µg/L have been reported from France, the Netherlands and the UK. All were isolated detections and none were considered significant, i.e. no reports of improvement measures being needed because of the presence of glyphosate in drinking water.

RMS Comment

No glyphosate exceedances of the individual pesticide standard for drinking water of 0.1 µg/L were reported from Belgium, Germany and Ireland, whereas concentrations > 0.1 µg/L have been reported from France, the Netherlands, Sweden and the UK. For AMPA, concentrations > 0.1 µg/L were reported from France and Sweden. Three of the four glyphosate exceedances in England and Wales were attributed to probable problems with the analysis, due to the occasional occurrences. Whilst there were no reported exceedances for glyphosate or AMPA in large public supplies in Denmark, there were some detections and exceedances in small private supplies. Special investigations revealed that all wells affected were abstracting shallow groundwater (probably supplied untreated) in conditions where there was rapid infiltration of surface water from nearby fields or run-off from treated court yards in the vicinity. A similar situation may be the case in Sweden, where glyphosate and AMPA exceedances were found in drinking water; these seemed to be mainly derived from groundwater, but no further sample details were available. Since the findings of glyphosate > 0.1 µg/L in the Netherlands were isolated exceedances (one sample only in each case), no further action, such as remedial measures, were deemed necessary. One of the glyphosate detections in France turned out to be for raw water rather than finished drinking water. Strangely, the majority of glyphosate detections in France were at sites where groundwater is used. Further investigations failed to establish any coherent relationships between these detections and factors, such as seasonal occurrence, raw water quality, type of aquifer, analysis and water treatment. In fact, several of the samples with glyphosate were found in chlorinated waters; although it has been shown that chlorine effectively remove glyphosate. Overall, the evidence points to isolated detections, most likely due to contamination at the sampling stage or problems with analyses, rather than any indication of a persistent presence in drinking water.

By the examination of the present study, it remains unclear if the findings can be correlated with the normal and proper use of glyphosate in the field. Nevertheless, the RMS considers that the findings of glyphosate and AMPA exceeding the pesticide standard for drinking water of 0.1 µg/L are giving cause for concern, especially since these compounds can be removed from raw water by drinking water treatment processes to a varying extent (see B.8.5.1). Therefore, the RMS recommends further investigation to seek clarification, such as more details of the sampling sites, type of groundwater and geological conditions, pathway into drinking water and removal during drinking water treatment.

B.8.6.4 Other studies on predicted environmental concentrations in surface water and in groundwater (PEC_{GW}; PEC_{SW}) (Review of open literature)

B.8.6.4.1 PEC_{GW} (open literature)

One article (de Paz and Rubio, 2006) was available on PEC_{GW} determination. It deals with the use of GIS-AF/RF system to compile herbicide pollution-risk maps for a citrus-growing area in eastern Spain. The article can be considered as additional information. No modification of the PEC_{GW} calculation is needed.

For a more detailed descriptions and the evaluation of the open literature studies, please refer to B.8.10. The respective references are listed there as well.

B.8.6.4.2 PEC_{SW} (open literature)

Three articles are available on PEC_{SW} determination and the calculation of indicators for surface water contamination. The articles can be considered as additional information, as the approaches cannot be compared to standard FOCUS modeling. No modification of the PEC_{GW} calculation is needed.

For a more detailed descriptions and the evaluation of the open literature studies, please refer to B.8.10. The respective references are listed there as well.

B.8.6.4.3 Monitoring Data (open literature)

Publications which are available in open literature mostly deal with glyphosate concentrations in runoff water rather than are derived from comprehensive monitoring programs of surface waters.

Out of the screened open literature, ~~12~~ ~~14~~ 19 citations deal with glyphosate and AMPA concen-trations in runoff water within the EU, ~~7~~ 11 are related to non-EU sites. They include analyses of storm water, urban runoff, roof and railways runoff as well as runoff from specific agri-culturally used sites such as vineyards. Concentrations in rainwater, information on bulk atmospheric deposition (wet and dry) are published as well. Some of the publications present results obtained from field studies and thus do not present results from monitoring campaigns in their common sense.

~~9~~ 20 citations show results on European surface water (e.g. streams, rivers, small creeks) monitoring, ~~8~~ 13 give results on monitoring outsid ~~the EU~~ Europe. The design and also the presentation of the results obtained from monitoring campaigns are quite heterogeneous. Information is rather incomplete. Also, 2 different publications might deal with the same campaign (Ludvigsen and Lode, 2001; Ludvigsen and Lode, 2002) but publish slightly different information. Furthermore, a few references focus on modeling aspects, PEC determination, risk assessment using the monitoring data published elsewhere and risk mitigation. In one case, glyphosate is discussed only but without publication of decent data. In one case information was extracted from a presentation, and graphs only were given. Exact data were not obtained from the graphs.

Maximum glyphosate and AMPA concentrations in European surface waters as measured in comprehensive monitoring campaigns (Horth, 2012, BVL no 2310291; see point B.8.6.3) range between 1.3 - 370 µg/L and 0.22 - >200 µg/L for glyphosate and AMPA, respectively. Compared to these findings, the maximum concentrations which were published in open literature are rather low, namely in the range of ~~0.4 – 1.37~~ 0.21 - 7.2 µg/L and 0.2 - 13 µg/L for glyphosate and AMA, respectively. Therefore, information published in open literature does not really modify the already existing assessment of glyphosate and AMPA occurrence in surface water (see B.8.6.3).

Groundwater monitoring data were obtained for ~~4~~ 6 EU-countries (Spain, Denmark, Norway, Hungary, Sweden and Germany), whereas ~~one~~ two citations ~~was~~ were found for ~~a~~ non-EU sites (Canada). As for surface water, information is rather heterogeneous. Maximum glypho-

sate and AMPA concentrations in either groundwater or drinking water well in the 4 EU-countries named above are in the range of 0.02 - 2.56 µg/L and 0.02 - 0.45 µg/L, respectively, whereas maximum glyphosate and AMPA concentrations as obtained from comprehensive monitoring programs (Horth, 2012, BVL no 2310291; see point B.8.6.3) are in the range of 0.01 -24 µg/L and 0.02 - 19 µg/L, respectively. Therefore, information published in open literature does not really modify the already existing assessment of glyphosate occurrence in groundwater (see B.8.6.3).

Moreover, there are a few results available from open literature studies (see B.8.10) investigating the behaviour of glyphosate in railway systems in Switzerland (Brauchli-Theotokis, 2004). These investigations show that after the application of glyphosate in railway systems, glyphosate concentrations up to 100 µg/L are detected in the drainage water of the experimental set-ups. During normal operation, glyphosate concentrations along railway tracks reached values up to 10 g/L in the drainage water. Similar concentrations were also detected in drainage ditches alongside railway lines in England (Heather et al., 1999). There, about 1800 g/ha glyphosate were applied experimentally and 12 µg/L glyphosate was measured in run-off water. According to the representative GAP applications on railway facilities are not indented and therefore, not addressed in the risk assessment. Generally, the RMS considers that additional specific information in order to assess the potential contamination of surface water by runoff as well as the potential contamination of groundwater via run-off in surface water with subsequent bank filtration are required, if applications of glyphosate on railway facilities are intended at the national level.

For a more detailed descriptions and the evaluation of the open literature studies, please refer to B.8.10. The respective references are listed there as well.

B.8.7 Fate and behaviour in air (Annex IIA 7.10; Annex IIIA1 9.9)

B.8.7.1 Volatilisation

Glyphosate has low vapour pressure (1.31 x 10^{-5} Pa at 25°C, (Robson, 1991), see IIA 2.3.1) and therefore, significant concentrations are not expected to be found in air through volatilisation following the use of the compound according to the proposed GAP. No volatilisation of glyphosate from plant and soil surfaces could be detected in one laboratory and one field study (Schulz, 1995, BVL no 1552734; Guth et al, 1993, BVL no 1939443) evaluated during the 2001 EU approval of glyphosate thus confirming the assumption. During the 2001 EU glyphosate evaluation it was concluded that:

> ”glyphosate can be classified as not volatile based on its Henry’s law constant and on volatilisation experiments from soil and plants with no significant rates. Due to no significant UV-absorption, direct photolysis in air will not occur. Once in the atmosphere rapid photochemical oxidative degradation of glyphosate will occur.”

Results from two additional studies (De Vries, 1997, Schneider, 1996,) available from GTF members did also not show any detectable volatilisation in a laboratory and one field study. It should be noted, that the studies De Vries (1997) and Schneider (1996) were not presented to the RMS and therefore could not be checked, however, the results presented in the dossier of the Notifier are in line with the conclusion of the 2001 EU glyphosate evaluation and can thus be considered plausible.

B.8.7.2 Photochemical oxidative degradation in air

No new studies in regard to the rate and route of degradation in air for glyphosate or the metabolite AMPA were conducted.

An atmospheric oxidation rate estimation for the active substance glyphosate based on a calculation procedure by means of quantitative structure reactivity relations (QSAR) developed by Atkinson was evaluated for the 2001 EU approval in two studies (Hayes, 2000, BVL no 2154359 and De Vries, 1995, BVL no 2006983). The study results are provided in the original EU monograph. A tropospheric half-life of 1.6 hours was estimated in both studies.

A re-calculation with the latest version of the program AOPWIN (version 1.92) is provided by the RMS. AOPWIN calculates the rate constant for the reaction of the pesticide with hydroxyl radicals. This calculation confirms the previous results (see Table B.8.7-1).

Table B.8.7-1: Estimated atmospheric degradation rate for glyphosate (RMS)

AOPWIN version	1.92
Concentration of OH radicals	1.5 x10^6 cm^{-3}
Time window	12 hours
k_{OH}	E-12 cm^3/molecule-sec
DT_{50}	1.6 hours

B.8.8 Predicted environmental concentration in air (PECA) (Annex IIIA1 9.9)

Glyphosate has low vapour pressure (1.31 x 10^{-5} Pa at 25 °C, (Robson, 1991), see IIA 2.3.1) and therefore, significant concentrations are not expected to be found in air through volatilisation following the use of the compound according to the proposed GAP. The calculated atmospheric life time of glyphosate is < 2 days, thus long range transport via air can be excluded.
As the expected distribution to the atmosphere by glyphosate is likely to be extremely low in field use based on very low vapour pressure, no estimates of environmental concentrations expected in air were provided by the Notifier.

B.8.9 Definition of the residue (Annex IIA 7.3)

B.8.9.1 Soil

The major residues in soil from the environmental fate point of view are:
- Glyphosate (parent)
- Aminomethylphosphonic acid (AMPA) (max. occurrence in soil 53.8 % AR, see Table B.8.1-118)

B.8.9.2 Water

B.8.9.2.1 Groundwater

The major residues in soil from representative environmental fate studies are:
- Glyphosate (parent)
- Aminomethylphosphonic acid (AMPA) (max. occurrence in soil 53.8 % AR, see Table B.8.1-118)

In all simulations the 80th percentile PEC_{GW} values of glyphosate acid at 1 m soil depth are below the groundwater threshold value of 0.1 µg/L. Due to the reason that the 80th percentile PEC_{GW} values of AMPA at 1 m soil depth do not exceed 0.1 µg/L, the definitive relevant residue for further groundwater assessment is defined as glyphosate (parent compound).

B.8.9.2.2 Surface water

The major residues in surface water and sediment, relevant for further risk assessment from the environmental fate point of view, are:
- Glyphosate (parent)
- Aminomethylphosphonic acid (AMPA) (max. occurrence in the water phase 15.7 % AR, max. occurrence in the sediment pahse 18.7 %, see Table B.8.4-2)
- Hydroxymethylphosphonic acid (HMPA) (max. occurrence in the water phase up to 10.0 % AR, see Table B.8.4-2)

B.8.9.3 Air

Not defined.

B.8.10 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[2]
KIIA 2.10	Hayes, S.E.	2000	Glyphosate Acid: Calculation of Half- Life by Reaction with Atmospheric Hydroxyl Radicals 46852/01 Date: 2000 GLP: yes Not published 2154359	Y	SYN

[2] Only Notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[2]
KIIA 2.10	De Vries	1995	Estimation of the photochemical-oxidative degradation of Glyphosate in the atmosphere NOTOX Project 136384 1995-03-28 GLP not applicable Not published 2006983	N	AGR
IIA 7.1.1/01 *- newly submitted with renewal dossier*	Ponte, M.	2010	Rate and route of degradation of [^{14}C]-glyphosate in one soil incubated under aerobic conditions Report No.: PTRL1923W-1 (study) MSL0023070 (sponsor) Date: October 6, 2010 GLP: yes Not published 2310242	Y	EGT
IIA 7.1.1/02 *- newly submitted with renewal dossier*	Goodyear, A.	1996	(^{14}C)-glyphosate: Aerobic Soil Metabolism Report No.: 1413/1-1015 (study) Date: July 11, 1996 GLP: yes Not published 2310246	Y	NUF
IIA 7.1.1/03 *- newly submitted with renewal dossier*	McLaughlin, S., Schanné, C.	1996	[^{14}C]-Glyphosate: determination of soil degradation, bio-transformation and metabolism under aerobic conditions Report No.: 96-120-1020 (study) Date: June 14, 1996 GLP: yes Not published 2310250	N	SIN
IIA 7.1.1/04 *- newly submitted with renewal dossier*	Esser, T.	1996	[P-Methylene-^{14}C]glyphosate acid: aerobic soil metabolism Report No.: PTRL548W-1 (study) RR 96-027B (sponsor) Date: July 11, 1996 GLP: yes Not published 2310248	N	SYN

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[2]
IIA 7.1.1/05 *- newly submitted with renewal dossier*	Dean, G. M.	1995	Rate and route of degradation of [^{14}C]glyphosate in one soil incubated under aerobic conditions Report. No.: SNY 333/951445 (study) Date: December 1, 1995 GLP: yes Not published 2310244	Y	ALS
IIA 7.1.1	Galicia, H. Morgenroth, U.	1993	Degradation and metabolism of 14C-Glyphosate in soil incubated under aerobic conditions RCC project No: 246486 Date: April 6, 1993 GLP: .Yes. Not published 1932059	N	MON
IIA 7.1.1 IIA 7.2.1	Matla, Y.A. Vonk, J.W.	1993	Rate of degradation and metabolism of [14C]-glyphosate in soil under aerobic conditions Report No: IMW-92-0022-01 IMW-R 93/047 Date: April 13, 1994 GLP: Yes Not published 2151389	N	MON, ADM
IIA 7.1.1	Honegger, J.L.	1992	Review of the aerobic metabolism of [14C]-Glyphosate in soil. Addendum to Monsanto Report No PTRL 368 Date: January 1992 GLP: No Not published 2325652	N	MON
IIA 7.1.1	Kesterson, A.L. Atkins, R.H.	1991	Aerobic metabolitsm of [14C]-Glyphosate in sandy loam and silt loam soils with biometer flask Report No. MSL-10578 Date: January 1, 1991 GLP: Yes Not published 1932061	N	MON

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[2]
IIA 7.1.1	McBain, J.B.	1985	Metabolism of SC-0224 in soil: Fate of the anion moiety Report no PSM 186 DOC 100465 MRC 85-11 Date: 1985 GLP: No Not published 1052660	N	SYN
IIA 7.1.1	McBain, J.B. Benedikt, S.K. Dennison, J.E. Ichien, J.A.	1985	Metabolism of SC-0224 in soil: Fate of the cation moiety Report no PSM 179 DOC 100464 MRC 85-10 Date: 1985 GLP: No Not published 1052661	N	SYN
IIA 7.1.2– *new study (not submitted)-*	McEwen, A.	2004*	[14C]-Glyphosate: Anaerobic soil metabolism (rate and route of degradation in a sandy loam soil) Report No.: SNN/05 Date: July 19, 2004 GLP: yes Not published	Y	SIN
IIA 7.1.2/01 *- newly submitted with renewal dossier*	Lowrie, C., Clayton, M.A., Paterson, K.	2003	The degradation of [14C]-glyphosate in soil under anaerobic conditions Report No.: 22581 (study); MSL-18018 (sponsor) Date: July 08, 2003 GLP: yes Not published 2310253	Y	MON
IIA 7.1.2– *new study (not submitted)-*	Knoch, E.	2003*	Route and rate of anaerobic soil degradation of glyphosate according to SETAC, Part 1, 1.2 (March 1995) Report No.: IF-02/00005224 Date: February 7, 2003 GLP: yes Not published	N	ALS
IIA 7.1.2	Keirs, D.C. Mackie, J.A.	2000	The degradation of [14C]-Glyphosate in soil under anaerobic conditions, 25 July 2000 Inveresk no. 395941 BOD2000-1308 Date: February 7, 2003 GLP: Yes Not published 1939595	N	MON

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[2]
IIA 7.1.2	McBain, J.B.	1987	SC-0224: Anaerobic soil metabolism study: Fate of the Carboxymethylaminomethyl-phosphonic acid moiety Report No: PSM 217 Date: 1987 GLP: No Not published 1031560	N	SYN
IIA 7.1.3	Rueppel, M.L. Brightwell, B.B.	1972	The degradation and metabolism of MON-0573 in soil Report No AgRR269 Date: October 26, 1972 GLP: N Not published 1932065	N	MON
IIA 7.1.3 – *new study (not submit-ted)-*	Esser	1996*	P-Methylene-14C] Glyphosate Acid: Photo-degradation in-on Soil by Natural Sunlight (WRC-96-066) ASF71/0159 ! RR 96-046B GLP: Yes not published 2154348	N	SYN
IIA 7.1.3	Van Dijk, A. Burri, R.	1993	Photodegradation study of 14C-Glyphosate on soil Report No RCC 315764 Date: 1993 GLP: Yes Not published 1932050	N	MON
IIA 7.1.3	Shepler, K. McGovern, P.A.	1989	Photodegradation of [^{14}C]-Glyohosate in/ on soil by natural sunlight MSL-9271 ! No. 972 Date: 1989 GLP: Yes Not published 1932096	N	MON
IIA 7.1.3	McGahen, L.	1983	The photodegradation of SC-0224 applied to soil PSM 137 BOD95-00420 Date: 1983 GLP: No Not published 1052662	N	SYN

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[2]
IIA 7.1.3	Brightwell, B.B.	1978	Photodegradation an anaerobic aquatic metabolism of Glyphosate, N-phosphonomethylglycine Report No MSL-0598 Date: 1978 GLP: No Not published 1932052	N	MON
IIA 7.2.1/01 *- newly submitted with renewal dossier*	Ponte, M.	2010	Rate of degradation of [^{14}C]glyphosate in three soils incubated under aerobic conditions Report No.: PTRL1946W-1 (study); MSL0023071 (sponsor) Date: October 6, 2010 GLP: yes Not published 2310255	Y	EGT
IIA 7.2.1/02 *- newly submitted with renewal dossier*	Mamouni, A.	2002	First amendment (addendum) to report - Degradation of ^{14}C-glyphosate in three soils incubated under aerobic conditions RCC Study No. : 271618 Date: June 3, 2002 GLP: No Not published 2437068	Y	CHE
IIA 7.2.1/03 *- newly submitted with renewal dossier*	Dorn, S.	2012	Kinetic modelling analysis of the degradation behaviour of glyphosate and its metabolite AMPA from aerobic laboratory soil degradation studies Report No.: 303604-1 Date: May 3, 2012 GLP: no (kinetic evaluation: does not contain laboratory work) Not published 2315991	Y	EGT
IIA 7.2.1	Galicia, H. Flückiger, J.	1993	Degradation of ^{14}C-glyphosate in three soils incubated under aerobic conditions Report NO RC 271618 Date: April 7, 1993 GLP: yes Not published 1932046	N	MON CHE

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[2]
IIA 7.2.1	Schneider, E.	1991	Behaviour of Glyphosate in water and soil, Part 5 Degradation in soil Report no: PR93/009 Date: 1991 GLP: yes Not published 1062355	N	ADM
IIA 7.2.1	Lewis, C.J. Turnbull, G.	1992	Glyphosate-Trimesium: Soil dissipation study and Glyphosate-Trimesium: Soil dissipation study (inkl. Addendum to final report) Study No: 7043-38/165 Date: April 1992 GLP: No Not published 1052659	N	SYN
IIA 7.2.1	Runnalls, J.K.	1991	Glyphosate-Trimesium: Laboratory degradation in four soils Report no: RJ1064B Date: 1991 GLP: No Not published 2154349	N	SYN
IIA 7.3.1/01 - *newly submitted with renewal dossier*	Kreschnak, C	2012	Kinetic modelling analysis of the degradation behaviour of glyphosate and its metabolite AMPA in field soil dissipation studies Report No.: 303604-2 Date: April 27, 2012 GLP: no (kinetic evaluation: does not contain laboratory work) Not published 2315993	Y	EGT
IIA 7.3.1	Schulz, H.	1992	Field soil dissipation rate determination of Glyphosate 360 (Diegten, Switzerland) RCC 273565 BOD95-00515 GLP: yes Not published 1932128	Y	MON CHE
IIA 7.3.1	Schulz, H	1992	Field soil dissipation rate determination of Glyphosate 360 (Ekergingen, Switzerland RCC 280416 BOD95-00514 GLP: yes Not published 1932131	N	MON CHE

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[2]
IIA 7.3.1	Schulz, H	1992	Field soil dissipation rate determination of Glyphosate 360 (Bad Krozingen, Germany) RCC 280427 BOD95-00512 GLP: yes Not published 1932073	N	MON CHE
IIA 7.3.1	Schulz, H	1992	Field soil dissipation rate determination of Glyphosate 360 (Menslage, Germany) RCC 280438 BOD95-00513 GLP: yes Not published 1932133	N	MON CHE
IIA 7.3.1	Hill, S.E.	1992	Glyphosate-Trimesium: Soil dissipation study (Germany 1990 – 1992) RJ1294B BOD95-00424 GLP: yes Not published 2154352	N	SYN
IIA 7.4.1/01 *- newly submitted with renewal dossier*	van Noorloos, B., Slangen, P.J.	2001	Adsorption/desorption of glyphosate on soil Report No.: 320164 (study) Date: December 10, 2001 GLP: yes Not published 2310257	Y	AGC
IIA 7.4.1/02 *- newly submitted with renewal dossier*	Thomas, P.K., Lane M.C.G.6	1996	Glyphosate acid: adsorption and desorption properties in 5 soils Report No: RJ2152B Date: September 12, 1996 GLP: yes Not published 2310260	N	SYN
IIA 7.4.1/03 *- newly submitted with renewal dossier*	van der Kolk, J.	1996	Glyphosate: determination of adsorption and desorption properties based on the OECD method 106 Report No.: 95-111-1020 (study) Date: April 26, 1996 GLP: yes Not published 2310258	Y	SIN

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[2]
IIA 7.4.1/04 Additionally included to the assessment by the RMS	Schneider, E.	1993	Glyphosate isopropylamine salt adsorption/desorption PR93/017 Date: June 17, 1993 GLP: yes Not published 1027844	N	ADM
IIA 7.4.1	Waring, D.M.	1992	14C-Glyphosate : Adsorption/desorption in soil Report 7180 GLP: Yes Not published 1932008	N	MON CHE
IIA 7.4.1	Livingston, C.L. Chott, K.H. Schafer, T.R.	1986	Australian notification base testing requirements for N- (Phosphonomethyl) Iniodiacetic Acid (Glaphosate Intermediate), Part II: Adsoption/Desoption Data. MSL-5393 ! Report 7863 GLP: N Not published 2325589	N	MON
IIA 7.4.2/01 *- newly submitted with renewal dossier*	Knoch, E.	2003	Aminomethylphosphonic acid: adsorption-desorption Report No.: IF-02/00005220 (study) Date: February 07, 2003 GLP: yes Not published 2310262	N	ALS
IIA 7.4.2/02 *- newly submitted with renewal dossier*	Wittig, A.	2002	Adsorption/desorption behaviour of AMPA on soil according OECD 106 (adopted January 2000) Report No.: PR02/007 (study) Date: June 24, 2002 GLP: yes Not published 2310264	N	ADM
IIA 7.4.2/03 *- newly submitted with renewal dossier*	Muller, K., Lane, M.C.G.	1996	Glyphosate acid: adsorption and desorption properties of the major metabolite, AMPA, in soil Report No: RJ2129B Date: August 27, 1996 GLP: yes Not published 2310266	N	SYN

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[2]
IIA 7.4.2	Weeden, D.M.	1993	Aminomethylphosphonic acid – Determination of the sorption and desorption properties. MSL-12703 Date: 1992 GLP: Yes Not published 2325586	N	MON
IIA 7.4.3	Burgener, A.	1992	Leaching characteristics of formulated 14C-Glyphosate in three soils RCC 281430 Date: 1992 GLP: Yes Not published 1932122	N	MON CHE
IIA 7.4.3	McGinley, A.M.	1992	Glyphosate-Trimesium: Leaching of material in soil columns. RJ247B, BOD95-00422 Date: 1992 GLP: Yes Not published 1052676	N	SYN
IIA 7.4.3	Schneider, E.	1991	Behaviour of Glyphosate in water and soil, Part 4 Leaching behaviour, second performance PR90/002 Date: 1991 GLP: Yes Not published 1062361	N	ADM
IIA 7.4.3	Brightwell, B.B. Malik, J.M.	1978	Solubility, volatility, adsorption and partition coefficients, leaching and aquatic metabolism of MON 0573 and MON 0101 MSL-0207 Date: 1978 GLP: Yes Not published 1932009	N	MON
IIA 7.4.5/01 *- newly submitted with renewal dossier*	McLaughlin, S.	1996	Determination of the mobility of aged[^{14}C]-glyphosate residues in one soil Springborn Laboratories, Horn, Switzerland Report No.: 96-121-1020 (study) Date: June 14, 1996 GLP: yes Not published 2310268	Y	SIN

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[2]
IIA 7.4.5	Waring, A.R. Purser, D.	1992	(14C)-Glphoysate-Trimesium: Aged soil leaching 7113-38/172 Datw: 1992 GLP: No Not published 1052677	N	SYN
IIA 7.4.9 *new study (not submit-ted)-*	De Vries, R.	1997*	Determination of the rate of volatilization of glyphosate from soil and plant surface (french beans) Report No.: 191071 Date: 1997 GLP: yes Not published	Y	AGC
IIA 7.4.9 *new study (not submit-ted)-*	Schneider, E.	1996*	Glyphosate: Determination of volatilisation - Field study Report No.: PR94/032 (study); Date: 1996 GLP: yes Not published	N	ADM
IIA 7.4.9	Guth, S., Knack-er, Th., Schall-naß, H., Steiper, M	1993	Determination of the volatilization of Glyphosate 360 SL from soil and plants Study no. BE_EA-49-92-01-Vol-1 Date: 1993 GLP: No Not published 1939443	N	MON
IIA 7.4.9	Schulz, J	1995	Final report - About testing volatilization behavior of TAIFUN forte in bush beans under field conditions Report No.: AGR/RV-95/ ADM Date: 1995 GLP: Yes Not published 1552734	N	ADM
IIA 7.5	Burgener, A.	1990	Hydrolysis determination of ^{14}C-glyphosate (PMG) at different pH values RCC 238500 Date: 1990 GLP: Yes Not published 2442046	N	MON

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[2]
IIA 7.5	Myers, H.W. Lee, K.S. Patchett, G.G Katague, D.B.	1983	Hydrolysis and photolysis degradation studies of SC-0224 WRC 83-53 Date: 1983 GLP: No Not published 1031582	N	SYN
IIA 7.6	Van Dijk, A.	1992	Photodegradation study of 14C-Glyphosate in wate rat pH 5, 7 and 9 RCC 250751 Date: 1992 GLP: Yes Not published 2252558	N	MON
IIA 7.6	Ericson, L.J.	1992	Glyphosate-Trimesium- Aquous photolysis. RR91-065B Date: 1992 GLP: No Not published 1052518	N	SYN
IIA 7.7/ 01 *new study (not submitted)*	Feil, J.	2009*	Ready biodegradability of glyphosate in a manometric respirometry test Report No.: 53981163 Date: December 10, 2009 GLP: yes Not published	Y	NUF
IIA 7.7	Carrick, T.R.	1991	A study to evaluate ready biodegradability of Glyohosate technical FH-OECD-09RB Date. 1991 GLP: Yes Not published 2325628	N	MON
IIA 7.7	Wüthrich, V.	1990	Glyphosate technical: Inherent biodegradability, “Modified Zahn-Wellens test” RCC 271653 Date: 1990 GLP: Yes Not published 1934369	N	MON
IIA 7.7	Anonymous	1990	Glyphosate COD and biodegradability Report No.: n.a. Date: 1990 GLP: No Not published 1934372	N	MON

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[2]
IIA 7.7	Brightwell, B.B., Malik, J.M.	1978	Solubility, volatility, adsorption and partition coefficients, leaching and aquatic metabolism of MON 0573 and Mon 0101 Report No: MSL-0207 Date: 1978 GLP: No Not published 1932009	N	MON
IIA 7.7	Henshall, A., Brightwell, B.B	1972	The degradation and metabolism of MON 0573 in river and lake bottom sediments and surface water Report No. 276 Date: 1972 GLP: No Not published 1934355	N	MON
IIA 7.8.3/01-- *newly submitted with renewal dossier*	Feser-Zügner, W.	2002	Aminomethylphosphonic acid: fate and behaviour in water-sediment A&M Labor für Analytik und Metabolismusforschung Service GmbH, Bergheim, Germany Report No.: A &M 01-106 (study) Date: November 12, 2002 GLP: yes Not published 2310270	N	ADM
IIA 7.8.3/02 - *newly submitted with renewal dossier*	Knoch, E.	2003c	Aerobic aquatic degradation of aminomethylphosphonic acid according to SETAC, part 1.8.2 (March 1995) CALLIOPE S.A.S. (sponsor) Institut Fresenius Chemische und Biologische Laboratorien AG , Herten, Germany Report No.: IF-02/00005222 (study) Date: February 07, 2003 GLP: yes Not published 2310273	N	ALS
IIA 7.8.3/03- *newly submitted with renewal dossier*	McEwen, A.	2004b	[^{14}C]-AMPA: Degradation and fate in water/sediment systems BioDynamics Research Limited, Northhamptonshire, UK Report No.: SNN/03 (study) Date: June 7, 2004 GLP: yes Not published 2310275	Y	SIN

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[2]
IIA 7.8.3/04-	Heintze, A.	1996*	Degradation and metabolism of glyphosate in two water/sediment systems under aerobic conditions - Laboratory test Report No.: 96138/01-CUWS (study) Date: December 16, 1996 GLP: yes Not published 1939626	Y	MON
IIA 7.8.3/05	Partsch, S.	2012	Kinetic modelling analysis of the disappearance behaviour of glyphosate and its metabolite AMPA in water-sediment studies Report No.: 303604-3 Date: April 30, 2012 GLP: no (kinetic evaluation: does not contain laboratory work) Not published 2316005	Y	EGT
IIA 7.8.3	Bowler, D.T. and Johnson, J. A.	1999	Glyphosate-trimesium: Degradation of 14C-PMG labelled compound in nautral water-sediment systems under laboratory conditions Report No.: RR99-039B (study) Date: November 4, 1999 GLP: yes Not published 2154357	N	SYN
IIA 7.8.3	Knoch, E. and Spirlet, M.	1999	Aminomethylphosphonic acid: Water/sediment Metabolism Report No.: IF-98114727 -00 (study) Date: September 20,1999 GLP: yes Not published 1934122	N	MON
IIA 7.8.3	Steginsky, C.A. and Powell, J.M.	1995	Determination of the degradability and persistence of 14C-Glyphosate in the water/sediment-system (Amendment) Report No.: ET01SE01 (study) Date: August 1, 1995 GLP: yes Not published 1934389	N	MON

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[2]
IIA 7.8.3	Möllerfeld, J. and Römbke, J.	1993	Determination of the degradability and persistence of ^{14}C-Glyphosate in the water/sediment-system Report No.: ET01SE01 (study) Date: May 17, 1993 GLP: yes Not published 193,4113	N	MON
IIA 7.8.3	Muttzall, P.I.	1993	Water/sediment biodegradation of [14C]-glyphosate Report No.: IMW-92-0022-02 Date: March 18, 1993 GLP: yes Not published 1982136	N	ADM AGC LUX
KIIIA1 9.6.4/01 - *newly submitted with renewal dossier*	Jönsson, J.	2012	Review of sustainable water treatment UC8408v2 MON GLP: N, published: N 2316001 /	Y	EGT
KIIIA1 9.6.4/ 02- *newly submitted with renewal dossier*	Jönsson, J., Camm, R.	2010	Removal of Glyphosate and AMPA by water treatment UC8164v2 MON GLP: N, published: N 2316003 /	Y	EGT
IIIA 9.4/01 IIIA 9.5/01 newly submitted with renewal dossier	Anyusheva, M.	2012	Predicted environmental concentrations of glyphosate and its metabolite AMPA in soil (PEC_s) following application to various crops in the EU Report No.: 303605-1 Date: April 25, 2012 GLP: no (modelling study: does not contain laboratory work) Not published 2315997	Y	EGT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[2]
IIIA 9.6/01 newly submitted with renewal dossier	Anyusheva, M.	2012	Predicted environmental concentrations of glyphosate and its metabolite AMPA in groundwater (PEC_{gw}) using FOCUS PEARL 4.4.4 and FOCUS PELMO 4.4.3 following application to various crops in the EU Report No.: 303605-2 Date: April 25, 2012 GLP: no (modelling study: does not contain laboratory work) Not published 231599	Y	EGT
IIIA 9.7/01 IIIA 9.8/01 newly submitted with renewal dossier	Anyusheva, M.	2012	Predicted environmental concentrations of glyphosate and its metabolites AMPA and HMPA in surface water (PECsw) and sediment (PECsed) following application to various crops in the EU Report No.: 303605-3 Date: April 27, 2012 GLP: no (modelling study: does not contain laboratory work) Not published 2316007	Y	EGT
IIA 7.12/01 newly submitted with renewal dossier	Calliera, M., Ferrari, F., Lamastra, L.	2011	Investigation of the potential glyphosate groundwater contamination in Lombardia region (North Italy) Aeiforia Srl, Fidenza, Italy Report No.: - Date:20 October 2011 GLP: no (literature study: does not contain laboratory work) Published 2310280	N	LIT
IIA 7.12/02 newly submitted with renewal dossier	Schmidt, B., Reichert, N.	2006	Clarification of well-related findings of glyphosate and AMPA in groundwater SGS Institut Fresenius GmbH, Taunusstein, Germany Report No.: IF-06/00603024 (study) Date: 14 December 2006 GLP: no (literature study: does not contain laboratory work) Not Published 2310282	N	MON

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[2]
IIA 7.12/03 newly submitted with renewal dossier	Franke, A.C., Groeneveld, R.M.W., Kempenaar, C.	2010	Evaluatie van metingen van glyfosaat en AMPA in grondwater in Nederland (Evaluation of glyphosate and AMPA measurements in groundwater in The Netherlands) Plant Research International, Wageningen UR, The Netherlands Report No.: 354 Date: October 2010 GLP: no (literature study: does not contain laboratory work) Not Published 2310284	N	LIT
IIA 7.12/04 newly submitted with renewal dossier	Carter, A., Pepper, T.	2005	An investigation of reported borehole contamination in the Vemmenhög Catchment, Sweden ADAS UK Ltd, Nottinghamshire, England Report No.: - Date: December 2005 GLP: no (literature study: does not contain laboratory work) Not Published 2310285	N	MON
IIA 7.12/05 newly submitted with renewal dossier	Anonymous	2012	Analysis of groundwater contamination with glyphosate/AMPA SCE Aménagement et Énvironnement, Nantes, France Report No.: - Date: February 2012 GLP: no (desk study: does not contain laboratory work) Not published 2310289	N	EGT
IIA 7.12/06 newly submitted with renewal dossier	Horth, H.	2012	Survey of glyphosate and AMPA in groundwaters and surface waters in Europe HoHQ, UK Report No.: - GLP: no (desk study: does not contain laboratory work) Not published 2310291	N	EGT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[2]
IIA 7.12/- Additionally included to the assess-ment by the RMS	Sanchís, J., Kantiani, L., Rubio, M.L.F., Ginebreda, A., Fraile, J., Gar-rido, T., Farré, M.**	2012	Erratum to:Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry Anal Bioanal Chem 404, 617 GLP: N, published: Y 2537361	N	LIT
IIA 7.12/- Additionally included to the assess-ment by the RMS	Anonymous**	2011	Berichte zu Pflanzenschutzmitteln 2009 - Jahresbericht Pflanzenschutz-Kontrollprogramm BVL-Reporte 5, 1-33 GLP: N, published: Y 2537364	N	LIT
IIA 7.12/- Additionally included to the assess-ment by the RMS	Anonymous**	2012	Berichte zu Pflanzenschutzmitteln 2010 - Jahresbericht Pflanzenschutz-Kontrollprogramm BVL-Reporte 6, 1-37 GLP: N, published: Y 2537365	N	LIT
IIA 7.12/07 newly sub-mitted with renewal dossier	Horth, H., Gen-debien A.	2008	Review of glyphosate and AMPA in drinking water in selected European countries WRc Swindon, Wiltshire, UK Report No.: UC7729.04 Date: September 2008 GLP: no (literature review: does not contain laboratory work) Published 2310278	N	EGT

* not submitted by the applicant
** open literature

Codes of owner

AGC	AgriChem B.V.
AGR	Agrilex (UK) LTD.
ALS	Alschu-Chemie GmbH
CHE	Cheminova A/S
EGT	European Glyphosate Task Force AIR 2
ADM	ADAMA Agan Ltd
LIT	Published literature
LUX	LUXAN B.V.
MON	Montedison (Deutschland) Chemie Handels GmbH
NUF	Nufarm GmbH & Co KG
SIN	SINON EU CORPORATION
SYN	Syntana Handelsgesellschaft

The open literature is listed in B.8.11.

Renewal Assessment Report

18 December 2013

Revised
29 January 2015
10 February 2015

Glyphosate

Volume 3

Annex B.8 (Appendix)

Evaluation of open literature regarding environmental fate and behaviour

RMS: Germany **Co-RMS: Slovakia**

Version history (Volume 3, chapter B.8 (Appendix))

Date	**Reason for revision**
19 December 2013	Initial Renewal Assessment Report
29 January 2015	Updated following RAR peer review, taking into account the reporting table, the evaluation table and additional data
10 February 2015	Updated taking into account further information from the open literature provided by the applicant (and not listed in the official EFSA Additional information request)

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B.8 Environmental fate and behaviour

B.8.11 Evaluation of open literature regarding environmental fate and behaviour

B.8.11.1 Purpose and matter of subject of the literature survey

Background

Article 8.5 of the regulation 1107/2009/EC stipulates the addition of open, peer-reviewed scientific literature with a submission of a dossier for annex-I inclusion of an active substance. Literature that was published within the last 10 years before the submission should be included.

In the course of the registration process of the herbicide glyphosate the Notifier (Glyphosate Task Force) as well as other organisations have compiled and assessed open publications and further statements for inclusion in the monograph. Among others around 300 publications and statements concerning environmental fate and behaviour have been submitted.

Applying the ~~following~~ procedure described further below these publications were re-classified, the data quality was assessed, results were compared to those obtained by standard studies and general conclusions on the fate and behaviour of glyphosate as based on the articles were drawn.

Process of retrieving literature by the Notifier - search strategy and data bases

The Notifier has been conducting routine surveillance of technical literature for glyphosate-related publications in a structured fashion since early 1997. During the period from 1997 to the present time, the search process and the literature databases used have been modified as new resources and technology became readily available. The technical databases that are used for the search include: Web of ScienceSM, BIOSIS Previews®, CAB Abstracts® (CABI), MEDLINE®, and CA Plus (Chemical Abstracts Plus). The searches are done on glyphosate acid, glyphosate salts (including isopropyl amine, potassium, ammonium, and methylamine), and AMPA, and their related chemical names and CAS numbers.

Starting from the ongoing literature database, all the peer-reviewed publications covering the time period from 2001 through 2011 that relate to the four key disciplines addressing exposure and hazard (toxicology, ecotoxicology, residues and environmental fate) were assessed within the appropriate discipline for inclusion in the literature review for the submission. Some publications address more than one discipline, and are included in each relevant discipline. More recent publications have continued to be reviewed up to shortly before submission, and selected publications have been included.

Additional publications cited in a recent document prepared by Earth Open Source have also been included in the literature review. Many of the cited peer-reviewed publications were already included, but others were not within the scope of this literature review, primarily because the publication date was prior to 2001. The additional peer-reviewed publications have been included and are discussed within the appropriate discipline.

Numerous publications are available in which various aspects of the environmental fate and behaviour of glyphosate and AMPA in soil, water, air, and plants are discussed. These publications cover a very wide range of study types, ranging from traditional study types

conducted under laboratory conditions to outdoor field studies, monitoring the fate of glyphosate and AMPA in the environment over the span of several years.

Procedure of publications classification and data quality assessment by the RMS

The Notifier mainly based the reliability and quality assessment on Klimisch et al. (1997). Klimisch et al. (1997) present a systematic approach to document and evaluate the quality of toxicological and ecotoxicological data for their inclusion in the risk assessment and in databases, e.g. IUCLD database. Categories of reliability are defined and annotated by a code:

Code	Category	Description
1	Reliable without restrictions	Studies carried out according to guideline, preferably under GLP, test parameter documented comprehensively
2	Reliable with restrictions	Studies not totally comply with test guideline, mostly not under GLP
3	Not reliable	Interferences between measuring system and the test substance are not relevant in relation to the exposure of the organisms
4	Not assignable	Not sufficient experimental data are included but the article is a review and data are cited only

The publication, Klimisch et al. (1997) has been modified, extended and adopted to upcoming demands. Küster et al. (2009) adopted the classification scheme to regulatory demands for the environmental risk assessment of pharmaceuticals. The authors basically defined three categories, namely: category I (reliable without restrictions according to the instructions of EMEA guideline), category II (reliable with restrictions according to the instructions of EMEA guideline), and category III (not reliable and not usable for environmental risk assessment). The detailed descriptions of the categories are comparable to those shown for Klimisch et al. (1997).

Küster et al. (2010) further developed their approach of 2009 by a reliability and relevance evaluation scheme. The scheme comprises several criteria which describe the reliability (such as: purpose, protocol, test compound, test environment, controls/replicates, statistical design) and the relevance of published data. They furthermore suggested how to use the evaluated (eco)toxicological data in the process of risk assessment. This is by differentiation between:

low weight in risk assessment	(low reliability plus low relevance),
supporting evidence in risk assessment	(high reliability plus low relevance or low reliability plus high relevance),
high weight in risk assessment	(high reliability plus high relevance).

Kase et al. (2012) suggested a so called Klimisch 2.0 scheme which improves the transparency of the system by Klimisch et al. (1997). Mainly based on Klimisch et al. (1997) and Küster et al. (2010) the authors presented a reliability check list, a relevance check list as well as a plausibility and weight of evidence check list.

In EFSA (2011) guidance is given on how to compile publicly accessible data bases, to design searches, and to evaluate the obtained data for further use. Since articles were already available and not further data base search was performed, EFSA (2011) was not taken into consideration.

As the described approaches to evaluate literature data focus on ecotoxicological data the approaches had to be adopted for use of data on fate and behaviour. Basically, the approaches by Küster et al. (2010) and by Kase et al. (2012) were used.

The following scheme was general applied for the presentation and evaluation of the articles provided by the Notifier:

Table B.8.11–1: Scheme for the presentation and evaluation of the articles provided by the Notifier

Reliability	
Endpoint	• description of endpoints • clarity of reporting
Protocol	• standard method/modified standard (e.g. OECD-guideline) • performed under GLP
Test compound	• identification (name, CAS-number), purity, source • active ingredient or formulated product
Test system and conditions	• Short description of the test system • concentration of test substance, mode of application • appropriate test conditions (e.g. pH, organic carbon-content, temperature) • controls (e.g. sterile samples, solvent controls; only if applicable)
Statistical design	• replicates and number thereof • description of statistical method used • description of other mathematical equations to assess the experimental data (e.g. Freundlich equation to describe adsorption) • description of models to assess and aggregate experimental data (e.g. kinetic models to describe $DegT_{50}$)
Relevance	
Environmental relevance	• Is the substance tested representative and relevant for the substance being assessed?
	• Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature)?
Weight of evidence	
"Positive" evidence	• Do other reliable studies support the results?
"Negative" evidence	• Do other reliable study results are in conflict with the shown results? • Are other "confounding" factors influencing the study result, e.g. hydrolysis of test chemicals?

By applying the evaluation scheme the published data were evaluated qualitatively as of:

- "high", "medium" or "low" reliability
- "relevance" or "no relevance"
- "positive evidence" or "not positive evidence"
- "negative evidence" or "not negative evidence".

Based on the results of such data evaluation the type of information was classified as critical, high/low weight, supporting or additional. In case of high weight of information, supportive information and high reliability the results and test parameter are presented in detail. Otherwise, a short summary and conclusions only were given.

Furthermore, a proposal on how to proceed with the data within the scope of the environmental risk assessment process was presented. The proposal was shortly summarized as: "to be considered, because..." or "not to be considered, because…" Argumentation for consideration and inclusion in the risk assessment process was

a) based on the above described evaluation scheme,
   and furthermore
b) oriented at the requirements of the respective endpoint under consideration as well as of European legislation.

In the following an argumentation as well as a comparison with the articles which were discussed in detail by the Notifier is given for the relevant sub-section of ´Environmental fate and behaviour` where open literature was available is given.

Soil Photolysis

Argumentation:

- To be considered as supportive information in case the standard test was followed.

Comparison of evaluation:

Notifier	Current evaluation
No articles discussed in detail	Echavia et al. (2009) briefly summarized but not discussed in detail
	Kirmser et al. (2010) briefly summarized but not discussed in detail
	Xu et al. (2011) briefly summarized but not discussed in detail

Rate of degradation in soil – Laboratory studies

Argumentation:

- To be considered for the calculation of $DegT_{50}$ for PEC-calculations if the following criteria were fulfilled:

Following criteria were used when selecting appropriate studies to be used for the calculation of $DegT_{50}$ for PEC-calculations:

1. The analysis of the degradation rate in the publication is in line with the guidance given in FOCUS Degradation kinetics with regard to the kinetic models used for the data analysis. Consequently, only following models were accepted: SFO (Single First Order), FOMC (First Order Multi Compartment), HS (Hockey-Stick), DFOP (Double First Order in Parallel).
2. The evaluation had to follow also the principles recommended in FOCUS Degradation kinetics (e.g. the use of HS and DFOP instead of FOMC if the residues at the end of the studies exceed 10 %.)
3. Only publications were accepted in which sufficient raw data is presented (e.g. time dependent residues in tables, or recommended quality criteria like FOCUS chi^2-test).

4. Only publications were accepted which either present normalised $DegT_{50}$ values according to FOCUS (2006) or at least contain sufficient information to perform the normalisation of presented $DegT_{50}$ values.

- To be considered as supportive information if the study is plausible and reliable, and performed according to standard guideline
- Not to be considered for an endpoint or PEC-recalculation if non-European soils were used
- Not to be considered for an endpoint or PEC-recalculation if validity cannot be proven due to lack of raw data

Comparison of evaluation:

Notifier	Current evaluation
No articles discussed in detail	Ghafoor et al. (2011) presented in detail but not considered for endpoint or PEC-recalculation
	Jacobsen et al. (2008) presented in detail but not considered for endpoint or PEC-recalculation
	Simonsen et al. (2008) presented in detail but not considered for endpoint or PEC-recalculation

Rate of degradation in soil – Field studies

Argumentation:

- For field studies in addition to the above list for laboratory studies sufficient information had to be provided that makes it possible to perform time-step normalisation of the degradation according to FOCUS degradation kinetics unless no normalised values are presented in the publication.
- Not to be considered for an endpoint or PEC- recalculation if non-European soils and sites, respectively, were used
- Not to be considered for an endpoint or PEC- recalculation if validity cannot be proven due to lack of raw data

Comparison of evaluation:

Notifier	Current evaluation
No articles discussed in detail	No articles discussed in detail

Studies on adsorption and desorption

Argumentation:

- To be considered as supportive information if the study is plausible and reliable, and performed according to standard guideline
- Not to be considered for an endpoint or PEC- recalculation if non-European soils were used
- Not to be considered for an endpoint or PEC- recalculation if validity cannot be proven due to lack of raw data

Comparison of evaluation:

Notifier	Current evaluation
Dion et al. 2001 discussed in detail, Klimisch: reliable with restrictions	Not discussed in detail
De Jonge et al. 2001 discussed in detail, Klimisch: reliable with restrictions	De Jonge et al. (2001) presented in detail but not considered for endpoint or PEC-recalculation
Laitinen et al. 2008 discussed in detail, Klimisch: not reliable	Not discussed in detail
Gimsing et al. 2004 discussed in detail, Klimisch: not reliable	Not discussed in detail
Not discussed in detail	Albers et al. (2009) presented in detail but not considered for endpoint or PEC-recalculation
Not discussed in detail	Al-Rajab et al. (2009) presented in detail but not considered for endpoint or PEC-recalculation
Not discussed in detail	Autio et al. (2004) presented in detail but not considered for endpoint or PEC-recalculation
Not discussed in detail	Mamy and Barriuso (2005) presented in detail but not considered for endpoint or PEC-recalculation
Not discussed in detail	Prata et al. (2003) presented in detail but not considered for endpoint or PEC-recalculation
Not discussed in detail	Jacobsen et al. (2008) presented in detail but not considered for endpoint or PEC-recalculation

Column leaching studies

Argumentation:

- To be considered as supportive information if the study is plausible and reliable, and performed according to standard guideline
- Not to be considered for endpoint- or PEC-recalculation if non-European soils were used
- Not to be considered for endpoint- or PEC-recalculation because sufficient data are available on adsorption and desorption

Comparison of evaluation:

Notifier	Current evaluation
No study discussed in detail	No study discussed in detail

Lysimeter studies or field leaching studies

Argumentation:

- To be considered as supportive information if the study is plausible and reliable, and performed according to standard guideline
- Not to be considered for endpoint- or PEC-recalculation if non-European soils or sites were used

- Not to be considered for endpoint- or PEC-recalculation because sufficient data are available on adsorption and desorption and on degradation
- To be considered as additional information since sufficient data are available on adsorption and desorption and on degradation

Comparison of evaluation:

Notifier	Current evaluation
Grundmann et al. 2008 discussed in detail, Klimisch: reliable with restrictions	No study discussed in detail but presented as additional information
Formsgaard et al. 2003 discussed in detail, Klimisch: reliable with restrictions	
Bergström et al. 2011 discussed in detail, Klimisch: reliable with restrictions	
Gjettermann et al. 2011, discussed in detail, Klimisch: not reliable	
Riedl et al. 2005, discussed in detail, Klimisch: reliable with restrictions	
Kjaer et al. 2005, discussed in detail, Klimisch: not reliable	
Landry et al. 2005, discussed in detail, Klimisch: not reliable	
Kjaer et al. 2011, discussed in detail, Klimisch: not reliable	
Laitinen et al. 2009, discussed in detail, Klimisch: not reliable	
Dousset et al. 2007, discussed in detail, Klimisch: not reliable	
Torstensson et al. 2005, discussed in detail, Klimisch: not assignable	

Studies on photochemical degradation

Argumentation:

- To be considered as supportive information if the study is plausible and reliable, and performed according to standard guideline

Comparison of evaluation:

Notifier	Current evaluation
No study discussed in detail	No study discussed in detail

Water/sediment studies

Argumentation:

- To be considered as supportive information if the study is plausible and reliable, and performed according to standard guideline

- Not to be considered for endpoint- or PEC-recalculation because sufficient data are available on adsorption and desorption

Comparison of evaluation:

Notifier	Current evaluation
No study discussed in detail	No study discussed in detail

Studies on impact on water treatment procedures

Argumentation:

- To be considered as additional information because sufficient information on water treatment procedures is available.
- To be considered as additional information because no fate related endpoint or any PEC-calculation is affected by the results of the article

Comparison of evaluation:

Notifier	Current evaluation
No study discussed in detail	No study discussed in detail

Studies on predicted environmental concentrations in surface water and groundwater

Argumentation:

- To be considered as additional information because sufficient reliable calculations on PEC_{GW} and PEC_{SW} are available

Comparison of evaluation:

Notifier	Current evaluation
No study discussed in detail	No study discussed in detail

Monitoring data

Argumentation:

- To be considered as additional information because sufficient reliable calculations on PEC_{GW} and PEC_{SW} are available.
- To be listed in a summarizing table for the purpose of additional information in case sufficient raw data are available
- Not to be considered as monitoring is outside the EU.

Comparison of evaluation:

Notifier	Current evaluation
No study discussed in detail	No study discussed in detail but studies with sufficient data are listed for the purpose of

| additional information

Other studies

83 articles obtained from open literature can be subsumed under the heading "other studies". They either focus on other topics than those given in the points B.8.11.2 to B.8.11.6 or present reviews on fate and behaviour in the environment.

The listed articles are valuable and might be used as additional information. However, they are not taken into account for any endpoint- or PEC-calculation. Consequently, they are not evaluated following the approach as it has been done for the studies from point B.8.11.2 to B.8.11.6 and the criteria "relevance" and "evidence" are characterised as "not applicable". The proposed action is: "not to be considered as publication does not focus on an environmental fate-related endpoint".

References

EFSA (2011): Submission of scientific peer-reviewd open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 9(2): 2029 [49 pp.]. doi:10.2903/j.efsa.2011.2092

FOCUS (2006): Work Group on Degradation Kinetics. Guidance document on estimating persistence and degradation kinetics from environmental fate studies on pesticides in EU registration. Sanco/10058/2005, version 2.0, June 2006

Kase R. et al. (2012): Klimisch 2.0 – Raising the bar to increase the transparency of environmental risk assessments. Poster SETAC 22nd Annual Meeting Berlin, Germany 20-24 May 2012

Klimisch, H-J, Andreae, M and Tillmann, U (1997): A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory Toxicology and Pharmacology 25, pp. 1-5

Küster A, Bachmann J, Brandt U, Ebert I, Hickmann S, Klein-Goedicke J, Maak G, Schmitz S, Thumm E and Rechenberg B (2009): Regulatory demands on data quality for the environmental risk assessment of pharmaceuticals. Regulatory Toxicology and Pharmacology 55 (2009) 276-280

Küster A, Agerstrand M, Bachmann J, Breitholz M, Ebert I, Rudén C and Rechenberg B (2010): Proposal for a new reliability and relevance evaluation scheme for ecotoxicity data. Poster SETAC Europe 20th Annual Meeting Seville, Spain 23-27 May 2010.

B.8.11.2 Route and rate of degradation in soil

B.8.11.2.1 Route degradation in soil

Soil photolysis

Summary

Three articles are available on the behaviour of glyphosate when subjected to irradiation in the presence various catalysts in or on a solid phase (solid TiO_2, thermally activated peroxydisulfate, $Fe_3O_4/SiO_2/TiO_2$). Test results are of minor relevance as they are not obtained by a standard test procedure on photodegradation in soil. They can be assessed as additional information only.

To sum up, Echavia et al. (2009) observed a complete (100 %) decomposition of glyphosate within 60 min of irradiation when irradiated in the presence of TiO_2. From photochemical activation of peroxydisulfate in flash-photolysis experiments, the bimolecular rate constants for the reaction of sulfate radical with glyphosate ($1.6 \cdot 10^8$ M^{-1} s^{-1}) was obtained by Kirmser et al. (2010). Photocatalytic degradation of glyphosate in soil by the photocatalyst $Fe_3O_4/SiO_2/TiO_2$ under solar irradiation was investigated by Xu et al. (2011). Results show that 0.5% $Fe_3O_4/SiO_2/TiO_2$ has the best photocatalytic activity. Degradation efficiency of glyphosate reaches 89 % in 2 h when the dosage of photocatalyst is 0.4 g/100 g (soil), and it increased slowly when more photocatalyst was used.

Detailed description of open literature on soil photolysis

Echavia et al. (2009)

Title Photocatalytic degradation of organophosphate and phosphonoglycine pesticides using TiO_2 immobilized on silica gel	
Author: Glory Rose Mangat Echavia, Fumiko Matzusawa, Nobuaki Negishi	
Reference: Chemosphere 76: 595-600.	
Year: 2009	
Results and conclusion: Complete (100%) decomposition of glyphosate was attained within 60 min of irradiation.	
Proposed action: Consider as additional information as no standard test design was followed. Recalculation of endpoints on stability in soil is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Photodegradation of glyphosate using TiO_2 immobilized on silica gel
Protocol	Non-GLP study
Test compound	Glyphosate (99%) purchased from Wako Pure Chemical Industries, Ltd., Japan (CAS 1071-83-6)
Test system and conditions	The photocatalyst used in the study was the commercial HQC-22 TiO_2 obtained from Shinto V Cerax Company, Japan. The batch photocatalytic reactor or photo reactor consisted of a spiral glass tube packed with 14.0 g of the TiO_2 photocatalyst and wound around a 6Wblack light fluorescent UV lamp. The photo reactor also included a 250 mL glass container that served as a reservoir

	for the pesticide solutions. The UV lamp emits a wavelength centred mostly at 365 nm with a light intensity of 1.4mWcm^{-2} (measured 1 cm away from the UV lamp by UV Caremate PRO produced by Fuji Xerox Co.). The external surface of the photo reactor chamber was covered with aluminium sheet to prevent dissipation of UV light.
Statistical design	No information
Relevance	
Environmental relevance	The relevance is low due to the artificial system.
Weight of evidence	
"Positive"/"Negative" evidence	The results of the experiments cannot be compared with standard testing.

Kirmser et al. (2010)

Title Degradation of the Herbicides Clomazone, Paraquat, and Glyphosate by Thermally Activated Peroxydisulfate	
Author: Elena M. Diaz Kirmser, Daniel O. Martire, Monica C. Gonzalez, and Janina A. Rosso	
Reference: J. Agric. Food Chem. 2010, 58, 12858–12862	
Year: 2010	
Results and conclusion: From photochemical activation of peroxydisulfate in flash-photolysis experiments, the bimolecular rate constants for the reaction of sulphate radical with glyphosate ($1.6 \cdot 10^8$ M^{-1} s^{-1}) was obtained. Thermal activation of peroxydisulfate was shown to degrade the herbicide glyphosate. Although the herbicide degradation was observed to take place in less than 1 h, the mineralization of the organic carbon required longer reaction times, because of the formation of stable organic intermediates.	
Proposed action: Consider as additional information as no standard test design was followed. Recalculation of endpoints on stability in soil is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Photodegradation of glyphosate using activation of peroxydisulfate in flash-photolysis experiments
Protocol	Non-GLP study
Test compound	
Test system and conditions	Not relevant
Statistical design	
Relevance	
Environmental relevance	The relevance is low due to the artificial system.
Weight of evidence	
"Positive"/"Negative" evidence	The results of the experiments cannot be compared with standard testing.

Xu et al. (2011)

Title Degradation of Glyphosate in Soil Photocatalyzed by Fe3O4/SiO2/TiO2 under Solar Light	
Author: Xuan Xu , Fangying Ji, Zihong Fan and Li He	
Reference: Int. J. Environ. Res. Public Health 2011, 8, 1258-1270; doi:10.3390/ijerph8041258	
Year: 2011	
Results and conclusion: Photocatalytic degradation of glyphosate in soil by these photocatalyst under solar irradiation was investigated. Results show that 0.5% $Fe_3O_4/SiO_2/TiO_2$ has the best photocatalytic activity. The best moisture content of soil is 30%~50%. Degradation efficiency of glyphosate reaches 89% in 2 h when the dosage of photocatalyst is 0.4 g/100 g (soil), and it increased slowly when more photocatalyst was used. Degradation of glyphosate is not obviously affected by sunlight intensity when the intensity is below 6 mW/cm2 or above 10 mW/cm^2, but it is accelerated significantly when the sunlight intensity increases from 6 mW/cm^2 to 10 mW/cm^2.	
Proposed action: Consider as additional information as no standard test design was followed. Recalculation of endpoints on stability in soil is not necessary..	
Type of information (critical, high/low weight, supporting, additional): Low weight	
Reliability	
Endpoint	Degradation of Glyphosate in Soil Photo catalyzed by $Fe_3O_4/SiO_2/TiO_2$ under Solar Light
Protocol	Non-GLP study
Test compound	Non-labelled: Glyphosate (CAS 1071-83-6)
Test system and conditions	Soil for experiments was typical red loam, which was collected from the Banan District, Chongqing, China.
Statistical design	Three replicates
Relevance	
Environmental relevance	There is little relevance for this analysis as the results cannot be compared with reliable studies.
Weight of evidence	
“Positive”/”Negative” evidence	The results cannot be directly compared with standard studies.

References

Echavia G.R. et al. 2009. Photocatalytic degradation of organophosphate and phosphonoglycine pesticides using TiO_2 immobilized on silica gel. Chemosphere 76: 595-600.

Kirmser E. et al. 2010. Degradation of the Herbicides Clomazone, Paraquat, and Glyphosate by Thermally Activated Peroxydisulfate. J. Agric. Food Chem. 2010, 58, 12858–12862

Xu X. et al. 2011. Degradation of Glyphosate in Soil Photocatalyzed by $Fe_3O_4/SiO_2/TiO_2$ under Solar Light. Int. J. Environ. Res. Public Health 2011, 8, 1258-1270; doi:10.3390/ijerph8041258

B.8.11.2.2 Rate of degradation

Laboratory studies

Summary

The degradation of glyphosate in soil under aerobic conditions has been investigated by several authors. It should be noted that most of these publications do not provide sufficient experimental details to evaluate the data quality and validity in these manuscripts. Those publications that do provide details clearly show deficiencies according to the guidance provided by FOCUS (2006, 2011) regarding data quality and data handling that are relevant to the kinetic analysis. These deficiencies include: low or no reported mass balance, lack of duplicate sampling and insufficient valid sampling times. In addition, the majority of these publications do not provide much information in regard to the procedures used for deriving degradation rates that have been reported. Consequently, the results of open literature were not used to derive endpoints or to calculate PEC's.

However, the review of open literature shows that glyphosate is degraded through soil microorganisms. Main metabolic pathway involves the formation of CO_2 and aminomethylphosphonic acid (AMPA) which is further degraded to CO_2. AMPA typically represents 15-25% of the applied glyphosate. Glyphosate mineralization varies extensively, depending on the soil type and biological activity. Glyphosate half-lives (DT_{50}) in laboratory experiments were investigated by several authors. When only those studies are considered studies, which were conducted in line with reliable standard GLP-studies, the DT_{50} values ranged from 9 to 115 days. AMPA is also biodegraded in soil. The DT_{50} values range from 25-75 days under laboratory conditions. In detail, glyphosate degradation in soil is a microbiological process and often shows a high degree of mineralization. The differences in glyphosate mineralization rate can be attributed to differences in the biological activity of the soils. Gimsing et al. (2004) observed a good correlation between the mineralization of glyphosate and the abundance of Pseudomonas spp bacteria in soil; although a poor correlation was observed with the overall soil respiration activity. Several authors also reported an inverse correlation with the strength of soil adsorption hypothesizing that if glyphosate is strongly adsorbed to soil, it is not bioavailable for degradation. Several authors have also investigated the mineralization of glyphosate in deeper soil layers. Jacobsen et al. (2003) observed that the soil below the ploughing layer had a low potential to mineralize glyphosate (< 10 % CO_2).

Detailed description of open literature on rate of degradation – Laboratory studies

Accinelli et al. (2006)

Title: Influence of Cry1Ac Toxin on Mineralization and Bioavailability of Glyphosate in Soil
Author: CESARE ACCINELLI, WILLIAM C. KOSKINEN, AND MICHAEL J. SADOWSKY
Reference: J. Agric. Food Chem. 2006, 54, 164-169
Year: 2006
Results and conclusion: Results from laboratory investigations indicate that soil incorporation of purified Cry1Ac toxin in the range of 0.25-1.0 µg g^{-1} does not influence glyphosate mineralization or its sorption in soil. These results are in contrast to results obtained in previous investigations done using a mixture of Cry toxins at a concentration of 10 µg g^{-1}. The concentration of Cry toxins in soil occurring during the growing season has been estimated not to exceed 1

µg g^{-1}, based on the average concentrations of Cry toxin in crop residues incorporated into the top soil or left at the soil surface. On the basis of these estimates and the results obtained here, the data indicate that concentrations of Cry1Ac comparable to those encountered under field conditions do not have the potential to increase persistence and sorption of glyphosate in soil. Following K_{foc}-values were determined for glyphosate: sandy loam (Italy): 6230 L/kg and 6408 L/kg (US soil).	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation or sorption is not necessary	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	$DegT_{50}$ in soil and sorption in soil
Protocol	Non-GLP standard degradation study modified OECD Guideline 307 and 106
Test compound	CAS 1071-83-6 Unlabelled glyphosate (chemical purity > 98%) ^{14}Clabeled glyphosate (N-phosphonomethyl-2-^{14}C-glycine; radiopurity > 99%, specific activity) 1.18 106 MBq g^{-1})
Test system and conditions	Two soils with different physicochemical properties, taken from areas of the Po Valley (Italy) and of south central Minnesota, were selected for this study. The Italien soil (IT, 0.7 % OC) and the American soil (MN, 0.94 % OC) were both classified as sandy loam At both locations, the soil was collected from fields that had not received glyphosate applications within the previous 5 years. A portion of the IT and MN soils was mixed with Cry1Ac toxin powder to obtain a final concentration of 100 µg g^{-1} soil. Aliquots of these two amended soils were mixed with a sufficient mass of IT and MN soils to obtain final soil concentrations of 0.25, 0.5, and 1.0 µg Cry1Ac toxin g^{-1} soil (air-dried basis). The soil moisture in treated soil samples was adjusted to the gravimetric content at -33 kPa using distilled water and incubated in the dark at 25 °C. Isotherms for sorption of glyphosate to IT and MN soils containing different Cry1Ac toxin concentrations were determined using the batch equilibrium method 20 °C for 14 h.
Statistical design	Three replicates were prepared for each soil type and toxin concentration, and controls consisted of soils with no toxin addition.
Relevance	
Environmental relevance	The sorption studies are in principle performed considering the current guidance documents and can be considered for the calculation of sorption parameters. The degradation studies are not documented well enough to be considered further.
Weight of evidence	
“Positive”/”Negative” evidence	The results on degradation and sorption are principally supported by other reliable studies.

Accinelli et al. (2004)

Title: Influence of insecticidal toxins from Bacillus thuringiensis subsp. kurstaki on the degradation of glyphosate and glufosinate-ammonium in soil samples	
Author: Cesare Accinelli, Claudio Screpanti, Alberto Vicari, Pietro Catizone	
Reference: Agriculture, Ecosystems and Environment 103 (2004) 497–507	
Year: 2004	
Results and conclusion: The influence of insecticidal toxins on the persistence of herbicides in soil is analysed. The persistence of GLYP and GLUF was enhanced by the addition of a high rate of Btk. insecticidal crystal toxins extracted and purified from the commercial formulation Dipel 2×. Since no influence of Btk toxins on SMC of the two soils was observed and a rapid decrease of the insecticidal activity of the added Btk toxins was estimated during the 28-day incubation period, the observed increase of GLYP and GLUF persistence was presumably due to the reduction of bio-availability of the two herbicides, modification of the soil nutritive status or other not measured properties, such as soil microbial activity.	
Proposed action: Not be considered for recalculation of endpoints since the study design did not completely follow standard testing (e.g. no radio-labelling)	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	$DegT_{50}$ in soil
Protocol	Non-GLP standard degradation study modified OECD Guideline 307
Test compound	Test compounds: CAS 1071-83-6 (GLYP) and 40465-66-5 (GLUF), Glyphosate [N-(phosphonomethyl)glycine] (GLYP) and glufosinate-ammonium [the ammonium salt of dl-homoalanin-4-yl(methyl)phosphinic acid] (GLUF) commercial formulation of Roundup Bioflow (Monsanto Agricoltura Italia S.p.A., Lodi, Italy; isopropylamine salt of GLYP, suspension concentrate, containing 360 g active ingredient, a.i., l−1 formulation), Basta (Bayer CropScience S.r.l., Milano, Italy; ammonium salt of GLUF, suspension concentrate, containing 120 g a.i. 1-1 formulation)
Test system and conditions	Surface (0-20 cm) soil samples were taken from two agricultural areas of the Po Valley (Italy), Cadriano and Ozzano. Cadriano soil was classified as a loam (Udic Ustochrepts, fine silty, mixed, mesic) and Ozzano soil as a sandy loam (Udertic Ustochrepts, fine, mixed, mesic). In both the locations, soils were collected from fields with no pesticide application during the last 5 years. Before the beginning of the experiment, soil moisture was adjusted to the gravimetric content at -33 kPa using ultrapure water. Soil samples were kept in the dark in a climatic chamber at 25 °C ± 0.5 for 10 days. The conditioning period of 10 days allowed the soil to establish a steady-state level of microbial activity. Conditioned soil samples were treated with water solutions of the commercial formulation. For herbicide half-life estimation, sampling times were 0, 3, 7, 14, 21 and 28 days after treatment.
Statistical design	Three replicate samples
Relevance	

Environmental relevance	The investigations dealing with the persistence of glyphosate and glufosinateammonium in soil in the presence of insecticidal toxins is not of preliminary interest, but can be considered as additional information.
Weight of evidence	
"Positive"/"Negative" evidence	Though the results did not completely follow the standard procedure the results are in line with results of other reliable studies.

Alexa et al. (2009)

Title: DYNAMIC OF GLYPHOSATE MINERALIZATION IN DIFFERENT SOIL TYPES	
Author: Ersilia Alexa, Mihaela Bragea, Renata Sumalan, Aurel Lazureanu, Monica Negrea, Stancu Iancu	
Reference: ROMANIAN AGRICULTURAL RESEARCH, Number 26/2009, 57-60	
Year: 2009	
Results and conclusion: The experimental results indicate a high microbial degradation of the glyphosate herbicide. The $^{14}CO_2$ quantity accumulated following glyphosate biodegradation under the microorganism action is higher in all 4 analyzed soils, comparing with blind sample (untreated soil). The experimental results show that the rate of glyphosate degradation in time is higher in the firsts 5 days, than the velocity decreases until the curves attained plateaus. The initial rapid phase of degradation was attributed to microbial action on the free glyphosate while the slower phase was due to the subsequent attack on the adsorbed glyphosate.	
Proposed action: Consider as supporting information. Recalculation of endpoints on degradation is not necessary	
Type of information (critical, high/low weight, supporting, additional): Supporting	
Reliability	
Endpoint	$DegT_{50}$ in soil
Protocol	Non-GLP standard degradation study modified OECD Guideline 307
Test compound	Glyphosate-phosphonomethyl-^{14}C-labeled with specific activity 2.2 mCi/mmol (CAS 1071-83-6)
Test system and conditions	Four types of soils were taken under study: black chernozem, vertisol, gleysol and phaeozom with different characteristics. Four types of soils were taken under study: black chernozem, vertisol, gleysol and phaeozom with different characteristics. The soil was onditioned by being moistened to 85% of the field water capacity. The soils were incubated at 20°C, for 40 days. The mineralization curves of $^{14}CO_2$ accumulated were compared during 40 days.
Statistical design	Not reported
Relevance	
Environmental relevance	The degradation studies are in principle performed considering the current guidance documents. However, the studies are not documented well enough to be considered further (e.g. time dependent residues not given).
Weight of evidence	
"Positive"/"Negative"	The results are principally supported by other reliable studies.

evidence	

Alexa et al. (2010)

Title: Studies on the biodegradation capacity of ^{14}C-labelled glyphosate in vine plantation soils	
Author: Ersilia Alexa, Renata Sumalan, Monica Negrea, Mihaela Bragea, Mariana-Atena Poiana, Isidora Radulov and Aurel Lazureanu	
Reference: Journal of Food, Agriculture & Environment, Vol.8 (3&4)	
Year: 2010	
Results and conclusion: The aim of this paper was to study the biodegradation capacity of glyphosate in soil samples prelevated from vine plantation from Timis county, Romania, belonging to Banat's University of Agricultural Science, Timisoara, in presence of organic and inorganic supplement, at different concentration levels. After addition of glyphosate-phosphonomethyl-^{14}C-labeled, the accumulated $^{14}CO_2$ (as % of total ^{14}C) was monitored during 44 days. Investigated soil shows a high degradation capacity of over 85% of total radioactivity after 44 days from the treatment application. Addition of inorganic supplement causes a decrease of glyphosate biodegradation capacity to 10.77-12.87% of total radioactivity, while in presence of straw the accumulated $^{14}CO_2$ (as % of total ^{14}C) during the 44 days ranged between 59.97 and 87.58%. The amount of $^{14}CO_2$ released reached the highest level in the first 4 days after herbicide application, both in control and experimental variants with organic and inorganic supplement (from 2.61 to 30.27% of total radioactivity). By glyphosate addition the growth and multiplication of soil microorganisms, whose biomass is digested in the range of 9-12 days of treatment, according to the daily mineralization rate (DMR) values, is stimulated. Our results on the activity of microorganisms showed that glyphosate degradation in soil is mainly performed by micromyces.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	$DegT_{50}$ in soil
Protocol	Non-GLP degradation study under laboratory conditions
Test compound	Labelled compound: ^{14}C-glyphosate
Test system and conditions	The soils were incubated at 20°C, to dark for 44 days. In order to evaluate the biodegradation of ^{14}C-labeled glyphosate during the incubation period, samples were taken every 4 days.
Statistical design	Not reported
Relevance	
Environmental relevance	Low relevance as important environmental parameters influencing the degradations were not reported (e.g. moisture)
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with other studies as important environmental parameters were not determined.

Alexa et al. (2010)

Title: RESEARCH ON THE WEED CONTROL DEGREE AND GLYPHOSATE SOIL BIODEGRADATION IN APPLE PLANTATIONS (PIONEER VARIETY)

Author: Ersilia ALEXA, Roxana MICU, Monica NEGREA, Renata SUMALAN, Olimpia IORDANESCU	
Reference: Analele Universitatii din Oradea - Fascicula Biologie Tom. XVII/1, 2010, pp. 5-8	
Year: 2010	
Results and conclusion: The experimental results indicate a high microbial degradation of the glyphosate herbicide. Glyphosate mineralization curve reveals two phases of CO_2 release, the first rapid phase, followed by a slow phase, when the mineralization curve reaches a steady plateau. According to the authers initial rapid phase covers a period of approximately 20 days from the beginning of the experiment and is attributed to the action of microorganisms on free glyphosate from soil, while the second phase is attributed to slow action of microorganisms on glyphosate adsorbed on soil components.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation or sorption is not necessary	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	$DegT_{50}$ in soil
Protocol	Non-GLP degradation study under outdoor conditions
Test compound	Labelled compound: Roundup 3 l/ha (glyphosate isopropyl amine salt 360 g/l), C^{14} marked to fosfonometil group with 37kBq (CAS 38641-94-0)
Test system and conditions	A field study was performed deteriming the mineralisation of glyphosate in soil.
Statistical design	Not reported
Relevance	
Environmental relevance	Low relevance as important environmental parameters influencing the degradations were not reported (moisture, temperature)
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with other studies as important environmental parameters were not determined.

Alexa et al. (2008)

Title: RESEARCHES REGARDING THE MICROORGANISMS INFLUENCE ON GLYPHOSATE BIODEGRADATION
Author: Alexa Ersilia, Sumalan Renata, Negrea Monica
Reference: Journal of Agroalimentary Processes and Technologies 14 (2) 2008
Year: 2008
Results and conclusion: The degradation capacity is influenced by the micro-biological soils particles and leads to the glyphosate primary metabolite formation, aminomethyl-phosphonic acide (AMPA). For determination of CO_2 content, it shows the microorganisms action over the free glyphosate in soil. The experimental results show microbial bio-degradation of glyphosate and of his metabolite AMPA after 96 hours (4 days) since the treatment application, when the CO_2 quantity release is maximum. The released CO_2 quantity grows until day six, than it reaches a constant level regarding the glyphosate degradation, and the mineralization speed decrease. Regarding the herbicide quantity added, it discovers that the free glyphosate from soil is

directly and rapidly degraded by micro-organisms and not affect the microbiological activity, even at the high concentrations applied, double comparing with the quantity used in field.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation or sorption is not necessary	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Principally $DegT_{50}$ in soil (mineralisation), but not explicitly calculated
Protocol	Non-GLP degradation study
Test compound	Non-Labelled glyphosate (CAS 38641-94-0) and AMPA (CAS: 74341-63-2)
Test system and conditions	Four types of soils have been taken under study: Black Chernozem, Vertisol, Gleysol and Phaeozem with different characteristics. The analyzed soils have been taken from horizon A, from a depth of 10 cm. In order to obtain a representative sample, the samples have been taken from different points by splitting the surface in quarters, diagonally and on rows, through the carrots.
Statistical design	Not reported
Relevance	
Environmental relevance	Low relevance as the study was not evaluated according to standard procedures (e.g. FOCUS Deg Kin).
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with other studies as the endpoints was not calculated adequately.

Al-Rajab et al. (2010)

Title: Degradation of ^{14}C-glyphosate and aminomethylphosphonic acid (AMPA) in three agricultural soils
Author: Abdul Jabbar Al-Rajab, Michel Schiavon
Reference: Journal of Environmental Sciences 2010, 22(9) 1374–1380
Year: 2010
Results and conclusion: Laboratory degradation studies were performed showing an immediate and high rate of glyphosate degradation after its application on soil. Mineralization of glyphosate after 17 days of incubation reached 32.2% to 39.7% of the initial amount applied to the two soils (sandy loam (pH 5.1) or silt clay loam (pH 6.3)). However, the mineralization rate was more rapid and intense for the clay loam soil (pH 7.9) with 48.4% reached by 12 days of incubation. Thereafter, the mineralization of glyphosate declined gradually for all three soils. The endogenous activity of mineralization was comparable for the three investigated soils. The fast mineralization of glyphosate in clay loam soil appears due exclusively to a bioavailability more important than in other two soils. The analysis of water extracts by HPLC showed the appearance of two degradation products of glyphosate AMPA. The appearance of AMPA during incubation varied significantly depending on the speed of mineralization of glyphosate in each soil (Table 2). In sandy loam soil, there was only 12.7% of AMPA present on day 3 after treatment, whereas 87.3% of the initial radioactive glyphosate was present on the same day.
Proposed action:

Consider as supporting information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Supporting	
Reliability	
Endpoint	$DegT_{50}$ in soil
Protocol	Non-GLP standard degradation study modified OECD Guideline 307
Test compound	Glyphosate (CAS 1071-83-6) [Phosphonomethyl-^{14}C]-glyphosate was obtained from ARC-ISOBIO (Belgium) diluted in water. Its specific radioactivity was 385 GBq/mmol and its radiochemical purity 99%. Non-radioactive glyphosate (purity 98.5%) was obtained from CIL Cluzeau (France). AMPA (CAS 74341-63-2), 10 ng/µL in water, was obtained from Dr. Ehrenstorfer GmbH (Germany).
Test system and conditions	Three cultivated soils from the Lorraine region in eastern France were selected on the basis of their texture and pH. None of these soils had ever been exposed to glyphosate. The jars were incubated in the dark at 20°C for 80 days. Analyses were performed in triplicates and one control of unspiked soil per type of soil was considered.
Statistical design	Analyses were performed in triplicates and one control of unspiked soil per type of soil was considered.
Relevance	
Environmental relevance	The degradation studies are in principle performed considering the current guidance documents. However, the studies are not documented well enough to be considered further.
Weight of evidence	
"Positive"/"Negative" evidence	The results are principally supported by other reliable studies.

Andréa et al. (2003)

Title Influence of repeated applications of glyphosate on its persistence and soil bioactivity
Author: Mara Mercedes de Andréa, Terezinha Bonanho Peres, Luiz Carlos Luchini, Sheila Bazarin, Solange Papini, Marcus Barifouse Matallo and Vera Lucia Tedeschi Savoy
Reference: Pesq. agropec. bras., Brasília, v. 38, n. 11, p. 1329-1335, nov. 2003
Year: 2003
Results and conclusion: Degradation of isopropylamine salt of glyphosate after repeated applications was analysed in the laboratory. The ^{14}C-glyphosate applied to soil was immediately mineralized since the first application, mainly in the first week after each treatment. The amounts mineralized in the first week after each application were 31.70±4.29%, 20.65±3.89%, 22.03±1.02% and 14.50±0.99%, respectively. The immediate mineralization seem to decrease with increasing number of treatments. The detected amounts of $^{14}CO_2$ were formed from the degradation of glyphosate to AMPA, as well as from degradation of AMPA and express really complete degradation of the pesticide molecule.
Proposed action: Consider as supporting information. Recalculation of endpoints on degradation is not nec-

essary	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation in soil after repeated applications (no explicit DT_{50} calculation)
Protocol	Non-GLP study
Test compound	CAS 38641-94-0 [^{14}C]-glyphosate solution containing 372 mg and 26.8 kBq mL^{-1} by mixture of Nortox® formulated glyphosate certified by the Environmental Chemistry Laboratory of Instituto Biológico as 480 g L^{-1} of the isopropylamine salt of glyphosate, and [N-(^{14}C-phosphonomethyl)-glycine] from Amersham International with 2.70 GBq $mmol^{-1}$.
Test system and conditions	An Ultisol soil sample from the Centro Experimental do Instituto Biológico, Campinas, SP, Brazil, was collected from 0 to 15 cm of the soil profile from an area without occurrence of pesticide applications Each treatment consisted in 3.0 mg glyphosate and 0.22 kBq of ^{14}C-glyphosate per g soil, which agrees with the interval of recommended doses. Soil moisture was 60% WMHC. The flasks were maintained in the dark at 25ºC during all the experimental time.
Statistical design	Not reported
Relevance	
Environmental relevance	The relevance is small since the DT_{50} were not calculated and the provided information is not sufficient to analyse the results further.
Weight of evidence	
"Positive"/"Negative" evidence	The amount of degradation is principally in line with other reliable studies performed. The effect after repeated applications seems questionable.

Assalin et al. (2010)

Title Studies on degradation of glyphosate by several oxidative chemical processes: Ozonation, photolysis and heterogeneous photocatalysis
Author: MARCIA R. ASSALIN, SANDRA G. DE MORAES, SONIAC.N.QUEIROZ, VERA L. FERRACINI and NELSON DURAN
Reference: Journal of Environmental Science and Health Part B (2010) 45, 89–94
Year: 2010
Results and conclusion: Degradation of aqueous solutions containing glyphosate was observed by oxidative advanced processes. Processes based on the formation of hydroxyl radical, such as Ti/UV and O_3/pH 10, were effective for the degradation of glyphosate and its degradation intermediates, AMPA, after a short treatment time. Under the experimental conditions used in this study the degradation of glyphosate followed a pseudo first-order kinetic law for both processes studied. The half–lives obtained for glyphosate degradation were 1.8 and 6.2 minutes for O_3/pH 10 and TiO_2/UV, respectively.
Proposed action: Consider as supporting information. Recalculation of endpoints on degradation is not necessary.

Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation by oxidative chemical processes
Protocol	Non-GLP study
Test compound	Non-labelled glyphosate (CAS 1071-83-6, purity 99.8 %) andAM-PA(CAS 1066-51-9 purity 99.1%) obtained from Monsanto and used without futher purification.
Test system and conditions	A stock solution containing 1000 mg/L of glyphosate was prepared in deionised water and diluted to the required concentration (42.275 mg/L) for the degradation experiments. The original pH of this solution was about 6.5. The pH was adjusted to 10 by the addition of a NaOH solution for the ozonation experiment. An ozone concentration of 14 mg/L was applied for 30 minutes in a batch reactor. Samples (42.275 mg/L glyphosate solution, 400 mL) were submitted to ozonation at pH 6.5 and at pH 10 (pH adjusted with a sodium hydroxide solution) at room temperature. Titanium dioxide (80% anatase and 20% rutile, average particle size of 30 nm and BET Method–Brunauer, Emmett and Teller [BET] surface of 50 ± 15 m2/g) was used without any pre-treatment. Illuminationwas provided by a high-pressure mercury lamp (Philips HPL-N, 125 W; λ > 290 nm) with the glass bulb removed. The lamp was fixed in the center of the reactor and cooled by a water jacket, at room temperature. The suspension was bubbled with oxygen (through a sintered glass disk placed in the bottom of the reactor) at a flow rate of about 6 ± 0.2 L/h for 30 minutes. Mineralization was followed by measuring the total organic carbon (TOC).
Statistical design	Not reported
Relevance	
Environmental relevance	The relevance is small since no environmental relevant DT_{50} were obtained due to the artificial experimental conditions.
Weight of evidence	
"Positive"/"Negative" evidence	The amount of degradation observed cannot be compared with the results of standard tests.

Barrett and Mc Bride (2005)

Title Oxidative Degradation of Glyphosate and Aminomethylphosphonate by Manganese Oxide
Author: K. A. BARRETT AND M. B. MC BRIDE
Reference: ENVIRONMENTAL SCIENCE & TECHNOLOGY, VOL. 39, NO. 23, 9223-9228
Year: 2005
Results and conclusion: The authors were unable to measure significant glyphosate and AMPA degradation in the presence of Mg^{2+}
Proposed action: Consider as supporting information. Recalculation of endpoints on degradation is not necessary.

Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation by oxidative chemical processes
Protocol	Non-GLP study
Test compound	Non-labelled glyphosate (CAS 1071-83-6, 96% purity) and AM-PA(CAS 1066-51-9 purity 99%)
Test system and conditions	Glyphosate reagent containing 10.5 mg/L glyphosate and 0.5 mM $MnCl_2$ was prepared in a background electrolyte solution of 0.01M$NaNO_3$. On a molar basis, Mn was present at approximately 8 times the concentration of glyphosate. The effect of solution pH was assessed by adjusting the pH to 5.0, 6.0, or 7.0 with NaOH. Controls were prepared with no $MnCl_2$. Degradation of AMPA (99% purity) in the presence of the manganese oxide was similarily studied.
Statistical design	Not reported
Relevance	
Environmental relevance	The relevance is small since no environmental relevant DT_{50} were obtained due to the artificial experimental conditions.
Weight of evidence	
"Positive"/"Negative" evidence	The amount of degradation observed cannot be compared with the results of standard tests.

Bazot and Lebeau (2008)

Title Oxidative Simultaneous mineralization of glyphosate and diuron by a consortium of three bacteria as freeand/or immobilized-cells formulations	
Author: S. Bazot & T. Lebeau	
Reference: Appl Microbiol Biotechnol (2008) 77:1351–1358	
Year: 2008	
Results and conclusion: Synthetic culture medium: glyphosate was mineralized between 72 and 480 h Sediment extract medium: no mineralisation of glyphosate	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation in soil
Protocol	Non-GLP study
Test compound	Non-labelled glyphosate (CAS 1071-83-6) obtained from Fluka, France, no information about purity
Test system and conditions	A bacterial consortium able to mineralize two herbicides, glyphosate (Pseudomonas 4ASW) and diuron (Arthrobacter sp. N4 and Delftia acidovorans), was cultivated in both a synthetic culture medium without phosphate and a sediment extract medium. In the aim at optimizing glyphosate and diuron mineralization, all the combinations, i.e., free and/or immobilized cells in Ca-alginate beads were tested.
Statistical design	Three replicates

Relevance	
Environmental relevance	The relevance is small since no environmental relevant DT_{50} were obtained due to the artificial experimental conditions.
Weight of evidence	
"Positive"/"Negative" evidence	The amount of degradation observed cannot be compared with the results of standard tests.

Bonfleur et al. (2011)

Title Mineralization and degradation of glyphosate and atrazine applied in combination in a Brazilian Oxisol	
Author: ELOANA J. BONFLEUR, ARQUIMEDES LAVORENTI and VALDEMAR L. TORNISIELO	
Reference: Journal of Environmental Science and Health Part B (2011) 46, 69–75	
Year: 2011	
Results and conclusion: Glyphosate mineralization rate was slightly higher in the presence of one dose of atrazine when compared with glyphosate alone. However, no significant differences were found when half or twice the atrazine dose was applied, meaning that differences in glyphosate mineralization rates cannot be attributed to the presence of atrazine	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation in soil (mineralisation) in the presence of atrazine
Protocol	Non-GLP study, modified OECD Guideline 307
Test compound	^{14}C- glyphosate purity 94 %, 0.2333 mCi mg^{-1} respectively. (CAS 1071-83-6)
Test system and conditions	Samples site National Center for Research on Beef Cattle (CNPGC - Embrapa) in Campo Grande, State of Mato Grosso, Brazil. soil type: dark red dystrophic Oxisol, clayey texture, maintained 16 years under cultivated pasture (Brachiaria brizantha). No information about previous applications of glyphosate. Soil samples were collected at 0-10 cm depth, Experiment conducted at 25 ± 2◦C, humidity weekly adjusted to 60 % of the retention capacity. Soil treatments consisted of the combination of a field dose of glyphosate (2.88 kg ha^{-1}) with 0, 1/2, 1 and 2 times a field dose of atrazine (3.00 kg ha^{-1}) and a field dose of atrazine with 0, 1/2, 1 and 2 times a field dose of glyphosate.
Statistical design	Four replicates
Relevance	
Environmental relevance	The relevance is small since atrazine is not used in the EU for many years.
Weight of evidence	
"Positive"/"Negative" evidence	The results are in the range of values obtained with standard GLP studies.

Castro et al. (2007)

Title Biodegradation of the herbicide glyphosate by filamentous fungi in platform shaker and batch bioreactor	
Author: JOAO V. CASTRO, JR, MARIA C.R. PERALBA and MARCO A.Z. AYUB	
Reference: Journal of Environmental Science and Health Part B (2007) 42, 883–886	
Year: 2007	
Results and conclusion: The study demonstrated that cultures of the filamentous fungi Fusarium oxysporum can degrade the herbicide glyphosate, even at high concentration. The metabolism presented by growth of Fusarium strains in consortium was similar to the fungi in pure culture. The biodegradation conducted in the bioreactor was more efficient than in the platform shaker. All strains tested showed no improvement on biodegradation by changing the rate of oxygen. The metabolite AMPA was not observed in any of the assays studied, probably indicating the formation of other metabolites during the degradation of glyphosate	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation by Microorganisms (mineralisation) Czapeck medium
Protocol	Non-GLP study
Test compound	95% pure glyphosate (N-phosphonomethylglycine) obtained from Milenia agricultural (CAS 1071-83-6)
Test system and conditions	Studies of the biodegradation of glyphosate as a sole source of phosphorous by fungal strains were carried out in 300mL Erlenmeyer flasks containing 100mL of Czapeck medium. The cultures were inoculated with a spore suspension (2.6×10^{7} spores.mL^{-1}) and incubated at 30°C on a shaking platform at 150 rev.min^{-1}. The fungal strains were inoculated as pure and consortium cultures. Samples were taken during the experiments to quantify the residue of herbicide. As control for non-biological degradation, assays were conducted without addition of microorganisms in the same way as for the fungal degradations above. All the assays were done in duplicate.
Statistical design	No information
Relevance	
Environmental relevance	The relevance is small due to the artificial environment.
Weight of evidence	
“Positive”/”Negative” evidence	The results cannot be compared with standard testing.

Doublet et al. (2009)

Title Delayed degradation in soil of foliar herbicides glyphosate and sulcotrione previously absorbed by plants: Consequences on herbicide fate and risk assessment
Author: Jérémy Doublet, Laure Mamy, Enrique Barriuso
Reference: Chemosphere 77 (2009) 582-9.

Year: 2009	
Results and conclusion: The fate in soil of herbicides residues in plants was different from that of control. Mineralization in soil of glyphosate in crops decreased compared to control, and amounts of ^{14}C-extractable residues, mainly composed by the metabolite aminomethylphosphonic acid (AMPA), and non-extractable residues (NER) increased. The experiments with contaminated plant parts incorporated show significantly reduced rates but not outside the known range.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation of glyphosate in soil depdent on incorporated contaminated plant parts
Protocol	Non-GLP study, modified OECD 307 (adding contaminated plants)
Test compound	[Methyl-^{14}C]glyphosate purchased from Sigma Chemicals (St. Louis, USA; 81 MBq mmol^{-1}, 99.2% purity, CAS 1071-83-6)
Test system and conditions	Soil samples were taken from the top layer (0–10 cm) of a French experimental site (Dijon, Burgundy). The soil is a clay-loam calcareous Cambisol with 37.7% of clay (<2 µm), 29.6% of silt (2–50 µm), 15.2% of sand (50–200 µm), 16.7% of CaCO3, 1.63% of organic carbon, pH in water of 8.2, and water field capacity of 26.1% (determined at -1000 hPa). Ten 5 µL droplets of glyphosate or sulcotrione solutions were applied on the second youngest leaf of oilseed rape and/or maize plants. Seven DAT, treated leaves were washed (see above), then different aerial parts of plants (lamina, apex, petiole and stem) were incorporated into 11.4 or 57 g soil corresponding to 10 or 50 g dry soil (11.4 g for oilseed rape apex, 57 g for oilseed rape lamina and petiole and maize lamina and stem). Soil treated directly with herbicides was used as control.
Statistical design	No information
Relevance	
Environmental relevance	The results show an interesting degradation dependency which is not considered in the standard testing. However, the results can to some extend explain degradation variability.
Weight of evidence	
“Positive”/”Negative” evidence	The control experiments showed degradation in the range known from standard testing. The experiments which contaminated plant parts incorporated show significantly reduced rates but still in the range of results of reliable (standard) studies.

Ermakova et al. (2008)

Title Microbial Degradation of Organophosphonates by Soil Bacteria
Author: I. T. Ermakova , T. V. Shushkova, and A. A. Leont’evskii
Reference: Microbiology, 2008, Vol. 77, No. 5, pp. 615–620
Year: 2008

Results and conclusion: Bacteria that can utilize glyphosate (GP) or methylphosphonic acid (MPA) as a sole phosphorus source have been isolated from soil samples polluted with organophosphonates (OP). No matter which of these compounds was predominant in the native habitat of the strains, all of them utilized methylphosphonate. Some of the strains isolated from GP-polluted soil could utilize both phosphorus sources. Strains growing on glyphosate only were not isolated. The isolates retained high destructive activity after long-term storage of cells in lyophilized state, freezing to -20°C, and maintenance on various media under mineral oil. When phosphorusstarved cells (with 2% phosphorus) were used as inoculum, the efficiency of OP biodegradation significantly increased (1.5-fold).	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation of glyphosate in a mineral MS1 medium
Protocol	Non-GLP study
Test compound	Glyphosate (CAS 1071-83-6) no further information
Test system and conditions	The bacteria were cultivated in a mineral MS1 medium, sodium glutamate (Difco, United States) was used as the carbon source (10 g/l). Concentration of inorganic phosphorus was determined spectrophotometrically by formation of a complex of phosphomolibdate and malachite green in an acidic medium. Total phosphorus was determined by the same method after organophosphonate hydrolysis with ammonium persulfate. The content of MPA and GP in the culture liquid was calculated as the difference between total and inorganic phosphorus by introducing respective conversion rates for each compound.
Statistical design	No information
Relevance	
Environmental relevance	The relevance of the results is low due to the artificial environment.
Weight of evidence	
“Positive”/”Negative” evidence	The results cannot be compared with standard laboratory testing.

Getenga and Kengara (2004)

Title: Mineralization of Glyphosate in Compost-Amended Soil Under Controlled Conditions
Author: Z. M. Getenga, F. O. Kengara
Reference: Bull. Environ. Contam. Toxicol. (2004) 72:266-275
Year: 2004
Results and conclusion: All the mineralization curves for the soils with different treatments exhibited same patterns. The mineralization curves had only two phases, the initial rapid phase followed by a slow final phase, when the curves attained plateaus. The rapid phase lasted for about 20 days. The initial rapid phase phase of degradation was attributed to microbial action on the free glyphosate while the slower phase was due to the subsequent attack on the adsorbed glyphosate. The study showed that compost did not stimulate intense mineralization of

glyphosate by microbes. The authors did not calculate the $DegT_{50}$, but according to the residue they can be estimated to be in the range of 50 to 100 d (which is rather low for glyphosate).	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Degradation of glyphosate in soil where compost has been added
Protocol	Non-GLP study
Test compound	Glyphosate-phosphonomethyl-^{14}C-labeled (International Isotopes, Munich) with specific activity of 52mCi/mmol, and radiochemical purity of 99% (CAS 1071-83-6)
Test system and conditions	The soil was conditioned by being moistened to 75% of the field water capacity before they were incubated at 30°C in the darkness under aerobic conditions. Compost made from urban solid organic waste was added
Statistical design	Three replicates
Relevance	
Environmental relevance	Though the experiments do not follow standard testing the results are of relevance since in the agricultural environment soils are mixed with compost.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard laboratory testing.

Ghafoor et al. (2011)

Title: Measurements and modeling of pesticide persistence in soil at the catchment scale
Author: A. Ghafoor, N.J. Jarvis, T. Thierfelder, J. Stenström
Reference: Science of the Total Environment 409 (2011) 1900–1908
Year: 2011

Results and conclusion:
The purpose of the study was to study the influence of various soil physical, chemical and microbiological characteristics on pesticide persistence in the contrasting cultivated soils found in a small (13 km²) agricultural catchment in Sweden and to develop and test a simple model approach that could support catchment scale modelling. Persistence of glyphosate was investigated in laboratory incubation experiments. Degradation rate constants were highly variable with coefficients of variation of 42% for glyphosate. The degradation rates (0.006–0.05 day^{-1}) The mean rate constant was found to be 0.028 d^{-1} ($DegT_{50}$: 24.7 d). Results for sorption of glyphosate not adequately described. Multiple linear regression analysis and Mallows Cp statistic were employed to select the best set of independent parameters accounting for the variation in degradation.
Detailed results are:

Soil characteristics

Soil No	Textural class	pH	Sand (%)	Silt (%)	Clay (%)	OC (%)	$CaCO_3$	Total N
1	Loam	7.6	49	32	18	1.6	9.2	0.16
2	Sand	6.2	87	8	4	1.2	0.1	0.08
3	Clay loam	7	43	27	30	2.3	0.1	0.22

4	Sandy loam	7.1	58	25	17	2.1	0.4	0.21
5	Sandy loam	6.9	68	17	15	2.1	0.2	0.21
6	Sandy loam	6.5	70	21	9	1.1	0.1	0.09
7	Loamy sand	6.5	85	9	6	1.6	0.1	0.15
8	Sandy loam	7.6	55	28	17	2.6	0.2	0.25
9	Silty clay	6.4	12	45	44	6.7	0.3	0.54
10	Silty clay	6.9	17	54	29	10.2	0.5	0.87
11	Clay	6.9	22	33	45	2.5	0.9	0.25
12	Sandy loam	7.3	56	24	20	5.4	0.4	0.53
13	Sandy loam	6	63	27	10	0.9	0.1	0.08
14	Loamy sand	6.1	83	11	6	1.3	0.1	0.13
15	Clay loam	7.5	35	39	25	3	0.1	0.28
16	Clay loam	7.1	31	40	29	1.9	0.2	0.19

Results (20°C and pF 2)

Soil No	**k (day-1)**	**r^2**
1	0.044±0.006	0.93
2	0.018±0.001	0.95
3	0.032±0.002	0.86
4	0.046±0.002	0.88
5	0.031±0.001	0.87
6	0.033±0.003	0.97
7	0.024±0.000	0.95
8	0.050±0.001	0.89
9	0.017±0.004	0.98
10	0.006±0.001	0.95
11	0.013±0.001	0.96
12	0.029±0.001	0.96
13	0.022±0.000	0.95
14	0.027±0.000	0.97
15	0.028±0.001	0.96
16	0.032±0.004	0.98

Proposed action:
No action since documentation of results insufficient (no raw data presented, no evaluation according to FOCUS degradation kinetics)

Type of information (critical, high/low weight, supporting, additional):
Supporting information, since the experiments were performed in principal similar to standard degradation studies but documentation of results (e.g. time dependent residues, kinetic analyses) is insufficient.

Reliability

Endpoint	$DegT_{50}$ in soil
Protocol	Non-GLP standard degradation study OECD Guideline 307 adsorption study OECD 106 guideline
Test compound	Test compound: CAS 1071-83-6 Unlabelled: Glyphosate (N-(phosphonomethyl)glycine, 98% purity) [P-methylene-^{14}C]glyphosate (5.155 MBq mg−1, purity N99%)
Test system and conditions	Degradation: Incubation experiments for each soil/pesticide combination were carried out on two replicate samples. Water contents were adjusted to and maintained at pF 2 throughout the experiment by the addition of de-ionized water as necessary. The samples were incubated in aerated glass tubes in the dark at 20°C for 64 days. Duplicate

	samples (5 g) were taken after 0, 2, 4, 8, 16, 32 and 64 days of incubation for measurement of the residual concentrations of glyphosate. Sorption: Soil (four grams d.w. for glyphosate) was shaken to pre-equilibrate with 0.01 M $CaCl_2$ (39 mL) for 24 h at 20°C.
Statistical design	Two replicate samples
Relevance	
Environmental relevance	The study was carried out using only soils from Östergötland, southern Sweden. However, the studies are performed close to standard testing guidelines on degradation and can be considered to calculate endpoints.
Weight of evidence	
"Positive"/"Negative" evidence	The results on degradation and sorption are supported by other reliable studies.

Gimsing et al. (2004)

Title: Chemical and microbiological soil characteristics controlling glyphosate mineralisation in Danish surface soils	
Author: Anne Louise Gimsing, Ole Kragholm Borggaard, Ole Stig Jacobsen, Jens Aamandb, Jan Sørensen	
Reference: Applied Soil Ecology 27 (2004) 233–242	
Year: 2004	
Results and conclusion: Glyphosate was rapidly adsorbed to iron and aluminium oxides, but were later released from these pools during mineralisation. In soils with high mineralisation rates the metabolite AMPA was formed and adsorbed. The rate of mineralisation was best correlated with the population size of Pseudomonas spp. bacteria in the soils. Phosphate addition had a stimulating effect on glyphosate degradation in soils with low mineralisation rates, but no effect or a negative effect on mineralisation in soils with high mineralisation rates. Finally, mineralisation rates were higher in soils from organically managed soils than in soils from conventional farming. The results indicate that the activity of glyphosate mineralising bacteria (e.g. Pseudomonas spp.) was a major factor controlling the fate of glyphosate in the soils.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary	
Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Degradation of glyphosate in soil (mineralisation)
Protocol	Non-GLP study modified OECD 307
Test compound	Glyphosate-monoisopropylamine solution (40 wt.%, density 1.218, Aldrich Chemical Company, 38641-94-0) ^{14}C glyphosate solution (7.40MBqml−1, 3.78 mM, Amersham Pharmacia Biotech, Hørsholm, Denmark, CAS 1071-83-6) water.
Test system and conditions	The soils used are from the A-horizon of five Danish agricultural soils representing the majority of soil types found in Denmark The glyphosate solution was mixed well into the soil, and a small test tube with 1 ml 1M NaOH was placed in a incubator at 15°C.
Statistical design	Three replicates
Relevance	
Environmental relevance	The experiments are close to standard testing studies. However, no DT_{50} were calculated for the compounds.
Weight of evidence	
“Positive”/”Negative” evidence	The results can be compared with standard laboratory testing. However, they were not evaluated according to the standard procedures.

Grundmann et al. (2008)

Title: Mineralization and Transfer Processes of ^{14}C-labeled Pesticides in Outdoor Lysimeters
Author: Sabine Grundmann & Ulrike Dörfler & Bernhard Ruth & Christine Loos & Tobias Wagner & Heidrun Karl & Jean Charles Munch & Reiner Schroll

Reference: Water Air Soil Pollut: Focus (2008) 8:177–185	
Year: 2008	
Results and conclusion: The glyphosate mineralization curves showed no lag phase – the microorganisms were able to mineralize glyphosate immediately. The cumulated amounts of mineralized ^{14}C-glyphosate amounted to 32–39%. No accumulation of residues in the soil and no leaching of the residues to deeper soil layers could be observed after three applications. Glyphosate was rapidly degraded to AMPA in the soil. Glyphosate and AMPA were accumulated in soy bean nodules.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary	
Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Degradation of glyphosate in soil (mineralisation) Accumulation and leaching
Protocol	Non-GLP study
Test compound	CAS 1071-83-6 ^{14}C-glyphosate [N-(phosphonomethyl)glycine] had the ^{14}C-labeling on the phosphonomethyl group and was purchased from PerkinElmer, Rodgau, Germany (purity >98%). Non-labeled glyphosate and metabolites were purchased from Dr. Ehrenstorfer (Augsburg, Germany)
Test system and conditions	The lysimeters consist of soil columns of 2 m height and a surface area of 1 m^2. To detect and quantify gaseous ^{14}C-losses from soil and plant surfaces, a two-chamber-system with special trapping facilities was designed. The chambers are placed on the surface of the lysimeters – a soil chamber and a plant chamber. Glyphosate was applied three times, in spring 2004 and in spring and autumn 2005 in an amount of 1 kg a.i. ha^{-1} (1.92 MBq mg^{-1}). During the experiment, mineralization and volatilization of the herbicides from soil and plants were measured during a time period of about 2–3 months after application
Statistical design	No information
Relevance	
Environmental relevance	The experiments are relevant though not performed close to standard lysimeter studies. Additionally, not sufficient information is provided to describe the situation (e.g. weather, irrigation).
Weight of evidence	
"Positive"/"Negative" evidence	The results support the information known for glyphosate from standard tests.

Haney et al. (2002)

Title: Bioremediation and Biodegradation: Effect of Roundup Ultra on Microbial Activity and Biomass from Selected Soils
Author: R. L. Haney, S. A. Senseman, and F. M. Hons
Reference: J. Environ. Qual. 31:730–735 (2002).
Year: 2002

Results and conclusion: Roundup Ultra appeared to be rapidly degraded by soil microbes regardless of soil type or organic matter content, even at high application rates, without adversely affecting microbial activity.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary	
Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Degradation of glyphosate in soil (mineralisation)
Protocol	Non-GLP study
Test compound	CAS 38641-94-0 Isopropylamine salt of glyphosate as Roundup Ultra (480 g active ingredient L^{-1})
Test system and conditions	Nine soils from Georgia and Texas were used in this study. The soils varied in soil pH (4.7 to 8.2), soil organic C (4.1 to 52.3 g C kg^{-1} soil), and clay content (6 to 45%). The isopropylamine salt of glyphosate as Roundup Ultra (480 g active ingredient L^{-1}) was added to soil at a rate of 234 mg kg^{-1}. This amount was based on the recommended rate of RU being 0.84 kg ha^{-1}. Final moisture content was 20 % w/w at 30°C
Statistical design	Three replicates
Relevance	
Environmental relevance	The experiments are relevant though not performed with radio labelled compound. The authors describe the degradation only qualitatively (no DT_{50} calculated).
Weight of evidence	
“Positive”/”Negative” evidence	The results support the information known for glyphosate from standard tests.

Haney et al. (2002)

Title: Soil carbon and nitrogen mineralization as affected by atrazine and glyphosate	
Author: R. L. Haney,· S. A. Senseman, L. J. Krutz, F. M. Hons	
Reference: Biol Fertil Soils (2002) 35:35–40	
Year: 2002	
Results and conclusion: Soil C and N mineralization was sensitive to the addition of atrazine as well as atrazine mixed with glyphosate. The addition of low C:N ratio herbicides stimulates microbial activity and enhances the eventual mineralization of these compounds.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Degradation of glyphosate in soil (mineralisation) when atrazine is present
Protocol	Non-GLP study

Test compound	CAS 38641-94-0 Isopropylamine salt of glyphosate as Roundup Ultra (480 g active ingredient L^{-1})
Test system and conditions	The soil used was a Weswood silt loam (fine, mixed, thermic Fluventic Ustochrept) with soil pH of 8.3 (1:2 soil/water), soil organic matter content of 10.6 g kg^{-1} soil, 115 g sand kg^{-1}, 452 g silt kg^{-1}, 310 g clay kg^{-1}. The isopropylamine salt of glyphosate as RoundUp Ultra (480 g active ingredient L^{-1}) was added to soil at rates of 2× (94 mg kg^{-1}), 4× (188 mg kg^{-1}), and 6× (282 mg kg^{-1}).
Statistical design	Three replicates
Relevance	
Environmental relevance	The experiments are relevant though not performed with radio labelled compound. The authors describe the degradation only qualitatively (no DT_{50} calculated for glyphosate).
Weight of evidence	
"Positive"/"Negative" evidence	The results support the information known for glyphosate from standard tests.

Helander et al. (2012)

Title: Glyphosate in northern ecosystems	
Author: Marjo Helander, Irma Saloniemi and Kari Saikkonen	
Reference: Trends in Plant Science, October 2012, Vol. 17 (10): 569-575	
Year: 2012	
Results and conclusion: Glyphosate is the main nonselective, systemic herbicide used against a wide range of weeds. Its worldwide use has expanded because of extensive use of certain agricultural practices such as no-till cropping, and widespread application of glyphosate-resistant genetically modified crops. Glyphosate has a reputation of being nontoxic to animals and rapidly inactivated in soils. However, recent evidence has cast doubts on its safety. Glyphosate may be retained and transported in soils, and there may be cascading effects on nontarget organisms. These processes may be especially detrimental in northern ecosystems because they are characterized by long biologically inactive winters and short growing seasons. In this opinion article, we discuss the potential ecological, environmental and agricultural risks of intensive glyphosate use in boreal regions. The authors state that the half-life time of glyphosate may be much longer in northern ecosystems than generally presumed.	
Proposed action: Consider as additional information as the article does not present experimental data but is a review article. No raw data and/or experimental design are given.	
Type of information (critical, high/low weight, supporting, additional): Low weight, as it is a review article no new experimental data are presented but cited and discussed.	
Reliability	
Endpoint	Sorption, degradation
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; AMPA, CAS-no.: 1066-51-9
Test system and conditions	Not applicable
Statistical design	Not applicable

Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Jacobsen et al. (2008)

Title: Variation of MCPA, metribuzine, methyltriazine-amine and glyphosate degradation, sorption, mineralization and leaching in different soil horizons

Author: Carsten S. Jacobsen, Peter van der Keur, Bo V. Iversen d, Per Rosenberg, Heidi C. Barlebo c, Søren Torp d, Henrik Vosgerau e, Rene´ K. Juhler a, Vibeke Ernstsen, Jim Rasmussen, Ulla Catrine Brinch, Ole Hørbye Jacobsen

Reference: Environmental Pollution 156 (2008) 794–802

Year: 2008

Results and conclusion:

Glyphosate (K_d values determined in the range of 200 L/kg to 4000 L/kg) does not follow the simple rule that increased organic matter leads to increased sorption. The two most important component determining glyphosate sorption in the A-horizon is gravel and organic matter (the latter being negative). Glyphosate was often higher in the inorganic subsoil samples compared to the A-horizon samples. No calculated DT_{50} values were provided.

Detailed results on sorption are:

Soil characterisation:

Soil	Soil type	Sand (%) (fine + coarse)	Clay (%)	C.E.C. (in meq/100g)
Nedre Julianhede		69.7	3.8	
Nörlund		77.9	3.1	
Stubkaer		54.6	3.8	
Söbjerg		76.0	4.2	
Ruskaer		84.9	3.5	
Ilskov		83.3	4.4	
Skaaphusgaard		83.7	3.9	
Roejen Mosegard		80.1	3.4	
Röjen Kaer		82.0	3.3	
Röjen		79.8	3.3	
Sneptrup		86.3	3.0	
Simmelkjaer		88.9	3.4	
Neder Simmelkjaer		88.2	3.6	
Ommose		85.6	3.5	
Hallundbaek		86.8	3.8	

Adsorption

Soil type	OC %	pH ($CaCl_2$)	K_d	K_{oc}	K_f	K_{foc}	1/n
Nedre Julianhede	2.8	4.9	867				
Nörlund	2.1	5.3	237				
Stubkaer	4.8	5.8	1858				
Söbjerg	1.8	6.3	871				
Ruskaer	4.1	4.2	3758				
Ilskov	3.9	5.2	342				
Skaaphusgaard	2.6	5.1	n.a.				
Roejen Mosegard	6.4	5.6	108				
Röjen Kaer	2.3	4.9	690				
Röjen	2.7	4.7	656				
Sneptrup	2.2	4.5	400				

Simmelkjaer	1.8	4.1	586
Neder Simmelkjaer	2.6	5.2	366
Ommose	4.3	4.6	551
Hallundbaek	1.6	5.5	257

Proposed action:
Not to be considered further as the authors only provided K_d-values

Type of information (critical, high/low weight, supporting, additional):
Supporting information

Reliability	
Endpoint	Sorption of glyphosate in top soil and sub soil
Protocol	Non-GLP studies modified OECD 106 and OECD 307
Test compound	CAS 38641-94-0 ^{14}C-glyphosate (CAS 1071-83-6)
Test system and conditions	Sampling was performed at 15 locations placed on a 28 km long transect of the Karup outwash plain in northwest Jutland, Denmark OC content and particle size distribution were determined for the A, B and C horizons. A sample to solution ratio of 1:10 was used for glyphosate because this herbicide is highly adsorbed. The flasks were incubated on an orbital shaker at 10 °C for 96 h. Mineralization experiments were performed by adding ^{14}C-labelled pesticides in a total concentration of 1.0 mg pesticide kg^{-1} (dry weight) soil and incubating at 10 °C in the dark.
Statistical design	Three replicates
Relevance	
Environmental relevance	The experiments are principally relevant. Unfortunately, the authors did not calculate DT_{50} for glyphosate.
Weight of evidence	
"Positive"/"Negative" evidence	The sorption studies support the information known for glyphosate from standard tests.

Karpouzas and Singh (2008)

Title: Microbial Degradation of Organophosphorus Xenobiotics: Metabolic Pathways and Molecular Basis

Author: Dimitrios G. Karpouzas and Brajesh K. Singh

Reference: ADVANCES IN MICROBIAL PHYSIOLOGY VOL. 51, 119-225

Year: 2008

Results and conclusion:
In the present article, the microbial degradation and metabolic pathways for some OP compounds are reviewed. The chemical and molecular basis of OP degradation by microbes and the evolution and distribution of genes/enzymes are also reviewed. This article also examines applications and future use of OP-degrading microbes and enzymes for bioremediation, treatment of OP poisoning, and as biosensors.

Proposed action:
Not to be considered further

Type of information (critical, high/low weight, supporting, additional):
Supporting information

Reliability	
Endpoint	Degradation of glyphosate and other organophosphorus xenobiot-

	ics
Protocol	No study
Test compound	Organophosphorus xenobiotics
Test system and conditions	-
Statistical design	-
Relevance	
Environmental relevance	The review is not suitable for considering in the dossier.
Weight of evidence	
"Positive"/"Negative" evidence	The conclusions cannot be compared with standard testing.

Kim et al. (2011)

Title: EFFECT OF SOIL METAL CONTAMINATION ON GLYPHOSATE MINERALIZATION: ROLE OF ZINC IN THE MINERALIZATION RATES OF TWO COPPER-SPIKED MINERAL SOILS	
Author: BOJEONG KIM, YOUNG SIK KIM, BO MIN KIM, ANTHONY G. HAY, and MURRAY B. MCBRIDE	
Reference: Environmental Toxicology and Chemistry, Vol. 30, No. 3, pp. 596–601, 2011	
Year: 2011	
Results and conclusion: For all but the highest Cu treatments (400 mg kg^{-1}) in the coarse-textured Arkport soil, mineralization began without a lag phase and declined over time. No inhibition of mineralization was observed for Zn up to 400 mg kg^{-1} in either soil, suggesting differential sensitivity of glyphosate mineralization to the types of metal and soil. Interestingly, Zn appeared to alleviate high-Cu inhibition of mineralization in the Arkport soil. The protective role of Zn against Cu toxicity was also observed in the pure culture study with Pseudomonas aeruginosa, suggesting that increased mineralization rates in high Cu soil with Zn additions might have been due to alleviation of cellular toxicity by Zn rather than a mineralization specific mechanism. Extensive use of glyphosate combined with its reduced degradation in Cu-contaminated, coarse-textured soils may increase glyphosate persistence in soil and consequently facilitate Cu and glyphosate mobilization in the soil environment,	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Degradation of glyphosate in soil (mineralisation) when heavy metals are present
Protocol	Non-GLP study
Test compound	CAS 1071-83-6 labelled glyphosate (N-phosphono[^{14}C]methylglycine; specific activity 9.3 $mCimM^{-1}$; Sigma) and 0.1 mg unlabelled glyphosate to yield 10 mg kg^{-1} total glyphosate based on soil dry weight
Test system and conditions	An Arkport fine sandy loam and a Hudson silty clay loam, collected from the surface layer of uncontaminated agricultural research fields on the Cornell University campus (Ithaca, NY, USA) in May, 2003, were used for the present study. Calibrated amounts of

	$CuSO_4$ and $ZnSO_4$ solutions were sprayed on the soil samples to achieve target concentrations of 0, 100, 200, and 400 mg kg^{-1} of Cu and Zn both singly and in all combinations of these levels, based on soil dry weight. The data presented are the average values of individual measurements from triplicate samples. Glyphosate-treated soil samples were allowed to incubate for 80 d at 20°C in the dark with the desired moisture level maintained (FC).
Statistical design	Three replicates
Relevance	
Environmental relevance	The conditions do not reflect the conditions for standard degradation experiments. The authors describe the degradation only qualitatively (no DT_{50} values calculated for glyphosate).
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard tests.

Klimek et al. (2001)

Title: Metabolism of the phosphonate herbicide glyphosate by a non-nitrate-utilizing strain of Penicillium chrysogenum	
Author: Magdalena Klimek, Barbara Lejczak, Pawel Kafarski and Giuseppe Forlani	
Reference: Pest. Manag. Sci 57:815-821	
Year: 2001	
Results and conclusion: Microbial activities were found to lead to a rapid and complete mineralisation of glyphosate in soil.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation of glyphosate by bacteria
Protocol	Non-GLP study
Test compound	Technical glyphosate (CAS 1071-83-6, acc. to HPLC >99 % pure)
Test system and conditions	The bacteria were cultivated in a mineral Czapek-Dox.
Statistical design	No information
Relevance	
Environmental relevance	The relevance of the results is low due to the artificial environment.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard laboratory testing.

Liu et al. (2010)

Title: The Environmental Risk Assessment of Herbicide Glyphosate on Various Chinese Cultivated Soils
Author: Yihua Liu, Xiaoguang Wu, Mei Yang, Guonian Zhu

Reference: Pest. Manag. Sci 57:815-821	
Year: 2001	
Results and conclusion: Laboratory experiments were performed to evaluate the degradation, adsorption, and leaching behavior of glyphosate in three agricultural soils with high sand content and different soil organic carbon content. Glyphosate degraded very fast in soils, the half-lives of glyphosate for the three soils were between 3.3 d-6.9 d, and the main metabolite of glyphosate was amino methylphosphonic acid (AMPA). The adsorption coefficient (KF) values for the three soils were were 93.99 (loam), 89.31 (clay) and 61.05 (sand), which may mean that the organic matter is not the key for glyphosate adsorption on soil. Leaching tests, performed in manually packed soil glass-plate, indicated that glyphosate moved very slowly on the three types of soil thin layer, it mainly stayed at the zone of 0-2 cm. Thus, the leaching behavior of glyphosate coincided well with the results of the batch sorption and degradation experiments. All the data showed that glyphosate had a low potential threat to groundwater.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation, adsorption, and leaching behavior of glyphosate in soil
Protocol	Non-GLP study
Test compound	Glyphosate isopropyl ammonium (95%, technical grade), glyphosate standard (purity 99%), Zhejiang Xinan chemical company (Hangzhou, China)
Test system and conditions	*Degradation studies* All the working solutions were prepared by dissolving glyphosate isopropyl ammonium in distilled water. A quotient 20 g of each of the three types of soil was weighed and placed in a 250 mL flat-bottomed flask. The soil was commixed thoroughly with 1 mL working solution (200 mg/L). Distilled water was added to the point 60% of the maximal holding capacity (MHC). 30 repetitions were set for each treatment. The flasks were covered with cotton plugs and placed at 25±1℃ in dark. Samples were taken according to a pre-determined schedule. *Adsorption studies* The triplicate samples of 5 g sieved soils were added to 250 mL conical flasks containing 50 mL glyphosate solution at concentrations of 2, 10, 50 and 100 mg/L (0.01 M $CaCl_2$ solution), respectively. After the addition of soil samples, the reaction mixtures were shaken on a horizontal shaker at 200 rpm at 25±1℃ until the equilibrium was established. After the desired time (24 h), 20 mL of sample was collected from each flask, centrifuged at 4000 rpm for 5 min and the supernatant was collected for pesticide residue analysis. *Mobility studies* 10 g soil was spreaded on the surface of a glass plate of 2×75×200

	mm to prepare a thin layer of 0.50~0.75 mm. The plate was held at room temperature for 24 h to let dry. 500 µL of working solution (10 mg/L) was spotted in line at a site 1.5 cm away from one end of the plate. The plate was placed in a water-contained glass tank (holding 30º angle) until front boundary of the mobile phase (water) came to a location 13 cm away from the "starting line" (1 cm). After the water evaporated, the soil layer was cut into six sections and the glyphosate residue was then measured.
Statistical design	The pesticide degradation data were modeled using a simple first-order model: $Ct = C0e{-}kt$, where Ct is the glyphosate concentration at time t, $C0$ is the initial concentration and k is the rate constant. The adsorption data were fitted to the Freundlich model.
Relevance	
Environmental relevance	The results are of relevance and were performed close to standard testing.
Weight of evidence	
"Positive"/"Negative" evidence	The results are in line with other reliable studies. No negative evidence.

Moneke et al. (2010)

Title: Biodegradation of glyphosate herbicide in vitro using bacterial isolates from four rice fields	
Author: A. N. Moneke, G. N. Okpala and C. U. Anyanwu	
Reference: African Journal of Biotechnology Vol. 9 (26), pp. 4067-4074, 28 June, 2010	
Year: 2010	
Results and conclusion: Time course of growth of the isolates on mineral salt medium containing glyphosate showed that both grew significantly (P < 0.05). The comparative effects of glyphosate as carbon and/or phosphorus source on the growth of the isolates showed that there was significant (P < 0.05) growth in the medium containing glucose and glyphosate. The effects of different concentrations of glyphosate on the growth of the isolates (P. fluorescens and Acetobacter sp) were evaluated. Significant (P < 0.05) growth was observed at lower concentrations (7.2-25 mg/ml) of glyphosate. No inhibition of growth was observed at high concentrations (100 - 250 mg/ml), indicating that the isolated bacteria can tolerate up to 250 mg/ml of glyphosate.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Glyphosate degradation in water using H_2O_2 and UV radiation
Protocol	Non-GLP study
Test compound	Isopropylamine salt of glyphosate, product Roundup® (containing 360 g active ingredient/L of glyphosate, Monsanto) CAS 38641-94-0
Test system and conditions	Soil samples were obtained from four rice fields located in Nigeria. These rice fields are known to have been previously exposed to glyphosate- for long periods of time. Soil samples were collected from depths of 0 - 15 cm from three different sites in each of the

	four locations. These rice fields are known to have been previously exposed to glyphosate-based formulation (Roundup®) for long periods of time. Soil samples were collected from depths of 0 - 15 cm from three different sites in each of the four locations. Inoculate used for the study were prepared by inoculating isolates into nutrient broth and incubated at 30°C for 24 h using sterile normal saline; the cells from the above cultures were re-suspended to a 0.5 McFarland nephelometer standard (Optical density of 0.17 at 660 nm).
Statistical design	Three replicates
Relevance	
Environmental relevance	The results are of minar relevantce since the experiments do not describe any of the standard endpoints.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared directly with standard tests.

Pipke et al. (1988)

Title: Degradation of the phosphonate herbicide Glyphosate by Arthrobacter atrocyaneus ATCC 13752	
Author: Pipke, R., Amrhein, N.	
Reference: Applied and Environmental Microbiology (1988) Vol 54 (5): 1293-1296	
Year: 1988	
Results and conclusion: Arthrobacter atrocyaneus metabolized glyphosate to aminomethylphosphonic acid. The carbon of aminomethylphosphonic acid was entirely converted to CO_2. This is the first report on glyphosate degradation by a bacterial strain without previous selection for glyphosate utilization as a source of phosphorus.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Degradation of glyphosate and AMPA by soil bacteria
Protocol	Non-GLP study
Test compound	^{14}C Glyphosate
Test system and conditions	The ability of Arthrobacter atrocyaneus ATCC 13752 to degrade glyphosate was evaluated in vitro. Samples were taken at the beginning of the experiments and after 80 h.
Statistical design	No information
Relevance	
Environmental relevance	The results cannot be used because the system is not comparable to standard tests.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared directly with standard tests.

Pizzul et al. (2009)

Title: Degradation of glyphosate and other pesticides by ligninolytic enzymes	
Author: Leticia Pizzul Æ Marı´a del Pilar Castillo Æ John Stenström	
Reference: Biodegradation (2009) 20:751–759	
Year: 2009	
Results and conclusion: In the presence of laccase and ABTS (Lac 2), 40.9% of the glyphosate disappeared after 24 h, whereas 62.8% of the glyphosate was degraded when Mn^{2+} and Tween 80 were added together with the enzyme (Lac 3). A synergistic effect of ABTS, Mn^{2+} and Tween 80 (Lac 4) was observed, where 90.1% of glyphosate disappeared after 24 h. The metabolite AMPA was detected in all the cases where degradation of glyphosate occurred. No other metabolites were analysed in the present work, but the equal stoichiometry between AMPA formed and glyphosate degraded suggests that AMPA was not degraded and that there was no or negligible formation of other compounds. No degradation of glyphosate was observed with laccase alone.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Glyphosate degradation by ligninolytic enzymes
Protocol	Non-GLP study
Test compound	Non-labelled Glyphosate [N-(phosphomethyl)glycine] (CAS 1071-83-6), non labelled AMPA (CAS 1066-51-9) both supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany) labelled glyphosate (2-^{13}C, 99%; 15N, 98%) and AMPA (^{13}C, 99%; 15N, 98%; methylene-D2, 98%) were supplied by LGC Standards (Borås, Sweden).
Test system and conditions	Horseradish peroxidase (EC 1.11.1.7), lignin peroxidase (EC 1.11.1.14) and laccase from Trametes versicolor (1.10.3.2) were purchased from Sigma–Aldrich (Steinheim, Germany). Manganese peroxidase (EC 1.11.1.13) from Nematoloma frowardii was obtained from JenaBios (Jena, Germany). The potential of MnP to degrade glyphosate was evaluated in vitro. All reactions were conducted in sterile, loosely capped, 8-ml glass vials. The vials were placed on a rotary shaker (150 rpm) at 35°C and samples were taken at the beginning of the experiments and after 1, 4 and 7 days.
Statistical design	No information
Relevance	
Environmental relevance	The results cannot be used because the system the endpoint is not comparable to standard tests.
Weight of evidence	
“Positive”/”Negative” evidence	The results cannot be compared directly with standard tests.

Reimer et al. (2005)

Title: Effect of Manure on Glyphosate and Trifluralin Mineralization in Soil

Author: M. Reimer, A. Farenhorst, and J. Gaultier	
Reference: Journal of Environmental Science and Health Part B, 40:605–617, 2005	
Year: 2005	
Results and conclusion: Experiments were conducted with glyphosate in soil microcosms in the laboratory for a total of 332 days. The rate and amount of mineralization of glyphosate were significantly influenced by the additions of fresh manure to soil in the laboratory and by the history of manure applications in the field. However, the maximum difference in herbicide mineralization between soils that were free of manure application and those amended with manure in the field or in the laboratory was only 7.3% of that initially applied. Therefore, it is conclude that liquid hog manure application to soil will have no significant effect on the mineralization of glyphosate and trifluralin under field conditions. Half lives were found for the degradation of glyphosate in the range of 18 to 34 d.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Glyphosate degradation by ligninolytic enzymes
Protocol	Non-GLP study
Test compound	Labelled compound: [*phosphonomethyl*-^{14}C] glyphosate (specific activity 89 MBq mmol^{-1}; Sigma-Aldrich Co., St. Louis, MO) Non-labelled compound: analytical-grade glyphosate (99% purity; Chem Service,West Chester, PA) (CAS 1071-83-6)
Test system and conditions	Surface soils (0–10 cm) of well-drained Orthic Black Chernozems (Canadian soil classification system) were collected from three study sites in western Manitoba, identified here by their vicinity to the nearest town: Birtle (101°05_W, 50°42_N), Decker (100°78_W, 50°27_N), and Neepawa (99°47_W, 50°23_N). For each site, representative soil samples were collected from lower slope positions in two adjacent fields: one field with a long-term history of hog manure applications and one field that had never received manure. Soil organic carbon and total nitrogen contents were determined. Fresh liquid hog manure was obtained from a farm near Fannystelle, MB. Microcosms were incubated at 20◦C and soil was brought to 70% field capacity. Herbicide mineralization rate constants were calculated assuming first-order kinetics.
Statistical design	Three replicates
Relevance	
Environmental relevance	The results are of relevance, but due to the manure applications they cannot be directly compared with standard testing.
Weight of evidence	
“Positive”/”Negative” evidence	The results cannot be compared directly with standard tests The results are principally supported by other reliable studies.

Roffignac et al. (2008)

Title Efficiency of a bagasse substrate in a biological bed system for the degradation of glyphosate, malathion and lambda-cyhalothrin under tropical climate conditions

Author: Laure de Roffignac, Philippe Cattan, Julie Mailloux, David Herzog and Fabrice Le Bellec	
Reference: Pest Manag Sci 64:1303–1313 (2008)	
Year: 2008	
Results and conclusion: Results showed that more than 99% of glyphosate were degraded in 6months. In the biological bed, the DT_{50} value for glyphosate 33 days. The degradation rate of aminomethylphosphonic acid (AMPA) residues from the degradation of glyphosate was slower than that of the other pesticides (DT_{50} 69 days).	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation of glyphosate in a biobed under tropical conditions
Protocol	Non-GLP study
Test compound	Glyphosate (product Glyfos, 360 gL^{-1}) (CAS 1071-83-6)
Test system and conditions	The experiment took place at the CIRAD (Centre de Cooperation Internationale en Recherche Agronomique pour le D´eveloppement) research station at Vieux-Habitants, 97 119 Guadeloupe, France. The biological bed was a hole dug in the ground with concrete walls coated with impermeable paint and topped with a metal roof. The internal dimensions were 2m long, 1m wide and 0.80m deep. The biological bed was filled with biomix to a height of 65 cm, which is equivalent to a volume of 1300 L. The biomix used was a mixture of soil and bagasse in a proportion of 1 volume of soil to 3 volumes of bagasse. Glyphosate was sprayed in the biological bed at 295×10^3 µg kg^{-1}.
Statistical design	No information
Relevance	
Environmental relevance	The relevance is small due to the tropical conditions.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard testing.

Rueppel et al. (1977)

Title: Metabolism and Degradation of Glyphosate in Soil and Water
Author: Rueppel, M.L., Brightwell, B.B., Schaefer, J., Marvel, J.T.
Reference: J. Agric. Food Chem., Vol. 25 (3): 517-528
Year: 1977
Results and conclusion: Using soil/water shake flasks, up to 50% of each carbon of l-^{14}C was evolved as $^{14}C0_2$ in 28 days. In two of the three soils examined, glyphosate was 90% dissipated in less than 12 weeks. Aminomethylphosphonic acid, the only significant soil metabolite of glyphosate, also undergoes rapid degradation in soil. Short-term shake flask metabolism experiments with both ^{13}C and ^{14}C-labeled glyphosate were carried out in order to permit facile, unequivocal spectral identification of glyphosate and its transient metabolite aminomethylphosphonic acid. Comparison of the metabolic samples to both reference stand-

ards and the spiked controls by means of ^{1}H, ^{31}P, and ^{13}C NMR, mass spectral analysis, ion-exchange chromatography, and thin-layer chromatography has unequivocally characterized both bound and unbound glyphosate and aminomethylphosphonic acid in soil. The parent herbicide glyphosate has also been shown to be stable to sunlight,nonleachable in soil, to have a low propensity for runoff, and to have a minimal effect on microflora.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Degradation of glyphosate and AMPA in a soil/water shake flasks system
Protocol	Non-GLP study
Test compound	^{13}C and ^{14}C Glyphosate
Test system and conditions	Each flask contained 4.5 g (dry weight) of the appropriate soil, 1 mg of the desired radioactive compound, and distilled water (100 mL). Incubation time was 28 d. After the desired period (normally 7 days) of metabolism on an incubator shaker at 30°C, flasks were flushed with air for 1.25 h to collect the evolved $C0_2$. The flask contents were then transferred to 250-mL centrifuge bottles, centrifuged at 8000 rpm for 15 min, and the supernatant transferred to a 100-mL volumetric flask for analysis by liquid scintillation counting and TLC/beta camera. The soil was washed once with 25 mL of H20, centrifuged, and the supernatant removed for analysis for radioactivity. The ^{14}C content of the soil was analyzed by combustion.
Statistical design	No information
Relevance	
Environmental relevance	The results cannot be used because the system is not comparable to standard tests.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared directly with standard tests.

Santos et al. (2009)

Title: Biodegradation of glyphosate in rhizospheric soil cultivated with Glycine max, Canavalia ensiformis E Stizolobium aterrimum
Author: SANTOS J.B., FERREIRA, E.A., FIALHO, C.M.T., SANTOS, E.A., GALON, L.; CONCENÇO, G.; ASIAZÚ, I. and SILVA, A.A.
Reference: Planta Daninha, Viçosa-MG, v. 27, n. 4, p. 781-787, 2009
Year: 2009
Results and conclusion: Biodegradation of glyphosate was evaluated in rhizospheric soil cultivated with Glycine max (soybean, var. BRS245-RR), Canavalia ensiformis and Stizolobium aterrimum. After these species were cultivated for 60 days, soil samples were collected, placed in flasks and treated with 14C-glyphosate. After 30 days of incubation, the total release rate of C-CO2 was determined along with microbial biomass (MBC), metabolic quotient (qCO2), and degradation percentage of the radio-labeled glyphosate released as 14C-CO2. A higher mass of rhizosphere-associated microorganisms was verified in the soil samples from pots

cultivated with soybean, regardless of glyphosate addition. However, in the presence of the herbicide, this characteristic was the most negatively affected. Microorganisms from the C. ensiformis rhizosphere released a lower amount of 14C-CO2, while for those originated from S. aterrimum, the amount released reached 1.3% more than the total carbon derived from the respiratory activity. The rhizospheric soil from S. aterrimum also presented higher glyphosate degradation efficiency per microbial biomass unit. However, considering qCO2, the microbiota of the rhizospheric soil cultivated with soybean was more efficient in herbicide degradation.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Dependence of glyphosate degradation in soil from the presence of Glycine max, Canavalia ensiformis and Stizolobium aterrimum cultivations
Protocol	Non-GLP study
Test compound	Non-labelled compound: glyphosate (CAS 1071-83-6),
Test system and conditions	In the laboratory, the soil samples were sieved, shade-dried for 24 h, weighed (150 g) and then placed in 300 mL glass flasks. Sample moisture was adjusted to 70% of field capacity and a glyphosate solution at the concentration of 1.76 mg kg-1 equivalent to 3.36 kg ha-1 of the technical product was added. Eight treatments (soil samples from soils cultivated with Glycine max, Canavalia ensiformis, Stizolobium aterrimum and non-cultivated soil) were evaluated, with these samples being treated and non-treated with glyphosate. The experiment was arranged in a completely randomized design in a factorial scheme (4 x 2) with four repetitions. The flasks were incubated for 32 days for evaluation of the C-CO2 release rate, quantified every eight days. To determine soil basal respiration rate after incubation of the samples, the CO2 evolved was captured in flasks containing 100 mL of NaOH (0.25 mol L-1) under a continuous air flow system (free of CO2 and moisture). After each incubation period, indirect titration of NaOH with HCl (0.25 mol L-1) was carried out and the excess NaOH that did not react to evolved CO2 was quantified (Anderson, 1982). At the end of the incubation period, the microbial biomass carbon was determined.
Statistical design	Four replicates
Relevance	
Environmental relevance	The results are of relevance, but due cannot be directly compared with standard testing.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared directly with standard tests.

Schnurer et al. (2006)

Title: Effects of Surface Sorption on Microbial Degradation of Glyphosate
Author: YLVA SCHNURER, PER PERSSON, MATS NILSSON, ANDERS

NORDGREN, AND REINER GIESLER	
Reference: Environ. Sci. Technol. 2006, 40, 4145-4150	
Year: 2006	
Results and conclusion: Additions of glyphosate, in combination with glucose and N, did not change the respiration rate in comparison with the same treatment but without glyphosate. In contrast, glyphosate additions combined with glucose and P decreased microbial growth, whereas the combination with goethite counteracted the negative effect. The results suggest that glyphosate was de-carboxylated in the sorbed state. Stimulating microbial growth by the addition of glucose and nitrogen resulted in further oxidation of glyphosate and only phosphate was detectable on the goethite surface after 13 days incubation. The results show that sorbed glyphosate is microbial degradable, and it retards microbial activity.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Effects of sorption on microbial degradation of Glyphosate
Protocol	Non-GLP study
Test compound	Non-labelled compound: glyphosate (CAS 1071-83-6),
Test system and conditions	The soil was collected in the Nyänget catchment (64°15N, 19°45E), Svartberget Experimental Forest, Vindeln, northern Sweden. The mean annual precipitation (1980-1995) was 590 mm, of which 40% was snow. The mean annual temperature was 1.4°C, with a January mean of -10.6°C and a July mean of 14.3°C. The soil moisture content of the samples was adjusted to about 270% of the organic matter (w/w) to optimize conditions for microbial growth. Respiration kinetics were recorded hourly at 20°C, using a 96-unit respirometer (18, 21) with 250-mL plastic jars. Different treatments were examined using attenuated total reflectance Fourier transform (ATR-FTIR) spectroscopy.
Statistical design	Five replicates
Relevance	
Environmental relevance	The results are of relevance, but due cannot be directly compared with standard testing.
Weight of evidence	
“Positive”/”Negative” evidence	The results cannot be compared directly with standard tests.

Schroll et al. (2006)

Title: Quantifying the Effect of Soil Moisture on the Aerobic Microbial Mineralization of Selected Pesticides in Different Soils
Author: REINER SCHROLL, HANSHEINRICH BECHER, ULRIKE DÖRFLER, SEBASTIA GAYLER, SABINE GRUNDMANN, HANSPETER HARTMANN, AND JÜRGEN RUOSS
Reference: Environ. Sci. Technol. 2006, 40, 3305-3312
Year: 2006
Results and conclusion:

At soil water potential <20 MPa minimal pesticide mineralization occurred; a linear correlation (P < 0.0001) exists between increasing soil moisture (within a soil water potential range of -20 and -0.015 MPa), and increased relative pesticide mineralization; optimum pesticide mineralization was obtained at a soil water potential of-0.015 MPa, and when soil moisture approximated water holding capacity, pesticide mineralization was considerably reduced	
Proposed action: Consider as supporting information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Effects of soil moisture on microbial degradation of Glyphosate
Protocol	Non-GLP study
Test compound	Labelled compound: phosphonomethylene-^{14}C >98% purity (CAS 1071-83-6)
Test system and conditions	The agricultural soils used were characterized by large variations in soil texture (sand content 4-88%) and organic matter content (0.97-2.70% org. C. Soil-water retention curves were determined at the Institute for Soil Science of Technical University Munich. Biodegradation of ^{14}C-labeled chemicals was studied in a discontinuously aerated laboratory system. Soils were incubated in 100 mL double-wall flasks in the dark at 20 °C (1°C.). Twelve different soil moistures ranging from 0.01 and 0.25 g g^{-1} (equivalent to 5 up to 100% WHC) were selected to study the effect of increased soil water content on glyphosate mineralization.
Statistical design	Four replicates
Relevance	
Environmental relevance	The results are of relevance, but due cannot be directly used as the rate constants were not provided.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared directly with standard tests.

Shushkova et al. (2012)

Title: Biodegradation of Glyphosate by Soil Bacteria: Optimization of Cultivation and the Method for Active Biomass Storage
Author: T. V. Shushkova, I. T. Ermakova1, A. V. Sviridov, and A. A. Leontievsky
Reference: Microbiology, 2012, Vol. 81, No. 1, pp. 44–50
Year: 2012
Results and conclusion: Conditions for obtaining the active biomass of *Ochrobactrum anthropi* GPK 3 and *Achromobacter* sp. Kg 16, bacteria which are able to degrade the herbicide glyphosate (N-phosphonomethylglycine), were investigated. In the batch culture, degradation was most effective in the medium with pH 6.0–7.0 and aeration at 10–60% of air saturation supplemented with glutamate and ammonium chloride as sources of carbon and nitrogen, respectively. Due to the adaptation of the cells and induction of the relevant enzymatic systems, the inoculum grown in the presence of glyphosate exhibited 1.5–2-fold higher efficiency of xenobiotic degradation than that grown with other sources of phosphorus (orthophosphate and methylphosphonic acid). The efficiency of the toxicant decomposition increased with

an increase in a specific load of glyphosate, which the cells were subjected to during the initial stage of growth. The specific load was regulated both by the initial cell concentration and the concentration of the phosphorus source, and the effect was probably determined by its availability to microorganisms. Storage of the liquid biopreparation as a paste with stabilizers (ascorbate, thiourea, and glutamate) at room temperature for 50 days resulted in high level of bacteria viability and a degrading activity approximately equal to that obtained when the bacteria were maintained on the agar medium containing glyphosate at 4°C with monthly transfers to the fresh culture medium.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Low weight	
Reliability	
Endpoint	Influence of different cultivation conditions on the degradation of glyphosate by soil bacteria
Protocol	Non-GLP
Test compound	Glyphosate (500 mg/L) as the herbicide GroundBio (36% aqueous solution of the isopropylamine salt, Technoexport, Russia)
Test system and conditions	The goal of the present work was to select optimal conditions for the cultivation of Ochrobactrum anthropi GPK 3 and Achromobacter sp. Kg 16, providing maximal effectiveness of the herbicide degradttion, as well as to work out the storage conditions for the biomass intended for introduction into the soil.
Statistical design	No information
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard studies.

Sillanpää et al. (2011)

Title: Degradation of chelating agents in aqueous solution using advanced oxidation process (AOP)
Author: Mika E.T. Sillanpää, Tonni Agustiono Kurniawan, Wai-hung Lo
Reference: Sillanpää, M.E.T., et al. Degradation of chelating agents in aqueous solution using advanced oxidation process (AOP). Chemosphere 83(11): 1443-1460. doi:10.1016/j.chemosphere.2011.01.007 (2011)
Year: 2011
Results and conclusion: The performance of individual AOP is compared. The selection of the most suitable AOP seems to depend on the characteristics of effluents, technical applicability, discharge standard, regulatory requirements and environmental impacts.
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.
Type of information (critical, high/low weight, supporting, additional): Low weight
Reliability

Endpoint	Degradation of chelating agents in aqueous solution using advanced oxidation process
Protocol	Review
Test compound	No chelating agents
Test system and conditions	Review
Statistical design	No information
Relevance	
Environmental relevance	The results have no relevance for pesticide registration.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard studies.

Simonsen et al. (2008)

Title: Fate and availability of glyphosate and AMPA in agricultural soil

Author: LOUISE SIMONSEN, INGE S. FOMSGAARD, B. VENSMARK and NIELS HENRIK SPLIID

Reference: Journal of Environmental Science and Health Part B (2008) 43, 365–375

Year: 2008

Results and conclusion:

The disappearance of glyphosate and the formation and disappearance of AMPA were monitored. The resulting curves were fitted based on FOCUS degradation kinetics. The best fit of the glyphosate degradation data was obtained using a first-order multi compartment (FOMC) model. DT_{50} values of 9 days (glyphosate) and 32 days (AMPA) indicated relatively rapid degradation.

Detailed results are:

Soil characteristics

Parameter	**Value**
% Clay (<2 μm)*a*	13.3
% Silt (2–20μm)*a*	16.2
% Coarse silt (20–63 μm)*a*	24.7
% Fine sand (63–200 μm)*a*	26.9
% Coarse sand (200–2000 μm)*a*	16.2
% Humus *a*	2.7
Density (g/cm3)	2.626
% $CaCO_3$	n.d.
pH-H_2O	6.5
P (Al)*b*	7
K (Al)*b*	20
Mg (Al)*b*	10
Ca (Al)*b*	140
P (HCl)*b*	39.9

Quantities of glyphosate and AMPA in blank soil samples found by extraction with borate solution (50 % MWHC, 14.3 °C

Time (days)	***Glyphosate (ng/g dry soil)***	***AMPA (ng/g dry soil)***
0	0.81	10.46
7	0.61	7.33
14	0.50	5.86
21	0.41	5.59
35	0.46	5.03

49	0.41	4.04
77	0.32	3.87
105	0.31	4.45
179	0.19	1.16

Model parameters ± confidence interval and DT_x values for glyphosate fitted with first order multi component (FOMC) and the decline of AMPA fitted with single first order (SFO).

Compound	***Parameter***	***Value***
Glyphosate	M_0	7.8 ± 0.58 nmol/g dry soil
	A	0.85 ± 0.28
	β	7.1 ± 4.7
	DT_{50}	9 days
	DT_{90}	101 days
	M_0	4.2 ± 0.28 nmol/g dry soil
AMPA	k	0.022 ± 0.0038 $days^{-1}$
	DT_{50}	32 days
	DT_{90}	106 days

Proposed action:
Consider as supporting information because it was not totally performed in line with FOCUS degradation kinetics (no DFOP or HS kinetics performed though residues > 10 % at the end of the study).

Type of information (critical, high/low weight, supporting, additional):
Supporting information

Reliability	
Endpoint	Glyphosate degradation by ligninolytic enzymes
Protocol	Non-GLP study
Test compound	Labelled compound ^{14}C-glyphosate solution (110 µL) and 13C-15N-glyphosate solution (180 µL) (CAS 1071-83-6)
Test system and conditions	The soil was sampled from the 0–2.5 cm layer of a field, where reduced tillage had been practiced, in Sandved (eastern Denmark). ^{14}C-glyphosate solution (110 µL) and ^{13}C-^{15}N-glyphosate solution (180 µL) were applied to each of 50 samples of 5 g dry soil.
Statistical design	Six replicates
Relevance	
Environmental relevance	The results are of relevance and were performed close to standard testing.
Weight of evidence	
"Positive"/"Negative" evidence	The results are principally supported by other reliable studies.

Stenrød et al. (2006)

Title: Spatial variability of glyphosate mineralization and soil microbial characteristics in two Norwegian sandy loam soils as affected by surface topographical features

Author: Marianne Stenrød, Marie-Paule Charnay, Pierre Benoit, Ole Martin Eklo

Reference: Soil Biology & Biochemistry 38 (2006) 962–971

Year: 2006

Results and conclusion:
Considerable spatial heterogeneity in the degradation rate of glyphosate and general carbon utilization exists even across small areas within a single agricultural field. This horizontal variability was observed over several spatial scales, and could not be clearly explained. It evidently arose from differences in environmental factors affecting microbial activity and

growth, and topographical features controlling redistribution of water and matter flow patterns were correlated to the investigated soil microbial variables.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Spatial variability of glyphosate mineralization
Protocol	Non-GLP study
Test compound	Non-labelled aqueous solutions of glyphosate were prepared from Roundup Ecow (30.05% glyphosate active ingredient, density *r*Z1198 g lK1) (Monsanto Crop Sciences, Norway). Labelled: [P-methylen ^{14}Cglyphosate](radiochemical activity 5.155 MBq mgK1, radiochemical purity >99%) (Institute of Isotopes, Budapest, Hungary) to give 84.85 kBq mL^{-1} (CAS 1071-83-6)
Test system and conditions	Soil was sampled from agricultural fields in Grue (South-East Norway) at N608280 E128020, and Malselv (North Norway) at N698150 E188330. Both locations had alluvial sandy loam soils, with potatoes as a main crop in rotation with barley. Two sites were used with two different sampling strategies to catch the spatial variability at two different scales. The site at Grue was used for assessing the variability at a metre scale and to observe horizontal variations. The site at Malselv was used for a decimetre scale study to evaluate horizontal and vertical variations in a soil profile. The statistical processing of the results included analysis of variance (ANOVA), principal component analysis (PCA), linear regression analysis and Tukey's test for comparison of means. A significance level of 5% was used for hypothesis testing.
Statistical design	Complex statistical analysis
Relevance	
Environmental relevance	There is relevance is as they point out the variability of degradation even on a small scale.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard testing.

Stenrød et al. (2005)

Title: Effect of freezing and thawing on microbial activity and glyphosate degradation in two Norwegian soils
Author: Marianne Stenrød, Ole Martin Eklo, Marie-Paule Charnay and Pierre Benoit
Reference: Pest Manag Sci 61:887–898 (2005)
Year: 2005
Results and conclusion: The glyphosate mineralization pattern was comparable with the overall microbial activity in the soils. Observed different levels of diversity might explain some of the difference in total glyphosate mineralization between soils. Organic C mineralization was found to be a good predictor of glyphosate mineralization for each soil individually, supporting other investigations. The two freeze–thaw treatments gave a similar total amount of glyphosate mineralized during the 84-day period but less than in the +5°C treatment.

Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Glyphosate mineralization under cold conditions
Protocol	Non-GLP study
Test compound	Non-labelled aqueous solutions of glyphosate were prepared from Roundup Ecow (30.05% glyphosate active ingredient, density *r*Z1198 g lK1) (Monsanto Crop Sciences, Norway). [P-methylene-^{14}C]glyphosate (radiochemical activity 5.155MBqmg^{-1}, radiochemical purity >99%; Institute of Isotopes, Budapest, Hungary) to give 8.00 kBqmg^{-1} (CAS 1071-83-6)
Test system and conditions	Soil was sampled from agricultural fields in Grue (South-East Norway) at N608280 E128020, and Malselv (North Norway) at N698150 E188330. Both locations had alluvial sandy loam soils, with potatoes as a main crop in rotation with barley. Bulk soil samples were taken from the top 10cm of the Ap-horizon in mid-October 2002. Bulk soil samples of both soils were adjusted to 70% of WHC. The soil samples were subjected to one of four winter temperature simulation regimes; constant thaw (control at +5°C), constant freezing (control at -5°C), short-term temperature fluctuations (24 h at -5°C followed by 24 h at +5°C), and long-term temperature fluctuations (3 weeks at -5°C followed by 3weeks at +5°C) for a total of 12 weeks. Glyphosate mineralization during the incubation was monitored by measuring the $^{14}CO_2$ in the NaOH traps by LSC (Packard Tri-Carb 2900TR), using Hionic- Fluor as liquid scintillation cocktail (Packard).
Statistical design	Three replicates
Relevance	
Environmental relevance	There is relevance is as they point out the dependency of degradation in cold winter periods.
Weight of evidence	
"Positive"/"Negative" evidence	The results are in line with other reliable studies.

Sviridov et al. (2011)

Title: New Approaches to Identification and Activity Estimation of Glyphosate Degradation Enzymes
Author: A. V. Sviridov, N. F. Zelenkova, N. G. Vinokurova, I. T. Ermakova, and A. A. Leontievsky
Reference: Biochemistry (Moscow), 2011, Vol. 76, No. 6, pp. 720_725.
Year: 2011
Results and conclusion: Glyphosate degradation can follow different pathways depending on physiological characteristics of metabolizing strains: in Ochrobactrum anthropi GPK3 the initial cleavage reaction is catalyzed by glyphosateoxidoreductase with the formation of ami-

nomethylphosphonic acid and glyoxylate, whereas Achromobacter sp. MPS12 utilize C-P lyase, forming sarcosine. The proposed methodology has several advantages as compared to others described in the literature.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Analysis of processes involved in microbial glyphosate degradation
Protocol	Non-GLP study
Test compound	Non-labelled glyphosate (CAS 1071-83-6),
Test system and conditions	Two strains of bacteria that degrade phosphonates: Ochrobactrum anthropi GPK3, isolated from soils contaminated with GP, and Achromobacter sp. MPS12A, isolated from sites of contamination with methylphosphonic acid (MPA) and adapted to growth in GP-containing medium. The organisms were under periodic cultivation in liquid mineral medium MS1 without phosphates. Sodium glutamate at concentration of 55 mM was used as a source of carbon. 3 mM GP as a Roundup component was used as a sole phosphorus source.
Statistical design	No information
Relevance	
Environmental relevance	The relevance is less relevant since the authors did not calculate any DT_{50} values.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with reliable studies.

Yang et al. (2013b)

Title: Mild salinization stimulated glyphosate degradation and microbial activities in a riparian soil from Chongming Island, China
Author: C. Yang, S. Shen, M. Wang and J. Li
Reference: Journal of Environmental Biology, Vol. 34, 367-373, 2013
Year: 2013
Results and conclusion: An incubation experiment was conducted to investigate the effects of simulated saltwater treatment with different percentages of artificial sea water on degradation dynamics of herbicide glyphosate and microbial activities in a riparian soil in Chongming Island, China. The results showed that 10% sea water treatment showed significantly enhancing effects on degradation efficiency of glyphosate with the lowest residual concentration among all the treatments. However, glyphosate degradation was markedly decreased in the riparian soil with 20% and 50% sea water treatments. The half-lives for 20% and 50% seawater treatments were prolonged by 12.1 and 39.0%, respectively, as compared to control. Microbial investigation indicated that 10% seawater treatment significantly stimulated microbial activities in the glyphosate-spiked riparian soil throughout the incubation period. At 42 day of incubation experiment, flourescein diacetate (FDA) hydrolysis rate, microbial adenosine triphosphate (ATP), and basal soil respiration (BSR) in the glyphosate-spiked riparian soil with 10% seawater were 59.2, 42.5 and 31.8%

higher than those with no saltwater treatment, respectively. In contrast, saltwater treatment with 50% sea water significantly inhibited microbial activities. Especially, FDA hydrolysis rate, microbial ATP and BSR were decreased by 66.4, 58.6 and 66.8%, respectively, as compared to control. The results indicate that levels of simulated saltwater can exert variable effects on herbicide degradation dynamics and microbial parameters in the riparian soil. In the following the half-life's of glyphosate biodegradation in riparian soils affected by saltwater treatments are given:

Salt water treatments [%]	Half life [d]	R^2
0	14.1	0.9877
10	10.5	0.9386
20	15.8	0.9976
50	19.6	0.9898

Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.

Type of information (critical, high/low weight, supporting, additional):
Additional information

Reliability	
Endpoint	Glyphosate biodegradation in riparian soils affected by saltwater treatments and effect of saltwater microbial parameters in a riparian soil
Protocol	Not given
Test compound	Glyphosate
Test system and conditions	Site description : The study was conducted in Chongming Island of Yangtze River Estuary, located in the East of China, where frequent seawater incursion results in an increase in salinity of inland freshwater, especially in the Northeast next to the East China Sea. Collection and pre-treatment of the riparian soil for incubation experiment: Non-contaminated soil was obtained from the 0-30 cm depth' of a riparian wetland (121.26° E, 31.55° N) in the Southwest of Chongming Island, where soil salinity is relatively slight due to receiving less seawater incursion. Present research aims at the overall effects of saltwater treatment on biodegradation dynamics of glyphosate and the related microbial parameters. Accordingly, the riparian soil for incubation experiment was spiked with glyphosate only at the concentration of about 5 mg kg^{-1}, which is slightly higher than the maximum of field concentration in the riparian soils. The collected riparian soil was spiked with glyphosate following the modified procedure Brinch et al., 2002. The prepared contaminated soil with glyphosate was stored at 4 °C in an airtight container for laboratory incubation experiment. Soil incubation and saltwater treatment: 200g dry weight equivalent glyphosate-contaminated riparian soil was weighed into a 500-ml flask and statically incubated at 30 °C in an incubator without illumination. Artificial seawater was prepared. Saltwater additions with 0, 10, 20 and 50% seawater were made once per day for 10 days, and simultaneously the flasks were kept submerged with 2 cm depth above the soil surface. After that, the soil moisture was

	adjusted to 85-90% of the maximum water holding capacity (WHC, which was 654 g kg^{-1} for the investigated soil) with addition of deionized water by weight method until the end of the incubation experiment. The incubation experiments were set up with three replicates per treatment. The incubation experiment ran for 50 d. Soil sample collection and analysis: Soil sampling was carried out at intervals of I, 7, 14, 21, 28, 35, 42, 50, 60 d of incubation period. The collected field-moist soil samples were homogenized and passed through a 2 mm sieve. A portion of the soil samples were stored in 4 'C for analysis for the microbial parameters, and the other portion of soil samples were freeze-dried for determination of the concentrations of the residual glyphosate as well as other chemical parameters. Residual glyphosate in riparian soil was determined following the method of Hu et al. (2008) with some modifications. Briefly, 10 g of frozen-dried soil sample were weighed into a stoppered centrifuge tube and extracted with 2 M NH_4OH under microwave assistance, derivatized by trifluoroacetic anhydride (TF AA) and trifluoroethanol (TFE), and then determined by gas chromatography with a nitrogen-phosphorus detector (GC-NPD). The glyphosate degradation kinetics in the riparian soil with simulated saltwater treatments was described by a first-order kinetic model. Soil microbial biomass (MBC) was determined by fumigation with ethanol free CHCI, and extraction with 0.5m K_4SO_4. Basal soil respiration (BSR) was determined by measuring CO_2 evolution in the aerobic condition.
Statistical design	All analyses were performed in triplicate. Data were analyzed statistically by analysis of variance (ANOVA). Duncan's New Multiple Range Test (DMRT) was employed to assess differences between the treatment means. The effects of simulated saltwater incursion with different seawater addition levels on glyphosate degradation and related microbial parameters were declared as significant at 5% probability levels. Standard errors were calculated for mean values of all determinations. All statistical analyses were performed with SPSSI 2.0software.
Relevance	
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	No negative evidence.

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Field Studies

Summary

The dissipation of glyphosate under field conditions has been investigated in several studies. However none of these studies fulfilled the quality criteria of FOCUS degradation kinetics or

even the EFSA guidance on soil persistence. Therefore, the results of open literature were not used to derive endpoints or to calculate PEC's.

In detail, the studies performed under outdoor conditions confirm that glyphosate is degraded through the action of soil microorganisms. Main metabolic pathway involves the formation of CO_2 and AMPA, which is further degraded to CO_2. The DT_{50} under typical field conditions ranged from 3-30 days. However, Laitinen et al. (2006) studied the fate of glyphosate in two Finnish sugar beet cultivated fields for 26 months. The results from this field study demonstrate overwinter persistence of glyphosate residues from an autumn treatment.

Detailed description of open literature on rate of degradation – Field studies

Adams et al. (2007)

Title: The Absence of Glyphosate Residues In Wet Soil and the Adiacent Watercourse after a Forestry Application in New Brunswick	
Author: Gregory W. Adams, Troy Smith, and J. David Miller	
Reference: NORTH. J. APL. FOR. 24(3)	
Year: 2007	
Results and conclusion: In August 2005, 3 years after herbicide application, the site was fully occupied by a mix of vegetation similar to the vegetation before spraying. Glyphosate concentrations in all water samples were below the detection limit. Average moisture content of the soil samples was 84.2 ± 0.5%. Recoveries from the site soil samples averaged 42.5 ± 2.9%. One replicate from one of the three soil collection sites, collected 1 year after application, was positive at the detection limit (0.40 ± 0.20 µL/g; uncorrected value). The remaining samples were negative. In this study, we found that the time taken to 50% degradation of the glyphosate (DT_{50}) in the water-saturated sediments was faster than expected (approximately 1 day considering the detection limit and recoveries).	
Proposed action: No action since documentation of results insufficient. Furthermore, the publication focuses on a site outside the EU.	
Type of information (critical, high/low weight, supporting, additional): Supporting information, since the experiments were performed in principal similar to standard degradation studies but documentation of results (e.g. time dependent residues, kinetic analyses) is insufficient.	
Reliability	
Endpoint	DT_{50}, PEC_{SW} and PEC_{Soil}
Protocol	Non-GLP, no standard laboratory studies
Test compound	Test compound: CAS 40465-66-5 Unlabelled: Glyphosate as a formulation, Vision concentrate (PCP 19899; Monsanto Canada, Winnepeg, MB, Canada)
Test system and conditions	Field study to analyse stream concentration after application of glyphosate. The application rate was 1.67 kg/ha glyphosate; the application took 2.5 hours. Water collections were taken at three locations along a stream 65 m from the perimeter of the treatment area spaced out along the perimeter of the treatment area. Three soil sample collection sites were identified in the treated area. These were all approximately 4 m², were comprised mainly of clay soil and organic matter, and were located where surface water would accumulate depending on the time of year. The use of GC-MS with single ion monitoring ensured a reliable detec-

	tion of glyphosate residues. For water samples, the limit of quantification was 25 µg/L (25 ppb). Glyphosate recoveries from three water samples averaged 130 +/- 6.5% at the limit of quantification (mean ± SE). Recoveries from the sand/organic soil laboratory method studies were 90.6 ± 9.2% and the detection limit was 0.40 µg/g.
Statistical design	No information
Relevance	
Environmental relevance	The study is principally relevant since the experimental design is close to the scenarios considered in the registration procedure (Drift as entry route). However, no sufficient information about raw data is given. It is further not clear whether the DT_{50} are simply caused by advection in the fresh water system.
Weight of evidence	
	The results may be influenced by advection in the surface water.

Grey et al. (2009)

Title: Herbicide Dissipation from Low Density Polyethylene Mulch	
Author: Timothy L. Grey, William K. Vencill, Theodore M. Webster, and A. Stanley Culpepper	
Reference: Weed Science 2009 57:351–356	
Year: 2009	
Results and conclusion: Data indicated that glyphosate dissipation was rapid following irrigation. Glyphosate DT_{50} was 1 h in the irrigated study, but 84 and 32 h for the dry scenario, respectively. This indicated that glyphosate could be removed from LDPE mulch with rainfall or irrigation, primarily due to their high water solubility.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Degradation of glyphosate in mulch
Protocol	Non-GLP study
Test compound	Roundup WeathermaxTM, glyphosate, EPA Reg. No. 524-537, Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, MO 63167. 23 P. (CAS 1071-83-6) unlabelled
Test system and conditions	Field studies conducted in Ty, GA to evaluate the dissipation of herbicides from LDPE mulch. The dry experiments did not receive irrigation or rainfall. For the irrigated experiments, samples were collected at 1 HAT, irrigated at 3 HAT with 1 cm of water using an overhead irrigation system, then sampled at 5 HAT. This washing and sampling procedure was then repeated at 24, 48, 72, and 96 HAT. Irrigation water pH samples were periodically collected and ranged from 7.0 to 8.1.
Statistical design	Four Three replicates
Relevance	

Environmental relevance	The environmental relevance is limited since mulch was tested instead of soil.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard laboratory testing.

Laitinen et al. (2006)

Title: Fate of the herbicides glyphosate, glufosinate-ammonium, phenmedipham, ethofumesate and metamitron in two Finnish arable soils	
Author: Pirkko Laitinen, Katri Siimes, Liisa Eronen, Sari Rämo, Leena Welling, Seija Oinonen, Leona Mattsoff and Marja Ruohonen-Lehto	
Reference: Pest Manag Sci 62: 473–491	
Year: 2006	
Results and conclusion: Glyphosate had been used in the sandy loam site the previous autumn and about one-third of the applied dose was still detected in the following May. Our results from the field study also show clear overwinter persistence for glyphosate. About 10–20% of applied glyphosate was detected in the subsequent June in both field sites, demonstrating that the time for 90% (DT_{90}) dissipation of glyphosate in our study was about 11 months. Glyphosate had been used in the clay soil site 1.5 years prior to our study. This gave no background signal, corresponding to less than 10% of the applied amount of glyphosate. However, AMPA was detected in both background samples and at the end of our field study, indicating that it is more persistent than glyphosate	
Proposed action: Consider as supporting information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Degradation of glyphosate in soil under Finish conditions
Protocol	Non-GLP study
Test compound	CAS 40465-66-5 Glufosinate-ammonium CAS 1071-83-6 Glyphosate
Test system and conditions	Field trials were carried out for 26 months starting in May 1999 at two different geographical sites in southern Finland. The soil in Perni was a clay soil according to FAO texture classes, while the soil in Janakkala was a sandy loam soil. Both fields were intensively drained (at 1m depth, tiles about 10m apart). Air temperature and cumulative precipitation were recorded at both geographical sites during the growing seasons. Relative soil moisture was measured at three depths (8, 25 and 40 cm). At both geographical sites, two of the four 200m2 plots were sown with glufosinate-ammonium-resistant sugar beet. Soil samples were taken from the tillage layer (0–28 cm) and subsoil (28–50 and 50–70 cm).
Statistical design	Eight to ten samples were collected into a single jar for each analysis.
Relevance	
Environmental relevance	The conditions in Finland do not reflect the conditions for standard degradation experiments and normalisation to standard conditions was not performed.

	However, the results are nevertheless of relevance as they describe glyphosate degradation over a cold winter period.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard tests.

Lane et al. (2011)

Title: Microbial activity, community structure and potassium dynamics in rhizosphere soil of soybean plants treated with glyphosate	
Author: Matthew Lane, Nicola Lorenz, Jyotisna Saxena, Cliff Ramsier, Richard P. Dick	
Reference: Pedobiologia - International Journal of Soil Biology (2010), doi:10.1016/j.pedobi.2011.12.005	
Year: 2011	
Results and conclusion: Glyphosate caused a significant decrease in the total microbial biomass in 46 soybean rhizosphere soil that had no previous exposure to glyphosate, at 7 days after glyphosate application. However, no significant changes were observed in the overall microbial community structure. In conclusion, the glyphosate application lowered the total microbial biomass in the 49 GR soybean rhizosphere soil that had no previous exposure to glyphosate, at 7 days after glyphosate application; caused no changes in the microbial community structure; and did not reduce the plant available K (soil exchangeable or plant tissue).	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary. Furthermore, the experimental site was outside the EU.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Microbial activity and K dynamics after glyphosate treatment
Protocol	Non-GLP study
Test compound	Unlabelled glyphosate, CAS 1071-83-6
Test system and conditions	Field study and Greenhouse-experiment: Two soil with similar physical and chemical characteristics, yet different levels of previous exposure to glyphosate were used in this study. Both sites were within an eleven kilometre radius in eastern Delaware County, Ohio. The climate is characterized in winter with an average temperature of – 2.8 °C and an average daily minimum temperature is -7.8 °C. In summer, the average 136 temperature is 21.1 °C and the average daily maximum temperature is 27.8 °C. The total annual precipitation is 941 mm. Of this, 536 mm, or about 58 percent, usually falls in April through September. Glyphosate was applied up to three times a year while growing soybeans, and once a year while cultivating corn, for an average of two yearly glyphosate applications.
Statistical design	No information
Relevance	
Environmental relevance	The results are of miner relevance since the experiments do not describe any of the standard endpoints.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared directly with standard tests.

Newton et al. (2008)

Title: Dissipation of four forest-use herbicides at high latitudes	
Author: Mike Newton & Elizabeth C. Cole & Ian J. Tinsley	
Reference: Environ Sci Pollut Res DOI 10.1007/s11356-008-0039-7	
Year: 2008	
Results and conclusion: Dissipation rates did not follow first-order rates because freezing conditions slowed most microbial activity. All products dissipated to close to or below detection limits within the time of the study.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary. Furthermore, the experimental site is an extreme site outside the EU.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Glyphosate degradation in soil under extremely cold conditions
Protocol	Non-GLP study
Test compound	Non-labelled glyphosate CAS 1071-83-6
Test system and conditions	Test plots were in upland and river bottom sites at 65°N and 58°N latitudes. The northern site has extremely cold winters, with soils that freeze to a depth of 1-2 m, and precipitation of 275 mm/year. The southern site has heavy rain and snowfall, amounting to 2,250 mm/year evenly distributed. Soil seldom freezes deeply. On each test plot, glyphosate was applied at twice the normal operational use rate to facilitate detection.
Statistical design	No information
Relevance	
Environmental relevance	The results cannot be used because the climatic conditions extreme and not comparable to the standard degradation studies. Also no normalisation to standard conditions was done.
Weight of evidence	
“Positive”/”Negative” evidence	The results cannot be compared directly with standard tests.

Siimes et al. (2006)

Title: Comparison of the behaviour of three herbicides in a field experiment under bare soil conditions
Author: K. Siimes, S. Rämö b, L. Welling, U. Nikunen, P. Laitinen
Reference: agricultural water management 84 (2006) 53 – 64
Year: 2006
Results and conclusion: Field study: Herbicides were detected mainly in the topsoil (0-3 cm). The field dissipation half-life time of glufosinate-ammoniumin the topsoil was about 1 week, whereas that of ethofumesate was over 10 weeks. Glyphosate analyses from soil media failed because organic fertilizer caused similar peaks in chromatography.
Proposed action: Consider as supporting information. Recalculation of endpoints on degradation is not necessary.
Type of information (critical, high/low weight, supporting, additional):

Supporting information	
Reliability	
Endpoint	Degradation of Glyphosate and glufosinate-ammonium under bare soil conditions
Protocol	Non-GLP study
Test compound	Non-labelled compound: glyphosate (CAS 1071-83-6), glufosinate-ammonium (CAS 40465-66-5)
Test system and conditions	The experiment was carried out on a leaching field plot (width 51 m, mean length 70 m) located in Toholampi in Finland. After 3 years of barley and 1 year of potato cultivation, no crop was cultivated on the plot during the study. Ethofumesate, glyphosate or glufosinate-ammonium had never been used before on the study plot. The three herbicides, as commercial herbicide products, were sprayed on bare soil on 8th July 1999 using the maximum recommended rates for single application
Statistical design	No replicates
Relevance	
Environmental relevance	The results are of relevance, but due cannot be directly used as they were not analysed considering FOCUS deg kinetics.
Weight of evidence	
"Positive"/"Negative" evidence	The results for glufosinate ammonium are in line with reliable studies.

Torstensson et al. (2005)

Title: Efficacy and fate of glyphosate on Swedish railway embankments
Author: Lennart Torstensson, Elisabet Börjesson and John Stenström
Reference: Pest Manag Sci 61:881–886
Year: 2005
Results and conclusion: The persistence of glyphosate in the embankments investigated was studied for two application rates of RoundUp Bio (3 and 5 litre ha^{-1}). 50% disappearance times (DT_{50}) for the different rates of glyphosate during the years of investigation were 3 ± 1 month. The appearance of glyphosate and its metabolite AMPA [(aminomethyl)phosphonic acid] in the embankment, e.g. mobility and persistence, was also studied. Mobility was low in most cases, the main proportion of both glyphosate and AMPA being found in the upper 30-cm layer although minor amounts penetrated to lower depths. The 50% disappearance time of glyphosate was generally <5months in railway embankments but cases with longer persistence were found. Transport to the groundwater was observed for glyphosate and AMPA in groundwater pipes along tracks. Downward transport appears to be dependent on the application rate, which should not exceed 3 litre ha^{-1} of RoundUp Bio to avoid groundwater contamination. A lower rate of glyphosate mixed with a low rate of another herbicide may achieve acceptable weed control and be environmentally safer.
Proposed action: Consider as supporting information. Recalculation of endpoints on degradation is not necessary.
Type of information (critical, high/low weight, supporting, additional): Supporting information

Reliability	
Endpoint	Analysis of processes involved in microbial glyphosate degradation
Protocol	Non-GLP study

Test compound	Non-labelled glyphosate (CAS 1071-83-6), Non-labelled AMPA (CAS 1066-51-9)
Test system and conditions	The railway embankments used in this investigation are located across Sweden. Most of the embankments are constructed of gravel and coarse and finer sand. For studies of the presence of glyphosate and AMPA in the embankment, samples were taken from randomly chosen areas (25 × 40cm^2) within the experimental plot, in one of the four replicates. The uppermost layer (0–10 cm) was sampled by cutting a sample from an area 9cm × 9 cm and 10cm depth. After that, the whole 10-cm layer within the sample area (25 × 40cm^2) was removed. The procedure was repeated for each of the remaining layers to be sampled.
Statistical design	Four replicates
Relevance	
Environmental relevance	There is relevance for this specific type of application in railway tracks. However, the authors did not calculate any DT_{50} values according to the recommended FOCUS procedure.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be directly compared with standard studies.

Vinther et al. (2008)

Title: Field-Scale Variation in Microbial Activity and Soil Properties in Relation to Mineralization and Sorption of Pesticides in a Sandy Soil
Author: F. P. Vinther, U. C. Brinch, L. Elsgaard L. Fredslund B.V. Iversen and S. Torp C. S. Jacobsen
Reference: J. Environ. Qual. 37:1710–1718
Year: 2008
Results and conclusion: As for the soil properties and the microbial parameters, the pesticide parameters exhibited a considerable higher variation in the Bs horizon than in the Ap horizon. The mineralization was quite low for all three pesticides (0.8 to 12.8%). The use of contour maps along with descriptive statistics, including CVs, may give a good impression of the spatial variation and distribution within a field. The results of this study indicate that spatial variation of soil properties, and in particular the content of soil organic C, has a major influence on the spatial variability of microbial parameters and parameters elated to glyphosate degradation and sorption in the soil. The local-scale variations within 100 m^2 areas were two to three times lower than the field-scale variation within the entire field of about 4 ha.
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.
Type of information (critical, high/low weight, supporting, additional): Additional information
Reliability

Endpoint	Analysis of processes involved in microbial glyphosate degradation
Protocol	Non-GLP study
Test compound	Labelled: (N-(phosphonomethyl)-glycine ^{14}C labelled at the P-

	methylene end to a specific activity of 172.1 µCi mg^{-1} (CAS 1071-83-6)
Test system and conditions	The study site was located in the northern part of Jutland (52°27′ E, 43°21′ N) on the Yoldia plains, composed mainly by deposits from the Yoldia. The soil texture (i.e., clay [<2 µm], silt [2–63 µm], and sand [63–500 µm]) was measured in the 102 individual soil samples. The moisture content was adjusted to 90% of the soil water-holding capacity. The microcosms were incubated at 10 °C
Statistical design	Four replicates
Relevance	
Environmental relevance	There is relevance for this analysis. However, the authors did not calculate any DT_{50} values according to the recommended FOCUS procedure.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be directly compared with standard studies.

Weaver et al. (2007)

Title Effects of glyphosate on soil microbial communities and its mineralization in a Mississippi soil	
Author: Mark A Weaver, L Jason Krutz, Robert M Zablotowicz and Krishna N Reddy	
Reference: Pest Manag Sci 63:388–393 (2007)	
Year: 2007	
Results and conclusion: Field study: Following the second in-season glyphosate application, the microbial community was not clearly separated by glyphosate treatment. Lab study: mineralization of glyphosate followed first-order kinetics, and estimates for parameters *a* and *k* were significantly different between treatments. After incubation for 42 days, 32–37% of the applied glyphosate was mineralized when applied at threefold field rates, with about 9% forming bound residues. These results indicate that glyphosate has only small and transient effects on the soil microbial community, even when applied at greater than field rates.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Analysis of processes involved in microbial glyphosate degradation
Protocol	Non-GLP study
Test compound	Non-labelled: Field study: Glyphosate-isopropylammonium (Roundup Ultra) (CAS 38641-94-0) Lab study: glyphosate (98% purity; Chem Service) and ^{14}C-labeled glyphosate (54 mCi $mmol^{-1}$ specific activity, 99% radiolabelled purity; Amersham Life Sciences) (CAS 1071-83-6)
Test system and conditions	Field study: Glyphosate-resistant soybean (AG 4702RR) was planted in Dundee silt loam (fine-silty, mixed, thermic Aeric Ochraqualf) on the Southern Weed Science Research farm in Stoneville, MS (USA).

	Glyphosate-isopropylammonium (Roundup Ultra) was applied at 4 weeks after Non-glyphosate treated plots were included as a control. Bulk surface soil (0–2.5 cm) was collected at time of planting, before initial glyphosate application and 14 days after each glyphosate application. Mineralization of ^{14}C-glyphosate was evaluated.
Statistical design	Four replicates
Relevance	
Environmental relevance	There is relevance for this analysis. However, the authors did not calculate any DT_{50} values according to the recommended FOCUS procedure.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be directly compared with standard studies.

Wang et al. (2010)

Title: Residue and field decline study of glyphosate-ammonium in ramie field	
Author: Wang Yan-hui (wangyh1984@163.com); Li Xin; Zhou Xiao-mao (zhouxm1972@126.com); Bai Lian-yang; Cai Hai-lin	
Reference: Nongyaoxue Xuebao Volume: 12 Issue: 2 Pages: 201-206 Published: JUN 2010	
Year: 2010	
Results and conclusion: A simple, sensitive and selective method using gas chromatography equipped with flame photometric detector (GC-FPD) was developed to determine residues of glyphosate-ammonium in soil and ramie root. Soil samples were extracted with 0.01 mol/L sodium hydroxide and other samples were extracted with water and acetone. Glyphosate was previously derived with trimethylorthoacetate (TMOA) in the presence of acetic acid. Combination of AG1-X8 anion exchange chromatography with Florisil cartridge cleanup process was favourable for the GCPFPD analysis. The recovery ranged from 73.6% to 102.6% and 85.9% to 105.1% with the relative standard deviations of 2.3% to 8.1% and 5.4% to 13.0%, respectively. The limit of detection (LOD) of the method was 0.5 x 10(-10) g. The limit of quantification (LOQ) was 0.05 mg/kg. The half-life of glyphosate-ammonium was 1.6 - 2.6 d, 1.0 - 1.8 d and 1.1 - 1.5 d in soil of Hunan, Guangxi and Fujian Province at two years, respectively. No glyphosate-ammonium residues were detected in ramie and soil samples at treatments of 2 250 3 375 g (a.i.) /ha at harvest season (60 days after the treatment).	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary. Furthermore, the experimental site is outside the EU.	
Type of information (critical, high/low weight, supporting, additional): Low weight	
Reliability	
Endpoint	Analysis of glyphosate ammonium residues in soil
Protocol	Non-GLP study
Test compound	Non-labelled: glyphosate-ammonium
Test system and conditions	Only the abstract given
Statistical design	No information given
Relevance	

Environmental relevance	The authors did not calculate any DT_{50} values according to the recommended FOCUS procedure.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be directly compared with standard studies.

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B.8.11.3 Adsorption, desorption and mobility in soil

B.8.11.3.1 Adsorption and desorption of the active substance and all relevant metabolites

Summary

~~62~~ 68 publications are available in open literature focusing on sorption and desorption properties of glyphosate. A reliability and validity assessment of the articles clarified that 16 articles

were plausible but important raw data, e.g. on mass balances and test item concentrations in the aqueous and solid phases, were missing. Consequently, the validity of these studies could not be proven and therefore, results were not used to derive endpoints or to calculate PEC's. Besides, there are numerous OECD-studies available that are considered to be plausible and valid. Thus, the existing endpoint calculation (k_{oc}, 1/n) is based on a comprehensive data base. In general the results of the studies named above are in agreement with those obtained by the OECD-studies; they confirm the strong binding potential of glyphosate to agriculturally used soils in the EU.

~~21~~ 27 articles focus on dependencies of glyphosate sorption on environmental parameters such as the amount of phosphate, copper, iron and aluminum oxide. Such basic research results give a more in-depth insight into sorption processes and furthermore are of importance for the discussion of the behaviour of glyphosate in soils. Last but not least such results give guidance on the interpretation of OECD studies as well as on a possible improvement of data requirements. Some of these articles also use material other than soil, e.g., charcoal, chitin, goethite and building material.

~~17~~ 19 studies have been performed using non-European soils. Therefore, their results are not taken into account to derive endpoints or to calculate PEC's. 7 publications are review articles and letters to the editor; they do not present single data and thus were not used. One of the articles focuses on risk mitigation rather than on results of a laboratory sorption study.

In detail, OECD-studies as well as publications obtained from open literature show that glyphosate sorption in soil is not influence by a single or a dominating soil property only but by many of these physico-chemical properties. Among these, of course the soil organic matter has to be mentioned. The adsorption to soil organic matter can be explained by hydrogen bonding among the various acidic and oxygen-containing groups in glyphosate and humic material (Albers et al., 2009).

Besides soil organic matter the adsorption of glyphosate is influenced by the clay content. The phosphonic moiety of the compound forms strong complexes with metal cations of clay minerals. Also, other metals such as Fe^{3+} and Al^{3+} increase glyphosate sorption (Gimsing et al., 2004), and a positive correlation between the chemical and the iron content in the soil can be observed (Jacobsen et al., 2003).

An inverse correlation between soil pH-value and the sorption of glyphosate is described by Gimsing et al., 2004; Wang et al., 2005; Al-Rajab et al., 2008 and Zhao et al., 2009. This might be due to more net negative charge and the occurrence of electrostatically unfavourable conditions between negatively charged soil oxides and the negatively charged ionized glyphosate molecule. On the other hand De Jonge et al. (2001) described an increase of glyphosate sorption when the soil pH-value was increased by liming.

Many of publications (in particular: De Jonge et al., 2001; Dion et al., 2001; Prata et al., 2003; Wang et al., 2005; Laitinen et al., 2008 and Zhao et al., 2009) focus on the competitive adsorption between glyphosate and phosphate. Competition is plausible because of the similarity of their chemical structures and the fact, that both chemicals are strongly adsorbed by variable charge soil minerals such as aluminum and iron oxides. Generally it can be stated, that a reduction of glyphosate sorption can be observed in phosphate rich soils. However, extend of reduction depends on the soil.

This study published by De Jonge et al. (2001) quantified the variation in glyphosate sorption and desorption to a coarse sandy soil and to a sandy loam soil with varying phosphorus content and pH values. Using batch experiments, glyphosate adsorption and desorption isotherms were determined on soil samples taken from long-term field experiments that received different additions of phosphorus and lime. The isotherms were best fitted with an extended Freundlich model. The phosphate content in the soils had a significant influence on the sorption of glyphosate. With 0.5 M bicarbonate extractable P (pH 8.5) increasing from 6.2 to 58.7 in the loamy sand and 9.1 to 87.4 in the coarse sand, the extended Freundlich adsorption coefficient (K_f, MF, ads) decreased from 214.7 to 106 and from 154.0 to 83.5, respectively.

The paper of Wang et al. (2005) studied the effects of phosphate on the adsorption of glyphosate on three different types of Chinese soils including two variable charge soils and one permanent charge soil. The results indicated that Freundlich equations used to simulate glyphosate adsorption isotherms gave high correlation coefficients (0.990-0.998) with K values of 2751, 2451 and 166 for the three soils. The more the soil iron and aluminum oxides and clay contained, the more glyphosate adsorbed. The presence of phosphate significantly decreased the adsorption of glyphosate to the soils by competing with glyphosate for adsorption sites of soils.

Rampazzo et al. (2012) studied adsorption of glyphosate and aminomethylphosphonic acid in soils. The results showed that glyphosate is initially adsorbed mostly in the upper 2 cm. It is then transported and adsorbed after few days in deeper soil horizons with concomitant increasing content of its metabolite aminomethylphosphonic acid. Moreover, Fe-oxides seem to be a key parameter for glyphosate and aminomethylphosphonic adsorption in soils. This study confirmed previous studies: the analysis showed lower contents of dithionite-soluble and Fe-oxides for the Chernozem, with consequently lower adsorption of glyphosate and aminomethylphosphonic.

Ghafoor et al. (2012) investigated glyphosate sorption in the surface and subsurface soils of an agricultural catchment in southern Sweden. The authors showed that interactions between organic and inorganic sorbents affected glyphosate sorption and concluded that information on clay, iron and aluminium oxides and soil pH, in addition to organic carbon, is needed for accurate prediction of pesticide leaching. The variables f_{oc}, f_{clay} and pH are generally available, whereas measurements of oxides of Al and Fe are rarely reported. The authors therefore emphasise the need to measure and report contents of oxides of Al and Fe in soil survey databases, because small variations in their concentrations may contribute significantly to large variations in sorption, especially of ionisable pesticides.

Competitive adsorption of glyphosate and phosphate on goethite and gibbsite and on illite, montmorillonite and two kaolinites differing in surface area was evaluated by Gimsing et al. (2004). The results show that glyphosate and phosphate are competing for the adsorption sites, but the degree of competition depends on the adsorbent. On goethite the competition is very much in favor of phosphate, on gibbsite the competition is closer, but still phosphate is favored, while on illite, montmorillonite and kaolinite the competition is almost equal. Changes in the surface area did not affect the competition between glyphosate and phosphate for adsorption sites. The results indicate that differences among soils of different mineralogical composition regarding the adsorption of glyphosate and phosphate can be expected.

Zhao et al. (2009) showed that glyphosate adsorption consistently decreased with increase in system pH value. The effect of phosphate application on glyphosate mobility varied with soil type. The authors concluded that phosphate application can cause system pH value change

with various extents in the soil, which subsequently contribute to glyphosate mobility in different degree.

Borggaard (2011) concluded in his review that although several factors may control transport of glyphosate (and AMPA) from the terrestrial to the aquatic environment, the similarity between glyphosate and phosphate in relation to sorption processes strongly indicates competition between the two species for sorption sites on soil solids. This may lead to glyphosate leaching in phosphate-rich soils, where sorption sites are occupied by phosphate provided the sorption mechanisms of the two sorbates are identical. On the other hand, while sorption may protect the herbicide against microbial degradation, soil solution glyphosate is bioavailable and can be biodegraded. In addition to this indirect effect, phosphate may directly interfere with the microbial glyphosate degradation.

Literature data published on soil sorption and desorption properties of AMPA is rather scarce (Huang et al., 2004; Gjettermann, 2009; Mamy et al., 2010). However, published data show that the substance is strongly adsorbed to the soil. Published K_d-values are between 32 and 496 mL/g. This is in the same range as obtained from OECD-studies.

Detailed description of open literature on adsorption and desorption of the active substance and all relevant metabolites

Accinelli et al. (2006)

Title: Influence of Cry1Ac Toxin on Mineralization and Bioavailability of Glyphosate in Soil	
Author: CESARE ACCINELLI, WILLIAM C. KOSKINEN, AND MICHAEL J. SADOWSKY	
Reference: J. Agric. Food Chem. 2006, 54, 164-169	
Year: 2006	
Results and conclusion: Results from laboratory investigations indicate that soil incorporation of purified Cry1Ac toxin in the range of 0.25-1.0 µg g^{-1} does not influence glyphosate mineralization or its sorption in soil. These results are in contrast to results obtained in previous investigations done using a mixture of Cry toxins at a concentration of 10 µg g^{-1} (21). The concentration of Cry toxins in soil occurring during the growing season has been estimated not to exceed 1 µg g^{-1}, based on the average concentrations of Cry toxin in crop residues incorporated into the top soil or left at the soil surface (22). On the basis of these estimates and the results obtained here, the data indicate that concentrations of Cry1Ac comparable to those encountered under field conditions do not have the potential to increase persistence and sorption of glyphosate in soil. Following K_{foc}-values were determined for glyphosate: sandy loam (Italy): 6230 L/kg and 6408 L/kg (US soil).	
Proposed action: Consider as additional information. No standard test is performed but effects of Cry1Ac Toxin on the sorption behaviour of Glyphosate are reported. Though interpretation given in the publication is plausible, it needs further in depth investigations to assess the relevance for the endpoint mobility.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	High

Endpoint	$DegT_{50}$ in soil and sorption on soil
Protocol	Non-GLP, standard degradation study, modified OECD Guideline 307 and 106
Test compound	Unlabelled glyphosate (chemical purity > 98%), ^{14}Clabeled glyphosate (N-phosphonomethyl-2-^{14}C-glycine; radio-purity > 99%, specific activity: 1.18 106 MBq g^{-1}), CAS-no.: 1071-83-6
Test system and conditions	Two soils with different physicochemical properties, taken from areas of the Po Valley (Italy) and of south central Minnesota, were selected for this study. The Italian soil (IT, 0.7% OC) and the American soil (MN, 0.94% OC) were both classified as sandy loam At both locations; the soil was collected from fields that had not received glyphosate applications within the previous 5 years. A portion of the IT and MN soils was mixed with Cry1Ac toxin powder to obtain a final concentration of 100 µg g^{-1} soil. Aliquots of these two amended soils were mixed with a sufficient mass of IT and MN soils to obtain final soil concentrations of 0.25, 0.5, and 1.0 µg Cry1Ac toxin g^{-1} soil (air-dried basis). The soil moisture in treated soil samples was adjusted to the gravimetric content at -33 kPa using distilled water and incubated in the dark at 25°C. Isotherms for sorption of glyphosate to IT and MN soils containing different Cry1Ac toxin concentrations were determined using the batch equilibrium method 20 °C for 14 h.
Statistical design	Three replicates were prepared for each soil type and toxin concentration, and controls consisted of soils with no toxin addition.
Relevance	
Environmental relevance	The sorption studies are in principle performed considering the current guidance documents and can be considered for the calculation of sorption parameters. The degradation studies are not documented well enough to be considered further.
Weight of evidence	
"Positive"/"Negative" evidence	Partly positive evidence.

Accinelli et al. (2005)

Title: Effects of Incorporated Corn Residues on Glyphosate Mineralization and Sorption in Soil
Author: CESARE ACCINELLI, WILLIAM C. KOSKINEN, JEFFREY D. SEEBINGER, ALBERTO VICARI, AND MICHAEL J. SADOWSKY
Reference: J. Agric. Food Chem. 2005, 53, 4110-4117
Year: 2005
Results and conclusion: Addition of corn residues did not change the relative differences in the sorptive capacities between two soils, regardless of the incorporated amount. There were no differences between corn residues from the two isolines. Incorporation of low amount of corn residues did not affect sorption of [^{14}C]glyphosate to the soils. In contrast, incorporation of the highest corn residue amount reduced the sorption capacities of both soils for glyphosate. The observed decrease of herbicide sorption on soil mixed with the highest level of corn

residue is possibly due to coverage of the soil sorptive sites by the corn residues, and not due to a reduction of the sorptive capacity of the soil per se. It also was observed that the binding of glyphosate and its degradation products to corn residue increased over the incubation time. This might be attributed to decomposition of the wheat residues associated with a decline in cellulose concentration and an enrichment of lignin More detailed information on the effect of weathering on the sorptive properties of corn residues to glyphosate is needed.	
Proposed action: Consider as additional information. No standard test is performed but effects of incorporated corn on the sorption behaviour of Glyphosate are reported. Though interpretation given in the publication is plausible, it needs further in depth investigations to assess the relevance for the endpoint mobility.	
Type of information (critical, high/low weight, supporting, additional): Low weight, may be additional information to already existing. Though interpretation given in the publication is plausible, it needs further in depth investigations to assess the relevance for the endpoint mobility.	
Reliability	
Endpoint	K_f-values, 1/n, r^2
Protocol	Modified standard (OECD 106), corn added to soil followed by sorption study according to OECD 106. Non-GLP
Test compound	Test compound: Glyphosate (unlabeled, chemical purity > 98%) and [^{14}C]-labelled glyphosate (*N*-phosphonomethyl-2-^{14}C-glycine; radio purity > 99%, specific activity 1.18 106 MBq g^{-1}), CAS-no.: 1071-83-6
Test system and conditions	Corn residues of two different hybrids were incorporated into a sandy and sandy loam soil. Concentrations of corn were from 0.5 to 8 %.
Statistical design	Triplicates, two soils, two corn hybrids, isotherm-calculation, Freundlich-equation used.
Relevance	
Environmental relevance	Environmental relevance is given: Conservation tillage systems are characterized by a significant presence of crop residues at the soil surface so that glyphosate is applied to a soil matrix rich in poorly decomposed crop residues as corn might be left.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies with such a design not known. No negative evidence.

Albers eta al. (2009)

Title: The influence of organic matter on sorption and fate of glyphosate in soil – Comparing different soils and humic substances
Author: Christian N. Albers, Gary T. Banta, Poul Erik Hansen, Ole S. Jacobsen
Reference: Environmental Pollution 157, 2865–2870
Year: 2009
Results and conclusion: Three approaches were followed to investigate the dynamics between glyphosate and soil organic matter. 1) Sorption studies with seven purified soil humic fractions showed that these could sorb glyphosate and that the aromatic content, possibly phenolic groups, seems to aid the sorption. 2) Sorption studies with six whole soils and with SOM removed showed that several soil parameters including SOM are responsible for the strong sorption of glyphosate in

soils. 3) After an 80 day fate experiment, appr. 40% of the added glyphosate was associated with the humic and fulvic acid fractions in the sandy soils, while this was the case for only appr. 10% of the added glyphosate in the clayey soils. Glyphosate sorbed to humic substances in the natural soils seemed to be easier desorbed than glyphosate sorbed to amorphous Fe/Al-oxides.
Detailed results are:

Soil characterisation:

Soil	Soil type	Sand (%) Fine	Medium	coarse	Clay+silt (< 0.063mm) (%)
SIA	Clay soil	26	19	7	47
SIB	Clay soil	43	21	5	32
WZ	Dark clayey zone soil	28	16	3	54
FIA	Sandy soil	16	35	12	6
FIB	Sandy soil	11	74	12	4
FIC	Sandy soil	22	74	4	0.2

Adsorption

Soil type	SOM %	pH (H_2O)	K_d	K_{oc}	K_f	K_{foc}	1/n
SIA	2.8	7.0			22[1)]		
SIB	1.7	7.5			29[1)]		
WZ	5.2	6.3			87[1)]		
FIA	4.5	6.6			28[1)]		
FIB	1.6	6.5			28[1)]		
FIC	0.2	6.6			24[1)]		

1) read from figure in the publication; no data in table or other raw data available

Desorption

Soil type	OC (%)	pH ($CaCl_2$)	Desorption K_d	K_{oc}	K_f	K_{foc}	1/n
					39[1)]		
					82[1)]		
					119[1)]		
					26[1)]		
					36[1)]		
					42[1)]		

[1)] read from figure in the publication; no data in table or other raw data available

Proposed action:
Not to be considered for the endpoint sorption and mobility as raw data are not reported. Results are presented as figures only but are not reported as tabular numbers

Type of information (critical, high/low weight, supporting, additional):
High weight, supporting information to already existing.

Reliability	High
Endpoint	K_f-values, 1/n, R^2
Protocol	Modified standard (OECD 106), sorption to soils and soil organic matter Non-GLP.
Test compound	Glyphosate (purity >99%), ^{14}C-glyphosate (purity >99%), CAS-no.: 1071-83-6
Test system and conditions	Sorption studies with soils and soil organic matter, adsorption isotherms, substance 3 concentrations each, 70 h. single measurements; mineralisation determined for 80 days; 5 sampling points.
Statistical design	Single measurements, Freundlich isotherms

Relevance

Environmental relevance	Given. Parameter influencing endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	Partly positive evidence.

Alexa et al. (2008)

Title: Researches regarding extractable glyphosate residues from different soils	
Author: Ersilia ALEXA, Aurel LAZUREANU, Simion ALDA, Monica NEGREA and Olimpia IORDANESCU	
Reference: Comm. Appl. BioI. Sci, Ghent University. 73/4, 2008	
Year: 2008	
Results and conclusion: The extractable glyphosate residues from soil solution have been determined analytically (HPLC-FlD). Substrates used were Black Chemozem, Typical Gteysoil, Slight Vertisol, with moderate carbonatation. The glyphosate adsorption on the 3 soils is high (>80%), depends on their physico-chemical characteristics and it increases: Gleysol, Black Chernozem, Vertisol. The analyzed soils are characterized through a high content of clay (36,5-41,8%) and humus (3,35-4,09%) which enhances the adsorption capacity of the glyphosate on soil particles, the glyphosate forming stable complexes with clays, which immobilize the active substance deactivating it. The quantity of extractable residues from the soil is low (<20%), depends on the characteristics of the soil and decreases as follows: Gleysoil, Black Chernozem, Slight Vertisol. The glyphosate leaching capacity in the soil is reduced because of the intense adsorption of the herbicide molecules in the surface horizon (0-10 cm).	
Proposed action: Consider as additional information. Data quality and quantity are not sufficient, and raw data are not comprehensively reported. Thus, their use for endpoint and PEC-assessment is not possible.	
Type of information (critical, high/low weight, supporting, additional): Critical, no additional information to already existing.	
Reliability	Medium
Endpoint	Extractable amount of Glyphosate after several times of application.
Protocol	No standard test design, non-GLP
Test compound	Glyphosate, purity not given, CAS-no.: 1071-83-6
Test system and conditions	Active ingredient added to soils in concentration according to GAP. Incubation for 1, 3, 7, 14 and 21 days in air tight vessels. Extraction, determination of a.i. concentration in the extract
Statistical design	Not given in the paper, no Freundlich equation, no further information
Relevance	
Environmental relevance	Test substance representative and relevant; parameters influencing the endpoint have been considered adequately.
Weight of evidence	
"Positive"/"Negative" evidence	Minor positive evidence, no negative evidence.

Al-Rajab et al. (2008)

Title: Sorption and leaching of ^{14}C-glyphosate in agricultural soils

Author: Abdul Jabbar Al-Rajab, Samira Amellal, Michel Schiavon

Reference: Agron. Sustain. Dev. 28, 419–428

Year: 2008

Results and conclusion:

Aim: to assess the dynamic interactions between glyphosate sorption and leaching; and to identify the main factors that influence the two processes in three undisturbed agricultural soils using microlysimeters under outdoor conditions.

OECD 106: Glyphosate was strongly adsorbed, yielding empirical constants of Freundlich sorption isotherms (K_f) of 16.6 for the clay loam soil, 33.6 for the silt clay loam soil and 34.5 for the sandy loam soil, with nf close to 1 in all three cases. Glyphosate was also weakly desorbed, i.e. 5 to 24% (w) of initially sorbed glyphosate. Sorption and desorption were only pH-dependent.

Outdoor microlysimeter: nearly 70% of the initial glyphosate was present in the soil in a non-extractable form at the beginning of the experiment. Conversely, only less than 20% of the initial glyphosate is present in the soil in a non-extractable form after 11 months. These findings suggest that the non-extractable residues become available and take part in biodegradation and leaching. The amounts of ^{14}C-glyphosate derivatives leached were less than 0.28% of the initially applied glyphosate. AMPA metabolite generally represented up to 100% of the residues present in the leachates. The results of leaching were highly influenced by the hydrodynamic properties and the biodegradation capacities of the soils.

Detailed results are:

Soil characterisation:

Soil	Soil type	Sand (%)	Clay (%)	C.E.C. (in meq/100g)
A	Sandy loam		10.5	
B	Silt clay loam		30.6	
C	Clay loam		34.9	

Adsorption

Soil type	OC %	pH (H_2O)	K_d	K_{oc}	K_f	K_{foc}	1/n
A	0.82	5.1			16.6		0.9995
B	1.45	6.3			33.6		1.004
C	1.91	7.9			34.5		0.97

Desorption

Soil type	OC %	pH (H_2O)	Desorption % desorbed	K_{oc}	K_f	K_{foc}	1/n
A	0.82	5.1	6.58; 7.37[1)]				
B	1.45	6.3	5.13; 6.91[2)]				
C	1.91	7.9	21.52; 24.33[3)]				

[1)] first number: % desorbed at initially sorbed Glyphosate concentration of 3.18 µg/g; second number: % desorbed at initially sorbed Glyphosate concentration of 131.7 µg/g

[2)] first number: % desorbed at initially sorbed Glyphosate concentration of 3.18 µg/g; second number: % desorbed at initially sorbed Glyphosate concentration of 131.7 µg/g

[3)] first number: % desorbed at initially sorbed Glyphosate concentration of 2.86 µg/g; second number: % desorbed at initially sorbed Glyphosate concentration of 115.0 µg/g

Proposed action:

Not to be considered for the endpoint sorption and mobility. Raw data on mass balances and test item concentrations in the aqueous and solid phases are not reported. Though the study is plausible, the validity cannot be proven.

Type of information (critical, high/low weight, supporting, additional):

High weight, supportive information

Reliability	High
Endpoint	OECD 106: K_f-values, 1/n, R^2, % desorbed; Outdoor microlysimeter: amount in leachate and distribution in soil

	over time (11 months).
Protocol	Standard (OECD 106), non-GLP; outdoor lysimeter studies, non-GLP.
Test compound	OECD 106: [Phosphonomethyl-^{14}C]-glyphosate (purity: 99%); non-radioactive glyphosate (purity 98.5%) Outdoor microlysimeter: [Phosphonomethyl-^{14}C]-glyphosate diluted in Roundup Express (isopropylamine salt) and water CAS-no.: 1071-83-6
Test system and conditions	OECD 106: 7 concentrations for isotherms, 3 soils; undisturbed outdoor micro-lysimeter (diameter: 10 cm, length: 25 cm): duration: 11 months, 3 soils, 7 sampling points, 21 lysimeter in total
Statistical design	OECD 106: triplicates, lysimeter: single lysimeter per sampling and soil. Stat Box computer software; Comparison of means by Newman-Keuls test at levels of 0.05, 0.01 and 0.001.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence, no negative evidence.

Autio et al. (2004)

Title: Adsorption of sugar beet herbicides to Finnish soils

Author: Sari Autio, Katri Siimes, Pirkko Laitinen, Sari Rämö, Seija Oinonen, Liisa Eronen

Reference: Chemosphere 55, 215–226

Year: 2004

Results and conclusion:

Adsorption of glyphosate studied using the batch equilibrium method in 21 soil samples collected from different depths. None of the measured soil parameters could alone explain the adsorption mechanism of these five herbicides. The results can be used in model assessments of risk for leaching to groundwater resulting from weed control of sugar beet in Finland.

Detailed results are:

Soil characterisation:

Soil	Soil type	Silt (%) (0.002-0.02 mm)	Clay (%) (<0.002 mm)
Kokemäki (0-20 cm)	Silty loam	62	17
Kokemäki (20-40 cm)	Silty loam	66	17
Kotkanoja (0-20 cm)	Clay	15	46
Kotkanoja (20-40 cm)	Clay	13	58
Rehtijärvi (0-20 cm)	Sandy loam	8	13
Rehtijärvi (20-40 cm)	Sandy loam	3	4
Perniö (0-30 cm)	Clay	24	41
Perniö (30-60 cm)	Clay	28	47
Turenki (0-30 cm)	Sandy loam	15	4
Turenki (30-60 cm)	Sandy loam	13	4
Perniö (0-25 cm)	Clay	n.a.	41
Perniö (25-50 cm)	Clay	n.a.	(>30%)
Turenki (0-20 cm)	Sandy loam	n.a.	21
Turenki (20-45 cm)	Sandy loam	n.a.	8

Perniö (0-30 cm)	Clay	24	41
Turenki (0-30 cm)	Sandy loam	15	4
Toholampi (0-25 cm)	Silt loam	16	5
Toholampi (25-35 cm)	Silt loam	20	4
Toholampi (35-60 cm)	Silt loam	30	8
Jokioinen (0-30 cm)	Muddy clay	n.a.	57
Jokioinen (0-30 cm)	Organic soil	13	79

Adsorption

Soil type	OC %	pH ($CaCl_2$)	K_d	K_{oc}	K_f	K_{foc}	1/n
Kokemäki (0-20 cm)	2.42	5.4			-	-	-
Kokemäki (20-40 cm)	0.47	6.1			166	34926	0.97
Kotkanoja (0-20 cm)	2.88	5.8			55	1914	0.92
Kotkanoja (20-40 cm)	0.54	5.6			249	46436	0.91
Rehtijärvi (0-20 cm)	2.57	5.8			44	6039	0.90
Rehtijärvi (20-40 cm)	0.72	5.7			55	2139	1.00
Perniö (0-30 cm)	7.06	6.0			97	1374	1.03
Perniö (30-60 cm)	2.96	6.0			41	1370	1.02
Turenki (0-30 cm)	5.93	6.4			97	1643	0.85
Turenki (30-60 cm)	1.77	5.9			51	2900	0.86
Perniö (0-25 cm)	2.67	8.1			58	2193	0.93
Perniö (25-50 cm)	2.5	7.9			113	4500	0.87
Turenki (0-20 cm)	2.35	7.1			93	3946	0.90
Turenki (20-45 cm)	0.75	6.8			90	11986	0.86
Perniö (0-30 cm)	7.05	6.0			179	2544	1.26
Turenki (0-30 cm)	5.93	6.3			121	2045	0.98
Toholampi (0-25 cm)	7.90	5.4[1)]			159	2014	0.93
Toholampi (25-35 cm)	4.50	5.6[1)]			102	2273	1.05
Toholampi (35-60 cm)	1.30	5.4[1)]			37	2823	0.76
Jokioinen (0-30 cm)	12.60	6.9			84	664	0.91
Jokioinen (0-30 cm)	26.00	5.2			303	1165	1.14

[1)] pH-value (H_2O)

Proposed action:
Consider as additional information for the endpoint sorption and mobility. Raw data on mass balances and test item concentrations in the aqueous and solid phases are not reported. Furthermore, Freundlich isotherms are not presented for each soil but exemplary Though the study is plausible, the validity cannot be proven.

Type of information (critical, high/low weight, supporting, additional):
High weight, supportive information to already existing.

Reliability	High
Endpoint	K_f, K_{foc}, 1/n,
Protocol	Standard (OECD 106, version from 1981; exception: water used instead of $CaCl_2$), Non-GLP
Test compound	Glyphosate (CAS no1071-83-6), unlabelled compound (purity not given)
Test system and conditions	21 soils tested, 2 test concentrations, batch equilibrium method, recoveries according the guideline requirements
Statistical design	Duplicates, Freundlich equation
Relevance	
Environmental relevance	Given. Environmental parameter measured and reported.
Weight of evidence	
“Positive”/”Negative” evidence	Other reliable studies support the results. No negative evidence.

Barja and dos Santos Afonso (2005)

Title: Aminomethylphosphonic Acid and Glyphosate Adsorption onto Goethite: A Comparative Study	
Author: B. C. BARJA AND M. DOS SANTOS AFONSO	
Reference: Environ. Sci. Technol., 39, 585-592	
Year: 2005	
Results and conclusion: The adsorption isotherms and surface coverage of AMPA and glyphosate in aqueous suspensions of goethite as a function of pH were measured. Adsorption isotherms were calculated using a nonlinear regression fitting program (Solver, Excel 5.0) to approximate a Langmuir shape. The Langmuir constant and maximum coverage of every system were reported. Values for maximum coverage were normalized with the area of the goethite for a better comparison.	
Proposed action: Consider as additional information as no standard protocol has been followed but basic research is published.	
Type of information (critical, high/low weight, supporting, additional): Low weight, may be additional information to already existing	
Reliability	Low
Endpoint	Langmuir constant
Protocol	No standard, Non-GLP
Test compound	Glyphosate (99%), CAS-no.: 1071-83-6, AMPA (99%), CAS-no.: 1066-51-9
Test system and conditions	Suspensions of goethite brought to a fixed ionic strength and desired pH. Samples left to reach equilibrium for 24 h. A given number of microliters of 0.010M PMG or AMPA added to suspensions, pH readjusted until constant values were reached. Isotherms measured after 24 h.
Statistical design	No information on replicates, on tested concentrations to obtain isotherms.
Relevance	
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	Other studies with comparable design not known. No negative evidence.

Benetoli et al. (2010)

Title: ADSORPTION OF GLYPHOSATE IN A FOREST SOIL: A STUDY USING MÖSSBAUER AND FT-IR SPECTROSCOPY	
Author: Luís Otávio de B. Benetoli, Henrique de Santana, Cristine E. A. Carneiro e Dimas A. M. Zaia, Ailton S. Ferreira e Andrea Paesano Jr., Cássia Thaïs B. V. Zaia	
Reference: Quim. Nova, Vol. 33, No. 4, 855-859	
Year: 2010	
Results and conclusion: The adsorption of glyphosate onto mineral particles of the forest reserve soil could be occurred through the interaction of the GPS carboxylic group with the metals in soil. Furthermore, glyphosate interacts with Fe^{3+} in soil solution.	
Proposed action:	

Consider as additional information as no standard protocol has been followed but basic research is published.	
Type of information (critical, high/low weight, supporting, additional): Low weight, may be additional information to already existing	
Reliability	Medium
Endpoint	FT-IR data for adsorption of Glyphosate onto mineral particles of the soil
Protocol	Experimental setup partly similar to OECD 106, Non-GLP
Test compound	Glyphosate (analytical grade), CAS-no.: 1071-83-6
Test system and conditions	Study of adsorption of glyphosate onto soil mineral particles, using FT-IR and Mössbauer spectroscopy. Soil/KCl-solution, glyphosate added, shaken for 24 h, centrifuged; supernatant: voltammograms, solid: FT-IR and Mössbauer spectra.
Statistical design	2 soils tested, 4 replicates, no further information on statistics used.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Other studies with such a design not known. No negative evidence.

Borggaard (2011)

Title: Does Phosphate Affect Soil Sorption and Degradation of Glyphosate? – A Review	
Author: Ole K. BORGGAARD	
Reference: Trends Soil Sci Plant Nutr J, 2(1):16-27	
Year: 2011	
Results and conclusion: Although several factors may control transport of glyphosate (and AMPA) from the terrestrial to the aquatic environment, the similarity between glyphosate and phosphate in relation to sorption processes strongly indicates competition between the two species for sorption sites on soil solids. This may lead to glyphosate leaching in phosphate-rich soils, where sorption sites are occupied by phosphate provided the sorption mechanisms of the two sorbates are identical. On the other hand, while sorption may protect the herbicide against microbial degradation, soil solution glyphosate is bioavailable and can be biodegraded, i.e. blocking of sorption sites by phosphate may increase soil solution glyphosate, and hence degradation. In addition to this indirect effect, phosphate may directly interfere with the microbial glyphosate degradation. The review discusses the various processes.	
Proposed action: Consider as additional information as the article does not present experimental data but is a review article. No raw data and/or experimental design are given.	
Type of information (critical, high/low weight, supporting, additional): Low weight, as it is a review article no new experimental data are presented but cited and discussed.	
Reliability	
Endpoint	Sorption, degradation
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; AMPA, CAS-no.: 1066-51-9
Test system and conditions	Not applicable
Statistical design	Not applicable

Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Borggaard and Gimsing (2008)

Title: Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review	
Author: Ole K Borggaard and Anne Louise Gimsing	
Reference: Pest Manag Sci 64, 441–456	
Year: 2008	
Results and conclusion: The very wide use of glyphosate to control weeds in agricultural, silvicultural and urban areas throughout the world requires that special attention be paid to its possible transport from terrestrial to aquatic environments. The aim of this review is to present and discuss the state of knowledge on sorption, degradation and leachability of glyphosate in soils. Difficulties of drawing clear and unambiguous conclusions because of strong soil dependency and limited conclusive investigations are pointed out.	
Proposed action: Consider as additional information as the article does not present experimental data but is a review article. No raw data and/or experimental design are given.	
Type of information (critical, high/low weight, supporting, additional): Low weight. As it is a review article no data are published but cited from other publications and discussed. Additional information.	
Reliability	
Endpoint	
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; AMPA, CAS-no.: 1066-51-9
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Cáceres-Jensen et al. (2009)

Title: Adsorption of Glyphosate on Variable-Charge, Volcanic Ash–Derived Soils	
Author: L. Cáceres-Jensen, J. Gan, M. Báez, R. Fuentes and M. Escudey	
Reference: J. Environ. Qual. 38:1449–1457	
Year: 2009	
Results and conclusion: Three volcanic ash–derived soils were selected for Glyphosate adsorption studies. Glyphosate was rapidly and strongly adsorbed on the selected soils, and adsorption isotherms were well described by the Freundlich relationship with strong nonlinearity ($n_{fads} < 0.5$). The n_{fads}	

values were consistently higher than n_{fdes} values, suggesting strong hysteresis. Adsorption (K_{ads}) increased strongly when pH decreased. The presence of glyphosate changed the adsorption behaviour of phosphate at its maximum adsorption capacity. During the successive desorption steps, glyphosate at the highest level increased K_{ads} values for phosphate in the Andisol soils but had little effect in the Ultisol soil. This different behaviour was probably due to the irreversible occupation of some adsorption sites by glyphosate in the Ultisol soil attributed to the dominant Kaolinite mineral.	
Proposed action: To be considered as additional information as non-standard soils were used, but the influence of variable charge of the volcanic ash-derived soils on sorption were investigated.	
Type of information (critical, high/low weight, supporting, additional): Low weight; additional information as non-European soils were investigated.	
Reliability	Medium
Endpoint	K_f, 1/n
Protocol	Similar to OECD 106, non-GLP
Test compound	Glyphosate, CAS-no.: 1071-83-6; no further information on purity
Test system and conditions	Batch equilibrium experiments, 24 h shaking, room temperature
Statistical design	7 concentrations, duplicate measurements, 2 soils, adsorption isotherms of phosphate were fitted to the Langmuir model
Relevance	
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	Other reliable studies support the results, no negative evidence.

da Cruz et al. (2007)

Title: Adsorption of Glyphosate on Clays and Soils from Paraná State: Effect of pH and Competitive Adsorption of Phosphate
Author: Lútecia Hiera da Cruz, Henrique de Santana, Cássia Thaïs Bussamra Vieira Zaia and Dimas Augusto Morozin Zaia
Reference: BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY, Vol. 50 (3), pp. 385-394
Year: 2007
Results and conclusion: This work showed that the adsorption of glyphosate (GPS) depends on surface area for clays and amount of clays and CEC for soils. Organic matter (OM) had a secondary role in the adsorption of GPS on soils. The adsorption of GPS on soils from Londrina and Floraí counties and clays (montmorillonite, kaolinite) decreased when pH increased, however, for bentonite clay and soil from Tibagi county was kept constant. For the soils, the competitive adsorption between GPS and phosphate showed that displace of GPS by phosphate was related to the amount of clays, CEC and pH. GPS was not easily displaced by phosphate on the clays. The FT-IR spectra of the soils and clays showed that soil from Londrina resembled kaolinite. Thus, this could explain the results of adsorption of GPS and the competitive adsorption between GPS and phosphate.
Proposed action: Consider as additional information as the articles presents basic research. The OECD standard protocol is not followed in detail. No raw data are published, and thus the validity of the study cannot be proven.

Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Low
Endpoint	Amount of Glyphosate (µmol) adsorbed to soil
Protocol	Similar to OECD 106, non-GLP but basic research on adsorption processes
Test compound	Glyphosate (95%), CAS-no.: 1071-83-6
Test system and conditions	Small Eppendorf vials, small volumes tested, shaking for 24 h, ninhydrin-test for amount of absorbed Glyphosate, different pH-values; number of replicates not clearly reported.
Statistical design	The ANOVA test and Student-Newman-Keuls test (S-N-K test) were used for the comparisons between means at a significance level of $p<0.05$
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Positive relevance not clear; no negative relevance.

Damonte et al. (2007)

Title: Some aspects of the glyphosate adsorption on montmorillonite and its calcined form	
Author: Marina Damonte, Rosa M. Torres Sánchez, María dos Santos Afonso	
Reference: Applied Clay Science 36, 86–94	
Year: 2007	
Results and conclusion: Influences of pH and ionic strength on the aggregation of montmorillonite particles in the presence or absence of glyphosate (PMG) were studied. Adsorption isotherms and X-ray diffraction indicated that ligand exchange is the main mechanism of PMG adsorption. The surface coverage increased with the ionic strength and was more noticeable at high PMG concentration indicating inner-sphere surface complexation. At low PMG concentration the inner-sphere surface complexes are located of the external clay mineral surface while at high PMG concentration the surface complexes are also formed in the interlayer space.	
Proposed action: Not to be considered as no data are published but isotherms are reported graphically only. Thus, the validity and quality of data cannot be proven.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information, basic research on influences of environmental parameter on sorption processes	
Reliability	Low
Endpoint	Sorption isotherms, no numbers given but graphical reporting
Protocol	No standard protocol, non-GLP
Test compound	Glyphosate (purity not given), CAS-no.: 1071-83-6
Test system and conditions	Sorption isotherms measured using batch method and under modification of ionic strength and pH-values.
Statistical design	Number of replicates, and of concentrations used for isotherm determination not reported
Relevance	
Environmental relevance	Environmental parameter modified during the project, thus environmental relevance given.

Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence, no negative evidence.

de Jonge et al. (2001)

Title: GLYPHOSATE SORPTION IN SOILS OF DIFFERENT PH AND PHOSPHORUS CONTENT

Author: H. de Jonge, L.W. de Jonge, O.H. Jacobsen, T. Yamaguchi, and P. Moldrup

Reference: Soil Science; 166, 230–238

Year: 2001

Results and conclusion:

This study quantifies the variation in glyphosate sorption and desorption to a coarse sandy soil and to a sandy loam soil with varying phosphorus content and pH. Using batch experiments, glyphosate adsorption and desorption isotherms were determined on soil samples taken from long-term field experiments that received different additions of phosphorus and lime. The isotherms were best fitted with an extended Freundlich model. The phosphate content in the soils had a significant influence on the sorption of glyphosate. With 0.5 M bicarbonate extractable P (pH 8.5) increasing from 6.2 to 58.7 in the loamy sand and 9.1 to 87.4 in the coarse sand, the extended Freundlich adsorption coefficient (K_f, MF, ads) decreased from 214.7 to 106 and from 154.0 to 83.5, respectively.

Detailed results are:

Soil characterisation:

Soil [1)]	Soil type	Sand (%)	Clay (%)	C.E.C. (in meq/100g)
St. Jyndevad A	Coarse sand	89.6	4.2	
St. Jyndevad B	Coarse sand	89.6	4.2	
St. Jyndevad C	Coarse sand	89.6	4.2	
St. Jyndevad D	Coarse sand	89.6	4.2	
St. Jyndevad E	Coarse sand	89.6	4.2	
St. Jyndevad F	Coarse sand	89.6	4.2	
St. Jyndevad G	Coarse sand	89.6	4.2	
St. Jyndevad H	Coarse sand	89.6	4.2	
St. Jyndevad I	Coarse sand	89.6	4.2	
St. Jyndevad J	Coarse sand	89.6	4.2	
St. Jyndevad K	Coarse sand	89.6	4.2	
St. Jyndevad L	Coarse sand	89.6	4.2	
St. Jyndevad M	Coarse sand	89.6	4.2	
St. Jyndevad N	Coarse sand	89.6	4.2	
St. Jyndevad O	Coarse sand	89.6	4.2	
St. Jyndevad P	Coarse sand	89.6	4.2	
Askov A	Sandy loam	71.0	10.8	
Askov B	Sandy loam	71.0	10.8	
Askov C	Sandy loam	71.0	10.8	
Askov D	Sandy loam	71.0	10.8	
Askov E	Sandy loam	71.0	10.8	
Askov F	Sandy loam	71.0	10.8	
Askov G	Sandy loam	71.0	10.8	

[1)] Soils were from Danish long-term field experiments with varying phosphate additions. There differentiated herein as A, B, C, etc.. Jynvard soil: 0 – 15.6 kg P /ha y; Askov soil: 0 – 57 kg P /ha y

Adsorption

Soil type	OC %	pH ($CaCl_2$)	K_d	K_{oc}	$K_{f.\ MF.\ ads}$	N_{ads}	D_{ads}
St. Jyndevad A	1.32	3.7			107.4	0.61	0.07
St. Jyndevad B	1.06	3.6			80.6	0.62	0.07
St. Jyndevad C	1.28	3.6			83.5	0.66	0.07

St. Jyndevad D	1.06	3.8			79.4	0.67	0.08
St. Jyndevad E	1.2	4.2			121.2	0.60	0.06
St. Jyndevad F	1.33	4.3			141.2	0.63	0.07
St. Jyndevad G	1.3	4.2			118.1	0.66	0.08
St. Jyndevad H	1.4	4.3			111.5	0.61	0.06
St. Jyndevad I	1.26	4.5			126.8	0.56	0.06
St. Jyndevad J	1.26	4.7			120.0	0.57	0.05
St. Jyndevad K	1.21	4.6			92.0	0.59	0.04
St. Jyndevad L	1.36	4.9			116.2	0.60	0.05
St. Jyndevad M	1.33	5.2			154.0	0.61	0.07
St. Jyndevad N	1.14	5.5			138.8	0.65	0.07
St. Jyndevad O	1.29	5.4			136.6	0.62	0.06
St. Jyndevad P	1.2	5.5			119.8	0.62	0.05
Askov A	1.28	6.2			214.7	0.55	0.06
Askov B	1.26	6.2			165.1	0.60	0.05
Askov C	1.25	6.3			137.6	0.67	0.07
Askov D	1.23	6.4			106.4	0.70	0.06
Askov E	1.21	6.3			171.7	0.57	0.05
Askov F	1.40	6.3			144.0	0.59	0.03
Askov G	1.44	6.3			151.3	0.65	0.08
Desorption							
Soil type	OC %	pH	Desorption				
		($CaCl_2$)	K_d	K_{oc}	$K_{f.\ MF.\ des}$	N_{des}	D_{des}
St. Jyndevad A	1.32	3.7			364.9	0.17	0.06
St. Jyndevad B	1.06	3.6			246.7	0.28	0.12
St. Jyndevad C	1.28	3.6			312.0	0.18	0.06
St. Jyndevad D	1.06	3.8			286.3	0.21	0.09
St. Jyndevad E	1.2	4.2			415.1	0.17	0.08
St. Jyndevad F	1.33	4.3			458.0	0.18	0.09
St. Jyndevad G	1.3	4.2			404.5	0.20	0.09
St. Jyndevad H	1.4	4.3			367.6	0.22	0.10
St. Jyndevad I	1.26	4.5			367.8	0.21	0.10
St. Jyndevad J	1.26	4.7			361.1	0.23	0.10
St. Jyndevad K	1.21	4.6			290.3	0.25	0.10
St. Jyndevad L	1.36	4.9			355.0	0.23	0.10
St. Jyndevad M	1.33	5.2			436.7	0.23	0.09
St. Jyndevad N	1.14	5.5			418.3	0.24	0.09
St. Jyndevad O	1.29	5.4			409.2	0.27	0.10
St. Jyndevad P	1.2	5.5			317.1	0.27	0.10
Askov A	1.28	6.2			453.7	0.33	0.15
Askov B	1.26	6.2			394.0	0.25	0.03
Askov C	1.25	6.3			239.7	0.65	0.18
Askov D	1.23	6.4			240.6	0.52	0.13
Askov E	1.21	6.3			383.9	0.35	0.13
Askov F	1.40	6.3			362.0	0.34	0.11
Askov G	1.44	6.3			465.2	0.11	-0.08

MF = modified Freundlich; N, D = shape-governing parameters

Proposed action:
To be considered as supportive information. Adsorption is not described according to OECD 106 but by an empirical modified Freundlich-model. Thus, results are not completely comparable to those obtained by following OECD 106.

Type of information (critical, high/low weight, supporting, additional):
Low weight, supporting information, basic research on sorption competitive processes

Reliability	High
Endpoint	K_f, 1/n, R^2
Protocol	Comparable to OECD 106, non-GLP
Test compound	Glyphosate (labelled and un-labelled, purity not reported), CAS-no.: 1071-83-6

Test system and conditions	Similar to OECD 106
Statistical design	Two soils, 5 concentrations, triplicate measurements, Langmuir-isotherms, Freundlich-isotherms, modified Freundlich-isotherms
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence as results are supported by similar publications; no negative evidence.

de Miranda Colombo and Masini (2011)

Title: Developing a fluorimetric sequential injection methodology to study adsorption/ desorption of glyphosate on soil and sediment samples	
Author: Sandro de Miranda Colombo, Jorge C. Masini	
Reference: Microchemical Journal 98, 260-266	
Year: 2011	
Results and conclusion: This paper describes the development of a sequential injection method to automate the fluorimetric determination of glyphosate. The method was applied to study adsorption /desorption properties in a soil and in a sediment sample. Adsorption and desorption isotherms were properly fitted by Freundlich and Langmuir equations, leading to adsorption capacities of 1384±26 and 295±30 mg kg^{-1} for the soil and sediment samples, respectively. These values are consistent with the literature, with the larger adsorption capacity of the soil being explained by its larger content of clay minerals, while the sediment was predominantly sandy.	
Proposed action: To be considered as additional information as the study focused on the development of an analytical methodology but not on the standard testing of Glyphosate.	
Type of information (critical, high/low weight, supporting, additional): Low weight, supportive information because results fit into known data.	
Reliability	Low
Endpoint	K_f, 1/n, R^2
Protocol	Similar to OECD 106, however, focus of the study is to develop an analytical methodology
Test compound	Glyphosate (no further information on purity), CAS-no.: 1071-83-6
Test system and conditions	1 soil, 1 sediment tested, 7 Glyphosate concentrations, duplicates
Statistical design	Freundlich equation applied
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence, information supported by other reliable literature data.

de Santana et al. (2006)

Title: Effect in glyphosate adsorption on clays and soils heated and characterization by

FT–IR spectroscopy	
Author: Henrique de Santana, Luís R.M. Toni, Luís O. de B. Benetoli, Cássia T.B.V. Zaia, Maurilio Rosa Jr., Dimas A.M. Zaia	
Reference: Geoderma 136(3-4): 738-750. doi:10.1016/j.geoderma.2006.05.012	
Year: 2006	
Results and conclusion: The main achievement of this paper is the determination of mineral structural changes in soils from three different sites of Paraná State, Brazil caused by heating and their effect on glyphosate (GPS) adsorption. Changes in soil structure due to heating probably play an important role in GPS adsorption. The non-adsorption of GPS on soil after burning could be a problem, as the un-adsorbed GPS could either leach to groundwater or decrease the productivity of some crops.	
Proposed action: Consider as additional information as basic research is published. Data quality and quantity are not sufficient, and raw data are not comprehensively reported. Thus, their use for end-point and PEC-assessment is not possible.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because basic research is reported, influence of environmental parameter on sorption processes given, no K_f-values reported	
Reliability	Low
Endpoint	FT-IR-spectra
Protocol	Similar to OECD 106, non-GLP
Test compound	Glyphosate (analytical grade), CAS-no.: 1071-83-6
Test system and conditions	Similar to OECD 106, batch experiments at different temperatures (room temperature, 280°C, 650°C)
Statistical design	5 concentrations, single measurements only. ANOVA test and Student–Newman–Keuls test at a significance level of p =0.05 for comparison of results at different temperatures
Relevance	
Environmental relevance	Given as the influence of environmental parameter is measured.
Weight of evidence	
“Positive”/”Negative” evidence	Results supported by other publications; no negative evidence.

Dideriksen and Stipp (2003)

Title: The adsorption of glyphosate and phosphate to goethite: A molecular-scale atomic force microscopy study
Author: K. DIDERIKSEN and S. L. S. STIPP
Reference: Geochimica et Cosmochimica Acta, Vol. 67, No. 18, pp. 3313–3327
Year: 2003
Results and conclusion: The adsorption of glyphosate and phosphate to the goethite {010} surface (Pbnm notation) was studied using an atomic force microscope (AFM). The microscope was capable of producing molecular scale images of surfaces exposed to glyphosate, phosphate and nitric acid. The relative maximum adsorption density of phosphate and glyphosate on the {010} surface expected from the AFM data was in agreement with that determined with X-ray photoelectron spectroscopy (XPS).
Proposed action:

Consider as additional information since no OECD standard protocol was followed but the article focuses on basic research.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information on sorption processes, basic research	
Reliability	Medium
Endpoint	Sorption analysed using AFM
Protocol	No standard protocol, FT-IR-analysis of surface, no batch experiments, non-GLP
Test compound	Glyphosate (analytical grade), CAS-no.: 1071-83-6
Test system and conditions	No batch experiments but surface analysis using atomic force microscope.
Statistical design	Fourier transformation analysis
Relevance	
Environmental relevance	Given. The influence of environmental parameter was investigated.
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence, no negative evidence.

Dion et al. (2001)

Title: Competitive sorption between glyphosate and inorganic phosphate on clay minerals and low organic matter soils	
Author: H. M. Dion, J. B. Harsh, H. H. Hill Jr.	
Reference: Journal of Radioanalytical and Nuclear Chemistry, Vol. 249, No , 385–390	
Year: 2001	
Results and conclusion: Inorganic phosphate may influence the adsorption of glyphosate to soil surface sites. It has been postulated that glyphosate sorption is dominated by the phosphoric acid moiety; therefore, inorganic phosphate could compete with glyphosate for surface sorption sites. We examine sorption of glyphosate in low organic carbon systems where clay minerals dominate the available adsorption sites using ^{32}P-labeled phosphate and ^{14}C-labeled glyphosate to track sorption. We found glyphosate sorption strongly dependent on phosphate additions. Isotherms were generally of the L type, which is consistent with a limited number of surface sites. Most sorption on whole soils could be accounted for by sorption observed on model clays of the same mineral type as found in the soils.	
Proposed action: Consider as additional information since no OECD standard protocol was followed but the article focuses on basic research.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as focus was on adsorption influenced by inorganic phosphate	
Reliability	Medium
Endpoint	K-values obtained by Langmuir equation, 1/n
Protocol	Partly similar to OECD 106, non-GLP
Test compound	Unlabeled and ^{14}C-labeled glyphosate (98.7% purity), CAS-no.: 1071-83-6
Test system and conditions	Batch experiments, 3 soils and clay minerals
Statistical design	Langmuir-equation
Relevance	

Environmental relevance	Given as influence by environmental parameter was tested.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Farenhorst et al. (2009)

Title: Variations in soil properties and herbicide sorption coefficients with depth in relation to PRZM (pesticide root zone model) calculations	
Author: A. Farenhorst, D.A.R. McQueen, I. Saiyed, C. Hilderbrand, S. Li, D.A. Lobb, P. Messing, T.E. Schumacher, S.K. Papiernik, M.J. Lindstrom	
Reference: http://dx.doi.org/10.1016/j.geoderma.2009.02.002	
Year: 2009	
Results and conclusion: Soil profiles were obtained from three landform elements in a strongly eroded agricultural field and segmented. Soil samples were analyzed for glyphosate sorption by soil (K_d, K_{oc}). Considering all soil profiles, glyphosate K_d values ranged from 19 to 547 L kg^{-1} and were predominantly controlled by variations in soil pH and clay content. PRZM predicted that glyphosate would be immobile in soils even under an extreme rainfall scenario of 384 mm at one day after herbicide application. PRZM output was particularly sensitive to input values of K_d, relative to input values of soil properties. We conclude that, when pesticide fate models such as PRZM are being used in policy analyses at larger-scales, data on K_d values in different landform elements and at the soil horizon level could be important for strengthening pesticide leaching predictions.	
Proposed action: Consider as additional information since the publication focuses on a comparison of experimental and modelling results. Raw data on the experimental part are not sufficiently comprehensive for their use for endpoint and PEC-assessment.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information; PRZM-modelling results are additional as PRZM version 3.12.2 is not used in the EU for pesticide registration.	
Reliability	Low for sorption; medium for modelling
Endpoint	K_d-values, PRZM-modelling output
Protocol	Non-GLP, batch experiments comparable to tier II OECD 106
Test compound	Glyphosate, purity not given, CAS-no.: 1071-83-6
Test system and conditions	Batch experiments similar to OECD 106, 24 h, room temperature
Statistical design	Duplicate measurements, soils from 3 landscapes, depth dependent soil sampling resulting in 90 individual samples. PRZM (pesticide root zone model, version 3.12.2)
Relevance	
Environmental relevance	Given; in depth analyses of subsoil layers.
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence, is supported by other publications; no negative evidence.

Farenhorst et al. (2008)

Title: Herbicide Sorption Coefficients in Relation to Soil Properties and Terrain Attributes

on a Cultivated Prairie	
Author: A. Farenhorst, S. K. Papiernik,I. Saiyed, P. Messing, and K. D. Stephens, J. A. Schumacher, D. A. Lobb and S. Li, M. J. Lindstrom, T. E. Schumacher	
Reference: J. Environ. Qual. 37:1201–1208	
Year: 2008	
Results and conclusion: The sorption of glyphosate in soil was quantified for 287 surface soils (0–15 cm) collected in a 10 × 10 m grid across a heavily eroded, undulating, calcareous prairie landscape. Other variables that were determined included soil carbonate content, soil pH, soil organic carbon content (SOC), soil texture, soil loss or gain by tillage and water erosion, and selected terrain attributes and landform segments. Regression equations were generated to estimate herbicide sorption in soils. The variation of glyphosate sorption across the field (upper slope: K_{oc}=11182; mid-slope: K_{oc}=14863; lower slope: K_{oc}=10891) was not much dependent on our measured soil properties and calculated terrain attributes. We conclude that the integration of terrain attributes or landform segments in pesticide fate modelling is of not much advantage for herbicides such as glyphosate that are strongly bound to soil regardless of soil properties.	
Proposed action: Not to be considered as soils outside the EU are used.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information on pesticide fate modelling; K_{oc}-values are in the range of those given in the dossier.	
Reliability	Medium
Endpoint	Pesticide leaching (by modelling), K_d-values
Protocol	Batch experiments comparable to tier II OECD 106
Test compound	Unlabeled Glyphosate (99%) and ^{14}C-labeled Glyphosate (95%); CAS-no.: 1071-83-6
Test system and conditions	Batch equilibrium analysis, 24 h, and room temperature.
Statistical design	287 individual samples; duplicate measurements per soil sample. Assessment of effects of landscape segments by SPSS version 13.0 (2004, SPSS Inc.), Sigma Stat version 2.03 (1992–1997, SPSS Inc.), or SAS version 8.01 (2000, SAS Inst.). Nonparametric Kruskal Wallis ANOVA.
Relevance	
Environmental relevance	Given; influencing endpoints analysed and considered adequately
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence; no negative evidence.

Ghafoor et al. (2012)

Title: Modelling pesticide sorption in the surface and subsurface soils of an agricultural catchment
Author: A. Ghafoor, N. J. Jarvis and J. Stenström
Reference: Published online in Wiley Online Library: 21 December 2012, (wileyonlinelibrary.com) DOI 10.1002/ps.3453
Year: 2012
Results and conclusion: BACKGROUND: Sorption models that improve upon the k_{oc} concept are urgently needed

for reliable spatial modelling of pesticide leaching. Sorption of glyphosate, bentazone and isoproturon was measured in surface and subsurface soils to test an 'extended' partitioning model that also accounts for inorganic sorbents and pH. Best-subset regression and Akaike information criteria were used to justify the inclusion of predictors and identify suitable models. RESULTS: The extended partitioning model improved upon the k_{oc} concept for all three compounds: inorganic sorbents dominated sorption in subsurface soils, and their effects were only masked by organic matter in surface soils with organic carbon contents larger than ca 2%. Interactions between organic and inorganic sorbents affected glyphosate sorption, but apparently not that of bentazone or isoproturon. CONCLUSION: Information on clay, iron and aluminium oxides and soil pH, in addition to organic carbon, is needed for accurate prediction of pesticide leaching. The variables f_{oc}, f_{clay} and pH are generally available, whereas measurements of oxides of Al and Fe are rarely reported. The authors therefore emphasise the need to measure and report contents of oxides of Al and Fe in soil survey databases, because small variations in their concentrations may contribute significantly to large variations in sorption, especially of ionisable pesticides.	
Proposed action: To be considered as supportive information.	
Type of information (critical, high/low weight, supporting, additional): Supporting information, basic research on sorption	
Reliability	
Endpoint	Freundlich sorption parameters, the variation in k_{oc}, relationships between soil physicochemical properties and pesticide sorption and modelling results
Protocol	Non-GLP
Test compound	Unlabelled isoproturon {N,N-dimethyl-N'-[4-(1-methylethyl)-phenyl]urea; 99% purity}, bentazone [3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide; 97% purity] and glyphosate [N-(phosphomethyl)glycine, 98% purity] were used.
Test system and conditions	The study was carried out in the E21 monitoring catchment in Östergötland, southern Sweden. The total catchment area of 13 km^2 consists of 95% agricultural land, with main crops of winter and spring sown cereals, rape, potatoes and peas. The soils, which are derived from glacial and post-glacial fluvial sediments and glacial till (moraine), have a wide range of texture, from loamy sand to clay. Soil samples were collected from 60 locations in the catchment (one location every 20 ha) on a grid pattern. Five soil samples from each location and depth were taken in the surface 0–20 cm, 20–45 cm and 45–70 cm, bulked, homogenised by passing through a 2mm sieve, put into plastic bags and stored at 4° C until use. Sorption of the three test compounds was measured in topsoil samples at 16 of these locations, selected to cover the range of measured textures, organic matter contents and pH values. Sorption was also measured in samples taken from the two subsoil layers 20–45 and 45–70 cm at five of these 16 locations. Soil pH was measured on fresh samples after shaking the samples in deionised water (1:2.5) at room temperature. Particle size distributions were evaluated using the standard pipette method. Total organic C and N were measured using a Leco

	CN 2000 instrument (LECO Corp., St Joseph, MI). Ammonium lactate extractable phosphorus (PAL) was measured. Oxides of aluminium and iron (Al_{ox} and Fe_{ox}) were determined in oxalate extracts of soils by ICS-AAS. Adsorption experiments were carried out according to the OECD 106 guideline. The sorption measurements were fitted to the Freundlich equation using non-linear regression (the nls procedure in the R software package).
Statistical design	Two replicates according to OECD 106 guideline, Freundlich equation using non-linear regression
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Gimsing and Borggaard (2002)

Title: Competitive adsorption and desorption of glyphosate and phosphate on clay silicates and oxides	
Author: A. L. GIMSING AND O. K. BORGGAARD	
Reference: Clay Minerals, 37, 509–515	
Year: 2002	
Results and conclusion: Competitive adsorption of glyphosate and phosphate on goethite and gibbsite and on illite, montmorillonite and two kaolinites differing in surface area was evaluated. The results show that glyphosate and phosphate are competing for the adsorption sites, but the degree of competition depends on the adsorbent. On goethite the competition is very much in favour of phosphate, on gibbsite the competition is closer, but still phosphate is favoured, while on illite, montmorillonite and kaolinite the competition is almost equal. The amounts of glyphosate and phosphate, which can be adsorbed also depends on the adsorbent: the oxides adsorb more than the clay silicates. The amount adsorbed on kaolinite was dependent on the specific surface area. Changes in the surface area did not affect the competition between glyphosate and phosphate for adsorption sites. The results indicate that differences among soils of different mineralogical composition regarding the adsorption of glyphosate and phosphate can be expected	
Proposed action: Consider as additional information as data quality is low, no standard OECD protocol is followed and the validity of the study cannot be proven.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information on sorption processes	
Reliability	Low
Endpoint	Amount of absorbed as function of time
Protocol	No OECD guideline followed; non-GLP
Test compound	Glyphosate purified from a glyphosate concentrate (purity?) and ^{14}C-Glyphosate (purity?); CAS-no.: 1071-83-6
Test system and conditions	Various minerals used to which Glyphosate was added, stirred over a certain time period, filtered, Glyphosate concentration in filtrate determined (sorption kinetics)
Statistical design	Experiments in triplicate

Relevance	
Environmental relevance	Given as the influence of environmental parameters on sorption were investigated.
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence, no negative evidence.

Gimsing and Borggaard (2002)

Title: EFFECT OF PHOSPHATE ON THE ADSORPTION OF GLYPHOSATE ON SOILS, CLAY MINERALS AND OXIDES	
Author: ANNE LOUISE GIMSING and OLE K. BORGGAARD	
Reference: Intern. J. Environ. Anal. Chem., Vol. 82, No. 8–9, pp. 545–552	
Year: 2002	
Results and conclusion: The effect of phosphate (ortho-phosphate) on the adsorption of the widely used glyphosate herbicide was evaluated with three typical Danish agricultural soils as well as pure oxides (goethite, FeOOH and gibbsite, $Al(OH)_3$ and silicates (illite and montmorillonite), which are considered the most important glyphosate and phosphate adsorbents in soils. All experiments showed competition between phosphate and glyphosate for adsorption sites but the various adsorbents exhibited great variation in affinity for glyphosate and phosphate. The current studies showed that the competition in soils is almost equal, but still phosphate affects the sorption of glyphosate in soil. The amount of glyphosate and phosphate adsorbed by the various kinds of adsorbents was found to decrease in the order: oxides>silicates>soils. For the soils tested aluminium oxides, and to a lesser extent iron oxides seem the most important components in determining a soil's ability to absorb phosphate and glyphosate, whereas the clay content and clay type seem of minor or little importance for adsorption of these species.	
Proposed action: Consider as additional information as the article focuses on basic research. No information on isotherms is given. Data quality and quantity is not sufficient for their use in endpoint and PEC-assessment.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because basic research is published giving more in depth insight into sorption mechanisms	
Reliability	Medium
Endpoint	K_d-values
Protocol	Close to OECD 106, non-GLP
Test compound	Unlabeled Glyphosate (99–100 % pure), ^{14}C-labeled Glyphosate (purity?); CAS-no.: 1071-83-6
Test system and conditions	Batch experiments, kinetics, room temperature
Statistical design	Triplicate measurements, 3 soils, comparison of % sorption between phosphate and Glyphosate
Relevance	
Environmental relevance	Given as influence of environmental parameter such as pH, soil parameter etc. on sorption was measured.
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other publications; no negative evidence

Gimsing et al. (2004)

Title: Influence of soil composition on adsorption of glyphosate and phosphate by contrasting Danish surface soils	
Author: A. L. GIMSING, O. K. BORGGAARD & M. BANG	
Reference: European Journal of Soil Science, 55, 183–191	
Year: 2004	
Results and conclusion: Adsorption of phosphate and glyphosate to five contrasting Danish surface soils was investigated by batch adsorption experiments. The different soils adsorbed different amounts of glyphosate and phosphate, and there was some competition between glyphosate and phosphate for adsorption sites, but the adsorption of glyphosate and phosphate seemed to be both competitive and additive. The competition was, however, less pronounced than found for goethite and gibbsite in an earlier study. The soil's pH seemed to be the only important factor in determining the amount of glyphosate and phosphate that could be absorbed by the soils; consequently, glyphosate and phosphate adsorption by the soils was well predicted by pH, though predictions were somewhat improved by incorporation of oxalate-extractable iron. Other soil factors such as organic carbon, the clay content and the mineralogy of the clay fraction had no effect on glyphosate and phosphate adsorption. The effect of pH on the adsorption of glyphosate and phosphate in one of the soils was further investigated by batch experiments with pH adjusted to 6, 7 and 8. These experiments showed that pH strongly influenced the adsorption of glyphosate. A decrease in pH resulted in increasing glyphosate adsorption, while pH had only a small effect on phosphate adsorption	
Proposed action: Consider as additional information as the article focuses on influence of soil parameter on sorption. Quality of data is not sufficiently high for their use for endpoint and PEC-assessment. The validity of the study cannot be proven.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information only since basic research on competitive mechanisms is published.	
Reliability	Medium
Endpoint	K_d-values; correlation coefficients between glyphosate (Gly_{ads}) and phosphate adsorption (P_{ads}) and soil factors
Protocol	In analogy to OECD 106, non-GLP
Test compound	Un-labelled Glyphosate (99-100% purity); ^{14}C-labeled Glyphosate (purity ?); CAS-no.: 1071-83-6
Test system and conditions	5 soils tested, batch experiment, sorption kinetics; concentration of radioactivity in the supernatant measured
Statistical design	5 soils, triplicate measurements, correlations determined
Relevance	
Environmental relevance	Given as influence of environmental parameter such as pH, soil parameter etc. on sorption was measured.
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other publications; no negative evidence.

Gimsing et al. (2004)

Title: Modelling the Kinetics of the Competitive Adsorption and Desorption of Glyphosate and Phosphate on Goethite and Gibbsite and in Soils
Author: Anne Louise Gimsing, Ole Borggaard, Peter Sestoft
Reference: Environ. Sci. Technol. 38, 1718-1722

Year: 2004	
Results and conclusion: Kinetics of the Competitive Adsorption and Desorption of Glyphosate and Phosphate on Goethite and Gibbsite and in Soils: We present and evaluate six simple, kinetic models that only take time and concentrations into account. Three of the models were found suitable to describe the competition in soil. These three models all assumed both competitive and additive adsorption, but with different equations used to describe the adsorption. For the oxides, three additional models assuming only competitive adsorption were also found suitable. This is in accordance with the observation that the adsorption in soil is both competitive and additive, whereas the adsorption on oxides is competitive. All models can be incorporated in transport models such as the convection-dispersion equation.	
Proposed action: Consider as additional information as the article focuses on influence of soil parameter on sorption and their modelling. Quality of data is not sufficiently high for their use for endpoint and PEC-assessment.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because basic research on competitive mechanisms are published.	
Reliability	Medium
Endpoint	Glyphosate concentrations in $CaCl_2$-solution at various time points after start of experiment as related to applied (% adsorbed)
Protocol	In analogy to OECD 106, non-GLP
Test compound	Un-labelled Glyphosate (99-100% purity); ^{14}C-labeled Glyphosate (purity ?); CAS-no.: 1071-83-6
Test system and conditions	5 soils tested, batch experiment, sorption kinetics; concentration of radioactivity in the supernatant measured
Statistical design	5 soils, triplicate measurements, 2 scenarios modelled: a) phosphate added, equilibrium between sorbed and dissolved phosphate, thereafter Glyphosate added; b) Glyphosate added, equilibrium between sorbed and dissolved Glyphosate, thereafter Phosphate added
Relevance	
Environmental relevance	Low weight, additional information because basic research on competitive mechanisms are published.
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other publications; no negative evidence.

Gimsing et al. (2007)

Title: Sorption of glyphosate and phosphate by variable-charge tropical soils from Tanzania
Author: A.L. Gimsing, C. Szilas, O.K. Borggaard
Reference: Geoderma 138, 127–132
Year: 2007
Results and conclusion: Sorption of glyphosate and phosphate by four contrasting soils from Tanzania, an Andisol (Sasanda), two Oxisols (Lubonde, Mlingano) and an Ultisol (Nkundi), with variable-charge clay minerals was studied by batch sorption experiments, during which glyphosate and phosphate were added separately as well as together (competitive sorption). Agreement was found between glyphosate and phosphate sorption and between sorbed glypho-

sate/phosphate and contents of aluminium and iron extractable by oxalate and dithionite-citrate-bicarbonate (oxides and allophane/ imogolite). The Langmuir sorption maxima of glyphosate ranged from 15.5 mmol kg^{-1} (Nkundi) to 126 mmol kg^{-1} (Sasanda), while that of phosphate varied from 5.8 mmol kg^{-1} (Nkundi) to 78.5 mmol kg^{-1} (Sasanda). Additive as well as competitive sorption can dominate the reaction of variable-charge soils with glyphosate and phosphate.	
Proposed action: Not to be considered as soils outside the EU are used.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information only since basic research on competitive mechanisms is published.	
Reliability	High
Endpoint	Glyphosate and phosphate sorption isotherms, sorption maxima and affinities by means of the Langmuir equation
Protocol	Close to OECD 106, non-GLP
Test compound	Un-labeled Glyphosate (99-100% purity); ^{14}C-labeled glyphosate (purity ?); CAS-no.: 1071-83-6
Test system and conditions	5 soils tested, batch experiment, glyphosate concentrations in supernatant measured, sorption isotherms calculated
Statistical design	4 soils, number of Glyphosate concentrations not given, triplicate measurements, sorption data fitted to Langmuir equation, sorption maxima and affinities calculated using a least squares non-linear method
Relevance	
Environmental relevance	Given as influence of environmental parameter on sorption was measured.
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other publications; no negative evidence.

Jacobsen et al. (2008)

Title: Variation of MCPA, metribuzine, methyltriazine-amine and glyphosate degradation, sorption, mineralization and leaching in different soil horizons
Author: Carsten S. Jacobsen, Peter van der Keur, Bo V. Iversen d, Per Rosenberg, Heidi C. Barlebo c, Søren Torp d, Henrik Vosgerau e, Rene´ K. Juhler a, Vibeke Ernstsen, Jim Rasmussen, Ulla Catrine Brinch, Ole Hørbye Jacobsen
Reference: Environmental Pollution 156 (2008) 794–802
Year: 2008
Results and conclusion: Glyphosate (K_d values determined in the range of 200 L/kg to 4000 L/kg) does not follow the simple rule that increased organic matter leads to increased sorption. The two most important component determining glyphosate sorption in the A-horizon is gravel and organic matter (the latter being negative). Glyphosate was often higher in the inorganic subsoil samples compared to the A-horizon samples. No calculated DT_{50} values were provided. Detailed results on sorption are:

Soil characterisation:

Soil	Soil type	Sand (%) (fine + coarse)	Clay (%)	C.E.C. (in meq/100g)
Nedre Julianhede		69.7	3.8	
Nörlund		77.9	3.1	

Stubkaer			54.6	3.8			
Söbjerg			76.0	4.2			
Ruskaer			84.9	3.5			
Ilskov			83.3	4.4			
Skaaphusgaard			83.7	3.9			
Roejen Mosegard			80.1	3.4			
Röjen Kaer			82.0	3.3			
Röjen			79.8	3.3			
Sneptrup			86.3	3.0			
Simmelkjaer			88.9	3.4			
Neder Simmelkjaer			88.2	3.6			
Ommose			85.6	3.5			
Hallundbaek			86.8	3.8			
Adsorption							
Soil type	OC %	pH ($CaCl_2$)	K_d	K_{oc}	K_f	K_{foc}	1/n
Nedre Julianhede	2.8	4.9	867				
Nörlund	2.1	5.3	237				
Stubkaer	4.8	5.8	1858				
Söbjerg	1.8	6.3	871				
Ruskaer	4.1	4.2	3758				
Ilskov	3.9	5.2	342				
Skaaphusgaard	2.6	5.1	n.a.				
Roejen Mosegard	6.4	5.6	108				
Röjen Kaer	2.3	4.9	690				
Röjen	2.7	4.7	656				
Sneptrup	2.2	4.5	400				
Simmelkjaer	1.8	4.1	586				
Neder Simmelkjaer	2.6	5.2	366				
Ommose	4.3	4.6	551				
Hallundbaek	1.6	5.5	257				

Proposed action:
Not to be considered further as the authors only provided K_d-values

Type of information (critical, high/low weight, supporting, additional):
Supporting information

Reliability	
Endpoint	Sorption of glyphosate in top soil and sub soil
Protocol	Non-GLP studies modified OECD 106 and OECD 307
Test compound	CAS 38641-94-0 ^{14}C-glyphosate (CAS 1071-83-6)
Test system and conditions	Sampling was performed at 15 locations placed on a 28 km long transect of the Karup outwash plain in northwest Jutland, Denmark OC content and particle size distribution were determined for the A, B and C horizons. A sample to solution ratio of 1:10 was used for glyphosate because this herbicide is highly adsorbed. The flasks were incubated on an orbital shaker at 10 °C for 96 h. Mineralization experiments were performed by adding ^{14}C-labelled pesticides in a total concentration of 1.0 mg pesticide kg^{-1} (dry weight) soil and incubating at 10 °C in the dark.
Statistical design	Three replicates
Relevance	
Environmental relevance	The experiments are principally relevant. Unfortunately, the authors did not calculate DT_{50} for glyphosate.

Weight of evidence	
"Positive"/"Negative" evidence	The sorption studies support the information known for glyphosate from standard tests.

Jia et al. (2011)

Title: Adsorption of Glyphosate on Resin Supported by Hydrated Iron Oxide: Equilibrium and Kinetic Studies	
Author: Dongmei Jia, Chao Zhou, Changhai Li	
Reference: Water Environ. Res., 83, 784	
Year: 2011	
Results and conclusion: Hydrated iron oxide supported on a resin was prepared as a new sorbent for the removal of glyphosate from wastewater. Batch adsorption studies were performed on glyphosate aqueous solutions with different initial glyphosate concentrations and temperatures. Experimental data were analyzed using the Langmuir and Freundlich isotherms, and the adsorption data were best fit to the Langmuir isotherm model. The thermodynamic parameters DG, DH, and DS also were calculated for the adsorption processes. Adsorption rate constants were determined using the pseudo-first-order and pseudosecond-order rate equations and Kannan–Sundaram intraparticle diffusion models. Adsorption of glyphosate clearly followed the pseudo-second-order model and was controlled by both film diffusion and intraparticle diffusion.	
Proposed action: Not to be considered as a sorbent other than soil was used.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because a sorbent other than soil is used	
Reliability	Low
Endpoint	Langmuir and Freundlich isotherms for sorption on hydrated iron oxide supported on resin
Protocol	Similar to OECD 106, but sorbent other than soil, non-GLP
Test compound	Glyphosate (purity 98%, CAS 1071-83-6)
Test system and conditions	Batch experiments, 7 concentrations, 2 different temperatures, 1 sorbent
Statistical design	Langmuir and Freundlich isotherms
Relevance	
Environmental relevance	Given, influence of temperature and iron investigated.
Weight of evidence	
"Positive"/"Negative" evidence	Comparable other studies not known; no negative evidence.

Kah and Brown (2006)

Title: Adsorption of Ionisable Pesticides in Soils
Author: M. Kah and C.D. Brown
Reference: Rev Environ Contam Toxicol 188:149–217
Year: 2006
Results and conclusion: Review article: This review presents the state of knowledge on the adsorption of ionisable pesticides in soils. It first introduces the issues concerning adsorption and the characteristics of this particular kind of chemical. Subsequently, the review focuses on the influence

of soil properties on adsorption and on potential to predict the behaviour of ionisable pesticides in soils. The standardization of experimental settings and the application of approaches specific to a particular class of pesticide or different type of soil might be necessary to describe the complexity of interactions among ionisable molecules.	
Proposed action: Consider as additional information as the article is a review. No raw data are published.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because review article	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate; CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Keshteli et al. (2011)

Title: ADSORPTION BEHAVIOR OF GLYPHOSATE IN SOME CITRUS GARDEN SOILS OF IRAN	
Author: Rafiei Keshteli, M.*, Farahbakhsh, M., Savaghebi, G.R.	
Reference: EJEAFChe, 10 (2), 1943-1951	
Year: 2011	
Results and conclusion: This investigation was performed to study adsorption of glyphosate in six citrus garden soils of north of Iran. The soil samples were thoroughly characterized for their physical and chemical properties, particularly organic matter and iron and aluminum oxides. Both Langmuir and Freundlich isotherms represented the adsorption data well in all cases although Langmuir equation showed a better estimate of glyphosate adsorption. Amounts of Freundlich adsorption coefficient (K_f) are in the range of 42.52-77.46 LKg^{-1} and Langmuir absorption coefficient (K_L) in the range of 0.326-1.089 LKg^{-1}. Maximum absorption coefficient in the soils studied was the soil that had the highest organic carbon content. K_f and K_L had shown significant correlations with soil organic carbon	
Proposed action: Not to be considered as non-European soils were used.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because a non-European soil is used.	
Reliability	High
Endpoint	K_f, 1/n, R^2
Protocol	Batch experiment, OECD 106, non-GLP
Test compound	Glyphosate (96% purity); CAS-no.: 1071-83-6
Test system and conditions	Batch experiments under standard conditions, 2 replicates each, 6 soils, 5 concentrations
Statistical design	Freundlich and Langmuir isotherms
Relevance	

Environmental relevance	Given, influencing parameter adequately considered.
Weight of evidence	
"Positive"/"Negative" evidence	Other reliable studies support the results; no negative evidence.

Khenifi et al. (2010)

Title: Adsorption of Glyphosate and Glufosinate by Ni_2AlNO_3 layered double hydroxide
Author: A. Khenifi, Z. Derriche, C. Mousty, V. Prévot, C. Forano
Reference: Applied Clay Science 47 (2010) 362–371
Year: 2010
Results and conclusion: The removal of organophosphate and organophosphonate herbicides from aqueous solution by Ni_2Al LDH material was investigated. Batch adsorption studies were conducted to evaluate the effect of various parameters such as contact time and initial herbicides concentrations. The adsorption kinetics was tested for Elovich, intraparticle diffusion, pseudo-second-order, and pseudo-first-order reactions and rate constants of kinetic models were calculated. The equilibrium adsorption data were analysed by Freundlich, Langmuir, and Tempkin using linear regression technique. Langmuir isotherms best fitted the data for adsorption equilibrium for both herbicides. Structural and textural analysis (XRD, FTIR, MEB) of Ni_2AlNO_3 LDH at different rates of adsorption evidence a mechanism of adsorption via an anion exchange reaction, Glyphosate and Glufosinate being adsorbed, in a 1rst step, at the surface of the cristallites and then intercalated in the interlayer domains. In detail: Adsorption experiments examining the removal of the anionic pesticide Glyphosate and Glufosinate from aqueous solutions by NiAl-LDH materials indicated two distinguishable adsorption paths, external surface adsorption and interlayer anion exchange. This was confirmed by the structural and textural analysis (XRD, FTIR, MEB) of Ni_2AlNO_3 LDH at different rates of adsorption. Batch kinetic studies performed by LDH system data tended to fit well the second-order model. The intraparticle diffusion was not the only rate-limiting step; the surface adsorption and intraparticle diffusion were concurrently operating during the Glyphosate and Glufosinate interactions. The adsorption isotherms are of H and L types for Gly and Glu respectively and they were well described by Langmuir model. The result indicates an important role of NiAl-LDH materials as potential adsorbents for removal of organophosphate and organophosphonate pollutants from water. Freundlich isotherm constants for the adsorption of Glyphosate and Glufosinate by NiAl-NO_3 LDH: Glyphosate: K = 39.7 mg/g, n = 0.36, R^2= 0.91 Glyphosinate: K = 27.3 mg/g, n = 0.44, R^2= 0.94
Proposed action: Consider as additional information.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.

Reliability	
Endpoint	Adsorption of Glyphosate and Glufosinate by Ni_2AlNO_3 (Freundlich, Langmuir, and Tempkin)
Protocol	Not given
Test compound	Glyphosate and glufosinate (99% purity)
Test system and conditions	Ni2Al–NO3 LDH was prepared by the coprecipitation method under nitrogen atmosphere (in order to minimise the contamination with atmospheric CO_2) and vigorous magnetic stirring. An aqueous

	solution of Ni and Al nitrate with Ni/Al molar ratio equal to 2 and total metal ion concentration of 1 M was added drop-wise to a flask containing 100 mL of deionised water. A solution of sodium hydroxide (2 M) was simultaneously added to fix the pH of co precipitation at 10.0±0.1. The addition of the salt solution was completed in 5 h. The precipitate was washed by three dispersion and centrifugation cycles in deionised water, and finally air-dried. Elemental analyses (Ni, Al, P) were performed by ICP (inductively coupled plasma) emission spectrometry with a Perkin-Elmer Optima 3000XL atomic emission spectrometer. Water contents were determined using a TG-DTA92 thermogravimetric analyzer. The material displays the following chemical composition: $Ni_{2.07}Al(OH)_{6.14}NO_3.1.98H_2O$. Ni_2Al LDH intercalated with Glyphosate (Ni2AlGly) or Glufosinate (Ni2AlGlu) were prepared by coprecipitation method as described above except that an excess of 2.5 times equivalence of Glyphosate or Glufosinate over Al3+ content was initially added into the reactor. Finally, the obtained gelatinous precipitates were washed by three dispersion and centrifugation cycles in deionised water and air-dried. For comparison, anion exchange reactions were also performed on Ni_2AlNO_3 with Glyphosate and Glufosinate, using 1 mg/mL Ni_2AlNO_3 aqueous suspension in 300 mg/L of herbicides at a pH=7.0. Powder X-ray diffraction (PXRD) patterns were obtained with a Siemens D501 X-ray diffractometer using Cu Kα radiation (λ= 1.5415 A°) and fitted with a graphite back-end monochromator. The samples were scanned from 2° to 70° (2θ) using steps of 0.08° and a counting time of 4 s per step. The attenuated total reflectance infrared spectra (ATR-FTIR) were collected on a FTIR Nicolet 5700 (Thermo Electon Corporation) spectrometer equipped with a Smart Orbit accessory. Thermogravimetric analyses (TGA) were recorded on a Setaram TG-DTA92 thermogravimetric analyzer coupled with a mass spectrometry analyzer (Thermostar 300 Balzers Instruments) in the temperature range of 25–1100 °C, with a heating rate of 5 °C/min, under air flow in an alumina crucible. Scanning electron microscopy (SEM) images were obtained on a JEOL 5190 microscope operating at an acceleration voltage of 15 keV. Structural modelization of Glufosinate and Glyphosate molecules were performed using the semiempirical ChemBioDraw Ultra version 11.0 MM2 simulation program. The adsorption isotherms and the kinetic study were measured at 25 °C using the batch equilibrium method in open bottles. Each experiment was repeated at least three times. The suspensions were kept in a vessel with continuous shaking. To get a homogeneous dispersion, the samples were dispersed in 25 mL of deionised/ decarbonated water and stirred for 24 h before the Glyphosate or Glufosinate molecules were added. The pH value was adjusted (pH=7.0) by hydrochloric acid (0.1 M) or NaOH (0.1 M). Different experiments by varying the contact time and the initial herbicide concentration with a final volume of 50 mL were carried out. Fifty milligram of the LDH mass was used for different batch equilibri-

	um experiments. After a contact time of 24 h, the suspensions were centrifuged. The amount of Glyphosate or Glufosinate adsorbed by the LDH (Qe) was determined from the difference between the initial (Ci) and the final equilibrium concentration (Ce) per gram of adsorbent. The amount of Gly and Glu present in the supernatant was measured as elementary phosphorus by ICP. The typical experimental error is lower than 5% for all the experimental results.
Statistical design	Experiments were repeated three times. The equilibrium adsorption data were analysed by Freundlich, Langmuir, and Tempkin using linear regression technique.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Kjær et al. (2011)

Title: Reply to Comments on ''Transport modes and pathways of the strongly sorbing pesticides glyphosate and pendimethalin through structured drained soils'' by Petersen, C.T. and Hansen, S. [Chemosphere 84 (4) (2011) 471–479]	
Author: Jeanne Kjær, Vibeke Ernstsen, Lis Wollesen de Jonge, Preben Olsen	
Reference: Chemosphere 85, 1539–1541	
Year: 2011	
Results and conclusion: We appreciate the opportunity to respond to the comments of Dr. Petersen and Dr. Hansen (Petersen and Hansen, 2011) and to further elaborate on the modes and transport pathways of strongly sorbing pesticides such as glyphosate and pendimethalin. Please find our response to the specific comments of Petersen and Hansen (2011) (marked in Italics) outlined below.	
Proposed action: Not to be considered, no experimental results, but letter to the editor	
Type of information (critical, high/low weight, supporting, additional): Low weight, letter to the editor	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate; CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Kogan Marcelo et al. (2003)

Title: Adsorption of Glyphosate in Chilean soils and its relationship with unoccupied phosphate binding sites	
Author: Kogan Marcelo et al.	
Reference:	
Year: 2003	
Results and conclusion: Glyphosate adsorption by Chilean soils and its relationship with unoccupied binding sites for phosphate adsorption was investigated. Experimental maximum adsorption capacity was 15000, 14300, and 4700 µg/g for the three soils under consideration. Maximum adjusted adsorption capacity with the Langmuir model was 213884, 17874, and 5670 µg/g.	
Proposed action: Not to be considered as non-European soils are tested	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because Chilean soils are investigated	
Reliability	High
Endpoint	K_f, 1/n R^2
Protocol	Close to OECD 106, tier III, non-GLP
Test compound	Glyphosate; CAS-no.: 1071-83-6
Test system and conditions	3 soils, 4 concentrations, batch experiment
Statistical design	Linear, Freundlich, Langmuir
Relevance	
Environmental relevance	Given, parameter assessed adequately.
Weight of evidence	
“Positive”/”Negative” evidence	Other studies support the results; no negative evidence.

Lashermes et al. (2010)

Title: Sorption and mineralization of organic pollutants during different stages of composting
Author: G. Lashermes, S. Houot, E. Barriuso
Reference: Chemosphere 79, 455–462
Year: 2010
Results and conclusion: The potential for compost microflora to degrade organic pollutants (OP), and compost sorption properties, were characterized at different stages of composting. The highest level glyphosate mineralization was found during the thermophilic stage. Glyphosate mineralization was probably linked to total microbial activity. Sorption on compost was linked to hydrophobicity of Glyphosate. Moreover, sorption did not decrease as compost maturity increased. The sorption coefficient was positively correlated to mineralization kinetics parameters, suggesting a positive effect of sorption on increasing mineralization rates.
Proposed action: Not to be considered as the article focuses on basic research and a specific topic. Data are not comparable to other used for endpoint and PEC-assessment.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as such a specific issue is not addressed in the monograph

Reliability	High
Endpoint	K_d-values, concentration in the supernatant after equilibrium; mineralisation (% $^{14}CO_2$-formation)
Protocol	Sorption: close to OECD 106; mineralisation: similar to parts of OECD 307 but $^{14}CO_2$-formation only, non-GLP
Test compound	Unlabelled Glyphosate (98% purity), ^{14}C-Glyphosate (93.8% purity); CAS-no.: 1071-83-6
Test system and conditions	Composting first, compost taken at various stages of composting for sorption studies; 4 concentrations, batch experiments, 5 replicates per measurement.
Statistical design	Outliers were removed from the analytical replicates using the Dixon test. Variance homogeneity was checked with the Levene test on the mineralization potential of OP during 92-d incubations and K_d values prior to the performance of ANOVA and LSD ($P < 0.05$) test to evaluate the effect of compost maturity on the parameters measured. Pearson's correlations were also calculated between the biochemical properties, K_d, and kinetic parameters. All statistical analyses were performed using XLStat software.
Relevance	
Environmental relevance	Given, environmental parameter considered adequately.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies with comparable design not known; no negative evidence.

Lexow et al. (2005)

Title: Glyphosate mobility in piedmont soils of the Australes range in the south of Buenos Aires Province
Author: C. Lexow, I. Morell, A.G. Bonorino
Reference: Chapter 16, p 199-206, in: Groundwater and Human Development. IAH Selected Papers on Hydrogeology 6. Bocanega, Hernandez and Usunoff (Eds)
Year: 2005
Results and conclusion: This study of glyphosate soil adsorption took place on an experimental plot of an agricultural sector of Buenos Aires Province. By carrying out batch tests the partition coefficient K_d, which relates the concentration of glyphosate in the water phase to the adsorbed one in the soil, was obtained. This coefficient was standardized according to the organic matter content (K_{oc}), and optimized using models based on the Freundlich isotherm. The greatest degree of adsorption of glyphosate occurs at surface level and decreases with depth, owing more to variations in the structure and chemical composition of the clay sediments than to the effect of the organic matter. There is a very high adsorption of the glyphosate in the soil (K_f: 17.0 – 49.2) so it falls into the category of being non-leachable. This characteristic gives it potentially little impact as a polluting agent, provided the conditions of preferential flow that could significantly increase its mobility are not generated.
Proposed action: Not to be considered as the study was performed under outdoor conditions in Argentina; not representative for EU.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because study was performed under outdoor conditions

in Argentina	
Reliability	Low
Endpoint	K_d, K_{oc}, K_f, 1/n, R^2
Protocol	In analogy to OECD 106, non-GLP
Test compound	Glyphosate (purity not given), CAS-no.: 1071-83-6
Test system and conditions	Batch experiments, 6 concentrations, soil from 3 depths
Statistical design	Freundlich Isotherms, number of replicates not given
Relevance	
Environmental relevance	Environmental parameter presented and discussed, thus environmental relevance is given.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies confirm the results, positive evidence; no negative evidence.

Litz et al. (2011)

Title: Comparative studies on the retardation and reduction of glyphosate during subsurface passage	
Author: N.T. Litz , A. Weigert, B. Krause, S. Heise, G. Grützmacher	
Reference: Water Research 45, 3047 - 3054	
Year: 2011	
Results and conclusion: The herbicide Glyphosate was detected in River Havel (Berlin, Germany) in concentrations between 0.1 and 2 mg/L. Laboratory (sorption and degradation studies) and technical scale investigations (bank filtration and slow sand filter experiments) were carried out. Batch adsorption experiments with Glyphosate yielded a low K_f of 1.89 (1/n = 0.48) for concentrations between 0.1 and 100 mg/L. Degradation experiments at 8°C with oxygen limitation resulted in a decrease of Glyphosate concentrations in the liquid phase probably due to slow adsorption (half life: 30 days). During technical scale slow sand filter (SSF) experiments Glyphosate attenuation was 70-80% for constant inlet concentrations of 0.7, 3.5 and 11.6 mg/L, respectively. Relevant retardation of Glyphosate breakthrough was observed despite the low adsorption potential of the sandy filter substrate and the relatively high flow velocity. The VisualCXTFit model was applied with data from typical Berlin bank filtration sites to extrapolate the results to a realistic field setting and yielded sufficient attenuation within a few days of travel time. Experiments on an SSF planted with Phragmites australis and an unplanted SSF with mainly vertical flow conditions to which Glyphosate was continuously dosed showed that in the planted SSF Glyphosate retardation exceeds 54% compared to 14% retardation in the unplanted SSF. The results show that saturated subsurface passage has the potential to efficiently attenuate glyphosate, favourably with aerobic conditions, long travel times and the presence of planted riparian boundary buffer strips.	
Proposed action: To be considered as additional information for risk mitigation. Not to be considered for endpoint and PEC-assessment.	
Type of information (critical, high/low weight, supporting, additional): High weight, supporting information on risk mitigation strategies	
Reliability	High
Endpoint	K_f, K_d,1/n, concentrations of gylphosate and AMPA
Protocol	Batch experiments: according to OECD 106; degradation experiments: partly similar to OECD 307, enclosures and SSF experi-

	ments: no standard protocols available, non-GLP
Test compound	Glyphosate (98.7 % purity); CAS-no.: 1071-83-6
Test system and conditions	Laboratory batch, enclosure and slow sand filter tests, filter material used. Laboratory experiments: Degradation: partly reducing conditions, 5 sampling points. Batch experiments: 4 concentrations, number of replicates not given. Enclosures: area of 1m^2, height of 1.85m (filtration length 1.00 m), situated within an infiltration pond (area: 90m^2), 3 Glyphosate levels. SSF experiments: two vertical-flow experimental SSFs: one without vegetation cover (average area 60m^2, filter depth 0.8 m, filter volume 48m^3) and the other with a 3 year old vegetation cover of Phragmites australis (average area 68 m^2, filter depth 1.2 m, filter volume 81.6m^3)
Statistical design	VisualCXTFit model, Freundlich isotherms,
Relevance	
Environmental relevance	Given, influencing environmental parameter recorded and discussed.
Weight of evidence	
"Positive"/"Negative" evidence	Publication with identical experimental setup not known, however results are logically explained; no negative evidence.

Mamy and Barruiso (2006)

Title: Desorption and time-dependent sorption of herbicides in soils	
Author: Mamy L. and E. Barruiso	
Reference: European Journal of Soil Science; doi:10.1111/j.1365-2389.2006.00822.x	
Year: 2006	
Results and conclusion: Objective of the study was to maximise the exploitation of sorption and/or desorption data to characterise desorption along with the effect of ageing on retention. The experiments involved three soils and five herbicides (inter alia Glyphosate). Sorption isotherms were not linear and desorption was markedly hysteretic. Desorption was inversely related to adsorption, being small when sorption was great as it is the case for Glyphosate. Single, different desorption isotherms are obtained that depend on initial sorbed herbicide concentration. A theoretical approach allowed calculation of adapted desorption parameters for different sorption concentrations from only one desorption isotherm. Generalised equations were derived to describe sorption and desorption, and these equations could be implemented in pesticide-fate models to take into account sorption and desorption parameters as well as their time dependence.	
Proposed action: Not to be considered as desorption-constants are not routinely used in PEC-assessment.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as desorption isotherms are not needed for fate assessment	
Reliability	Medium
Endpoint	K_f, 1/n, desorption constants, desorption isotherms; desorption isotherms after several times of ageing.
Protocol	a) according to OECD 106; b) desorption after ageing not according to a protocol; non-GLP

Test compound	Glyphosate (purity not given); CAS-no.: 1071-83-6
Test system and conditions	a) batch adsorption and desorption studies; b) desorption studies after certain times of ageing.
Statistical design	3 soils; Freundlich; 7 time intervals for ageing experiment. Generalised equations to describe sorption and desorption.
Relevance	
Environmental relevance	Given, environmental parameter described.
Weight of evidence	
"Positive"/"Negative" evidence	Completely comparable publication not known, but results are plausible; no negative evidence.

Mamy and Barriuso (2005)

Title: Glyphosate adsorption in soils compared to herbicides replaced with the introduction of glyphosate resistant crops

Author: Laure Mamy, Enrique Barriuso

Reference: Chemosphere 61, 844–855

Year: 2005

Results and conclusion:

This work compares glyphosate adsorption in soil with that of other herbicides frequently used in rape (trifluralin and metazachlor), sugarbeet (metamitron) and corn (sulcotrione). Herbicide adsorption was characterised in surface soils and in the complete soils profiles through kinetics and isotherms using batch equilibration methods. Pedological and molecular structure factors controlling the adsorption of all five herbicides were investigated. Glyphosate was the most strongly adsorbed herbicide, thus having the weakest potential for mobility in soils. Glyphosate adsorption was dependent on its ionisable structure in relation to soil pH, and on soil copper, amorphous iron and phosphate content. Trifluralin adsorption was almost equivalent to glyphosate adsorption, whereas metazachlor, metamitron and sulcotrione adsorption were lower. Trifluralin, metazachlor and metamitron adsorption increased with soil organic carbon content. Sulcotrione was the least adsorbed herbicide in alkaline soils, but its adsorption increased when pH decreased. Ranking the adsorption properties among the five herbicides, glyphosate and trifluralin have the lowest availability and mobility in soils, but the former has the broadest spectrum for weed control.

Detailed results are:

Soil characterisation:

Soil	Soil type	Sand (%)	Clay (g kg^{-1})	C.E.C. (in cmol kg^{-1})
Châlons (0-10 cm. composite sample)			88	6.4
Châlons (0-10 cm)			93	7.1
Châlons (10-20 cm)			95	6.2
Châlons (20-30 cm)			91	4.6
Dijon (0-10 cm. composite sample)			376	17.8
Dijon (0-10 cm)			377	20.6
Dijon (10-20 cm)			327	27.0
Dijon (20-30 cm)			363	28.6
Dijon (30-60 cm)			396	31.7
Dijon (60-90 cm)			307	21.8
Toulouse (0-10 cm. composite sample)			274	16.4
Toulouse (0-10 cm)			235	15.9

Toulouse (10-20 cm)				222	15.3		
Toulouse (20-30 cm)				221	15.3		
Toulouse (30-40 cm)				236	14.9		
Toulouse (40-50 cm)				245	15.0		
Adsorption							
Soil type	OC ($g\ kg^{-1}$)	pH (H_2O)	K_d ($L\ kg^{-1}$)	K_{oc} ($L\ kg^{-1}$)	K_f	K_{foc}	n_f
Châlons (0-10 cm, composite sample)	18,6	8,4					
Châlons (0-10 cm)	20,0	8,2	31,1 +/- 2,1	1552 +/- 105	34,8 +/- 0,6		0,80 +/- 0,02
Châlons (10-20 cm)	17,8	8,3					
Châlons (20-30 cm)	13,2	8,5					
Dijon (0-10 cm, composite sample)	13,5	8,3					
Dijon (0-10 cm)	16,9	8,2	38,7 +/- 2,5	2375 +/- 153	41,9 +/- 0,5		0,80 +/- 0,02
Dijon (10-20 cm)	14,8	8,2					
Dijon (20-30 cm)	9,5	8,3					
Dijon (30-60 cm)	7,7	8,4					
Dijon (60-90 cm)	6,9	8,6					
Toulouse (0-10 cm, composite sample)	10,1	6,3					
Toulouse (0-10 cm)	9,6	7,6	427 +/- 31	44360 +/- 3341	276 +/- 13		0,77 +/- 0,02
Toulouse (10-20 cm)	10,1	7,7					
Toulouse (20-30 cm)	9,4	7,7					
Toulouse (30-40 cm)	8,6	7,9					
Toulouse (40-50 cm)	6,8	8,2					

Proposed action:
To be considered as additional information but not for endpoint and PEC-assessment. Raw data on mass balances and test item concentrations in aqueous and solid phases are not reported and thus, the validity of the study cannot be proven.

Type of information (critical, high/low weight, supporting, additional):
High weight, supportive information, K_f-values compared to those already known

Reliability	High
Endpoint	K_f, 1/n, K_{oc}, K_d
Protocol	According to OECD 106;
Test compound	[Methyl-^{14}C]glyphosate (97.7 % purity); CAS-no.: 1071-83-6
Test system and conditions	Batch method according to OECD 106
Statistical design	3 soils, up to 4 soil depths, 8 sampling times for kinetics, 2 replicates; 6 concentrations for isotherms, 2 replicates, Freundlich isotherms
Relevance	
Environmental relevance	Given, guideline requirements fulfilled.
Weight of evidence	
"Positive"/"Negative" evidence	K_f-values in the range of already known values; no negative evidence.

Morillo et al. (2002)

Title: The effect of dissolved glyphosate upon the sorption of copper by three selected soils
Author: E. Morillo, T. Undabeytia, C. Maqueda, A. Ramos
Reference: Chemosphere 47, 747–752
Year: 2002

Results and conclusion: The effect of the pesticide glyphosate (GPS) on adsorption processes of copper onto three soils of different characteristics has been studied. Cu adsorption decreases in general with increasing GPS concentration in solution, due principally to the lower equilibrium-pHs, although this is not the only variable affecting copper adsorption. For the same pH values, Cu adsorption is higher in two of the three soils in the presence of GPS, but for the third soil, Cu adsorption is higher in the absence of GPS. This behaviour is explained by the possibility of GPS adsorption on these soils and by the formation of Cu-GPS complexes in solution. The soils showing a higher Cu adsorption in the presence of GPS than in its absence for the same pH are able to adsorb this pesticide. In these soils, copper can be adsorbed directly on the soil surfaces, and also through the formation of bonds with GPS previously adsorbed. The third soil was not able to adsorb GPS. Consequently, all the pesticide remained in solution, forming strong Cu complexes with low tendency to be adsorbed on this soil. For this reason, the concentration of free Cu in solution is drastically reduced, and the adsorption of copper on this soil is lower.	
Proposed action: Not to be considered for endpoint and PEC-assessment as sorption of copper in combination with Glyphosate has been investigated.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information only	
Reliability	Low
Endpoint	Adsorption isotherms of copper
Protocol	Comparable to OECD 106, tier II
Test compound	Copper, Glyphosate was added (no purity given); CAS-no.: 1071-83-6
Test system and conditions	Batch equilibrium method
Statistical design	3 soils, 5 Glyphosate, 4 copper concentrations
Relevance	
Environmental relevance	Given, parameter are reported.
Weight of evidence	
“Positive”/”Negative” evidence	Not directly comparable to publications dealing with Glyphosate sorption; no negative evidence.

Nourouzi et al. (2010)

Title: Adsorption of glyphosate onto activated carbon derived from waste newspaper
Author: M. Mohsen Nourouzi, T.G. Chuah, Thomas S.Y. Choong
Reference: Desalination and Water Treatment 24, 321-326
Year: 2010
Results and conclusion: This paper investigates the ability of activated carbon derived from waste newspaper (WNAC) to remove pesticide glyphosate from aqueous solution. The influence of initial pH was first studied. It was found that the WNAC presented the highest uptake capacity at pH 2.5. Adsorption isotherm models such as Langmuir, Freundlich and Redlich-Peterson were used to describe the adsorption of glyphosate by WNAC. The results show that the Langmuir adsorption isotherm model best fits the experimental data. The maximum adsorption capacity of WNAC is found to be 48.4 mg/g.
Proposed action: Not to be considered for endpoint and PEC-assessment as activated carbon derived from

waste newspaper is used. Thus, no standard scenario for pesticide environmental risk assessment in the EU is presented.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information	
Reliability	Medium
Endpoint	Langmuir, Freundlich and Redlich-Peterson isotherms
Protocol	Comparable to OECD 106
Test compound	Glyphosate (analytical grade); CAS-no.: 1071-83-6
Test system and conditions	Batch equilibrium study using activated carbon derived from waste newspaper as a sorbent.
Statistical design	8 pesticide concentrations
Relevance	
Environmental relevance	Given; environmental parameter considered.
Weight of evidence	
"Positive"/"Negative" evidence	No comparable citations known, however, results are plausible; no negative evidence.

Ololade et al. (2014)

Title: Sorption of Glyphosate on Soil Components: The Roles of Metal Oxides and Organic Materials	
Author: I. A. OLOLADE, N. A. OLADOJA, F. F. OLOYE, F. ALOMAJA, D. D. AKERELE, J. IWAYE AND P. AIKPOKPODION	
Reference: Soil and Sediment Contamination, 23:571–585, 2014	
Year: 2014	
Results and conclusion: The sorption characteristics of glyphosate (GPS) on soil and their main components were investigated, indicating that the mineral phase is more important than the organic carbon in adsorption of GPS. Sorption isotherms were determined from each component using the batch equilibrium method at various concentrations (5, 10, 15, 20, 25, and 30 mg L^{-1}) and sorption affinity of GPS was approximated by the Freundlich equation. The sorption strength K_f [mg kg^{-1}(L $mg^{-1})^{-n}$] across the various components ranged from 2.1–134.9 while the organic carbon-normalized Freundlich sorption capacity values, K_{foc}, ranged from 1.28–3.53 mg kg^{-1}-OC/(mg $L^{-1})^n$. Infrared Fourier transform spectroscopy (FTIR) of the components showed significant structural differences. The results suggest that the presence of the oxides and hydroxides iron, in particular in soil solutions, enhanced GPS adsorption. They also suggest that reduction in OC% due to various treatments may enhance the remobilization of GPS into the aqueous phase (i.e., groundwater), though at different rates. Comparatively, contribution of surface area to the adsorption of GPS on the various components proved more significant than contents of organic carbon.	
Proposed action: Not to be considered as non-European soils were used.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because a non-European soil is used.	
Reliability	
Endpoint	K_f, k_{foc}
Protocol	Not given
Test compound	Glyphosate (N-(phosphono-methyl-glycine) with >98% certified purity
Test system and con-	Surface soil (top 1–5 cm) was collected from University Campus,

ditions	Ondo State, Nigeria, with a clean, methanol-rinsed, stainless-steel trowel. The soil samples were air-dried, passed through a 60-mesh screen, and stored in glass bottles for further use. The soil samples were characterized following the conventional methods (Tao et al., 2006). Soil samples were prepared with four different reagents to extract target components. 0.1 mol L^{-1} $NH_2OH{\cdot}HCl$ and 0.01 mol $L^{-1}HNO_3$ for 30 min were used to remove manganese oxides (Li et al., 2006). About 3.0 mL H_2O_2 (30%) heated to 40°C was employed to remove organic matters (OMs) based on a previous report (Mikutta et al., 2005). 0.2 mol L^{-1} $(NH_4)_2C_2O_4$ was buffered at pH 3.0 with H_2C2O4 and shaken in the dark for 4 h, then employed to extract both Fe and Mn hydrous oxides (Pei et al., 2006). All of the samples were centrifuged at 3800 rpm for 30 min, and supernatant was filtered (0.45 µm) into 50 mL polypropylene (PP) tube for the determination of Fe and Mn. The extracts were washed 3–4 times with distilled water and air-dried. Sorption capacity of GPS on the treated and untreated samples was determined using the batch equilibrium method. GPS is a non-residual herbicide with solubility in water of 12 g L^{-1} at 25°C. Briefly, triplicate adsorption experiments were done using PP centrifuge tube (50 mL capacity) by mixing 0.5 g of air-dried sample with 20 mL of 0.5mmol L^{-1} CaCl2 solutions containing various concentrations of GPS (5, 10, 15, 20, 25, and 30 mg L^{-1}). Thesamples were shaken for 24 h at 25 ± 1°C on a 2D-shaker at 250 rpm at pH maintained at 7± 0.1. The preliminary sorption kinetic test showed that the apparent sorption equilibrium was reached at 120 min for the original sample. The equilibrium pHs were maintained by adding aliquots of 0.1 M HCl or NaOH. The tubes were centrifuged at 9000 rpm for 30 min. The supernatant were taken out, filtered through a 0.22 µm nylon syringe filter, and analyzed by a UV-visible spectrophotometer (UV–VIS 1902PC, Searchtech Instrument). The glyphosate concentrations were determined by comparing them with the calibration graph obtained by preparing standards of glyphosate. The amount of GPS adsorbed was calculated from the differences between its concentration in solution before and after equilibration. Blanks and controls were prepared at the same time under the same conditions. Blanks were set up using the same solid-to-water ratios as the samples, but without adding GPS. One control sample with only the test substance in 0.5 M NaCl solution (without soil sample) was subjected to precisely the same steps as the test systems, in order to check the stability of the test substance in NaCl solution and its possible adsorption on the surfaces of the test vessels. All of the experiments, including controls and blanks, were carried out in duplicate. The GPS desorption experiment was performed on soil residues immediately after adsorption experiments. All of the supernatant solution was removed and replaced with 20 mL fresh 0.5 M NaCl prepared with distilled water. Soil residues were equilibrated using an end-over-end mechanical shaker for 120 min at 25 ± 1°C at pH = 7. Thereafter, desorbed GPS was measured in filtrates as de-

	scribed in adsorption experiments after centrifuging the sample at 9000 rpm for 30 min and filtering through a 0.22µm nylon syringe filters.
Statistical design	Freundlich equation
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence

Pessagno et al. (2008)

Title: Glyphosate behavior at soil and mineral - water interfaces	
Author: Romina C. Pessagno, Rosa M. Torres Sánchez, María dos Santos Afonso	
Reference: Environmental Pollution 153, 53-59	
Year: 2008	
Results and conclusion: Adsorption isotherms and surface coverage of glyphosate (PMG), in aqueous suspensions of three Argentine soils with different mineralogical composition were measured as a function of PMG concentration and pH. Zeta potential curves for PMG/soils system were also determined. PMG formed surface complexes on goethite, kaolinite, illite, montmorillonite and soils with similar maximum surface coverage, and the extent of the complexation was dependent on the ligand concentration in solution and pH. The extent of PMG adsorption onto iron oxides was higher than onto soils or clays. The adsorption behavior of PMG on minerals and soils in aqueous suspensions were analyzed as a function of pH and surface coverage. The results suggest that the phosphonate moiety of PMG coordinates to the external surface site of solids with similar structures as iron oxides. The formation of inner-sphere surface complexes is suggested. These results are potentially important to provide a fundamental understanding of the degradability and bioavailability of PMG in soils and natural waters. PMG complexation with metal ions and its adsorption onto mineral surfaces might affect its degradation, distribution, and bioavailability in soils and groundwater. The study of the properties of these soils and mineral surface complexes is of high importance in order to assess the implications for control of PMG contamination. PMG belongs to a unique class of strongly chelating agents and the adsorption process makes herbicide more persistent in soil.	
Proposed action: Not to be considered as non-European soils were used.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information, non-European soils	
Reliability	Medium
Endpoint	Langmuir isotherms, maximum adsorption densities, zeta potential,
Protocol	Similar to OECD 106, tier III
Test compound	Glyphosate (CAS-no.: 1071-83-6; 99% purity)
Test system and conditions	3 soils plus one after OM removal and iron removal, batch experiments,
Statistical design	Non-linear regression fitting program (Solver,Excel 10) to approximate a Langmuir shape
Relevance	

Environmental relevance	PH-dependencies and influence of soil material was tested, thus, influencing parameters were considered adequately; environmental relevance given.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Petersen and Hansen (2011)

Title: Letter to the Editor	
Author: Carsten T. Petersen, Søren Hansen	
Reference: Chemosphere 85, 1538	
Year: 2011	
Results and conclusion: Comments on ''Transport modes and pathways of the strongly sorbing pesticides glyphosate and pendimethalin through structured drained soils'' by J. Kjær, V. Ernsten, O.H. Jacobsen, N. Hansen, L.W. de Jonge, P. Olsen [Chemosphere 84(4) (2011) 471–479].	
Proposed action: Not to be considered; letter to the Editor	
Type of information (critical, high/low weight, supporting, additional): Low weight, letter to the Editor	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate (CAS-no.: 1071-83-6)
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Prata et al. (2003)

Title: GLYPHOSATE SORPTION AND DESORPTION IN SOILS WITH DISTINCT PHOSPHORUS LEVELS
Author: Fábio Prata; Vanessa Camponez do Brasil Cardinali; Arquimedes Lavorenti; Valdemar Luiz Tornisielo; Jussara Borges Regitano
Reference: Scientia Agricola 60 (1), 175-180
Year: 2003
Results and conclusion: Glyphosate sorption in the three studied soils was influenced by the soil P level, and the amount of sorbed glyphosate became substantially reduced at P levels starting from 1000 mg dm-3. This visualization of a reduction in glyphosate sorption, however lacks of practical importance, since these phosphorus levels would never be attained under field conditions in agricultural soils, which suggests that under this condition the competition between glyphosate and P for covalent binding sites in the soil must not occur. Thus, these results confirm that the extent of the binding forces in glyphosate is proportional to the soil capacity of adsorbing in-

organic phosphate. However, even as a secondary role, organic matter plays also a very important role for glyphosate retention, especially in oxide-poor soils. Glyphosate competes with phosphorus for specific sorption sites of the soil, but this competition becomes only important when the soil P levels reach very high values, which are not attained under agricultural field conditions. The herbicide extraction is low and increases with P levels in the soil. Glyphosate remains in the soil as a bound residue.
Detailed results are:

Soil characterisation:

Soil	Soil type	Sand (g kg^{-1})	Clay (g kg^{-1})	C.E.C. (in meq/100g)
Nvef	Rhodic Kandiudalf	250	550	
Law	Anionic Acrudox	590	350	
G	Typic Humaquept	200	540	

Adsorption[1)]

Soil type	OC (g kg^{-1})	pH (H_2O)	K_d	K_{oc}	K_f	K_{foc}	1/n
Nvef	27.5	6.1			184,3		
Law	18.9	5.5			172,3		
G	78.5	4.2			222,1		

1) Applied amount of phosphate: 0 – 50000 kg P_2O_5 ha^{-1}

Proposed action:
To be considered as non-European soils (Brasil) were used.

Type of information (critical, high/low weight, supporting, additional):
High weight, supportive information on Glyphosate sorption dependencies on soil parameter

Reliability	High
Endpoint	K_f-values, 1/n
Protocol	According to OECD 106, non-GLP
Test compound	Glyphosate (CAS-no.: 1071-83-6; purity not given)
Test system and conditions	3 (soils) × 5 (soil P levels) factorial experiment, three replicates, 5 Glyphosate concentrations, batch experiments, 25 +/- 2°C
Statistical design	Freundlich Isotherms; Regression analyses for: increasing levels of P and Freundlich constants, as well as for the total sorbed percentage. Analyses of variance and mean comparison tests (Tukey, $P < 0.05$) for the percentages of glyphosate extracted and desorbed
Relevance	
Environmental relevance	Influence of phosphate on sorption investigated; environmental relevance given.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies support the results; no negative evidence.

Prata et al. (2005)

Title: GLYPHOSATE BEHAVIOR IN A RHODIC OXISOL UNDER NO-TILL AND CONVENTIONAL AGRICULTURAL SYSTEMS
Author: Fábio Prata, Arquimedes Lavorenti, Jussara Borges Regitano, Harry Vereecken, Valdemar Luiz Tornisielo & Adelino Pelissari
Reference: R. Bras. Ci. Solo, 29:61-69
Year: 2005
Results and conclusion:

The behaviour of glyphosate in a Rhodic Oxisol, collected from fields under no-till (NT) and conventional (CON) management systems in Ponta Grossa, Parana state (Brazil) was investigated. Glyphosate mineralization, soil-bound forms, sorption and desorption kinetics, sorption/desorption batch experiments, and soil glyphosate phythoavailability (to Panicum maximum) were determined. Sorption: The glyphosate sorption kinetics was practically instantaneous (over 90 % sorbed within 10 min). For both the NT and CON systems, glyphosate presented a high sorption rate, which difficult its mineralization. The molecules remained in the soil as bound-residue. Mineralisation: The NT system contributed to the acceleration of glyphosate mineralization. The main metabolite resulting from glyphosate degradation was aminomethylphosphonic acid (AMPA).	
Proposed action: Not to be considered as non-European soils (Brasil) were used.	
Type of information (critical, high/low weight, supporting, additional): High weight, supportive information on Glyphosate behaviour in soils under different management systems.	
Reliability	High
Endpoint	$^{14}CO_2$-formation; sorption kinetics, Freundlich sorption and desorption constants
Protocol	According to OECD 106, non-GLP, close to OECD 307 but comprehensive for measurements mineralisation only, AMPA analysed but not used for kinetics
Test compound	Glyphosate (CAS-no.: 1071-83-6; purity not given), ^{14}C-labelled Glyphosate, 2 label positions (> 97% purity)
Test system and conditions	Mineralisation: completely randomized design with a 2 x 2 factorial scheme (two management systems and two ^{14}C radiolabelled positions in the glyphosate), with five replicates. $^{14}CO_2$ evolution measured in 7-day intervals during 63 days. Sorption: kinetics investigated in a batch experiment, 7 equilibration times up to 60 hours. Sorption/desorption using equilibrium batch experiments. Five different concentrations for sorption and one concentration for desorption. Same soils as for mineralisation.
Statistical design	Freundlich Isotherms; Regression analyses for results for the different endpoints and soil management systems. Analyses of variance and mean comparison tests (Tukey, $P < 0.05$).
Relevance	
Environmental relevance	Influence of soil management practices on mineralisation and sorption investigated; environmental relevance given.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies support the results; no negative evidence.

Pessagno (2005)

Title: N-(PHOSPHONOMETHYL)GLYCINE INTERACTIONS WITH SOILS
Author: Pessagno, R.C., dos Santos Afonso, M., Torres Sanchez, R.M.
Reference: The Journal of the Argentine Chemical Society, 93 (4/6), 97-108
Year: 2005
Results and conclusion: The adsorption isotherms and surface coverage of glyphosate (NPhosphonomethylglycine,

PMG) in aqueous suspensions of Argentine soils as a function of PMG concentration and pH were measured. Zeta potential curves for the PMG/soils system were also determined. The formation of inner sphere surface complexes of PMG on the soil surface, were analyzed as a function of pH and surface coverage.	
Proposed action: Not to be considered as a non-EU soila (Argentina) were tested.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information, non-European soils	
Reliability	Low
Endpoint	Maximum adsorption densities, Langmuir constants, zeta potential curves
Protocol	Similar to OECD 106, tier III
Test compound	Glyphosate (99% purity); CAS-no.: 1071-83-6
Test system and conditions	3 soils and Goethite tested, batch experiments, pH-dependencies
Statistical design	Number of concentrations not given, number of replicates not reported
Relevance	
Environmental relevance	Given as pH-dependencies are determined.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies support the results; no negative evidence.

Rampazzo et al. (2012)

Title: Adsorption of glyphosate and aminomethylphosphonic acid in soils
Author: N. Rampazzo, G. Rampazzo Todorovic, A. Mentler, and W.E.H. Blum
Reference: Int. Agrophys., 2013, 27, 203-209, doi: 10.2478/v10247-012-0086-7
Year: 2012
Results and conclusion: The results showed that glyphosate is initially adsorbed mostly in the upper 2 cm. It is than transported and adsorbed after few days in deeper soil horizons with concomitant increasing content of its metabolite aminomethylphosphonic acid. Moreover, Fe-oxides seem to be a key parameter for glyphosate and aminomethylphosphonic adsorption in soils. This study confirmed previous studies: the analysis showed lower contents of dithionite-soluble and Fe-oxides for the Chernozem, with consequently lower adsorption of glyphosate and aminomethylphosphonic as compared with the Cambisol and the Stagnosol. In detail: 1. No-tillage plots show a higher bulk density and a lower total porosity than conventionally tillaged plots as due to a natural settlement of particles free from tillage practices. 2. Shortly after Roundup Max application only a part of the applied glyphosate amount enter the upper 0-2 cm and is then transported and adsorbed in deeper horizons with time with concomitant increase of the aminomethylphosphonic acid content. 3. The results showed distinguished contents of glyphosate and aminomethylphosphonic acid in different soils at the same soil depth, according to their chemical-mineralogical adsorption properties, especially Fe-oxides (Fe_d and Fe_o). 4. Thus, iron-oxides in general seem to be a key parameter for glyphosate and aminomethylphosphonic acid adsorption in soils.
Proposed action: Consider as additional information.

Type of information (critical, high/low weight, supporting, additional): Supporting information.	
Reliability	
Endpoint	Adsorption of glyphosate and aminomethylphosphonic
Protocol	Not given
Test compound	Glyphosate (Roundup Max)
Test system and conditions	The experiments were carried out at agricultural experimental fields, where different tillage systems: no-tillage (NT), direct drill, no plough, with a winter green vegetation cover and maize crop in spring, and conventional tillage (CT), plough with or without a winter green vegetation cover in 3 field replications are tested since 2007 (Kirchberg, Styria), 1999 (Pyhra and Pixendorf, Lower Austria). Three soils under different climatic conditions and featuring different physico-mineral composition were investigated: a sandy stagnic Cambisol (WRB, 2006; Nestroy et al., 2000) at Kirchberg (Styria) fromtertiary carbonate free sediments, a loamy Stagnosol (WRB, 2006; Nestroy et al., 2000) from carbonate free sediments (flysch, sandstone) at Pyhra (Lower Austria) and aChernozem(WRB, 2006; Nestroy et al., 2000) from loess at Pixendorf (Lower Austria). Moreover, these three soil types were selected because of their contrasting physico-chemico-mineralogical parameters eg texture, carbonate content, pH-value, and Fe-oxides for a better understanding of their influence on the glyphosate behaviour and extraction from soils. The Roundup Max application was performed at all three sites according to the common agricultural practice ie 4 l Roundup Max (450 g glyphosate /l Roundup Max) were dissolved in 200 l of water and applied per ha (2%herbicide solution). This corresponds to an application of 1 800 g glyphosate ha^{-1} or 180 mg glyphosatem-2. The application was carried out at sunny and not windy weather at the NT-plots. Soil bulk samples from all plots (NT and CT) were taken for physico-chemico-mineralogical analysis at each site at two soil depths (0-5 and 5-20 cm), collected from 10 different points/field replication. The samples were air-dried and sieved at 2 mm size (fine earth). Moreover, for further physical analysis undisturbed samples (cylinders with 200 cm3) were taken separated from each NT and CT field replication at 5-15 cm soil depth each in 5 repetitions. In order to investigate the fate of glyphosate and AMPA in depth and time after Roundup Max application, soil bulk samples were taken at different time intervals after application at 10 points within each NT-field replication (pooled than to one sample per site). After each soil sampling soil samples were immediately transported to the laboratory in cooling boxes. In the laboratory all samples were stored at -18 °C until measurements. All physical, chemical and mineralogical analyses were carried out according to the standard methods.
Statistical design	Not given
Relevance	

Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Selim et al. (2010)

Title: The sorption of Glyphosate and its metabolite amino-methyl-phosphonic acid (AMPA) on biopolymer chitin	
Author: Shady Selim, A. Klik, B. Grillitsch, M. Fürhacker, and A. Mentler	
Reference: Report ALVA – Jahrestagung 2010, „Vom Lebensmittel zum Genussmittel – was essen wir morgen"?, May 31 - June, 1 2010, Austria	
Year: 2010	
Results and conclusion: The main objective of this study was to demonstrate that chitin has a considerable adsorption capacity for glyphosate and its main metabolite AMPA. In the present study, the adsorption of glyphosate and its metabolite aminomethyl phosphonic acid (AMPA) onto chitin was analyzed. Results showed that chitin had an adsorption capacity and the adsorptive coefficient of glyphosate is higher than that of AMPA. The Freundlich equation fits the adsorption behaviour of glyphosate and AMPA better than the Langmuir model. Values were: Glyphosat: 974.4 mg/g (S_{max}, r^2=0.972, Langmuir); 436.7 mg/g (K_f, r^2=0.979, Freundlich) AMPA: 673.8 4 mg/g (S_{max}, r^2=0.977, Langmuir); 13.26 mg/g (K_f, r^2=0.980, Freundlich).	
Proposed action: Not to be considered as no soils are used for sorption.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information on Glyphosate sorption to material other than soil	
Reliability	Low
Endpoint	Freundlich and Langmuir constants
Protocol	Close to OECD 106, non-GLP
Test compound	Glyphosate (CAS-no.: 1071-83-6; purity not given)
Test system and conditions	Batch method with concentration of Chitin 10 g L-1 < 1 mm and concentrations of glyphosate and AMPA varied from 1µg L-1 to 500 µg L-1 at 23 C° isotherm, no further data presented
Statistical design	Not specified
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Completely comparable publications not known, however, results are plausible; no negative evidence.

Shareef and Hamadamin (2009)

Title: Adsorption of Metalaxyl and Glyphosate on Six Erbilian Agricultural Soils
Author: KAPIA M. SHAREEF and SHIREEN I. HAMADAMIN
Reference: Asian Journal of Chemistry 21 (4), 2673-2683
Year: 2009
Results and conclusion: A study was conducted to determine the differences in the adsorption behaviour of two non-ionic pesticides, metalaxyl [N-(2,6-dimethyl phenyl)N-(methoxyacetyl)] and glyphosate [N-phosphonomethyl-glycine] on six agricultural soil

samples from Erbil governorate. Data from batch equilibrium method revealed that the adsorption of metalaxyl and glyphosate on the selected soil samples followed the first order rate law. Glyphosate exhibited the faster rate of accumulation with 76.53 % adsorption on the soil solid matrix after 0.5 h as compared to that for metalaxy 66.06 %. Linear, Freundlich and Langmuir models were used to describe the adsorption of both pesticides. Values of distribution coefficient (K_d) indicated moderate to strong adsorption of metalaxyl (mean calculated K_d: 5.963 mL g^{-1}) and very strong adsorption of glyphosate (mean calculated K_d: 703.716 mL g^{-1}) and consequently there is no considerable risk of groundwater contamination. Wide variation in adsorption affinities of the soils to both pesticides was observed, K_d values for metalaxyl varied between 2.93 and 9.97 mL g^{-1} and for glyphosate between 5.16 and 456.34 mL g^{-1}. A linear correlation was found between the values of adsorption coefficients of both pesticides and soil organic carbon (R^2: 0.61 and 0.69 for metalaxyl and glyphosate, respectively).	
Proposed action: Not to be considered as non-European soils are used.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information	
Reliability	Low
Endpoint	K_d, R^2, Langmuir and Freundlich isotherm
Protocol	Close to OECD 106, non-GLP
Test compound	Glyphosate (CAS-no.: 1071-83-6; purity > 99.2%)
Test system and conditions	Batch method, 6 soils, 7 time points for kinetics, 3 concentrations, number of replicates not given
Statistical design	Langmuir and Freundlich models; too less concentrations measured for reliable isotherms
Relevance	
Environmental relevance	Given; influencing parameter are reported.
Weight of evidence	
“Positive”/”Negative” evidence	Results supported by other publications; no negative evidence.

Sheals et al. (2002)

Title: Adsorption of Glyphosate on Goethite: Molecular Characterization of Surface Complexes
Author: Sheals J., Sjöberg S. and Persson P.
Reference: Environ. Sci. Technol. 36, 3090-3095
Year: 2002
Results and conclusion: The adsorption of Glyphosate (PMG) on goethite (R-FeOOH) has been studied as a function of pH and PMG concentration. Adsorption was investigated with batch experiments, attenuated total reflectance Fourier transform infrared spectroscopy (ATRFTIR), and X-ray photoelectron spectroscopy (XPS). A minor quantity of bidentate complexes is thought to form both at near-neutral pH and when the surface concentration of PMG is low. The findings show that goethite has a relatively large capacity for PMG adsorption and thus aids the removal of bioavailable PMG from soil solution. The phosphonate group binds to the goethite component of soil to form predominantly monodentate inner-sphere complexes while the carboxylate group remains relatively “free” from complexation with goethite, leaving it subject to degradation and/or complexation with metal ions.
Proposed action:

Not to be considered as Goethite and no soil was used.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional in-depth information on soil sorption processes	
Reliability	Low
Endpoint	surface concentration of PMG as a function of total PMG concentration at three constant pH-values
Protocol	Partly comparable to OECD 106, non-GLP
Test compound	^{14}C-Glyphosate (CAS-no.: 1071-83-6; purity 95%)
Test system and conditions	Batch method, different pH-values tested
Statistical design	No details given
Relevance	
Environmental relevance	Given; influencing parameter such as pH-value are tested.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Shushkova et al. (2009)

Title: Sorption and Microbial Degradation of Glyphosate in Soil Suspensions	
Author: T. V. Shushkova, G. K. Vasilieva, I. T. Ermakova, and A. A. Leontievsky	
Reference: Applied Biochemistry and Microbiology, Vol. 45, No. 6, pp. 599–603	
Year: 2009	
Results and conclusion: Sorption and microbial destruction of glyphosate, the active agent of the herbicide Ground Bio, in suspensions of sod-podzol and gray forest soils has been studied. According to the adsorptive values (3560 and 8200 mg/kg, respectively) and the Freundlich constants (K_f: 15.6 and 18.7, respectively), these soils had a relatively high sorption capacity for the herbicide. Inoculation of a native suspension of sod-podzol soil with cells of a selected strain-degrader *Ochrobactum anthropi* GPK 3 resulted in a 25.4% decrease in the total glyphosate content (dissolved and extractable), whereas in a non-inoculated suspension, the loss did not exceed 5.5%. The potential for the use of a selected bacterial strain in the glyphosate destruction processes in soil systems is demonstrated for the first time.	
Proposed action: Not to be considered as soils outside EU were tested.	
Type of information (critical, high/low weight, supporting, additional): High weight, supportive information on the lower range of K_f-values and on selected microbial strains degrading Glyphosate	
Reliability	Medium
Endpoint	Sorption: K_f, 1/n, R^2; dissipation: amount of Glyphosate in soil suspension
Protocol	Mineralisation: no standard protocol, adsorption isotherms: similar to OECD 106; non-GLP
Test compound	Glyphosate-isopropylammonium salt (CAS-no.: 8641-94-0; product: Ground Bio)
Test system and conditions	Sorption: Batch method, 2 soils, 8 concentrations Dissipation: soil suspensions (sterile, non-sterile; bacterial strain-degrader *O. anthropi* GPK 3 and indigenous microbial community.
Statistical design	Sorption: Freundlich Isotherms; Dissipation: triplicate measurements, percentage error less than 12%, P = 0.95, no DT_{50} calculat-

	ed
Relevance	
Environmental relevance	Given; influencing parameter such as soil properties reported.
Weight of evidence	
"Positive"/"Negative" evidence	No publication with same bacterial strain used is known, results are plausible; no negative evidence.

Si et al. (2013)

Title: Complex Interaction and Adsorption of Glyphosate and Lead in Soil

Author: Y.-B. SI, Y. XIANG, C. TIAN, X.-Y. SI, J. ZHOU AND D.-M. ZHOU

Reference: Soil and Sediment Contamination, 22:72–84, 2013

Year: 2013

Results and conclusion: In the study, the adsorption and co-adsorption of Pb and glyphosate were determined on two soils [a red (RS) soil, Udic Ferrisol, and a yellow-brown (YB) soil, Udic Luvisol] of distinctly different chemical characteristics at varying pH conditions. Results indicate that the adsorption of lead and glyphosate strongly depends on soil types: the RS soil, characterized by a relatively high iron/aluminum content but a low pH and organic matter content, shows a much lower adsorption capacity for Pb but a higher sorption for glyphosate than the YB soil. The co-existence of Pb and glyphosate in soils resulted in complex interactions among Pb, glyphosate, Pb-glyphosate complexes, and soil minerals. The presence of glyphosate decreased Pb adsorption on the two soils, which was attributed primarily to the formation of soluble Pb-glyphosate complexes having relatively low affinities to soil surfaces. On the other hand, addition of Pb increased the adsorption of glyphosate on both soils, which was attributed to: (1) a decreased solution pH due to the ion exchange between Pb^{2+} and H^{+} on soil surfaces; and (2) increased sorption sites where Pb was adsorbed and acted as a bridge between glyphosate and the soil. The present study illustrates that the complex interactions among glyphosate, Pb, and soil may have important implications for the mobility and bioavailability of Pb in soil and should thus be considered in future environmental risk assessments.
The Freundlich fitting parameters corresponding to glyphosate adsorption isotherms on the RS and YB soils in the absence or presence of Pb are given in the following:

Soil	**Pb^{2+} [mg/L]**	**k_f**	**1/n**	**R**
RS	**0**	**1304.60±75.03**	**0.308±0.016**	**0.987**
	50	**1341.60±54.82**	**0.313±0.011**	**0.993**
	200	**1420.30±39.13**	**0.315±0.008**	**0.998**
YB	**0**	**368.18±9.67**	**0.377±0.007**	**0.999**
	50	**376.73±10.40**	**0.387±0.008**	**0.999**

	200	**385.05±11.33**	**0.432±0.009**	**0.999**

Proposed action:
Not to be considered as non-European soils were used.

Type of information (critical, high/low weight, supporting, additional):
Low weight, additional information because a non-European soil is used.

Reliability	
Endpoint	K_f, 1/n
Protocol	Not given
Test compound	Glyphosate
Test system and conditions	A surface red soil (RS soil, Udic Ferrisol) and a surface yellow-brown soil (YB soil, Udic Luvisol) were used for the experiments and obtained at a depth of 0–20 cm from Yingtan County, Jiangxi Province, and Feidong County, Anhui Province, in China. Effect of Glyphosate and pH on Lead Adsorption on Soils: Lead adsorption isotherms were determined in batch experiments by mixing the soil (5.0 g) with the Pb stock solution in a background electrolyte concentration of 0.01 mol/L $NaNO_3$. The experiment was performed at three levels of glyphosate (0, 50, and 200 mg/L) at the same background solution. The added Pb concentrations varied from 0, 50, 100, 150, 200, 300, 400, to 500 mg/L. The final volume was made up to 25 mL, which gave a solid to solution ratio of 1 : 5 (w/v). All experiments were performed in duplicate. The sample tubes were subsequently shaken for 2 h at 25◦C, centrifuged and then filtered through a filter paper. Solution pH was measured following equilibrium. The Pb concentration in the centrifuged solution was determined by atomic absorption spectrophotometry (AAS) using a ThermoElemental SOLAAR M5 spectrometer (Thermo Electron Corp., Verona, WI, USA). The amount of Pb adsorbed was calculated by the difference between that added in the initial solution and that found after equilibrium. Similar experiments were performed to study the effect of pH on Pb adsorption in the presence or absence of glyphosate. In this case, a fixed, final Pb concentration of 200 mg/L was used, and the final solution pH was adjusted from 3 to 9 by adding different volumes of either 0.01 mol/L NaOH or 0.01 mol/L HNO_3 solution. Effect of Pb and pH on Glyphosate Adsorption on Soils: Glyphosate adsorption isotherms in the presence or absence of Pb were determined by mixing the glyphosate and the soil in a background electrolyte solution of 0.01 mol/L $NaNO_3$. The final volume was 25 mL, and the final glyphosate concentrations were 0, 200, 400, 600, 800, 1000, and 1200 mg/L, respectively. Three Pb concentration levels (0, 50, and 200 mg/L) were used to study the effect of Pb on glyphosate adsorption. All experiments were performed in duplicate. Following the equilibration at at 25◦C for 2 h, samples were centrifuged and filtrated through a filter paper. The clear supernatant solution was thus obtained, and pH determined

	following equilibrium. The glyphosate concentration in the supernatant solution was determined by an Agilent 1100 high-performance liquid chromatography (HPLC) (Agilent Technologies Co. Ltd., Santa Clara, CA, USA) using a C18 Hypersil ODS column (250 mm × 4.6 mm i.d., with a 5 µm particle size). In brief, 1.0 mL supernatant of glyphosate was first derived by reacting with 130 mmol/L p-toluene-sulphonyl chloride in acetonitrile (1:1 by volume) for 5 min at 50◦C. Derivatized samples (20 µL) were then injected into the chromatographic column. The mobile phase consisted of 50 mmol/L sodium phosphate (pH 2.3) in 15% (v/v) acetonitrile. The analysis proceeded at a flow rate of 1.0 mL/min, and glyphosate detected at 240 nm (Kawai et al., 1991; Abdullah et al., 1995; Forlani et al., 1999). The limit of detection was estimated to be 2×10−10 g, and the minimum determination concentration of glyphosate in soil samples was 0.02mg/kg. The ranges of average recoveries and coefficient variation of the method were 94.2%~98.3% and 0.66%~3.63%, respectively. The amount of glyphosate adsorbed was calculated by the difference between that added in the initial solution and that found after equilibrium. Similar experiments were performed to study the effect of pH on glyphosate adsorption in the presence or absence of Pb. In this case, a fixed, final glyphosate concentration of 200 mg/L was used, and the final solution pH was adjusted from 3 to 9 by adding different volumes of either 0.01 mol/L NaOH or 0.01 mol/L HNO_3 solution.
Statistical design	All experiments were performed in duplicate. The Freundlich equation was used to describe the adsorption data. Data from the recovery rate were analyzed by calculation of the means and standard deviations. All experimental data were processed using the statistical software SPSS 11.5. The speciations of Pb and glyphosate at varying pH conditions were calculated using the computer program WinSGW.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Sørensen et al. (2006)

Title: Sorption, desorption and mineralisation of the herbicides glyphosate and MCPA in samples from two Danish soil and subsurface profiles
Author: Sebastian R. Sørensen, Anne Schultz, Ole S. Jacobsen, Jens Aamand
Reference: Environmental Pollution 141, 184-194
Year: 2006
Results and conclusion: The vertical distribution of the sorption, desorption and mineralisation of glyphosate and MCPA was examined in samples from two contrasting soil and subsurface profiles, obtained from a sandy agricultural site and a non-agricultural clay rich site. The highest mineralisation of [^{14}C-methylen] glyphosate, with 9.3 - 14.7% degraded to $^{14}CO_2$ within 3 months, was found in the deepest sample from the clay site. In the deeper

parts of the sandy profile high sorption and low desorption of glyphosate coincided with no or minor mineralisation indicating a limited glyphosate bioavailability. The herbicide was not mineralised under anoxic conditions. Based on the present study the potential for natural attenuation of glyphosate is apparent in both profiles. In sandy locations, such as Fladerne Bæk, glyphosate will most likely not be mobile since preferential flow patterns probably are insignificant and sorption to matrix components will retain the herbicide in the top soil. If the herbicide should bypass the soil zone, however, and hence enter the deeper part of the tested profile, it appeared that the mineralisation potential may be low, but that the majority of the glyphosate will be associated with the matrix.	
Proposed action: Not to be considered as K_d-values for one concentration only are reported. No raw data on mass balances and concentrations in aqueous and solid phases are given, and thus the validity of the study cannot be proven.	
Type of information (critical, high/low weight, supporting, additional): High weight; supportive information on depth-dependent sorption and mineralisation of Glyphosate	
Reliability	Medium
Endpoint	Mineralisation: $^{14}CO_2$-formation. Sorption: K_d-values
Protocol	Mineralisation: similar to OECD 307 (but mineralisation tested only); sorption: according to OECD 106
Test compound	Glyphosate (CAS-no.: 1071-83-6; 97.5% purity); [^{14}C-methylen] Glyphosate (>95% radiochemical purity).
Test system and conditions	Mineralisation: airtight glass flasks containing a vial with NaOH to capture $^{14}CO_2$. The aquifer sediments saturated to water holding capacity with natural groundwater. Anoxic mineralisation experiments performed in butyl rubber sealed serum flasks equipped with a base trap similar to the aerobic experiments. Sorption: batch experiment, 96 h, one concentration
Statistical design	Number of replicates not reported; 2 soils sampled at 8 different depths.
Relevance	
Environmental relevance	Given; influencing parameter reported.
Weight of evidence	
“Positive”/”Negative” evidence	Supported by other publications; no negative evidence.

Spanoghe et al. (2005)

Title: Rainfastness and adsorption of herbicides on hard surfaces
Author: Pieter Spanoghe, Johan Claeys, Luc Pinoy and Walter Steurbaut
Reference: Pest Manag Sci 61:793–798
Year: 2005
Results and conclusion: Herbicides are still used to control weeds on hard surfaces, including municipal, private and industrial sites. In this study, three kinds of hard surface were evaluated: asphalt, concrete surface and gravel (fine and coarse). Three herbicides were applied: glyphosate, diuron and diflufenican. At different times after treatment with the herbicides, rainfall was simulated and substance concentration determined in run-off. After this run-off event, the materials were immersed in water to measure desorption which, together with the compound in the run-off, gave a measure of the dislodgable resi-

dues. The polar herbicide glyphosate lost 75% in run-off from asphalt but was adsorbed strongly to soil and concrete pavement.	
Proposed action: Not to be considered as building material and no soils were tested.	
Type of information (critical, high/low weight, supporting, additional): High weight; supportive information on behaviour of Glyphosate having been applied onto building material and subjected to run-off events.	
Reliability	High
Endpoint	Concentration in run-off; K_d-values for building material
Protocol	No standard protocol; non-GLP
Test compound	Glyphosate (CAS-no.: 1071-83-6; product Canyon® was applied).
Test system and conditions	a) runoff-experiments: The hard surfaces were treated with a suspension concentrate containing 112 g/L glyphosate, 71 g/L diuron and 15 g/L diflufenican (Canyon®). Rainfall was simulated and thereafter, run-off was collected from the drain. b) adsorption experiment: kinetics measured; Freundlich isotherm was obtained by adding building material to solutions of differing concentrations of the herbicide. Concentration in the supernatant was analysed. c) desorption experiment: building material obtained after a) was immersed in water for 72 h.
Statistical design	Number of replicates not reported; for Freundlich-isotherms 4 concentrations were measured.
Relevance	
Environmental relevance	Given; influencing parameter reported.
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other publications; no negative evidence.

Strange-Hansen et al. (2004)

Title: Sorption, mineralization and mobility of N-(phosphonomethyl)glycine (glyphosate) in five different types of gravel
Author: Rikke Strange-Hansen, Peter E Holm, Ole S Jacobsen and Carsten S Jacobsen
Reference: Pest Manag Sci 60:570–578
Year: 2004
Results and conclusion: Cumulative mineralization of [methyl-14C]glyphosate in batch studies was highest in coarse gravel, amounting to 14% after 4 days at 30°C and 32% after 31 days. Mineralization was slowest in the sandy reference soil, amounting to only 2% after 31 days. The adsorption coefficient (K_d) of glyphosate in gravel ranged from 62 to 164 litre kg^{-1}, while that in the sandy reference soil was 410 litre kg^{-1}. The results indicate that the relatively low K_d in gravel allows a relatively high rate of glyphosate mineralization by the biomass. When K_d is high, in contrast, mineralization is slow. Lowering the temperature to 10°C decreased mineralization by 50% in one of two gravels. The leaching of glyphosate was screened in simple columns of gravel or soil in which precipitation events (20mm over a 2-h period) were simulated on three occasions, starting either immediately after or 2 days after application of glyphosate. [^{14}C]Glyphosate was applied as a tracer mixed with the commercial product Roundup Garden at the recommended rate of 2.4 kg glyphosate ha^{-1}, equivalent to 1$\mu g g^{-1}$ soil. The highest concentration of [^{14}C] compounds (expressed in terms of glypho-

sate concentration) in leachate from the columns exceeded 1300 µg litre^{-1}, and was detected in rounded gravel after the first rain event. No glyphosate was detected in leachate from the sandy reference soil.	
Proposed action: Not to be considered as raw data on mass balances and on concentrations in the aqueous and solid phases are not reported. K_f-values cannot be calculated from the published data. Furthermore, the validity of the study cannot be proven.	
Type of information (critical, high/low weight, supporting, additional): Low, additional information	
Reliability	Medium
Endpoint	K_d = distribution coefficient; [^{14}C]carbon dioxide
Protocol	According to the OECD guidelines (106), but with a modified soil: solution ratio of 2 instead of 5; non-GLP,
Test compound	[methyl-^{14}C]Glyphosate (specific activity 1.08MBq mmol^{-1}; radiochemical purity >99%); unlabelled glyphosate (purity 98%); for column experiments, Roundup Garden (commercial SL formulation containing 120 g litre^{-1} glyphosate (isopropylamine salt)); five types of gravel and a sandy agricultural reference soil; CAS-no.: 1071-83-6
Test system and conditions	Batch studies: 1) Sorption: glyphosate/[^{14}C]glyphosate concentration of 0.6 mgkg^{-1} (192 Bq), rotated for 96 h; 2) Mineralization in flasks: glyphosate/[^{14}C]glyphosate concentration of 16.9mgkg^{-1} (0.1mM) and 833 Bq, final moisture level equivalent to 80% of WHC, incubated at 30 ◦C in the dark for 31 days, repeated on two types of gravel incubated at 10, 20 and 30 ◦C; 3) Leaching studies: Two columns for each substrate, and each column was exposed to two different simulated precipitation events (Table 3). [^{14}C]Glyphosate (1733 Bq in short columns, and 5666 Bq in tall columns) mixed with Roundup Garden (recommended rate of 2.4 kg glyphosate ha^{-1}), Over the next 6 days the columns were subjected to three simulated precipitation events at 20 ◦C, trapping the [^{14}C]carbon dioxide, total effluent collected 1 day after each precipitation event, determination of residual [^{14}C] compounds
Statistical design	Analyses in triplicate; K_d value represents one measurement of glyphosate in the solution, assuming linearity between glyphosate adsorption and glyphosate concentration in the solution
Relevance	
Environmental relevance	Given as influence by environmental parameter was tested.
Weight of evidence	
“Positive”/”Negative” evidence	Results supported by other publications.

Todorovic (2009)

Title: BEHAVIOR OF ORGANIC POLLUTANTS IN THE SOIL ENVIRONMENT. SPECIAL FOCUS ON GLYPHOSATE AND AMPA
Author: Gorana Rampazzo Todorovic
Reference: “Qualità of the Environment" series assembles the scientific communications presented during the "Air, Water, and Soil Quality" International Congress held at Imola

(Imola) on 24th and 25th of June 2009. ISBN 10: 88-901261-7-5 ISBN 13: 978-88-901261-7-8	
Year: 2009	
Results and conclusion: The aim of the meeting was to take account of the present quality of the air water-soil system, comparing Italian realities with those in other countries of the European Union and to make known the most efficient measures and instruments for fighting ecosystem degradation and the waste of resources. In this document, the state of the art regarding the main mechanisms, processes and factors governing the fate and behaviour of organic contaminants in the soil-groundwater system is reviewed. The behaviour of organic contaminants in soils is generally governed by a variety of complex dynamic physical, chemical and biological processes, including sorption–desorption, volatilization, chemical and biological degradation, uptake by plants, run-off, and leaching. These processes directly control the transport of contaminants within the soil and their transfer from the soil to water, air or food. The relative importance of these processes varies with the chemical nature of the contaminant and the properties of the soil. Both the direction and rate of these processes depend on the chemical nature of the organic contaminant and the chemical, biological, and hydraulic properties of the soil. Better understanding of the behaviour of glyphosate is needed (e.g. adsorption conditions, environmental influence, specific soil parameters, soil microbes behaviour) for a better risk assessment of environmental pollution.	
Proposed action: Not to be considered as the article is a review and no raw data are published.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information only.	
Reliability	Medium
Endpoint	Soil properties and the K_d-values for Glyphosate for different soils and silica Sand based on literature data
Protocol	-
Test compound	-
Test system and conditions	Information about: Dissipation ways of the organic pollutants; Soil parameters governing the glyphosate fate in environment; Glyphosate sorption in soil; Glyphosate sorption on iron oxides; Biodegradation of glyphosate; Glyphosate biodegradation pathways; Environmental fate of metabolites; Influence of glyphosate on shallow aquifers/aquatic/marine ecosystems
Statistical design	-
Relevance	
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	Summary of results of other publications.

Vereecken (2005)

Title: Review Mobility and leaching of glyphosate: a review
Author: Harry Vereecken
Reference: Pest Manag Sci 61:1139–1151

Year: 2005	
Results and conclusion: The purpose of this review is to present and discuss the state of knowledge with respect to the mobility and leaching of glyphosate from agricultural soils. Specific attention is given to the adsorption behaviour of glyphosate and the analysis of available studies on glyphosate transport. In addition, there are a number of experimental and numerical studies indicating that other strongly sorbing substances may be transported rapidly to the subsurface. The experimental studies analysed in the paper encompass column-, lysimeter and field-scale experiments on glyphosate transport. The experimental findings, combined with transport studies on other strongly sorbing pesticides in the literature, support the hypothesis that transport of glyphosate may be caused by an interaction of high rainfall events shortly after application on wet soils showing the presence of preferential flow paths. Concentrations of glyphosate in European groundwater have been reported occasionally but monitoring is still limited.	
Proposed action: Consider as additional information. The article presents an overview on the state of the art with respect to mobility and leaching of glyphosate in agricultural soils.	
Type of information (critical, high/low weight, supporting, additional): Medium; overview and summary of endpoints	
Reliability	Medium
Endpoint	Freundlich exponent, K_f; Freundlich distribution coefficient; summary data for field sites with respect to leaching of glyphosate
Protocol	-
Test compound	-
Test system and conditions	Information about: adsorption of Glyphosate (on clay minerals; on soil organic matter, on soil oxides and hydroxides); mobility and leaching of Glyphosate (on the laboratory scale, on the lysimeter scale, on the field scale); occurrence in groundwater
Statistical design	-
Relevance	
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	Results supported by other publications.

Waiman et al. (2012)

Title: A simple and rapid spectrophotometric method to quantify the herbicide glyphosate in aqueous media. Application to adsorption isotherms on soils and goethite
Author: Carolina V. Waiman, Marcelo J. Avena, Mariano Garrido, Beatriz Fernández Band, Graciela P. Zanini
Reference: Geoderma 170, 154–158
Year: 2012
Results and conclusion: This article presents a simple, fast and low cost UV–vis spectrophotometric method to quantify glyphosate. This method can be used to perform adsorption isotherms on soils and metal oxides. It comprises a derivatization step and further measurement of the absorbance at 265 nm. The trueness of the results is validated using Ultra Performance Liquid Chromatography with tandem mass spectrometry detection (UPLC-MS/MS) as a reference method. The proposed spectrophotometric method is able to quantify glyphosate in the concentra-

tion range from 0.084 to 21.8 mg L^{-1}. This range is suitable to construct reliable adsorption isotherms. Examples of adsorption isotherms on goethite at pH 4.5 and a soil sample at pH 4.5, 6.0 and 8.0 are given. Interferences caused by dissolved organic matter can be corrected at least up to an organic matter concentration of 12 mg L^{-1}.	
Proposed action: Not to be considered as the article focuses on method development. Raw data on mass balances and concentrations in the aqueous and solid phases are not sufficient; the validity of the results cannot be proven.	
Type of information (critical, high/low weight, supporting, additional): Low weight, may be additional information	
Reliability	Medium
Endpoint	K_f and 1/n
Protocol	Standard, OECD
Test compound	Analytical-standard glyphosate (PESTANAL, 99.729%), CAS-no.: 1071-83-6. Analytical reagent-grade disodium tetraborate decahydrate ($Na_2B_4O_7.10H_2O$), 9-fluorenyl methoxycarbonyl chloride (FMOC-Cl) for synthesis; one soil and goethite
Test system and conditions	Adsorption isotherms: batch equilibration technique at room temperature; concentration range from 7 mg L−1 to 190 mg L−1; Isotherms were performed at pH 4.5, 6.0 and 8.0. Quantification of glyphosate was performed by UV–vis spectrophotometry after a derivatization step with FMOC-Cl in alkaline media
Statistical design	Freundlich isotherms
Relevance	
Environmental relevance	Given. The parameters influencing the endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Waimann et al. (2013)

Title: A real time in situ ATR-FTIR spectroscopic study of glyphosate desorption from goethite as induced by phosphate adsorption: Effect of surface coverage
Author: C. V. Waiman, M. J. Avena, A. E. Regazzoni, G. P. Zanini
Reference: Journal of Colloid and Interface Science 394 (2013) 485–489
Year: 2013
Results and conclusion: The desorption of glyphosate from goethite as induced by the adsorption of phosphate was investigated by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy in combination with adsorption isotherms. Desorption of glyphosate was very low in the absence of phosphate. Addition of phosphate promoted glyphosate desorption. At low initial surface coverages, added phosphate adsorbed on free surface sites, mainly, displacing a small amount of glyphosate. At high initial surface coverages, on the contrary, phosphate adsorption resulted in a significant glyphosate desorption. In the latter conditions, the ratio desorbed glyphosate to adsorbed phosphate was 0.60. The desorption process can be explained by assuming that phosphate adsorbs first forming a monodentate mononuclear complex, which rapidly evolves into a bidentate binuclear complex that displaces glyphosate.
Proposed action: Consider as additional information.

Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	
Endpoint	Glyphosate desorption from goethite as induced by phosphate adsorption
Protocol	Not given
Test compound	Glyphosate, phosphate
Test system and conditions	Goethite synthesis and characterization: Goethite particles were synthesized as described by Puccia et al, following the methodology proposed by Atkinson et al. Adsorption isotherms: Glyphosate and phosphate adsorption isotherms were obtained by batch equilibration experiments. They were performed by adding 0.2 mL of the stock goethite suspension (22.10 g L^{-1}) to 15mL polypropylene centrifuge tubes, to which 9.8 mL of an aqueous solution of known concentration of either glyphosate or phosphate was added; the concentration of the background electrolyte (KCl) was 0.1 M. The pH of these dispersions was adjusted to 4.5 and kept constant by adding a few microliters of either KOH or HCl solutions. The tubes were shaken overnight with an end-overend rotator, and then, the supernatants separated by centrifugation. The concentration of glyphosate in the supernatants was measured by the UV-Vis spectrophotometric method proposed by Waiman et al. The concentration of phosphate was quantified by the molybdenum blue method proposed by Murphy and Riley. UV–Vis spectra were recorded with an Agilent 8453 UV–Vis diode array spectrophotometer equipped with a 1 cm Hellma quartz cell. The amount of glyphosate and phosphate adsorbed by goethite was calculated solving the mass balance of the systems. ATR-FTIR spectroscopy: ATR-FTIR spectra were obtained using a Nicolet Nexus 470 FTIR spectrometer equipped with a DTGS detector, a SMART-ARK ATR accessory and a ZnSe crystal (area: 10 × 72 mm, incident angle: 45º, total reflections: 12).
Statistical design	Not given
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Wang (2005)

Title: Effects of phosphate on the adsorption of glyphosate on three different types of Chinese soils
Author: WANG Yu-Jun, ZHOU Dong-Mei, SUN Rui-Juan
Reference: Journal of Environmental Sciences Vol. 17, No.5, 711-715
Year: 2005
Results and conclusion: This paper studied the effects of phosphate on the adsorption of glyphosate on three differ-

ent types of Chinese soils including two variable charge soils and one permanent charge soil. The results indicated that Freundlich equations used to simulate glyphosate adsorption isotherms gave high correlation coefficients (0.990-0.998) with K values of 2751, 2451 and 166 for the zhuanhong soil (ZH soil, Laterite), red soil (RS, Udic Ferrisol) and Wushan paddy soil (WS soil, Anthrosol), respectively. The more the soil iron and aluminium oxides and clay contained, the more glyphosate adsorbed. The presence of phosphate significantly decreased the adsorption of glyphosate to the soils by competing with glyphosate for adsorption sites of soils. Meanwhile, the effects of phosphate on adsorption of glyphosate on the two variable charge soils were more significant than that on the permanent charge soil. When phosphate and glyphosate were added in the soils in different orders, the adsorption quantities of glyphosate on the soils were different, meaning a complex interaction occurred among glyphosate, phosphate and the soils.	
Proposed action: Not to be considered as non-EU soils were tested.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as focus was on adsorption influenced by inorganic phosphate	
Reliability	Medium
Endpoint	K values obtained by Freundlich equations; correlation coefficients
Protocol	Partly similar to OECD 106, non-GLP
Test compound	Glyphosate (purity not given), CAS-no.: 1071-83-6; phosphate (analytical grade):
Test system and conditions	Batch experiments, 3 soils, Glyphosate adsorption isotherms on the soils with and without phosphate were performed
Statistical design	Measurements in duplicate; Freundlich isotherms
Relevance	
Environmental relevance	Applying glyphosate in soil containing higher content of phosphate will possibly increase the environmental risk of glyphosate transfening from soil to groundwater and surface water.
Weight of evidence	
“Positive”/”Negative” evidence	Results supported by other publications; no negative evidence.

Wang et al. (2006)

Title: Cosorption of zinc and glyphosate on two soils with different characteristics
Author: Yu-Jun Wang, Dong-Mei Zhou, Rui-Juan Sun, Long Cang, Xiu-Zhen Hao
Reference: Journal of Hazardous Materials A137, 76–82
Year: 2006
Results and conclusion: Cosorption of Zn and glyphosate on a Red soil (RS, Udic Ferrosols) and a Wushan soil (WS, Anthrosol) was studied. In comparison with the WS, the RS has less adsorption capacity for Zn and higher for glyphosate. The presence of glyphosate decreased Zn adsorption on the two soils, which are resulted from the decreased equilibrium solution pH caused by the added glyphosate, and also the formation of water-soluble complexes of glyphosate with solution Zn^{2+} that had lower affinity to soil surface in comparison with Zn^{2+} itself. Such effect is more significant on the RS than on the WS, mainly because of the less adsorption quantity of Zn on the former one. On the contrary, the presence of Zn increased the adsorption quantities of glyphosate on the RS and WS, which is resulted from the decreasing pH value of the equilibrium solution caused by Zn^{2+} exchange with H^{+} ions of soil surface. Such results suggest that glyphosate in field may increase the mobility and bioa-

vailability of Zn and correspondingly increase its environmental risk.	
Proposed action: Not to be considered as non-EU soils were tested.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as focus was on adsorption influenced by zinc	
Reliability	Medium
Endpoint	No K values, only adsorption isotherms
Protocol	Partly similar to OECD 106, non-GLP
Test compound	Glyphosate (purity not given); CAS-no.: 1071-83-6
Test system and conditions	Glyphosate adsorption isotherm on two soils in the absence and presence of Zn; 7 concentrations of glyphosate and 3 concentrations of Zn
Statistical design	Measurements performed in replicate, no further statistic
Relevance	
Environmental relevance	Parameter influencing endpoints are measured and reported.
Weight of evidence	
“Positive”/”Negative” evidence	Results supported by other publications; no negative evidence.

Wang et al. (2009)

Title: Adsorption Kinetics of Glyphosate and Copper(II) - Alone and Together on Two Types of Soils	
Author: Yu-Jun Wang, Yu-Xia Cui, Dong-Mei Zhou, Shen-Qiang Wang, An-Yun Xiao, Ru-Hai Wang	
Reference: Soil Sci. Soc. Am. J. 73(6): 1995-2001. doi:10.2136/sssaj2008.0360	
Year: 2009	
Results and conclusion: Adsorption kinetics of glyphosate and Cu(II) alone and together were studied using a continuous flow experimental setup on two soils with different characteristics at pH5.5. Four kinetic models, i.e., the Lagergren first-order, pseudo-second-order, Elovich, and power function equations, were successfully used to describe their adsorption kinetics. Among the four models, the Lagergren first-order kinetic model fit the experimental data of glyphosate and Cu(II) adsorption the best. Glyphosate significantly increased the adsorption quantity of Cu(II) on the Red soil (a Hapludult or Udic Ferrosol), due to the fact that Cu(II) was adsorbed on the sites where glyphosate had been strongly adsorbed. Glyphosate decreased the adsorption of Cu(II) on the Wushan soil (a Haplaquept or Anthrosol), however, because adsorption of glyphosate on this soil was weak and the complex of glyphosate and Cu(II) tended to be highly soluble in water, thus preventing Cu(II) from exchanging with Ca^{2+} and Mg^{2+} ions on the soil surface. On the other hand, the presence of Cu(II) decreased the adsorption of glyphosate on both soils, which may be attributed to the lower affinity of the Cu(II)–glyphosate complex to the soils than glyphosate alone.	
Proposed action: Not to be considered as non-EU soils were tested.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as focus was on adsorption influenced by inorganic Cu(II)	
Reliability	Medium
Endpoint	Correlation coefficients (R^2); Kinetic parameters and the normalized standard deviation
Protocol	Non-GLP
Test compound	Glyphosate (purity not given), CAS-no.: 1071-83-6
Test system and conditions	Column experiments, one glyphosate concentration; with or without Cu(II); soils: surface (0–20 cm) Red soil (low pH (4.95) and high Fe oxide content) and a surface (0–20 cm) Wushan soil (high organic matter content, CEC, and pH (7.20)
Statistical design	Lagergren first-order, pseudo-second-order, Elovich, and power function equations
Relevance	
Environmental relevance	Not given; parameter influencing endpoints are not measured and reported.
Weight of evidence	
“Positive”/”Negative” evidence	No negative evidence.

Xu et al. (2009)

Title: Land Use and Riparian Effects on Prairie Wetland Sediment Properties and Herbicide Sorption Coefficients
Author: Dani Xu, Sheila Meyer, Jeanette Gaultier, and Annemieke Farenhorst, Dan Pen-

nock	
Reference: J. Environ. Qual. 38:1757–1765	
Year: 2009	
Results and conclusion: Bottom sediments were sampled in 0- to 5- and 5- to 10-cm sections from 17 wetlands under five different land use classes. Sediments were analyzed for total organic carbon (TOC), total inorganic carbon (TIC), pH, electrical conductivity, exchangeable cations (EXCAT), total cation exchangeable capacity (CEC), and percent clay (%clay). Sediment herbicide sorption partition coefficient (K_d) was measured for trifluralin, atrazine, 2,4-D, and glyphosate. The sorption of the herbicides in the sediment increased in the order of 2,4-D < atrazine < glyphosate < trifluralin. The sorption of 2,4-D, atrazine, and trifluralin was positively correlated to TOC, EXCAT, and CEC but negatively correlated to %clay. Glyphosate sorption was negatively correlated to pH, TIC, EXCAT, and %clay. Overall, wetland sediments that were recently cultivated (ECNR and E4G) had lower TOC, TIC, EC, EXCAT, CEC, and K_d values (2,4-D, trifluralin, and atrazine) than sediments that had not been recently cultivated (ECR, E20G, and SP).	
Proposed action: Not to be considered as raw data on mass balances and concentrations in the aqueous and solid phases are not given. Thus, the validity of the study cannot be proven.	
Type of information (critical, high/low weight, supporting, additional): Medium weight, additional information about the influence of TOC etc of the sorption	
Reliability	High
Endpoint	Sorption partition coefficient K_d (L kg^{-1}); K_{oc}; Data as mean ± SE
Protocol	similar to OECD 106 , non-GLP
Test compound	Glyphosate (99% purity; Chem Service, West Chester, PA), and (phosphonomethyl-^{14}C) glyphosate (95% purity, specific activity 344.1 MBq $mmol^{-1}$; Sigma-Aldrich Co.); CAS-no.: 1071-83-6
Test system and conditions	Batch equilibrium experiments, set to 20°C until equilibrated (24 h)
Statistical design	In duplicate, Kolmogorov-Smirnov normality test, nonparametric Kruskal-Wallis test
Relevance	
Environmental relevance	Given. Parameter influencing endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Yu and Zhou (2005)

Title: Adsorption characteristics of pesticides methamidophos and glyphosate by two soils
Author: Ying Yu, Qi-Xing Zhou
Reference: Chemosphere 58, 811–816
Year: 2005
Results and conclusion: Contributions of organic matter and minerals in soil were evaluated by comparing changes in adsorption of methamidophos (MDP) and glyphosate (GPS) before and after removal of organic matter from argaltoll (mollisol) and typustalf (alfisol) soils. Adsorption isotherms of MDP and GPS by the two soils comforted to Freundlich equation, and the adsorption capacity of GPS by argaltoll soil was higher than that of MDP. Due to the removal of organic matter from soils, K_f values of MDP and GPS adsorbed by argaltoll soil, which were

calculated from Freundlich equations and the measure of adsorption capacity, decreased by 46.1% and 75.0%, and these by typustalf soil decreased by 34.9% and 52.5%, respectively. Results from this study suggested that soil organic matter made greater contributions to adsorption of GPS, but soil minerals could provide more available adsorption sites for MDP.	
Proposed action: Not to be considered as non-EU soils were tested.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information about the influence of organic matter	
Reliability	High
Endpoint	Freundlich coefficients K_f, n_f (measure of the nonlinearity of the isotherm; indicates concentration dependence of adsorption), correlation coefficient rf and standard error Se
Protocol	Similar to OECD 106, non-GLP
Test compound	Commercial products: the emulsified oil containing 40% of MDP (pH 6.4) and water agent containing 10% of GPS salt (pH 6.1) (from the Chemical Plant of the Zhejiang Technical University and the Jiangnan Chemical Plant in Zhenjiang, Jiangsu Province); CAS-no.: 1071-83-6
Test system and conditions	Batch-equilibration technique; two unpolluted surface soils; with or without organic matter; different concentration of MDP or GPS; shaken at 25 ± 1 °C for 24 h
Statistical design	Three replicates; Freundlich isotherms;
Relevance	
Environmental relevance	Given. Parameter influencing endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Yu et al. (2005)

Title: Effects of methamidophos and glyphosate on copper sorption-desorption behavior in soils
Author: YU Ying, ZHOU Qixing, HE Zhenli
Reference: Science in China Ser. C Life Sciences Vol.48 Supp. I 67—75
Year: 2005
Results and conclusion: A batch-equilibration technique was employed to study the impact of two organophosphorus pesticides methamidophos (MDP) and glyphosate (GPS) on copper (Cu^{2+}) sorption-desorption for phaeozem and burozem collected from Northeastern China. The addition of the two pesticides decreased Cu^{2+} sorption, increased Cu^{2+} desorption and prolonged the equilibrium time of Cu^{2+} sorption-desorption. But GPS appeared to exert a stronger influence on Cu^{2+} sorption-desorption due to its stronger complexion with Cu^{2+}. When MDP was added, Cu2+ sorption-desorption was linearly correlated with MDP treatment concentrations. But in the presence of GPS, Cu^{2+} sorption first underwent a rapid decrease period, and then slowly tended towards a steady period. The reverse pattern could be found for Cu^{2+} desorption in the presence of GPS. Without pesticides and with the existence of MDP, Cu^{2+} sorption-desorption kinetics was well conformed to two-constant equation and Elovich equation. But that was not the case for Cu^{2+} desorption kinetics in the presence of GPS although its sorption could be also described by these two equations.

Proposed action: Not to be considered as non-EU soils were tested.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information on complex reaction	
Reliability	Medium
Endpoint	Partition coefficient Kp ($L \cdot kg^{-1}$), Standard deviation
Protocol	Similar to OECD 106, non-GLP
Test compound	Emulsified oil containing 40% MDP (pH 6.37) (Zhejiang Industry University Chemical Plant); 10% GPS salt solution (pH 6.12) Jiangnan Chemical Plant); CAS-no.: 1071-83-6
Test system and conditions	Batch-equilibration technique; two unpolluted soils different concentrations of MDP or GPS; sorption of Cu^{2+}; shaken for 24 h; desorption experiment; kinetic study equilibrated for 1, 2, 5, 10, 15, 20, 40, 80, 120, 240 min
Statistical design	Three replicates
Relevance	
Environmental relevance	Given. Parameter influencing endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Zhao et al. (2009)

Title: Glyphosate mobility in soils by phosphate application: Laboratory column experiments
Author: Bingzi Zhao, Jiabao Zhang, Jiandong Gong, Hui Zhang, Congzhi Zhang
Reference: Geoderma 149: 290-297
Year: 2009
Results and conclusion: (1) phosphate application might induce system pH decrease, and (2) the overall glyphosate mobility by phosphate application might depend on the relative contribution of two competing processes: increase in glyphosate adsorption from pH decrease versus reduced glyphosate adsorption from competitive adsorption between phosphate and glyphosate for sorption sites available. To test these two hypotheses, laboratory batch and column experiments using a miscible displacement approach were conducted on two Primosols and one Anthrosols, to investigate, respectively, (1) pH-dependent glyphosate adsorption onto the three studied soils, and (2) glyphosate leaching and mobility in soil columns as influenced by phosphate application. Our results showed that glyphosate adsorption consistently decreased with increase in system pH. The effect of phosphate application on glyphosate mobility varied with soil type. We conclude that phosphate application can cause system pH change with various extents in the soil, which subsequently contribute to glyphosate mobility in different degree.
Proposed action: To be considered as additional information for the endpoint sorption and mobility. The article presents basic research and understanding of pH and phosphate influence. Furthermore, raw data on mass balances and concentrations in the aqueous and solid phases are not reported and thus, the validity cannot be proven.
Type of information (critical, high/low weight, supporting, additional): Low weight; additional information of mechanisms of glyphosate mobility in the presence of phosphate

Reliability	Medium
Endpoint	Breakthrough curves (BTCs)
Protocol	Partly similar to OECD 106, non-GLP
Test compound	Glyphosate (99.9% purity); CAS-no.: 1071-83-6
Test system and conditions	1) batch experiments, shaken for 20 h at 25 °C; three soils 2) column leaching experiments, 6 column experiments including with or without phosphate (two concentrations), and 3 levels of glyphosate introduced;
Statistical design	Duplicate samples
Relevance	
Environmental relevance	Given. Parameter influencing endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Zhou et al. (2004)

Title: Adsorption and cosorption of cadmium and glyphosate on two soils with different characteristics	
Author: Dong-Mei Zhou, Yu-Jun Wang, Long Cang, Xiu-Zhen Hao, Xiao-San Luo	
Reference: Chemosphere 57, 1237–1244	
Year: 2004	
Results and conclusion: Adsorption and cosorption of cadmium and glyphosate on a Wushan soil (WS soil, Anthrosol) and a Zhuanhong soil (ZH soil, Udic Ferrisol) as affect by solution pH were studied by means of batch adsorption experiments. It indicated that the adsorption quantity of Cd or glyphosate was highly relevant to soil characteristics. The WS soil had higher adsorption capacity of Cd than the ZH soil, due to its high organic matter content and cation exchange capacity (CEC). In contrast, the adsorption quantity of glyphosate on the WS soil was less than that on the ZH soil, because the WS soil has lower iron and aluminum oxides content but higher pH than the ZH soil. The herbicide glyphosate affected Cd adsorption on the two soils when they coexisted in a same soil solution, which was attributed to a glyphosate-induced pH-decrease and the corresponding decline in negative surface charges of the soil. Besides that, glyphosate reacted with solution Cd to form the water-soluble complexes that had lower affinity to soil surface in comparison with Cd itself. On the other hand, the presence of Cd in the soil solution also affected the adsorption of glyphosate on the soils. The presence of Cd increased adsorption quantity of glyphosate on the WS and ZH soils, which was resulted from the decrease of equilibrium solution pH caused by Cd2+ exchange with H+ ions of soil surface. In addition to that, glyphosate adsorption possibly takes place on sites where Cd was previously adsorbed and acted as a bridge between the soil and glyphosate.	
Proposed action: Not to be considered as non-European soils were tested.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as focus was on adsorption influenced by inorganic cadmium and by pH	
Reliability	High
Endpoint	Freundlich equations and their correlation coefficients
Protocol	Similar to OECD 106; non-GLP
Test compound	Glyphosate (purity not given); CAS-no.: 1071-83-6

Test system and conditions	1) Cadmium adsorption isotherms in the presence and absence of glyphosate; 2) Effect of pH on Cd adsorption in the presence and absence of glyphosate; 3) Glyphosate (different concentrations) adsorption isotherm on the soils in the presence and absence of cadmium, shaken for 2h at 25°C; 4) Effect of pH on glyphosate adsorption in the presence and absence of cadmium
Statistical design	Two replicates; Freundlich equations; two soils
Relevance	
Environmental relevance	Given; parameter influencing endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

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B.8.11.3.2 Mobility in soil

Column leaching studies

Summary

Several articles on soil column leaching can be found in literature. The results show that glyphosate and AMPA can only be found in groundwater under exceptional cases, usually in soil prone to preferential flow. Furthermore, it can be concluded from a soil column leaching study that glyphosate sorption does not necessarily result in phosphate release from soil.

In detail, 12 articles are available from open literature presenting information on glyphosate leaching in laboratory or outdoor soil columns. Either structured or undisturbed soil columns were used. Three representative articles out of the 12 publications are discussed:

Al-Rajab et al. (2008) performed a leaching experiment in undisturbed soil columns under outdoor conditions for 11 months. The authors suggest that non-extractable residues, which are formed at the beginning of the study, become available and take part in biodegradation and leaching. The amounts of ^{14}C-glyphosate derivatives leached were less than 0.28 % of the initially applied glyphosate. AMPA metabolite generally represented up to 100 % of the residues present in the leachates. The results of leaching were highly influenced by the hydrodynamic properties and the biodegradation capacities of the soils.

The leaching of glyphosate through structured soil columns was studied by Dousset et al. (2004) using a loamy sand and two sandy loams from sites currently under Christmas tree cultivation in the Morvan (France). After 160 mm of simulated rainfall applied over 12 days, less than 0.01 % of applied glyphosate appeared in the leachate. The mobility was greater in the soils with higher gravel contents, coarser textures, and lower organic carbon contents. Moreover, glyphosate migration seems negatively correlated not only to soil organic carbon, but also to aluminum and iron contents of soils.

Furthermore, Barrett et al. (2007) concluded that glyphosate sorption does not necessarily result in phosphate release from soil. The authors suggested that there is only limited competition between glyphosate and phosphate for sorption sites.

Detailed description of open literature on column leaching

Barrett and McBride (2007)

Title: PHOSPHATE AND GLYPHOSATE MOBILITY IN SOIL COLUMNS AMENDED WITH ROUNDUP	
Author: Katherine A. Barrett and Murray B. McBride	
Reference: Soil Science;172:17–26	
Year: 2007	
Results and conclusion: Sorption of glyphosate and competitive desorption of phosphate in soils has been measured in column leaching experiments. Soils representing a wide range of physical and chemical properties, including total and soluble P, were included in the study. The results suggest that glyphosate sorption does not necessarily result in PO_4^{3-} dissolution and that there is only limited competition for sorption sites between glyphosate and PO_4^{3-}. Strong glyphosate sorption on high-organic matter soils indicates bonding of this anion by a metal bridge to organic functional groups.	
Proposed action: To be considered as additional information only since the soil column leaching studies are not required. Data on sorption/desorption are sufficient for the assessment.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information to endpoint leaching, soil column leaching	
Reliability	Medium
Endpoint	Concentration of glyphosate in the leachate, total P and PO_4^{3-} in the leachate.
Protocol	No standard protocol followed, non-GLP
Test compound	Commercial Roundup (containing 27% active ingredient), CAS-no.:1071-83-6
Test system and conditions	Soil column studies with homogenized soils. Air-dried soil packed into polycarbonate tubes (width of 5.5 cm and height of 10 cm, with soil depth of approximately 6 cm). Application of roundup, ageing for 24 h, 100 mL leachate.
Statistical design	Duplicates, StatView software, $P < 0.05$ are considered to be significantly different.
Relevance	
Environmental relevance	Given. Influencing parameter are reported and considered.
Weight of evidence	
“Positive”/”Negative” evidence	Results of other studies with identical design not known. No negative evidence.

Barrett and McBride (2006)

Title: Trace Element Mobilization in Soils by Glyphosate
Author: K. A. Barrett and M. B. McBride
Reference: Soil Sci. Soc. Am. J. 70: 1882–1888
Year: 2006
Results and conclusion: No significant increases in elemental leaching were detected in mineral and organic soils with normal background concentrations of heavy metals and phosphor.

Proposed action: Not to be considered for the endpoint sorption and leaching as desorption of trace elements is measured.	
Type of information (critical, high/low weight, supporting, additional): Low weight may be additional information to already existing.	
Reliability	Medium
Endpoint	Trace element concentrations in soil column leachates.
Protocol	Non-GLP, no standard protocol
Test compound	Reagent-grade glyphosate [N-(phosphonomethyl)glycine, 96%), CAS-no.:1071-83-6
Test system and conditions	Tendency for glyphosate to mobilize Cu and other elements was tested in soil leaching experiments by applying glyphosate alone or complexed with Cu to mineral and organic soil columns (10 cm height, 1.8 cm i.d.) and measuring the concentrations of these elements in the leachates.
Statistical design	ANOVA analysis of variance, 6 soils tested, single test (?)
Relevance	
Environmental relevance	Given, environmental parameter reported.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies with identical design and objective not known. No negative evidence.

Candela et al. (2007)

Title: Laboratory studies on glyphosate transport in soils of the Maresme area near Barcelona, Spain: Transport model parameter estimation
Author: L. Candela, J. Álvarez-Benedí, M.T. Condesso de Melo, P.S.C. Rao
Reference: Geoderma 140, 8–16
Year: 2007
Results and conclusion: Batch and column experiments were performedbusing two soils collected from the Maresme area near Barcelona, Spain. Measured batch sorption isotherms for glyphosate conformed to the Freundlich model. The sorption coefficients ranged from 93 to 154, suggesting that it is strongly bound to soil. Glyphosate breakthrough curves measured during steady, saturated water flow in soil columns showed asymmetrical behavior, which was attributed to non-equilibrium sorption and the presence of a sink due to irreversible sorption loss. Loss of glyphosate increased with residence times, with longer columns or with slower pore-water velocities and breakthrough occurred earlier than predicted by retardation factors calculated from batch data. A two-site equilibrium/kinetic sorption model coupled with first-order sinks was fitted to the observed glyphosate breakthrough curves to estimate the rate parameters for non-equilibrium sorption and irreversible sorption. The transport parameters obtained by numerical simulation suggest that glyphosate sorption is a kinetic process depending on the pore-water velocities and the residence time of soil solution.
Proposed action: To be considered as additional information only since the soil column leaching studies are not required.
Type of information (critical, high/low weight, supporting, additional): Sorption experiments: low weight, no additional information to already existing. Column experiments: low weight, supportive information give in-sight into the sorption kinetic pro-

cesses	
Reliability	Medium
Endpoint	K_f-values, 1/n, R^2; column break through curves
Protocol	Adsorption batch experiment: comparable to OECD 106, however, OECD not cited; column experiment: similar to soil leaching (OECD 312), non-GLP
Test compound	Glyphosate, HPLC grade, CAS-no.:1071-83-6
Test system and conditions	Batch experiments comparable to OECD 106, shaking for 24 hours, 25°C. Column transport experiments: 5 columns, 2 different lengths, 2 soils, various pore water velocities.
Statistical design	Batch experiments: five concentrations, duplicate measurements, 2 soils. Sorption isotherms
Relevance	
Environmental relevance	Given, influencing parameter well considered.
Weight of evidence	
"Positive"/"Negative" evidence	Other reliable studies support the information on sorption behaviour. No negative evidence.

Dousset et al. (2004)

Title: Transfer of hexazinone and glyphosate through undisturbed soil columns in soils under Christmas tree cultivation	
Author: S. Dousset, C. Chauvin, P. Durlet, M. Thevenot	
Reference: doi:10.1016/j.chemosphere.2004.06.007	
Year: 2004	
Results and conclusion: The leaching of glyphosate (N-(phosphono-methyl-glycine)) through structured soil columns was studied using one loamy sand and two sandy loams from sites currently under Christmas tree cultivation in the Morvan. The three soils were cultivated sandy brunisol. After 160 mm of simulated rainfall applied over 12 days, less than 0.01% of applied Glyphosate appeared in the leachate. The mobility was greater in the soils with higher gravel contents, coarser textures, and lower organic carbon contents. Moreover, glyphosate migration seems negatively correlated not only to soil organic carbon, but also to aluminium and iron contents of soils. The surface water contamination with glyphosate via the horizontal subsurface flow in upper centimetres of soil appears unlikely.	
Proposed action: To be considered as additional information only since the soil column leaching studies are not required.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information on soil column leaching	
Reliability	Medium
Endpoint	Residues in leachate
Protocol	No standard protocol, non-GLP
Test compound	Glyphosate (> 98% purity), CAS-no.:1071-83-6, AMPA, CAS-N.:1066-51-9
Test system and conditions	Undisturbed soil column leaching experiments, 15 cm inner diameter, 20 cm length, leaching experiment 48 h after Glyphosate application
Statistical design	3 sampling sites, 2 columns per site

Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Information supported by other publications; no negative evidence.

Dousset et al. (2007)

Title: Facilitated Transport of Diuron and Glyphosate in High Copper Vineyard Soils	
Author: Sylvie Dousset, Astrid Jacobson, Jean Baptiste des Sogne, Nathalie Guichard, Philippe Baveye and Francis Andreux	
Reference: Environ. Sci. Technol., 41, 8056–8061	
Year: 2007	
Results and conclusion: The effect of copper on the leaching of glyphosate through a granitic and a calcareous soil was studied using sieved-soil columns. Each soil was enriched with copper sulphate to obtain soil copper concentrations of 125, 250, 500, and 1000 $mg \cdot kg^{-1}$. Glyphosate leaching was influenced by soil pH and copper concentration. In the calcareous soil glyphosate leaching decreased as copper levels increased. In the granitic soil glyphosate leaching increased as copper levels increased. The shapes of the copper elution curves in presence of glyphosate were similar to shapes of the glyphosate curves, suggesting the formation of Cu-glyphosate complexes that leach through the soil. Increasing copper concentrations reduce glyphosate leaching through calcareous soils, and conversely, increases glyphosate leaching through granitic soils. Our findings suggest that the risk of groundwater contamination by glyphosate increases in granitic soils with elevated copper concentrations.	
Proposed action: To be considered as additional information only since the soil column leaching studies are not required. Data on sorption/desorption are sufficient for the assessment .	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because leaching through soil columns is not needed in the monograph due to comprehensive information on sorption. K_d-values are in the same range as already obtained results; furthermore, no K_f-values have been measured.	
Reliability	Medium for soil leaching; low for sorption
Endpoint	K_d-values, concentrations in leachate, break-through curves
Protocol	Batch experiments: comparable to tier II of OECD 106; soil column leaching: comparable to OECD 312. All non-GLP.
Test compound	Glyphosate (N-(phosphono-methyl-glycine; >98%purity), ^{14}C-glypho-sate (99.7% radiochemical purity), CAS-no.:1071-83-6
Test system and conditions	Batch experiments to determine K_d-values for several Cu-concentrations; soil column leaching experiments using columns of 6 cm length and inner diameter of 6.8 cm.
Statistical design	2 soils, triplicate measurement per Cu-concentration for the batch-experiments; single measurements per Cu-concentration for soil column leaching experiments.
Relevance	
Environmental relevance	Given, influences of environmental variables on sorption and leaching are investigated.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Gjettermann et al. (2011)

Title: Kinetics of Glyphosate Desorption from Mobilized Soil Particles	
Author: B. Gjettermann, C. T. Petersen, S. Hansen, C. Bender Koch, M. Styczen	
Reference: Soil Sci. Soc. Am. J. 75 (2), 434–443	
Year: 2011	
Results and conclusion: Desorption of glyphosate [N-(phosphonomethyl)glycine] on mobilized particles from column leaching was investigated. Particles leached by free drainage from the bottom and particles mobilized by splash erosion and collected next to the top of the column. Glyphosate concentrations in the leachate were determined and values of the Damköhler number were estimated. It was concluded that desorption kinetics are important for evaluating the significance of dissolved and particle-facilitated transport of glyphosate. To quantify particle-facilitated glyphosate transport, the water and solid phases in the leachate should consequently be separated within a few minutes after leaching.	
Proposed action: To be considered as additional information only since the soil column leaching studies are not required. Data on sorption/desorption are sufficient for the assessment.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information, details on dissolved and particle bound transport are not needed for endpoint calculation	
Reliability	Medium
Endpoint	Desorption from leached particles, soil column leaching, desorption constant
Protocol	Soil column leaching partly comparable to OECD 312, particle transport, non-GLP
Test compound	Glyphosate solution: 4.4% ^{14}C-labeled glyphosate, 93.5% unlabeled glyphosate, (CAS-no.:1071-83-6) and 2.1% AMPA (CAS-no.: 1066-51-9); purities not given
Test system and conditions	Two soil columns (50-cm height, 30-cm diameter) each from tilled and non-tilled plots, fresh leachate samples investigated within 30 min of sampling, and desorption from splash-eroded particles in suspension followed for 48 h.
Statistical design	4 columns in total, desorption constants calculated, regression equations, Damköhler number (Da, measure of the relative importance of kinetics to equilibrium processes in transport)
Relevance	
Environmental relevance	Given, influencing parameter were tested and considered.
Weight of evidence	
“Positive”/”Negative” evidence	Other literature of same topic as published by the same authors in 2009 supports the results; no negative evidence.

Gjettermann et al. (2011)

Title: Evaluation of Sampling Strategies for Pesticides in a Macroporous Sandy Loam Soil
Author: B. GJETTERMANN, M. STYCZEN, C. B. KOCH, S. HANSEN, AND C. T. PETERSEN
Reference: Soil and Sediment Contamination, 20:986–994
Year: 2011

Results and conclusion: It is not straightforward to sample and demonstrate the presence and transport of pesticides in heterogeneous soil. Following leaching experiments with four differently structured 50-cm-long soil columns (tilled and untilled soil), the objective of this study as to investigate the extent that visual tracing of the dye Brilliant Blue could support in soil sampling for two strongly sorbing pesticides (^{14}C-labeled glyphosate and pendimethalin). About 830 samples were collected. No pesticide was found below 10–25 cm depth by random sampling, even though 0.21–0.31% of the applied amounts were leached, and 0.18% of the soil volume was sampled. With similar sampling efforts, the pesticides could generally be traced throughout the columns by sampling from stained soil volumes, only. None of the two particular sampling strategies for pesticides produced accurate mass balances or balances that were obviously better than the other. No pesticide was detected outside stained soil volumes, except for glyphosate in one sample. Below 30 cm, stained soil comprised on average 5% of the total soil volume, leaving 95% as expectedly pesticide-free. The results suggest that much more efficient sampling for sorbing pesticides can be obtained by using the dye and focusing on stained soil volumes.	
Proposed action: Not to be considered as publication deals with a specific analytical procedure to trace pesticides in loamy soils.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Low
Endpoint	Glyphosate concentration in soil from soil columns after leaching and Brillant Blue tracing
Protocol	No protocol
Test compound	Glyphosate (CAS-no: 1071-83-6, purities not given) and AMPA, Roundup Bio, ^{14}C-glyphosate, and blank formulation. Distribution: ^{14}C-glyphosate (4.4%), unlabeled glyphosate (93.5%), and AMPA (2.1%)
Test system and conditions	Soil column leaching experiments, analysis of Glyphosate in soil layers of the soil columns, visual tracing by Brillant Blue, collection of 830 soil samples, and application of both analytical methods, establishment of mass balances
Statistical design	Not further detailed
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	Supported by other experiments. No negative evidence.

Gjettermann et al. (2009)

Title: Particle-facilitated Pesticide Leaching from Differently Structured Soil Monoliths
Author: B. Gjettermann, C. T. Petersen, C. B. Koch, N. H. Spliid, C. Grøn, D. L. Baun, M. Styczen
Reference: J. Environ. Qual. 38:2382–2393
Year: 2009
Results and conclusion: The leaching of soil particles and surface applied ^{14}C-labeled glyphosate from intact soil columns were investigated, and the relative significance of particle-facilitated pesticide

transport was quantified. Pesticide leaching was driven by preferential water flow in macropores. For the plowed structure 10% of the leached glyphosate was bound to particles whereas significantly less glyphosate was bound to particles in leachate from minimally disturbed columns. Thus, the results suggest that soil structure affected the mode of transport of glyphosate. It is likely that glyphosate sorbed strongly when applied on recently plowed soil (K_d = 503 L kg^{-1} for the soil), and that it could be mobilized and transported independently of soil particles more easily when applied on the minimally disturbed soil covered in part with crop residues (K_d < 1 L kg^{-1} for straw). Significantly less amounts of soil particles were leached from minimally disturbed (119–247 mg) than from recently plowed (441–731 mg) columns.	
Proposed action: To be considered as it is supportive information for modelling purposes.	
Type of information (critical, high/low weight, supporting, additional): High weight, supportive information	
Reliability	High
Endpoint	K_d-values; soil column leaching, macropore leaching, preferential flow, relation to tillage practices;
Protocol	Sorption of Glyphosate and AMPA: based on OECD 106; Soil column leaching: no standard, partly comparable to OECD 112, non-GLP
Test compound	Glyphosate solution: 4.4% ^{14}C-labeled glyphosate, 93.5% unlabeled glyphosate, CAS-no.: 1071-83-6, and 2.1% AMPA (CAS-no.: 1066-51-9); purities not given
Test system and conditions	Sorption: same soils as for column leaching,; Soil columns (50-cm height, 30-cm diameter) from: recently plowed (four columns) and an untilled (five columns) sandy loam soil; Glyphosate concentration in filtered and non-filtered leachate analysed.
Statistical design	Sorption: 4 concentrations for isotherms; linear adsorption isotherms of sorbed chemical as function of chemical in solution; distribution coefficient (by slope of the isotherm) calculated by linear regression analysis. Colum leaching: 4 and 5 columns, respectively; relative significance of particle-facilitated transport (fraction of leached glyphosate being bound to soil particles)
Relevance	
Environmental relevance	Given, influencing parameter were tested and considered.
Weight of evidence	
"Positive"/"Negative" evidence	Other literature on preferential flow supports information; no negative evidence.

Magga et al. (2008)

Title: Soil column experiments used as a means to assess transport, sorption, and biodegradation of pesticides in groundwater
Author: ZOI MAGGA, DIMITRA N. TZOVOLOU, MARIA A. THEODOROPOULOU, THEODORA DALKARANI, KONSTANTINOS PIKIOS and CHRISTOS D. TSAKIROGLOU
Reference: Journal of Environmental Science and Health Part B 43, 732–741
Year: 2008

Results and conclusion: Soil column experiments are used to investigate the fate of three pesticides of high, intermediate, and low solubility in groundwater: N-phosphonomethyl glycine (glyphosate); O,O-diethyl-S-[(ethylthio) methyl] phosphorodithioate (phorate); (2,4-dichlorophenoxy)acetic acid (2,4-D). Feed solutions are prepared by adding each pesticide (100 mg/L glyphosate, 50µg/L phorate, 50 mg/L 2,4-D) along with conservative tracer, KBr, in synthetic groundwater. The concentration of the pesticides in effluents is detected by ion chromatography (glyphosate, 2,4-D) and GC-FID (phorate). The Br-breakthrough curves are employed to estimate the dispersion coefficient and mean pore velocity in each column. Solute transport and reactive models accounting for equilibrium/non-equilibrium sorption and biodegradation are coupled with inverse modelling numerical codes to estimate the kinetic parameters for all pesticides.	
Proposed action: To be considered as additional information only since the soil column leaching studies are not required. Data on sorption/desorption are sufficient for the assessment	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information in the context of discussing leaching modelling (e.g. FOCUS Pelmo) results, K_d-values estimated from the soil column leaching experiment, not directly measured as it is the case in a study according to OECD 106	
Reliability	Medium
Endpoint	Concentrations in leachate, dispersion coefficients, breakthrough curves, kinetic parameter e.g., rate constant of non-equilibrium sorption, half-saturation growth constant, estimated K_d-values
Protocol	Partly comparable to OECD 312, non-GLP
Test compound	Glyphosate (purity not given), CAS-no.:1071-83-6
Test system and conditions	Transport and leaching through packed soil columns (i.d. 5 cm, length 80 cm) under controlled laboratory conditions
Statistical design	One column
Relevance	
Environmental relevance	Given, environmental parameter discussed.
Weight of evidence	
“Positive”/”Negative” evidence	No contradiction to other results; no negative evidence.

Strange-Hansen et al. (2004)

Title: Sorption, mineralization and mobility of N-(phosphonomethyl)glycine (glyphosate) in five different types of gravel
Author: Rikke Strange-Hansen, Peter E Holm, Ole S Jacobsen and Carsten S Jacobsen
Reference: Pest Manag Sci 60:570–578
Year: 2004
Results and conclusion: Cumulative mineralization of [methyl-^{14}C]glyphosate in batch studies was highest in coarse gravel, amounting to 14% after 4 days at 30°C and 32% after 31 days. Mineralization was slowest in the sandy reference soil, amounting to only 2% after 31 days. The adsorption coefficient (K_d) of glyphosate in gravel ranged from 62 to 164 litre kg^{-1}, while that in the sandy reference soil was 410 litre kg^{-1}. The results indicate that the relatively low K_d in gravel allows a relatively high rate of glyphosate mineralization by the biomass. When K_d is high, in contrast, mineralization is slow. Lowering the temperature to 10°C decreased mineralization by 50% in one of two gravels. The leaching of glyphosate was screened in

simple columns of gravel or soil in which precipitation events (20mm over a 2-h period) were simulated on three occasions, starting either immediately after or 2 days after application of glyphosate. [^{14}C]Glyphosate was applied as a tracer mixed with the commercial product Roundup Garden at the recommended rate of 2.4 kg glyphosate ha^{-1}, equivalent to 1µgg^{-1} soil. The highest concentration of [^{14}C] compounds (expressed in terms of glyphosate concentration) in leachate from the columns exceeded 1300 µg litre^{-1}, and was detected in rounded gravel after the first rain event. No glyphosate was detected in leachate from the sandy reference soil.	
Proposed action: To be considered as additional information only since the soil column leaching studies are not required Data on sorption/desorption are sufficient for the assessment	
Type of information (critical, high/low weight, supporting, additional): Low, additional information	
Reliability	Medium
Endpoint	K_d = distribution coefficient; [^{14}C]carbon dioxide
Protocol	According to the OECD guidelines (106), but with a modified soil: solution ratio of 2 instead of 5; non-GLP,
Test compound	[methyl-^{14}C]Glyphosate (specific activity 1.08MBq mmol−1; radiochemical purity >99%); unlabelled glyphosate (purity 98%); CAS-no.:1071-83-6 for column experiments: Roundup Garden (commercial SL formulation containing 120 g litre−1 glyphosate (isopropylamine salt), CAS-no.: 38641-94-0
Test system and conditions	Batch studies: 1) Sorption: glyphosate/[^{14}C]glyphosate concentration of 0.6 mgkg^{-1} (192 Bq), rotated for 96 h; 2) Mineralization in flasks: glyphosate/[^{14}C]glyphosate concentration of 16.9mgkg^{-1} (0.1mM) and 833 Bq, final moisture level equivalent to 80% of WHC, incubated at 30 ◦C in the dark for 31 days, repeated on two types of gravel incubated at 10, 20 and 30°C; 3) Leaching studies: Two columns for each substrate, and each column were exposed to two different simulated precipitation events. [^{14}C]Glyphosate (1733 Bq in short columns, and 5666 Bq in tall columns) mixed with Roundup Garden (recommended rate of 2.4 kg glyphosate ha^{-1}), Over the next 6 days the columns were subjected to three simulated precipitation events at 20°C, trapping the [^{14}C]carbon dioxide, total effluent collected 1 day after each precipitation event, determination of residual [^{14}C] compounds. Five types of gravel and a sandy agricultural reference soil
Statistical design	Analyses in triplicate; K_d value represents one measurement of glyphosate in the solution, assuming linearity between glyphosate adsorption and glyphosate concentration in the solution.
Relevance	
Environmental relevance	Given as influence by environmental parameter was tested.
Weight of evidence	
“Positive”/”Negative” evidence	Results supported by other publications.

Vischetti et al. (2006)

Title: Biochemical parameter changes in urban-waste compost used as biofilter for pesticide decontamination	
Author: COSTANTINO VISCHETTI, PIERO PERUCCI, CRISTIANO CASUCCI, ELGA MONACI and STEFANO DUMONTET	
Reference: Intern. J. Environ. Anal. Chem. Vol. 86, Nos. 3–4, 15 March–10 April 2006, 195–205	
Year: 2006	
Results and conclusion: Here, water that was contaminated by three different pesticides, the insecticide chlorpyrifos (Chl), the fungicide metalaxyl (Meta) and the herbicide glyphosate (Gly), was percolated through 2 kg of UWC (Urban-waste compost) material. The pesticide residues in the leached water and the modifications induced in some of the UWC biochemical and microbiological parameters (including microbial biomass carbon (MBC) and nitrogen (MBN), and fluorescein diacetate (FDA) hydrolysis, alkaline monophosphatase (AMP) and dehydrogenase (DH) activities) were investigated over 2 months of incubation at 20°C. The UWC showed a good retention capacity towards the three pesticides tested, with the highest efficiency for Gly. Chl caused an initial detrimental effect on the MBC content and a decrease in the FDA hydrolysis capacity, while Meta and Gly increased the MBC content throughout the incubation. The results demonstrate that UWC can be successfully used as a biofilter to reduce pesticide spills and to clean up water contaminated with pesticides. The evaluation of the modifications induced on the UWC MBC and MBN, and FDA hydrolysis, AMP and DH activities suggest different biodegradation potentials of the UWC microorganisms vs. the three pesticides studied.	
Proposed action: Not to be considered; basic research, no endpoint correction needed	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information	
Reliability	Medium
Endpoint	Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN); Alkaline monophosphatase (AMP) activity; Dehydrogenase [DH] activity; recoveries of the pesticides
Protocol	Standard, non-GLP
Test compound	Analytical standard of glyphosate (Gly), (CAS-no.: 1071-83-6)
Test system and conditions	81 PVC columns, filled with 2 kg of UWC, treated twice a day with 4 L of deionised water containing Chl, Meta and Gly at different field doses; leached water was collected from each column after each leaching event and analysed to determine the pesticide residues. The modifications of the UWC microbiological and biochemical parameters induced by the pesticides were evaluated
Statistical design	Three replicates for each pesticide, for each sampling time; SYSTAT programme was used for the analysis of variance and Duncan's range test on the means
Relevance	
Environmental relevance	Given. Parameters influencing endpoints are measured.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence; results supported by other publications.

Zhao et al. (2009)

Title: Glyphosate mobility in soils by phosphate application: Laboratory column experiments	
Author: Bingzi Zhao, Jiabao Zhang, Jiandong Gong, Hui Zhang, Congzhi Zhang	
Reference: Geoderma; article in press	
Year: 2009	
Results and conclusion: (1) phosphate application might induce system pH decrease, and (2) the overall glyphosate mobility by phosphate application might depend on the relative contribution of two competing processes: increase in glyphosate adsorption from pH decrease versus reduced glyphosate adsorption from competitive adsorption between phosphate and glyphosate for sorption sites available. To test these two hypotheses, laboratory batch and column experiments using a miscible displacement approach were conducted on two Primosols and one Anthrosols, to investigate, respectively, (1) pH-dependent glyphosate adsorption onto the three studied soils, and (2) glyphosate leaching and mobility in soil columns as influenced by phosphate application. Our results showed that glyphosate adsorption consistently decreased with increase in system pH. The effect of phosphate application on glyphosate mobility varied with soil type. We conclude that phosphate application can cause system pH change with various extents in the soil, which subsequently contribute to glyphosate mobility in different degree.	
Proposed action: Not to be considered for the endpoint sorption and mobility as the study aims at basic research and understanding of pH and phosphate influence.	
Type of information (critical, high/low weight, supporting, additional): Low weight; additional information of mechanisms of glyphosate mobility in the presence of phosphate	
Reliability	Medium
Endpoint	Breakthrough curves (BTCs)
Protocol	Partly similar to OECD 106, non-GLP
Test compound	Glyphosate (99.9% purity); CAS-no.: 1071-83-6
Test system and conditions	1) batch experiments, shaken for 20 h at 25 °C; 2) column leaching experiments, 6 column experiments including with or without phosphate (two concentrations), and 3 levels of glyphosate introduced rate, three soils
Statistical design	Duplicate samples
Relevance	
Environmental relevance	Given. Parameter influencing endpoints are measured and reported.
Weight of evidence	
“Positive”/”Negative” evidence	Results supported by other publications; no negative evidence.

Zhou et al. (2010)

Title: Differential Transport of Atrazine and Glyphosate in Undisturbed Sandy Soil Column
Author: Y. ZHOU, Y. WANG, D. HUNKELER, F. ZWAHLEN AND J. BOILLAT
Reference: Soil and Sediment Contamination, 19:365–377
Year: 2010
Results and conclusion:

Laboratory studies were conducted to determine the behaviour of atrazine and glyphosate within the root zone of an undisturbed sandy soil in Jianghan Plain, central China. Chloride as a tracer for water movement was applied to the soil as KCl for 26 hours before pesticide application for another 160 hours. Glyphosate, atrazine, and Cl concentrations (conc.) were determined as a function of time in breakthrough curves (BTCs). Atrazine BTC was fitted better in convection-dispersion equation equilibrium model. For glyphosate, however, a two-site non-equilibrium model was chosen. Leaching rate of atrazine from sandy soil was much higher than that of glyphosate and it took longer for glyphosate to leach through the column due to stronger sorption and degradation to its major metabolite, AMPA (aminomethylphosphonic acid, CH_6NO_3P), which was detected (up to 8890 ng/l) in the final leachate.	
Proposed action: To be considered as additional information only since the soil column leaching studies are not required and data on sorption/desorption are sufficient for the assessment.	
Type of information (critical, high/low weight, supporting, additional): Low weight, supporting information of leaching of glyphosate and AMPA	
Reliability	High
Endpoint	Breakthrough curves BTC; kinetic parameters: retardation factor R and partitioning coefficient β
Protocol	Standard, non-GLP
Test compound	Glyphosate (CAS-no.: 1071-83-6) from Landi (Switzerland) with 360 g/l certified purity.
Test system and conditions	Leaching column experiment, Glyphosate, AMPA and atrazine concentrations in the leachate samples were analyzed
Statistical design	Breakthrough curves to a two-site (or bicontinuum) sorption model with degradation. The two-site model is a one-dimensional advective-dispersive transport model with a first-order bicontinuum-description of soil, which allows estimation of the rate parameters for non-equilibrium sorption and irreversible sorption.
Relevance	
Environmental relevance	Given. Parameter influencing endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

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Lysimeter studies or field leaching studies

Summary

Several articles on lysimeter and field leaching can be found in open literature. The results show that glyphosate and AMPA can only be found in groundwater under exceptional cases, usually in soil prone to preferential flow.

Grundmann et al. (2008) investigated the leaching of glyphosate in a lysimeter filled with sandy soil. Glyphosate was applied three times in two consecutive years: twice in the spring,

once in the fall. The results after two years of monitoring show that glyphosate and AMPA were only detected in the top soil layer of the lysimeter.

Leaching of glyphosate and its metabolite AMPA was studied by (Fomsgaard et al., 2003) in four lysimeters, two of them being replicates from a low tillage field, the other two being replicates from a normal tillage field. The mean yearly concentration of leached glyphosate and/or AMPA was significantly below 0.1 µg/l in both sets of lysimeters, and thus no significant difference between the two lysimeter sets was shown. However, in both sets of lysimeters several single findings at concentrations above 0.1 µg/l were seen, which might be due to the leaching of particle-bound compounds.

Malone et al. (2004) used four monolith lysimeters to investigate leaching of glyphosate. A high-intensity rainfall was applied shortly after herbicide application. Even with preferential flow, glyphosate was not transported to 2.4 m at concentrations approaching environmental concern.

The transport of glyphosate and AMPA has been studied by Candela et al. (2010) in the north of Barcelona, where groundwater is located at a depth of 5.5 m. After 69 days of application residues of glyphosate up to 73.6 µg/1 were detected till a depth of 0.5 m under irrigated conditions, AMPA, analyzed only in the irrigated plot was detected till a depth of 0.5 m. Such migration could be related to the low content of organic matter and clays in the soils; recharge generated by irrigation and heavy rain, and possible preferential solute transport and/or colloidal mediated transport.

Aronsson et al. (2011) conducted a field leaching study over a period of 2-year on two different soil types located at different sites in southwest Sweden. The results of the study confirmed that soil texture was the dominant factor for leaching of glyphosate. The immediate detection of glyphosate in the drain flow after application could be attributed to particle-facilitated preferential flow contributions, although the study did not distinguish between particle-bound and dissolved glyphosate.

Detailed description of open literature – Lysimeter studies or field leaching studies

Al-Rajab et al. (2008)

Title: Sorption and leaching of ^{14}C-glyphosate in agricultural soils
Author: Abdul Jabbar Al-Rajab, Samira Amellal, Michel Schiavon
Reference: Agron. Sustain. Dev. 28, 419–428
Year: 2008
Results and conclusion: Aim: to assess the dynamic interactions between glyphosate sorption and leaching; and to identify the main factors that influence the two processes in three undisturbed agricultural soils using microlysimeters under outdoor conditions. OECD 106: Glyphosate was strongly adsorbed, yielding empirical constants of Freundlich sorption isotherms (K_f) of 16.6 for the clay loam soil, 33.6 for the silt clay loam soil and 34.5 for the sandy loam soil, with n_f close to 1 in all three cases. Glyphosate was also weakly desorbed, i.e. 5 to 24% (w) of initially sorbed glyphosate. Sorption and desorption were only pH-dependent. Outdoor microlysimeter: nearly 70% of the initial glyphosate was present in the soil in a non-extractable form at the beginning of the experiment. Conversely, only less than 20% of the initial glyphosate is present in the soil in a non-extractable form after 11 months. These findings suggest that the non-extractable residues become available and take part in biodeg-

radation and leaching. The amounts of ^{14}C-glyphosate derivatives leached were less than 0.28% of the initially applied glyphosate. AMPA metabolite generally represented up to 100% of the residues present in the leachates. The results of leaching were highly influenced by the hydrodynamic properties and the biodegradation capacities of the soils.	
Proposed action: To be considered for endpoint sorption as it is supportive information though raw data in a common sense of GLP-study reports are not available. Information is highly reliable and plausible.	
Type of information (critical, high/low weight, supporting, additional): High weight, supportive information	
Reliability	High
Endpoint	OECD 106: K_f-values, 1/n, R^2, % desorbed; Outdoor microlysimeter: amount in leachate and distribution in soil over time (11 months).
Protocol	Standard (OECD 106), non-GLP; outdoor lysimeter studies, non-GLP.
Test compound	OECD 106: [Phosphonomethyl-^{14}C]-glyphosate (purity: 99%); non-radioactive glyphosate (purity 98.5%) Outdoor microlysimeter: [Phosphonomethyl-^{14}C]-glyphosate diluted in Roundup Express (isopropylamine salt) and water CAS-no.: 1071-83-6
Test system and conditions	OECD 106: 7 concentrations for isotherms, 3 soils; undisturbed outdoor micro-lysimeter (diameter: 10 cm, length: 25 cm): duration: 11 months, 3 soils, 7 sampling points, 21 lysimeter in total
Statistical design	OECD 106: triplicates,. Lysimeter: single lysimeter per sampling and soil. Stat Box computer software; Comparison of means by Newman-Keuls test at levels of 0.05, 0.01 and 0.001.
Relevance	
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	Positive evidence, no negative evidence.

Al-Salamah (2004)

Title: Simulating the fate and transport of pesticide in unsaturated soil: a case study with glyphosate-isopropylammonium
Author: I. S. Al-Salamah
Reference: Geo-Environmen J. F. Martin-Duque, C. A. Brebbia, A. E. Godfrey & J. R. Diaz de Teran (Editors) © 2004 WIT Press, www.witpress.com, ISBN 1-85312-723-X
Year: 2004
Results and conclusion: A simultaneous transport of water and glyphosate was studied experimentally and numerically. The glyphosate redistributed along the soil column experimentally and numerically. The predicted glyphosate concentrations were improved by increasing the dispersivity up to 75 mm. The observed glyphosate concentration proved that the mass flow mechanism is important for migration of the glyphosate in the sandy soil. The results of this study indicated that transport models need to include the effect of temperature and temperature gradient to describe the movement of water and glyphosate.

Proposed action: To be considered as additional information only since the climatic does not fit with European climatic conditions.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Experimental simulation of glyphosate isopropyl-ammonium leaching
Protocol	Non-GLP standard study
Test compound	Glyphosate-isopropylammonium (CAS 38641-94-0)
Test system and conditions	Soil materials were sampled from a surface layer (0.0-0.3 m depth) from the Agriculture and Veterinary Collage farm, King Saud University, Al-Qassim. The soil was composed of sand (96.3 % sand, 1.9 % clay, and 1.8 % silt) materials. Four soil columns were packed at bulk densities of 1514 kg m-3. The soil columns were buried vertically within a bare soil field with exposing the upper end to the natural atmosphere of Al-Qassim region. Water and glyphosate solution were poured at the open of soil column at different time. The soil temperatures and the soil moisture conditions at both ends of soil column were recorded.
Statistical design	Not reported
Relevance	
Environmental relevance	The relevance is small since the climatic does not fit with European climatic conditions.
Weight of evidence	
"Positive"/"Negative" evidence	The results are principally supported by other studies performed with leaching models

Aronsson et al. (2010)

Title: Leaching of N, P and glyphosate from two soils after herbicide treatment and incorporation of a ryegrass catch crop	
Author: H. Aronsson, M. Stenberg & B. Ulén	
Reference: Soil Use and Management; doi: 10.1111/j.1475-2743.2010.00311.x	
Year: 2010	
Results and conclusion: The overall aim was to determine the effects of different cropping systems with catch crops on losses of N, P and glyphosate. Soil type affected glyphosate leaching to a larger extent than the experimental treatments. Glyphosate was not leached from the sand at all, while it was found at average concentrations of 0.25 lg⁄L in drainage water from the clay soil on all sampling occasions.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, data on sorption/desorption is sufficient for the assessment.	
Type of information (critical, high/low weight, supporting, additional): High weight, may be supporting information to already existing	
Reliability	Medium
Endpoint	Glyphosate concentration in leachate (drainage)
Protocol	Non-GLP
Test compound	Site 1: glyphosate applied as Glyphomax Bio, 3.5 or 4.0 L⁄ha Site 2: glyphosate applied as Round-up Bio, 3.5 L⁄ha.

	CAS-no.: 1071-83-6
Test system and conditions	During 2005–2007, studies were carried out in two field experiments in southwest Sweden with separately tile-drained plots on a sandy soil (three replicates) and on a clay soil (two replicates). Drainage water was sampled continuously in proportion to water flow and analysed for N, P and glyphosate. Catch crops were sampled in late autumn and spring and soil was analysed for mineral N content. The yields of following cereal crops were determined.
Statistical design	Analysis of variance: mixed procedure in SAS 9.1 for the statistical analysis of differences in yields, catch crop biomass and N and P contents, soil mineral N, leaching of N and P and concentrations of glyphosate between treatments. The t-test at P = 0.05 was used for pair wise comparisons by the PDIFF statement. Block was used as the random variable in analysis of a single year.
Relevance	
Environmental relevance	Given; relevant substance assessed; field studies in southwest of Sweden, environmental parameter
Weight of evidence	
"Positive"/"Negative" evidence	Further leaching and monitoring studies support the results. No negative evidence.

Augustin and Seibel (2002)

Title: Herbicide treatment of urban areas _ a possible source of surface water contamination	
Author: Bernd Augustin and Helmut Seibel	
Reference: GESUNDE PFLANZEN, 54. Jahrg., Heft 7	
Year: 2002	
Results and conclusion: A rough concrete surface with an inclination of 1-2 % for rainwater elimination was treated with Roundup Ultra® (Glyphosat), Basta® (Glufosinate) and Vorox G® (Glyphosat-Diuron). Run-off-water was collected after artificial rain (2 mm) given in different periods after herbicide application (1 and 24 h; 10 days). Chemical analysis showed that the run-off-water contained considerable quantities of Glyphosate and Glufosinat even 10 days after herbicide treatment and 17 mm of artificial and natural rainfall. The results are discussed considering recent detection of Glyphosate contamination of surface water in Germany.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, data on sorption/desorption is sufficient for the assessment.	
Type of information (critical, high/low weight, supporting, additional): Low weight, may be additional information to already existing.	
Reliability	Low
Endpoint	Concentration in leachate
Protocol	For chemical analysis: DFG-method 405, non-GLP. General set-up without specific protocol but research project.
Test compound	Roundup Ultra® (Glyphosat), Vorox G® (Glyphosat-Diuron); CAS-no.: 1071-83-6
Test system and conditions	A rough concrete surface with an inclination of 1-2 % for rainwater elimination was treated with Roundup Ultra® (Glyphosat), Basta® (Glufosinate) and Vorox G® (Glyphosat-Diuron). Run-

	off-water was collected after artificial rain (2 mm) given in different periods after herbicide application (1 and 24 h; 10 days).
Statistical design	Not given
Relevance	
Environmental relevance	Relevant
Weight of evidence	
“Positive”/”Negative” evidence	Results support monitoring data. However, monitoring studies and campaigns are of more reliability and relevance. No negative evidence.

Barrett and McBride (2007)

Title: PHOSPHATE AND GLYPHOSATE MOBILITY IN SOIL COLUMNS AMENDED WITH ROUNDUP	
Author: Katherine A. Barrett and Murray B. McBride	
Reference: Soil Science;172:17–26	
Year: 2007	
Results and conclusion: Sorption of glyphosate and competitive desorption of phosphate in soils has been measured in column leaching experiments. Soils representing a wide range of physical and chemical properties, including total and soluble P, were included in the study. The results suggest that glyphosate sorption does not necessarily result in PO_4^{3-} dissolution and that there is only limited competition for sorption sites between glyphosate and PO_4^{3-}. Strong glyphosate sorption on high-organic matter soils indicates bonding of this anion by a metal bridge to organic functional groups.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, data on sorption/desorption is sufficient for the assessment.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information to endpoint leaching, soil column leaching	
Reliability	Medium
Endpoint	Concentration of glyphosate in the leachate, total P and PO_4^{3-} in the leachate.
Protocol	No standard protocol followed, non-GLP
Test compound	Commercial Roundup (containing 27% active ingredient); CAS-no.: 1071-83-6
Test system and conditions	Soil column studies with homogenized soils. Air-dried soil packed into polycarbonate tubes (width of 5.5 cm and height of 10 cm, with soil depth of approximately 6 cm). Application of roundup, ageing for 24 h, 100 mL leachate.
Statistical design	Duplicates, StatView software, $P < 0.05$ are considered to be significantly different.
Relevance	
Environmental relevance	Given; influencing parameter are reported and considered.
Weight of evidence	
“Positive”/”Negative” evidence	Results of other studies with identical design not known. No negative evidence.

Barrett and McBride (2006)

Title: Trace Element Mobilization in Soils by Glyphosate
Author: K. A. Barrett and M. B. McBride
Reference: Soil Sci. Soc. Am. J. 70: 1882–1888
Year: 2006
Results and conclusion: No significant increases in elemental leaching were detected in mineral and organic soils with normal background concentrations of heavy metals and phosphor.
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, data on sorption/desorption is sufficient for the assessment.

Type of information (critical, high/low weight, supporting, additional): Low weight, may be additional information to already existing.	
Reliability	Medium
Endpoint	Trace element concentrations in soil column leachates.
Protocol	Non-GLP, no standard protocol
Test compound	Reagent-grade glyphosate [N-(phosphonomethyl)glycine, 96%); CAS-no.: 1071-83-6
Test system and conditions	Tendency for glyphosate to mobilize Cu and other elements was tested in soil leaching experiments by applying glyphosate alone or complexed with Cu to mineral and organic soil columns (10 cm height, 1.8 cm i.d.) and measuring the concentrations of these elements in the leachates.
Statistical design	ANOVA analysis of variance, 6 soils tested, single test (?)
Relevance	
Environmental relevance	Given, environmental parameter reported.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies with identical design and objective not known. No negative evidence.

Bergström et al. (2011)

Title: Laboratory and Lysimeter Studies of Glyphosate and Aminomethylphosphonic Acid in a Sand and a Clay Soil	
Author: Lars Bergström, Elisabet Börjesson, and John Stenström	
Reference: J. Environ. Qual. 40:98–108	
Year: 2011	
Results and conclusion: Influence of adsorption on glyphosate degradation was confirmed: very slow degradation rate in clay soil (half-life 110–151 d). Kinetics of AMPA residues suggest that although AMPA is always more persistent than glyphosate when formed from glyphosate, its degradation rate can be faster than that of glyphosate. The kinetics also suggests that the sarcosine pathway can be just as significant as via AMPA. The long persistence of glyphosate was also confirmed in the lysimeter study, where glyphosate+AMPA residues constituted 59% of the initial amount of glyphosate added to the clay soil 748 d after application. Despite large amounts of precipitation in the autumn and winter after application, however, these residues were mainly located in the topsoil, and only 0.009 and 0.019% of the initial amount of glyphosate added leached during the whole study period in the sand and clay, respectively. No leaching of AMPA occurred in the sand, whereas 0.03 g/ha leached in the clay soil.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, data on sorption/desorption is sufficient for the assessment.	
Type of information (critical, high/low weight, supporting, additional): High weight, may be supporting information to already existing.	
Reliability	High
Endpoint	Lysimeter studies: concentrations in leachate and soil; adsorption: K_f, 1/n, R^2; degradation: DT_{50}
Protocol	Lysimeter studies: no guideline; adsorption: OECD 106; degradation: similar to OECD 307, Non-GLP
Test compound	Glyphosate, ^{14}C-Glyphosate, purity not given; CAS-no.: 1071-83-6

Test system and conditions	Batch laboratory and lysimeter transport studies were performed to assess the potential for leaching of the compounds in two agricultural soils. Unlabeled and ^{14}C-labeled glyphosate were added at a rate corresponding to 1.54 kg a.i./ha on undisturbed sand and clay columns. Leachate was sampled weekly during a period of 748 d for analyses of glyphosate, AMPA, total ^{14}C, and particle-bound residues. Topsoil and subsoil samples were used for determination of glyphosate adsorption, glyphosate degradation, and formation of AMPA and its degradation.
Statistical design	Lysimeter studies: 7 lysimeters, 2 soils; adsorption: two soils (top and subsoil), duplicates; degradation: 2 soils, duplicates, first order kinetics both for Glyphosate and AMPA. Least squares fits of data on adsorption and on residual values of glyphosate and AMPA were fitted to their respective equations by nonlinear regression.
Relevance	
Environmental relevance	Given; environmental parameter adequately tested.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies support information. No negative evidence.

Borggaard and Gimsing (2008)

Title: Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review	
Author: Ole K Borggaard and Anne Louise Gimsing	
Reference: Pest Manag Sci 64, 441–456	
Year: 2008	
Results and conclusion: The very wide use of glyphosate to control weeds in agricultural, silvicultural and urban areas throughout the world requires that special attention be paid to its possible transport from terrestrial to aquatic environments. The aim of this review is to present and discuss the state of knowledge on sorption, degradation and leachability of glyphosate in soils. Difficulties of drawing clear and unambiguous conclusions because of strong soil dependency and limited conclusive investigations are pointed out.	
Proposed action: Not to be used as it is a review article.	
Type of information (critical, high/low weight, supporting, additional): Low weight. As it is a review article no data are published but cited from other publications and discussed. Additional information.	
Reliability	
Endpoint	Review, no data published
Protocol	Not applicable
Test compound	Glyphosate (CAS-no.:1071-83-6), AMPA (CAS-no.: 1066-51-9)
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Given
Weight of evidence	

"Positive"/"Negative" evidence	Not applicable

Brown and van Beinum (2009)

Title: Pesticide transport via sub-surface drains in Europe	
Author: Colin D. Brown, Wendy van Beinum	
Reference: Journal of Environmental Pollution; doi:10.1016/j.envpol.2009.06.029	
Year: 2009	
Results and conclusion: Results of 23 field drainage experiments undertaken at sites across Europe were collated and analysed by residual maximum likelihood. Both maximum concentration of pesticide in drain-flow (n = 167) and seasonal loss of pesticide to drains (n = 97) were significantly related to strength of pesticide sorption to soil, half-life of the pesticide in soil, the interval between application and first drain-flow and the clay content of the soil. The statistical models accounted for 71% of the variability in both maximum concentration and seasonal load. Next, the dataset was used to evaluate the current methodology for assessment of aquatic exposure used in pesticide registration in Europe. Simulations for seven compounds with contrasting properties showed a good correspondence with field measurements. Finally, the review examines management approaches to reduce pesticide transport via sub-surface drains. Despite a large amount of work in this area, there are few dependable mitigation options other than to change application rate or timing or to restrict use of a compound in the most vulnerable situations.	
Proposed action: Not to be considered as it is a review article.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information only as article is a review	
Reliability	Low (review article)
Endpoint	Maximum concentration of pesticide in drain-flow and seasonal loss of pesticide to drains;
Protocol	No standard protocol
Test compound	Glyphosate (CAS-no.: 1071-83-6)
Test system and conditions	Review article on results of 23 field drainage experiments.
Statistical design	Statistical technique: residual maximum likelihood (REML). Similar to multiple regression the method identifies a combination of factors that best explains the values for maximum concentration and seasonal loss.
Relevance	
Environmental relevance	Given; influencing parameter are considered and discussed.
Weight of evidence	
"Positive"/"Negative" evidence	Review article analyzing the results of other experiments; no negative evidence

Candela et al. (2010)

Title: Glyphosate transport through weathered granite soils under irrigated and non-irrigated conditions — Barcelona, Spain	
Author: Lucila Candela, Juan Caballero, Daniel Ronen	
Reference: http://dx.doi.org/10.1016/j.scitotenv.2010.03.006	
Year: 2010	
Results and conclusion: The transport of Glyphosate and AMPA has been studied in the Mediterranean Maresme area of Spain, north of Barcelona, where groundwater is located at a depth of 5.5 m. The unsaturated zone of weathered granite soils was characterized in adjacent irrigated and non-irrigated experimental plots where 11 and 10 boreholes were drilled, respectively. At the non irrigated plot, the first half of the period was affected by a persistent and intense rainfall. After 69 days of application residues of Glyphosate up to 73.6 $\mu g g^{-1}$ were detected till a depth of 0.5 m under irrigated conditions, AMPA, analyzed only in the irrigated plot was detected till a depth of 0.5 m. According to the retardation coefficient of Glyphosate as compared to that of Br^- for the topsoil and subsoil (80 and 83, respectively) and the maximum observed migration depth of Br^- (2.9 m) Glyphosate and AMPA should have been detected till a depth of 0.05 m only. (Furthermore for Glyphosate: surface soil (K_f=93), subsoil (K_f=154)) Such migration could be related to the low content of organic matter and clays in the soils; recharge generated by irrigation and heavy rain, and possible preferential solute transport and/or colloidal mediated transport.	
Proposed action: Not to be considered for the endpoint sorption. Consideration for the monitoring chapter as it supports the information for soils of low content of organic matter and clays.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information to the endpoint sorption and mobility. Might be supportive information to the chapter “monitoring data”.	
Reliability	Medium
Endpoint	Glyphosate concentrations in deeper soil layers and leachate, mobility and leaching
Protocol	Standard, OECD
Test compound	Glyphosate (Roundup®, 36% p/v; CAS-no.: 1071-83-6), AMPA (CAS-no.: 1066-51-9)
Test system and conditions	Outdoor plot experiments under irrigated and non-irrigated conditions, experiments run for approximately 90 days. Vadose zone soil and water sampling. Glyphosate and AMPA residue analyses in soil and water. Extraction efficiency >95% for both analytes.
Statistical design	Not given in detail
Relevance	
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	Positive evidence, supportive information; no negative evidence.

Fomsgaard et al. (2003)

Title: Leaching of Pesticides Through Normal-Tillage and Low-Tillage Soil—A Lysimeter Study. II. Glyphosate
Author: Inge S. Fomsgaard, Niels Henrik Spliid and Gitte Felding

Reference: JOURNAL OF ENVIRONMENTAL SCIENCE AND HEALTH, Part B—Pesticides, Food Contaminants, and Agricultural Wastes, Vol. B38, No. 1, pp. 19–35	
Year: 2003	
Results and conclusion: Leaching of glyphosate and/or its metabolite AMPA was studied in four lysimeters, two of them being replicates from a low tillage field (lysimeter 3 and 4), the other two being replicates from a normal tillage field (lysimeter 5 and 6). The mean yearly concentration of leached glyphosate and/or AMPA was significantly below 0.1 mg/l from both sets of lysimeters, and thus no significant difference between the two lysimeter sets was shown. However, in both sets of lysimeters several single findings at concentrations above 0.1 mg/l were seen (Glyphosate: up to 0.52 µg/L; AMPA: up to 0.22 µg/L), which might be due to the leaching of particle-bound compounds. A significant difference between the soil residual concentrations of AMPA was seen, the higher concentration was found in the set of lysimeter where low-tillage had been practiced. This might be due to differences in extraction efficiencies or due to residues resulting from earlier, more frequent sprayings with Round Up in the low tillage soil.	
Proposed action: To be considered as supportive information, gives valuable information on leaching under outdoor conditions	
Type of information (critical, high/low weight, supporting, additional): High weight, supporting information	
Reliability	High
Endpoint	Concentration in lysimeter leachate
Protocol	Close to BBA test guideline (1990): guideline for the Testing of Plant Protection Products in Registration Procedure, Part IV, 4-3: Lysimeter tests for the translocation of plant protection products into the subsoil.
Test compound	Lysimeter 3 and 4 were sprayed with a mixture of ^{14}C-labelled glyphosate and unlabelled glyphosate (Roundup 2000 together with the additive Team Up), lysimeter 5 and 6 were sprayed with unlabelled glyphosate. Analysis for Glyphosate (CAS-no.:1071-83-6) and AMPA (CAS-no.: 1066-51-9)
Test system and conditions	The soil was a sandy loam soil with 13–14% clay. The lysimeters had a surface area of 0.5 m2 and a depth of 110 cm. The spraying took place September 18, 1997. The total amount of glyphosate sprayed onto each lysimeter was 40 mg, corresponding to 0.8 kg active ingredient per ha. The lysimeters were installed in an outdoor system in Research Centre Flakkebjerg and were thus exposed to normal climatic conditions of the area. A mean of 260 l drainage water were collected from lysimeter 3 and 4 and a mean of 375 litres from lysimeter 5 and 6.
Statistical design	2 soils, 2 lysimeters per soil
Relevance	
Environmental relevance	Given; environmental and climatic parameters were recorded.
Weight of evidence	
“Positive”/”Negative” evidence	Positive evidence, other studies are not in conflict to the results reported here; no negative evidence.

Grundmann et al. (2008)

Title: Mineralization and Transfer Processes of ^{14}C-labeled Pesticides in Outdoor Lysimeters	
Author: Sabine Grundmann & Ulrike Dörfler & Bernhard Ruth & Christine Loos & Tobias Wagner & Heidrun Karl & Jean Charles Munch & Reiner Schroll	
Reference: Water Air Soil Pollut: Focus (2008) 8:177–185	
Year: 2008	
Results and conclusion: The glyphosate mineralization curves showed no lag phase – the microorganisms were able to mineralize glyphosate immediately. The cumulated amounts of mineralized ^{14}C-glyphosate amounted to 32–39%. No accumulation of residues in the soil and no leaching of the residues to deeper soil layers could be observed after three applications. Glyphosate was rapidly degraded to AMPA in the soil. Glyphosate and AMPA were accumulated in soy bean nodules.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment and the study was not performed close to standard tests.	
Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Degradation of glyphosate in soil (mineralisation) Accumulation and leaching
Protocol	Non-GLP study
Test compound	^{14}C-glyphosate [N-(phosphonomethyl)glycine] had the ^{14}C-labeling on the phosphonomethyl group and was purchased from PerkinElmer, Rodgau, Germany (purity >98%). Non-labelled glyphosate and metabolites were purchased from Dr. Ehrenstorfer (Augsburg, Germany); CAS 1071-83-6
Test system and conditions	The lysimeters consist of soil columns of 2 m height and a surface area of 1 m2. To detect and quantify gaseous ^{14}C-losses from soil and plant surfaces, a two-chamber-system with special trapping facilities was designed. The chambers are placed on the surface of the lysimeters – a soil chamber and a plant chamber. Glyphosate was applied three times, in spring 2004 and in spring and autumn 2005 in an amount of 1 kg a.i. ha^{-1} (1.92 MBq mg^{-1}). During the experiment, mineralization and volatilization of the herbicides from soil and plants were measured during a time period of about 2-3 months after application
Statistical design	No information
Relevance	
Environmental relevance	The experiments are relevant though not performed close to standard lysimeter studies. Additionally, not sufficient information is provided to describe the situation (e.g. weather, irrigation).
Weight of evidence	
“Positive”/”Negative” evidence	The results support the information known for glyphosate from standard tests.

Hagner et al. (2013)

Title: The effects of biochar, wood vinegar and plants on glyphosate leaching and degrada-

tion	
Author: M. Hagner, O.-P. Penttinen, K. Tiilikkala, H. Setälä	
Reference: European Journal of Soil Biology 58 (2013) 1-7	
Year: 2013	
Results and conclusion: A pot experiment was established to explore the potential impacts of biochar, wood vinegar, and plants on the environmental fate of glyphosate. In the presence of plants (Lolium perenne), and irrespective of the presence of biochar or wood vinegar, leaching of glyphosate through the soil was multiple compared to the plant free systems. However, the addition of biochar to the soil decreased the leaching of glyphosate irrespective of plants. Soils treated with biochare-wood vinegar mixture showed the lowest glyphosate leaching, both with and without plants. Biochar, wood vinegar or plants, alone, had no effect on the degradation of glyphosate in soil. When the plants were present the degradation of glyphosate was highest in soils treated with biochare-wood vinegar mixture. The results imply that biochar in particular can be applied as a soil improving agent to reduce the potential environmental risks to aquatic environments caused by glyphosate.	
Proposed action: To be considered as additional information.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information from a field study	
Reliability	
Endpoint	Effects of plants, biochar and wood vinegar on the environmental fate of glyphosate (glyphosate leaching and degradation)
Protocol	GEP
Test compound	Glyphosate
Test system and conditions	The soil used in the experiment had no previous history of glyphosate application. It derived from an arable field used over 20 years in organic potato farming in Tammela, Finland. The biochar used here was derived from birch wood (incl. bark) and pyrolysed in a batch retort by Tisle Suomi Ltd. (Mikkeli, Finland) at 450 °C for a holding time of 23 h. The study was performed in a glasshouse at MTT Agrifood Research Finland in Jokioinen, Finland, in the summer of 2010. The experiment was conducted in 1500 ml flowerpots (Ø 11 cm, height 19 cm) with four holes (Ø 0.5 cm) at the bottom. The four treatments, each with 20 replicates consisted of soil mixed with 1) biochar, 2) wood vinegar, 3) biochar and wood vinegar, or 4) a control system with neither biochar nor wood vinegar. Coarse gravel (Ø 0.5e1.5 cm, 100 g dry) was put at the bottom of each pot to maintain capillary action and to prevent water holes from blocking up. The application rate of biochar to the pots corresponded to 51 t ha^{-1}, assuming 10 cm incorporation depth (3.3% biochar content by dry mass). Wood vinegar concentrations applied to the pots corresponded to 2000 L ha^{-1} (0.26%). To get data on the highest possible risks and benefits of the substances, relatively high concentrations of wood vinegar and biochar were used in the experiments. Before adding 800 g of treated soil to the pots, biochar (sieved through a 2 mm sieve) and wood vinegar were homogenously mixed with the soil in a bucket, and the water content of the mixture and that of the control soil was adjusted to 20% of wet mass. The pots were randomly placed on a moist filter bed that ensured constant soil moisture during incubation. To provide opti-

	mal growth conditions for Lolium perenne, the pots were kept in a glasshouse with constant air temperature (23 ± 2 °C) throughout the experiment. The experiment ran for 82 days, during which time soil and water leachate samples were taken three times: at 4, 46 and 80 days. After the first sampling, seeds of L. perenne were sown (150 per pot) in half of the pots of each treatment to determine the effects of plants on the fate of glyphosate. All the pots were covered with plastic film for 7 days to maintain soil moisture conditions. After seed germination, the plastic film was removed and all pots were fertilised dose of 100 kg ha^{-1}). When the grass reached 20 cm in height (Day 36), half of the pots of each treatment (with and without plants) were treated with glyphosate (Roundup Bio; Monsanto, Copenhagen, Denmark) mixed with water (1:100) corresponding to 2000 g active ingredient ha^{-1} (ca. 2000 mg/pot). Glyphosate was sprayed according to the Good Experimental Practice GEP protocol used by the Agrifood Research Finland (MTT). The GEP standard was adopted by EEC in the Directive 93/71/EEC. Annex II in this directive specifies the requirements that are referred to as GEP. The GEP standard suites well to a variety of agricultural practices and experimentations. Grasses in glyphosate treated pots withered and died during 3e21 days after spraying the glyphosate. As part of initially added wood vinegar was obviously degraded, four days after the addition of glyphosate, a second addition of wood vinegar (500 L ha^{-1}) was made for pots that already contained wood vinegar. This was done to ensure that enough wood vinegar is present in the soil to stimulate glyphosate degradation by soil microbes. The second and third sampling events (46 and 80 d) were made 10 and 44 days after the addition of glyphosate.
Statistical design	Repeated measures MANOVA was used to examine treatment effects of biochar, wood vinegar, vegetation and glyphosate on microbial respiration, numbers of nematodes, the soil C/N ratio and leachate properties (pH, conductivity, TOC) at the second and third sampling events. Soil and leachate samples were analysed separately. In case of leachates, a significant interaction effect was observed between time and biochar and the effects of biochar on pH, conductivity, TOC of leachates in different sampling events were studied separately using Simple-effects model. Transformations (log, ln) were used to normalised the data. Samples taken 5 days after the study started differed from those taken 40 and 80 d after the start due to the addition of grasses (Day 6) and glyphosate (Day 36) to some of the pots. Therefore the effects of biochar and wood vinegar on nematodes, microbial respiration, TOC, conductivity and pH of the samples taken during day 5 were performed separately using MANOVA. Soil and leachate samples were analysed separately. All analyses were carried out using SPSS c.15 for Windows. As glyphosate concentrations in the leachates and soils were calculated from pooled samples (representing average of five replicates), statistical analyses were not performed.

Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Kjær et al. (2011)

Title: Transport modes and pathways of the strongly sorbing pesticides glyphosate and pendimethalin through structured drained soils	
Author: Jeanne Kjær, Vibeke Ernsten, Ole H. Jacobsen, Nis Hansen, Lis Wollesen de Jonge, Preben Olsen	
Reference: Chemosphere 84, 471–479	
Year: 2011	
Results and conclusion: Leaching of glyphosate was evaluated in an 8-month field study focussing on preferential flow and particle-facilitated transport, both of which may enhance the leaching of such a pesticides in structured soils. Glyphosate mainly sorbs to mineral sorption sites. The pesticide was applied to a structured, tile-drained soil, and the concentration was then measured in drainage water sampled flow-proportionally. Glyphosate leached from the root zone, with the average concentration in the drainage water being 3.5 µg L^{-1}. Particle-facilitated transport (particles >0.24 µm) accounted for only a small proportion of the observed leaching (13-16%). Drain-connected macropores located above or in the vicinity of the drains facilitated very rapid transport of pesticide to the drains. That the concentration of glyphosate in the drainage water remained high (>0.1 µg/L) for up to 7 d after a precipitation event indicates that macropores between the drains connected to underlying fractures were able to transport strongly sorbing pesticides in the dissolved phase. Lateral transport of dissolved pesticide via such discontinuities implies that strongly sorbing pesticides such as glyphosate could potentially be present in high concentrations (>0.1 µg/L) in both water originating from the drainage system and the shallow groundwater located at the depth of the drainage system.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment and the studies were not performed close to standard tests.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information from a field study	
Reliability	Medium
Endpoint	Concentrations in drainage water, leaching pattern
Protocol	No standard protocol, field study, non-GLP
Test compound	Glyphosate (1.44 kg/ha active ingredient; 4.0 L/ha Roundup Bio), CAS-no.: 1071-83-6
Test system and conditions	For a period of 8 months following application of the glyphosate the concentration of the pesticide and bromide was measured on a weekly basis in drainage water sampled flow-proportionally. In addition, more intense sampling of drainage water was performed in connection with three flow events triggered by precipitation order to enable detailed description of the transport of water and pesticides. Sampling lasted for 2, 13 and 9 d, respectively.
Statistical design	Not applicable

Relevance	
Environmental relevance	Given; field study, environmental parameter recorded and reported.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies support the results; no negative evidence.

Kjær et al. (2005)

Title: Vadose Zone Processes and Chemical Transport: Leaching of Glyphosate and Amino-Methylphosphonic Acid from Danish Agricultural Field Sites	
Author: Jeanne Kjær, Preben Olsen, Marlene Ullum, and Ruth Grant	
Reference: J. Environ. Qual. 34 (2), 608–620	
Year: 2005	
Results and conclusion: The risk of leaching was evaluated for glyphosate and its degradation product AMPA under field conditions at one sandy and two loamy sites. Over a 2-yr period drainage water, groundwater, and soil water were sampled and analyzed for pesticides. At a sandy site, the strong soil sorption capacity and lack of macropores seemed to prevent leaching of both glyphosate and AMPA. At one loamy site, which received low precipitation with little intensity, the residence time within the root zone seemed sufficient to prevent leaching of glyphosate, probably due to degradation and sorption. Minor leaching of AMPA was observed at this site, although the concentration was generally low, being on the order of 0.05 µg/L or less. At another loamy site, however, glyphosate and AMPA leached from the root zone into the tile drains (1 m below ground surface [BGS]) in average concentrations exceeding 0.1 µg/L, which is the EU threshold value for drinking water. The leaching of glyphosate was mainly governed by pronounced macropore flow occurring within the first months after application. AMPA was frequently detected more than 1.5 yr after application, thus indicating a minor release and limited degradation capacity within the soil. Leaching has so far been confined to the depth of the tile drains, and the pesticides have rarely been detected in monitoring screens located at lower depths. This study suggests that as both glyphosate and AMPA can leach through structured soils, they thereby pose a potential risk to the aquatic environment.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, data on sorption/desorption is sufficient for the assessment	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information to already existing monitoring data	
Reliability	Low
Endpoint	Concentration in drainage water, groundwater, soil water
Protocol	Not applicable, non-GLP
Test compound	Glyphosate (CAS-no.: 1071-83-6), AMPA (CAS-no.: 1066-51-9)
Test system and conditions	Field trials
Statistical design	Mean values and maximum values are reported
Relevance	
Environmental relevance	Environmental data, e.g., climatic data reported; thus environmental relevance is given.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies report similar data, positive evidence; No negative evidence.

Klier et al. (2008)

Title: Modelling the Environmental Fate of the Herbicide Glyphosate in Soil Lysimeters	
Author: Christine Klier & Sabine Grundmann & Sebastian Gayler & Eckart Priesack	
Reference: Water Air Soil Pollut: Focus (2008) 8:187–207	
Year: 2008	
Results and conclusion: The results showed that the conducted laboratory experiments were useful to generate appropriate input values in dependence on environmental conditions for the subsequent fate modelling of glyphosate. Glyphosate transport measurements in the risk assessment study and the mathematical modelling results indicate that due to the high sorption of glyphosate to the soil matrix and the high microbial capacities for glyphosate degradation in the lysimeter soil, leaching risk can be considered to be low, but cannot be entirely excluded.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment and the studies were not performed close to standard tests.	
Type of information (critical, high/low weight, supporting, additional): Supporting	
Reliability	
Endpoint	Experimental simulation of glyphosate leaching
Protocol	Non-GLP standard study
Test compound	^{14}Cradiolabelled glyphosate (5 mCi; 108 mg m^{-2} a.i.) (CAS 1071-83-6)
Test system and conditions	Field study combined with modelling: Meteorological driving parameters used in the study from the years 2003 to 2005 were measured at the automatic weather station at the lysimeter facility. Soil moisture was controlled during the study by tensiometers. The soil is classified as Haplic Arenosol and soil origin was Neumarkt in middle Bavaria. In the glyphosate transport study soil water flow was simulated according to the model HYDRUS 6.0 The bottom boundary condition used in this application considers free drainage. Glyphosate fate was simulated using LEACHP. For the fate in plants the model PLANTX by Trapp was used.
Statistical design	Not reported
Relevance	
Environmental relevance	The relevance is high since the results are of comparable quality as standard FOCUS simulations.
Weight of evidence	
“Positive”/”Negative” evidence	The results are principally supported by other studies performed with leaching models.

Laitinen et al. (2009)

Title: Glyphosate and phosphorus leaching and residues in boreal sandy soil
Author: Pirkko Laitinen, Sari Rämö, Unto Nikunen, Lauri Jauhiainen, Katri Siimes, Eila Turtola
Reference: Plant Soil 323, 267–283
Year: 2009

Results and conclusion: Persistence and losses of glyphosate were monitored in a field with low phosphorus status and possible correlation between glyphosate and phosphorus leaching losses was studied. Glyphosate and its metabolite AMPA residues in soil samples were analysed after a single application in autumn. Twenty months after the application the residues of glyphosate and AMPA in the topsoil (0–25 cm) corresponded to 19% and 48%, respectively, of the applied amount of glyphosate, and traces of glyphosate and AMPA residues were detected in deeper soil layers (below 35 cm). These results indicate rather long persistence for glyphosate in boreal soils. Surface runoff and subsurface drain-flow were collected continuously all year round for 20 months and analysed for glyphosate, AMPA, dissolved phosphate, total phosphorus and total suspended solids. The glyphosate concentrations in the surface runoff water were highest, with 99% of the total leaching losses obtained, during the periods of snow melting and soil thawing in the first winter following the autumn application. The total leaching of glyphosate was 5.12 g ha^{-1} and that of AMPA 0.48 g ha^{-1}, corresponding to about 0.51% and 0.07%, respectively, of the applied amount of glyphosate. No residues of glyphosate and AMPA were detected in the subsurface drain-flow. The correlations between concentrations of glyphosate and dissolved orthophosphate as well as glyphosate and total phosphorus in surface runoff were significant ($p<0.01$).	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment and the studies were not performed close to standard tests.	
Type of information (critical, high/low weight, supporting, additional): High weight, supporting information on Glyphosate behaviour in the environment	
Reliability	High
Endpoint	Concentrations in Soil, water (drain-flow) and root samples
Protocol	Not applicable; field trial, non-GLP
Test compound	Commercial product Roundup containing glyphosate 360 g L−l Glyphosate
Test system and conditions	Experimental field study. 4 out of 16 plots were applied. Soil, water (drain-flow) and root samples analysed for Glyphosate (CAS-no.: 1071-83-6) and AMPA (CAS-no.: 1066-51-9)
Statistical design	Correlations between Glyphosate and phosphate
Relevance	
Environmental relevance	Depth dependent soil parameter reported; weather and other relevant environmental parameter recorded; thus environmental relevance given.
Weight of evidence	
“Positive”/”Negative” evidence	Supported by other studies; no negative relevance.

Laitinen et al. (2007)

Title: Glyphosate translocation from plants to soil – does this constitute a significant proportion of residues in soil?
Author: Pirkko Laitinen, Sari Rämö, Katri Siimes
Reference: Plant Soil 300 ,51–60
Year: 2007
Results and conclusion: Translocation of glyphosate (N-(phosphonomethyl) glycine) to plant roots and its impact on detected herbicide residues in sandy loam soil were studied in a glasshouse pot experi-

ment in Finland. Quinoa (Chenopodium quinoa, Willd) plants in two different growing phases (6–8 and 12–14 leaf stages, groups A and B, respectively) were sprayed with non-labelled glyphosate. Bare soil pots were included as controls (group C). Glyphosate and AMPA concentrations were measured in soil and plant roots at various times after application. Glyphosate fate was simulated with the PEARL 3.0 model. Simulated concentrations in bare soil pots were very close to the observed ones. However, the model lacks a process description for herbicide transport within a plant and, therefore, the observed and simulated glyphosate residues in soil after canopy applications did not correlate. Simulations highlight the importance of the translocation process in glyphosate fate. We conclude that also in field studies part of the detected glyphosate soil residues must originate from plant roots, and translocation process should be included both in leaching assessments and pesticide fate models.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment and the studies were not performed close to standard tests.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information on a possible realistic case but not a worst case for leaching and sorption	
Reliability	Low
Endpoint	Concentrations in soil, upper plant and roots
Protocol	No standard protocol; basic research, non-GLP
Test compound	Glyphosate
Test system and conditions	Pot trials, glasshouse
Statistical design	Parallel residue analyses for soil samples; single extraction for root samples due to limited availability of material. PEARL 3.0 model (FOCUSPEARL 3.3.3) for modelling
Relevance	
Environmental relevance	Given; parameter reported.
Weight of evidence	
“Positive”/”Negative” evidence	No negative evidence.

Landry et al. (2005)

Title: Leaching of glyphosate and AMPA under two soil management practices in Burgundy vineyards (Vosne-Romanée, 21-France)
Author: David Landry, Sylvie Dousset, Jean-Claude Fournier, Francis Andreux
Reference: Environmental Pollution 138, 191 - 200
Year: 2005
Results and conclusion: Some drinking water reservoirs under the vineyards of Burgundy are contaminated with herbicides. Thus the effectiveness of alternative soil management practices, such as grass cover, for reducing the leaching of glyphosate and its metabolite, AMPA, through soils was studied. The leaching of both molecules was studied in structured soil columns under outdoor conditions for 1 year. The soil was managed under two vineyard soil practices: a chemically treated bare calcosol, and a vegetated calcosol. After 680 mm of rainfall, the vegetated calcosol leachates contained lower amounts of glyphosate and AMPA (0.02% and 0.03%, respectively) than the bare calcosol leachates (0.06% and 0.15%, respectively).

No glyphosate and only low amounts of AMPA (< 0.01%) were extracted from the soil. Glyphosate, and to a greater extent, AMPA, leach through the soils; thus, both molecules may be potential contaminants of groundwater. However, the alternative soil management practice of grass cover could reduce groundwater contamination by the pesticide.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment and the studies were not performed close to standard tests.	
Type of information (critical, high/low weight, supporting, additional): High weight, supporting information for risk mitigation discussion	
Reliability	High
Endpoint	Concentrations in leachates; additionally: mineralisation of ^{14}C-glyphosate
Protocol	Mineralisation comparable to OECD 307; leaching: no standard protocol; non-GLP
Test compound	Unlabelled Glyphosate (98% certified purity), [^{14}C]Glyphosate (99.7% radiochemical purity), CAS-no.: 1071-83-6
Test system and conditions	Structured soil columns (15 cm inner diameter, 25 cm length) under outdoor conditions. Soil from 0 -20 cm layer of a) a grassed calcosol with rye-grass between the vine rows, b) chemically weeded bare calcosol. Duration: May 2001 – May 2002. Weekly rainfall recorded. Additionally: mineralisation studies with soil taken from the upper 30 cm, duration 42 days.
Statistical design	Mineralisation: 4 replicates for each soil and blank; leaching: one undisturbed soil column per field plot.
Relevance	
Environmental relevance	Given, influencing parameter recorded.
Weight of evidence	
"Positive"/"Negative" evidence	Completely comparable other studies not known; no negative relevance.

Litz et al. (2011)

Title: Comparative studies on the retardation and reduction of glyphosate during subsurface passage
Author: N.T. Litz , A. Weigert, B. Krause, S. Heise, G. Grützmacher
Reference: Water Research 45, 3047 - 3054
Year: 2011
Results and conclusion: The herbicide Glyphosate was detected in River Havel (Berlin, Germany) in concentrations between 0.1 and 2 mg/L. Laboratory (sorption and degradation studies) and technical scale investigations (bank filtration and slow sand filter experiments) were carried out. Batch adsorption experiments with Glyphosate yielded a low K_f of 1.89 (1/n = 0.48) for concentrations between 0.1 and 100 mg/L. Degradation experiments at 8°C with oxygen limitation resulted in a decrease of Glyphosate concentrations in the liquid phase probably due to slow adsorption (half life: 30 days). During technical scale slow sand filter (SSF) experiments Glyphosate attenuation was 70-80% for constant inlet concentrations of 0.7, 3.5 and 11.6 mg/L, respectively. Relevant retardation of Glyphosate breakthrough was observed despite the low adsorption potential of the sandy filter substrate and the relatively high flow velocity. The VisualCXTFit model was applied with data from typical Berlin

bank filtration sites to extrapolate the results to a realistic field setting and yielded sufficient attenuation within a few days of travel time. Experiments on an SSF planted with Phragmites australis and an unplanted SSF with mainly vertical flow conditions to which Glyphosate was continuously dosed showed that in the planted SSF Glyphosate retardation exceeds 54% compared to 14% retardation in the unplanted SSF. The results show that saturated subsurface passage has the potential to efficiently attenuate glyphosate, favorably with aerobic conditions, long travel times and the presence of planted riparian boundary buffer strips.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, data on sorption/desorption is sufficient for the assessment.	
Proposed action: To be considered as supplementary information for risk mitigation.	
Type of information (critical, high/low weight, supporting, additional): High weight, supporting information on risk mitigation strategies	
Reliability	High
Endpoint	K_f, K_d,1/n, concentrations of gylphosate and AMPA
Protocol	Batch experiments: according to OECD 106; degradation experiments: partly similar to OECD 307, enclosures and SSF experiments: no standard protocols available, non-GLP
Test compound	Glyphosate (98.7 % purity), CAS-no.:1071-83-6)
Test system and conditions	Laboratory batch, enclosure and slow sand filter tests, filter material used. Laboratory experiments: Degradation: partly reducing conditions, 5 sampling points. Batch experiments: 4 concentrations, number of replicates not given. Enclosures: area of 1m^2, height of 1.85m (filtration length 1.00 m), situated within an infiltration pond (area: 90m^2), 3 Glyphosate levels. SSF experiments: two vertical-flow experimental SSFs: one without vegetation cover (average area 60m^2, filter depth 0.8 m, filter volume 48m^3) and the other with a 3 year old vegetation cover of Phragmites australis (average area 68 m^2, filter depth 1.2 m, filter volume 81.6m^3)
Statistical design	VisualCXTFit model, Freundlich isotherms,
Relevance	
Environmental relevance	Given, influencing environmental parameter recorded and discussed.
Weight of evidence	
"Positive"/"Negative" evidence	Publication with identical experimental setup not known, however results are logically explained; no negative evidence.

Malone et al. (2004)

Title: Residual and Contact Herbicide Transport through Field Lysimeters via Preferential Flow
Author: R. W. Malone, M. J. Shipitalo, R. D. Wauchope, and H. Sumner
Reference: J. Environ. Qual. 33:2141–2148
Year: 2004
Results and conclusion:

Four monolith lysimeters (8.1 m^2 by 2.4 m deep) were used to investigate leaching of contact and residual herbicides under a worst-case scenario. Glufosinate, atrazine, alachlor, and linuron were applied in 1999 before corn (Zea mays L.) planting and glyphosate, alachlor, and metribuzin were applied in 2000 before soybean [Glycine max (L.) Merr.] planting. A high-intensity rainfall was applied shortly after herbicide application both years. Most alachlor, metribuzin, atrazine, and linuron losses occurred within 1.1 d of rainfall initiation and the peak concentration of the herbicides coincided (within 0.1 d of rainfall initiation in 2000). More of the applied metribuzin leached compared with alachlor during the first 1.1 d after rainfall initiation (2.2% vs. 0.035%, $P < 0.05$). In 1999, 10 of 24 discrete samples contained atrazine above the maximum contaminant level (atrazine maximum contaminant level [MCL] = 3 µg L^{-1}) while only one discrete sample contained glufosinate (19 µg L^{-1}, estimated MCL = 150µg L^{-1}). The results indicate that because of preferential flow, the break- through time of herbicides was independent of their sorptive properties but the transport amount was dependent on the herbicide proper- ties. Even with preferential flow, glyphosate and glufosinate were not transported to 2.4 m at concentrations approaching environmental concern.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment and the studies were not performed close to standard tests.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information, performed in USA	
Reliability	Medium
Endpoint	Concentration in leachate; preferential flow, transport through lysimeter
Protocol	Not according to BBA-guideline but different design
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Outdoor lysimeter study, 7 pesticides applied onto the same lysimeter, rainfall simulation experiments, concentration in leachate/percolate
Statistical design	4 lysimeters used
Relevance	
Environmental relevance	Given; weather conditions and artificial rainfall recorded.
Weight of evidence	
“Positive”/”Negative” evidence	No completely comparable publication known, but results are plausible; no negative concern.

Pappas and Smith (2007)

Title: Effects of dredging an agricultural drainage ditch on water column herbicide concentration, as predicted by fluvarium techniques
Author: E.A. Pappas and D.R. Smith
Reference: Journal of Soil and Water Conservation, Volume 62, Number 4
Year: 2007
Results and conclusion: In artificially drained agricultural areas, dredging of drainage ditches is often necessary to ensure adequate field drainage. Stream-simulator (fluvarium) experiments were performed to evaluate the potential of associated bed material changes to impact water column concentrations of atrazine, metolachlor, and glyphosate. In the first experiments, water having high herbicide concentrations flowed across bed sediment collected from a ditch immedi-

ately before or after dredging. Afterward, water having initially zero herbicide concentrations flowed across these sediments. Results indicate that the bed sediments remaining after dredging, which had coarser texture and lower organic matter, may contribute to overall higher water herbicide levels in the short term by removing significantly less glyphosate from contaminated water and contributing marginally higher sustained levels of herbicide to uncontaminated water, applicable where sediments exhibit similar dredging characteristic effects. In this case, dredging when herbicide levels are expected to be lowest can help minimize increased transport of some herbicides.	
Proposed action: Not to be considered as a specific hydraulic technique and its consequences for Glyphosate levels was investigated.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information	
Reliability	Medium
Endpoint	Glyphosate concentrations in water column and sediment
Protocol	No standard protocol, non-GLP
Test compound	Glyphosate (as roundup), CAS-no.: 1071-83-6
Test system and conditions	Stream simulator experiments (fluvarium run)
Statistical design	2 fluvarium runs each
Relevance	
Environmental relevance	Given, parameter are reported.
Weight of evidence	
“Positive”/”Negative” evidence	No other comparable publication known; no negative evidence.

Queiroz et al. (2011)

Title: GLYPHOSATE TRANSPORT IN RUNOFF AND LEACHING WATERS IN AGRICULTURAL SOIL	
Author: Gabriela Marina Pompeo Queiroz, Marcos Rivail da Silva, Renata Joaquim Ferraz Bianco, Adilson Pinheiro, Vander Kaufmann	
Reference: Quim. Nova, Vol. 34, No. 2, 190-195	
Year: 2011	
Results and conclusion: Glyphosate was determined in runoff and leaching waters in agricultural soil that received an application of active ingredient and was exposed to simulated intensive rain conditions. The concentrations decreased during the simulation period and the concentrations of the runoff were higher than those achieved in the samples of leaching waters. The concentrations were lower than the pattern in the Brazilian Regulation MS N. 518/2004 for drinking water. The transported load of the applied active ingredient by the leaching was of 15.4% (w/w) and for the runoff was of 1.7% (w/w).	
Proposed action: Not to be considered; non-European site.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additonal information on Glyphosate concentrations in runoff and leachate waters in Brasil	
Reliability	Low
Endpoint	Concentrations in leaching and runoff waters after application of Glyphosate
Protocol	No standard protocol, non-GLP

Test compound	Glyphosate (CAS-no.: 1071-83-6; purity 99.7 %)
Test system and conditions	Outdoor trials, 1m^2 plots, lysimeters
Statistical design	? (english summary only, text Spanish)
Relevance	
Environmental relevance	? (english summary only, text Spanish)
Weight of evidence	
"Positive"/"Negative" evidence	? (english summary only, text Spanish)

Schmidt and Boas (2006)

Title: Accompanying experiments on weed control on public footways using the roller wiper 'Rotofix'	
Author: Heinz Schmidt, Peter Boas	
Reference: Nachrichtenbl. Deut. Pflanzenschutzd., 58 (2), 46–49	
Year: 2006	
Results and conclusion: Accompanying investigations on the roller wiper 'Rotofix' were carried out in model and road trials to identify seepage of glyphosate in relation to different footway surfaces and joint width and to identify rain wash of the herbicide and its loss to road drainage in public road environment. The model trial used boxes with various footway surfaces (small set paving, pavement flags, water-bound surface). After treatment the plots were irrigated (8.5 and 14 mm) and the seeping water sampled at various time intervals and analysed for glyphosate and AMPA. Glyphosate peaked with 10.6 mg/l. The concentration was found in the seepage water from the flag-paved footway immediately after irrigation. Three days later, however, it had already decreased by more than three times. Results were different with the loamy footway (water-bound surface) and the small set paving. Peaks were identified on the third day after application, but were clearly below the pavement flag level. In the road trial, four samples were taken from road drainage. First results showed low concentrations of appr. 0.0002 mg/l glyphosate and up to 0.0005 mg/l AMPA. The higher AMPA level is assumed to be not only due to Rotofix application. It can be concluded that non-licensed herbicide applications on public and private roads and squares might result in risk to surface waters. However, the roller-wiper "Rotofix" can be used after licensed by local Authorities.	
Proposed action: Not to be considered as it presents risk management conclusions.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additonal information on Glyphosate concentrations in seeping water after application by a special technique on pavement	
Reliability	Low
Endpoint	Concentrations in seeping waters after application of Glyphosate
Protocol	No standard protocol, non-GLP
Test compound	Glyphosate (CAS-no.: 1071-83-6; 10% Roundup-Ultra)
Test system and conditions	Plot trials (worst-case scenarios), outdoor trials of selected roads (Berlin; after spring application performed by the city of Berlin), monitoring of selected receiving waters (Berlin)
Statistical design	Not specified
Relevance	
Environmental relevance	Given

Weight of evidence	
"Positive"/"Negative" evidence	Completely comparable publications not known, however, results are plausible; no negative evidence.

Spliid et al. (2006)

Title: Leaching and degradation of 21 pesticides in a full-scale model biobed	
Author: Niels Henrik Spliid, Arne Helweg, Kirsten Heinrichson	
Reference: Chemosphere 65 (2006) 2223–2232	
Year: 2006	
Results and conclusion: During the total study period of 563 days, no traces of 10 out of 21 applied pesticides were detected in the percolate. Glyphosate was not detected.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Glyphosate degradation by in a biobed
Protocol	Non-GLP study
Test compound	Labelled 2-13C, 15N-glyphosate was from Cambridge Isotope Laboratories (Andover, Massachusetts, US). (CAS 1071-83-6),
Test system and conditions	The degradation and leaching of 21 pesticides (5 g of each) was followed in an established full-scale model biobed. Percolate was collected and analysed for pesticide residues, and the biobed material was sampled at three different depths and analysed by liquid chromatography double mass spectrometry (LC-MSMS).
Statistical design	Six replicates
Relevance	
Environmental relevance	The relevance is low as the results are not relevant for standard PEC-simulations.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard testing.

Stadlbauer et al. (2005)

Title: Lysimeteruntersuchungen zur Verlagerung von Glyphosate im Lichte der Anwendung von Pflanzenschutzmitteln zur Beseitigung von winterharten Gründecken [Lysimeter investigations on the removal of glyphosate in the light of applications of pesticides for removal of winter green cover]
Author: Stadlbauer H, Fank J., Lorbeer G
Reference: paper read at: 11. Lysimetertagung, Lysimetrie im Netzwerk der Dynamik von Ökosystemen, 5. und 6. April 2005, Raumberg-Gumpenstein, Austria, 131-136
Year: 2005
Results and conclusion: Not to be considered as
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment and the studies were

not performed close to standard tests.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information on leaching behaviour	
Reliability	Low
Endpoint	Concentrations of Glyphosate and AMPA in soil and leachate
Protocol	No standard protocol given; non-GLP
Test compound	Glyphosate, isopropylammonium salt ("Round-Up", CAS-no: 38641-94-0), AMPA (CS-no.: 1066-51-9)
Test system and conditions	Lysimeter studies, size of lysimeter not given, application of Round-up (3.872 L/ha) applied in March 2002, duration until April 2004, leachate sampling, soil sampling in different depths shortly after application
Statistical design	1295 leachate samples analysed for Glyphosate and AMPA; soil samples from 3 depths at four sampling times analysed for Glyphosate and AMPA
Relevance	
Environmental relevance	Given, weather conditions and soil parameter recorded.
Weight of evidence	
"Positive"/"Negative" evidence	Results are supported by other studies; no negative evidence.

Stone and Wilson (2006)

Title: Preferential Flow Estimates to an Agricultural Tile Drain with Implications for Glyphosate Transport	
Author: Wesley W. Stone and John T. Wilson	
Reference: J. Environ. Qual. 35:1825–1835	
Year: 2006	
Results and conclusion: Agricultural subsurface drains, commonly referred to as tile drains, are potentially significant pathways for the movement of fertilizers and pesticides to streams and ditches in much of the Midwest. This paper uses chloride concentrations to estimate preferential flow contributions to a tile drain during two storms in May 2004. Chloride, a conservative anion, was selected as the tracer because of differences in chloride concentrations between the two sources of water to the tile drain, preferential and matrix flow. A strong correlation between specific conductance and chloride concentration provided a mechanism to estimate chloride concentrations in the tile drain throughout the storm hydrographs. A simple mixing analysis was used to identify the preferential flow component of the storm hydrograph. During two storms, preferential flow contributed 11 and 51% of total storm tile drain flow; the peak contributions, 40 and 81%, coincided with the peak tile drain flow. Positive relations between glyphosate [N-(phosphonomethyl)glycine] concentrations and preferential flow for the two storms suggest that preferential flow is an important transport pathway to the tile drain.	
Proposed action: Not to be considered as field trials were performed outside EU and focus on Chloride as tracer.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information only as publication focuses on Chloride as tracer	
Reliability	Low
Endpoint	Concentration of Glyphosate in drain flow

Protocol	No standard protocol available
Test compound	Glyphosate (CAS-no.: 1071-83-6)
Test system and conditions	Tests focused on Chloride as tracer; no detailed information on Glyphosate given
Statistical design	Not given for glyphosate
Relevance	
Environmental relevance	Not applicable as publication focuses on Chloride.
Weight of evidence	
"Positive"/"Negative" evidence	Not known as publication focuses on Chloride.

Strange-Hansen et al. (2004)

Title: Sorption, mineralization and mobility of N-(phosphonomethyl)glycine (glyphosate) in five different types of gravel	
Author: Rikke Strange-Hansen, Peter E Holm, Ole S Jacobsen and Carsten S Jacobsen	
Reference: Pest Manag Sci 60:570–578	
Year: 2004	
Results and conclusion: Cumulative mineralization of [methyl-^{14}C]glyphosate in batch studies was highest in coarse gravel, amounting to 14% after 4 days at 30°C and 32% after 31 days. Mineralization was slowest in the sandy reference soil, amounting to only 2% after 31 days. The adsorption coefficient (K_d) of glyphosate in gravel ranged from 62 to 164 litre kg^{-1}, while that in the sandy reference soil was 410 litre kg^{-1}. The results indicate that the relatively low K_d in gravel allows a relatively high rate of glyphosate mineralization by the biomass. When K_d is high, in contrast, mineralization is slow. Lowering the temperature to 10°C decreased mineralization by 50% in one of two gravels. The leaching of glyphosate was screened in simple columns of gravel or soil in which precipitation events (20mm over a 2-h period) were simulated on three occasions, starting either immediately after or 2 days after application of glyphosate. [^{14}C]Glyphosate was applied as a tracer mixed with the commercial product Roundup Garden at the recommended rate of 2.4 kg glyphosate ha^{-1}, equivalent to 1µgg^{-1} soil. The highest concentration of [^{14}C] compounds (expressed in terms of glyphosate concentration) in leachate from the columns exceeded 1300 µg litre^{-1}, and was detected in rounded gravel after the first rain event. No glyphosate was detected in leachate from the sandy reference soil.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, data on sorption/desorption is sufficient for the assessment.	
Type of information (critical, high/low weight, supporting, additional): Low, additional information	
Reliability	Medium
Endpoint	K_d = distribution coefficient; [^{14}C]carbon dioxide
Protocol	According to the OECD guidelines (106), but with a modified soil: solution ratio of 2 instead of 5; non-GLP
Test compound	[methyl-^{14}C]Glyphosate (specific activity 1.08MBq mmol^{-1}; radiochemical purity >99%); unlabelled glyphosate (purity 98%); for column experiments, Roundup Garden (commercial SL formulation containing 120 g litre^{-1} glyphosate (isopropylamine salt)); five types of gravel and a sandy agricultural reference soil, CAS-no.: 1071-83-6

Test system and conditions	Batch studies: 1) Sorption: glyphosate/[^{14}C]glyphosate concentration of 0.6 mgkg^{-1} (192 Bq), rotated for 96 h; 2) Mineralization in flasks: glyphosate/[^{14}C]glyphosate concentration of 16.9mgkg^{-1} (0.1mM) and 833 Bq, final moisture level equivalent to 80% of WHC, incubated at 30°C in the dark for 31 days, repeated on two types of gravel incubated at 10, 20 and 30°C; 3) Leaching studies: Two columns for each substrate, and each column was exposed to two different simulated precipitation events (Table 3). [^{14}C]glyphosate (1733 Bq in short columns, and 5666 Bq in tall columns) mixed with Roundup Garden (recommended rate of 2.4 kg glyphosate ha^{-1}), Over the next 6 days the columns were subjected to three simulated precipitation events at 20 ◦C, trapping the [^{14}C]carbon dioxide, total effluent collected 1 day after each precipitation event, determination of residual [^{14}C] compounds
Statistical design	Analyses in triplicate; K_d value represents one measurement of glyphosate in the solution, assuming linearity between glyphosate adsorption and glyphosate concentration in the solution.
Relevance	
Environmental relevance	Given as influence by environmental parameter was tested.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications.

Styczen et al. (2011)

Title: Macroscopic Evidence of Sources of Particles for Facilitated Transport during Intensive Rain
Author: Styczen M., Petersen C.T., Bender Koch C., Gjettermann B.
Reference: Vadose Zone J. 10:1151–1161
Year: 2011
Results and conclusion: Particulate transport of pesticides, heavy metals, and phosphate in soil is of interest when evaluating water contamination risk; however, researchers differ in their view of how and where the contaminated particles are mobilized. The main line of thought is that the particles originate in the soil, as a function of concentration differences between aggregate surfaces and surrounding immobile water, and move into mobile water by diffusion. Furthermore, material can be stored at the air–water interface. Low electrical conductivity enhances the formation of mobile particles. Other researchers consider the generation to take place close to the soil surface, as a side effect of splash erosion. By combining data on particle concentrations and the amounts of particle-bound glyphosate [N-(phosphonomethyl) glycine] from three leaching experiments with information about glyphosate on splashed material and the distribution of glyphosate in the soil, we concluded that the particles from the top 0.5 cm of the soil column contribute more than proportionally (up to 50%) to the particles in the leachate. The development in particle concentration with time and in columns with different properties indicated that particle generation took place both inside the column and as a result of the splash process. The leached particles that are generated inside the column probably stem from the flow-active part of the plow layer, a volume that differs from column to column and between tillage treatments.
Proposed action:

Not to be considered as basic research on sorption and transport mechanisms; will not lead to a modification of an endpoint	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information only as publication focuses on basic research on sorption and transport mechanisms	
Reliability	Low
Endpoint	Concentration of particle-bound Glyphosate on leached and splash-eroded particles; concentration in soil layers at the end of the study
Protocol	Similar to OECD 312
Test compound	Commercial product Roundup Bio (isopropylammonium salt?, CAS-no.: 38641-94-0), ^{14}C-labeled glyphosate (CAS-no.: 1071-83-6), and blank formulation plus AMPA (CAS-no.: 1066-51-9)
Test system and conditions	Undisturbed soil columns, 0 - 60 cm soil depth, 30 cm inner diameter, sampled from two experimental plots with different tillage treatments, product applied. Leachate sampling, soil subdivided into pieces at the end of the study
Statistical design	T-tests (two treatments with repetitions) were used to evaluate the amount of water draining from the columns as well as to compare particle concentrations and amounts for the two treatments.
Relevance	
Environmental relevance	Given; influencing parameter are considered and discussed.
Weight of evidence	
"Positive"/"Negative" evidence	Other publication support the results, no contradictions; no negative evidence.

Tesfamariam et al. (2009)

Title: Glyphosate in the rhizosphere – Role of waiting times and different glyphosate binding forms in soils for phytotoxicity to non-target plants
Author: Tsehaye Tesfamariam, S. Bott, I. Cakmak, V. Römheld, G. Neumann
Reference: Europ. J. Agronomy 31, 126–132
Year: 2009
Results and conclusion: Contradictory results are reported concerning the bio-availability of glyphosate residues in soils and the potential risks for intoxication of non-target organisms. This study addresses the question whether plant residues of glyphosate-treated weeds or direct soil application of glyphosate bears an intoxication risk for subsequently cultivated sunflower seedlings. Also the potential role of different waiting times between glyphosate application and sunflower cultivation was considered. Generally, the detrimental effects were more pronounced after glyphosate weed application (90% biomass reduction) compared with direct soil application (55–70% biomass reduction) at waiting time 0 d. The inhibitory effects on seedling growth were associated with a corresponding increase in shikimate accumulation in the root tissue as physiological indicator for glyphosate toxicity. Glyphosate intoxication of sunflower seedlings was also associated with an impairment of the manganese-nutritional status, which was still detectable after a waiting time of up to 21 d, particularly on the Arenosol in the variants with glyphosate weed application. These findings indicate an important and yet un-investigated role of glyphosate in plant residues in determining the risk of non-target plant intoxication.
Proposed action: Not to be considered as article aims at role of Glyphosate in plant residues and thus no need

for recalculation of an environmental fate endpoint is needed.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information only as article aims at role of Glyphosate in plant residues	
Reliability	Low
Endpoint	Plant growth
Protocol	Similar to OECD 208
Test compound	Roundup Ultramax® glyphosate formulation (CAS-no.: 1071-83-6)
Test system and conditions	Treated weeds: model plant perennial rye grass, Lolium perenne L.; soil application; subsequently cultivated after Glyphosate application: sunflower (Helianthus annuus L.), The experiments were conducted as greenhouse studies on two soils with contrasting properties (acidic, sandy Arenosol, calcareous loess subsoil)
Statistical design	All treatments comprised 4 replicates and pots were arranged in the greenhouse in a completely randomized block design. Analysis of variance was performed with SPSS statistics software package (SPSS Inc., IL, USA).
Relevance	
Environmental relevance	Given; influencing parameter are considered and discussed.
Weight of evidence	
"Positive"/"Negative" evidence	Other publication support the results, no contradictions; no negative evidence.

Ulén et al. (2012)

Title: Particulate-facilitated leaching of glyphosate and phosphorus from a marine clay soil via tile drains
Author: B. ULÉN, G. ALEX, J. KREUGER, A. SVANBÄCK & A. ETANA
Reference: Acta Agriculturae Scandinavica Section B - Soil and Plant Science, 2012; 62: Supplement 2, 241-251
Year: 2012
Results and conclusion: The objective of the present study was to quantify and evaluate leaching of glyphosate (Gly) in parallel with P. Leaching losses of autumn-applied Gly (1.06 kg ha^{-1}) via drainage water were examined by flow-proportional sampling of discharge from 20 drained plots in a field experiment in eastern Sweden. Samples were analysed for Gly in particulate-bound (PGly) and dissolved (DGly) form. The first 10 mm water discharge contained no detectable Gly, but the following 70 mm had total Gly (TotGly) concentrations of up to 6 µg L^{-1}, with 62% occurring as PGly. On average, 0.7 g TotGly ha^{-1} was leached from conventionally ploughed plots, compared with 1.7 g TotGly ha^{-1} from shallow-tilled plots (cultivator to 12 cm working depth). Higher Gly losses occurred in snowmelt periods in spring, but then with the majority (60%) as DGly. All autumn concentrations of PGly in drainage water were significantly correlated ($p<0.001$) to the concentrations of particulate-bound phosphorus (PP) lost from the different plots (Pearson correlation coefficient 0.84), while PP concentrations were in turn significantly correlated to water turbidity (Pearson correlation coefficient 0.81). Leaching losses of TotGly were significantly lower (by 1.3 g ha^{-1}; $p<0.01$) from plots that had been structure-limed three years previously and ploughed thereafter than from shallow-tilled plots. Turbidity and PP concentration also tended to be lowest in discharge from structure-limed plots and highest from shallow-tilled plots. This difference in TotGly leaching between soil management

regimes could not be explained by differences in measured pH in drainage water or amount of discharge. However, previously structure-limed plots had significantly better aggregate stability, measured as readily dispersed clay (RDC), than unlimited plots. The effects of building up good soil structure, with strong soil aggregates and an appropriate pore system in the topsoil, on mitigating Gly and P losses in particulate and dissolved form should be further investigated.	
Proposed action: To be considered as additional information.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information from a field study	
Reliability	
Endpoint	Leaching of glyphosate and phosphorus
Protocol	Not given
Test compound	Glyphosate (Glypro Bio, 1.06 kg ha^{-1} active substance)
Test system and conditions	Experimental plots and soil characteristics: In 2006, an experimental field with a sub-surface drainage water collection system was constructed on a flat plain close to the Lake Bornsjön reservoir by Stockholm Water Company. It encompasses 28 drained plots, 20 of which were used in the present experiment. In order to match the experimental plots to farm machinery, their dimensions are 20 m × 24 m (0.048 ha) and the drains are placed centrally, with 8 m spacing, in order to effectively drain the soil. Drainage water flows to a sampling and measuring station and is recorded with tilting vessels and data logger. The data logger controls the flow-proportional sampling by means of small tube pumps in the basement of the station. After a certain volume of water has passed, the suction tube is first cleaned by reverse pumping and thereafter a small volume is sampled. The flow-proportional (composite) sampling took place in dark glass vessels (2.5 L) at relatively cold temperature and in darkness for a maximum of one week prior to freezing the water samples and transport to the laboratory before analysis. Glyphosate application and cultivation practices: In preceding years the crops were: winter wheat in 2007, spring barley in 2008 and 2009 and oats in 2010. No Gly had been applied to the actual experimental plots for the previous three years. Phosphorus fertilization (mean year 1988-2006) was 11 kg ha^{-1} $year^{-1}$, always applied in mineral form in spring. This is a moderate load, since the area has special restrictions. When starting the experiment the aim was to avoid P limitation of the crop and therefore 20 kg ha^{-1} $year^{-1}$were applied in 2007- 2011 for all plots except four. Glyphosate was applied on 22 September 2010 as the commercial product Glypro Bio, at a rate equal to 1.06 kg ha^{-1} active substance. This amount, which represents a normal dose in Swedish production systems, was applied in evening at air temperature 11ºC and no wind. Twelve days later, the conventional and structure-limed plots were stubble-harrowed and eight plots were shallow-tilled (12 cm) twice and reconsolidated with a rib-roller. After a further 10 days, the conventionally ploughed plots (8) and the structure-limed plots (4) were mouldboard-ploughed and the soil

	was inverted to a depth of 23 cm. Sampling and analysis: On 28 March, 186 days after glyphosate application in autumn, turbidity was observed once again in the flow-proportionally sampled water and additional water was collected for Gly analysis, which was performed on the 14 most turbid samples. The same analytical procedure was used for both PGly and DGly and involved ion-exchange and derivatization, using a modified version of Mogadati et al. (1996), followed by final identification and quantification by gas chromatography-mass spectrometry (GC-MS). The limit of detection (LOD) was 0.03, 0.1 and 0.2 mg L^{-1} for DGly, PGly and AMPA, respectively, with occasional higher LODs due to background interference. The limit of quantification (LOQ) was 2-3 times higher.
Statistical design	The mean and standard deviation were calculated for the experimental parameters determined in all flow-proportional samples from replicate plots for the different treatments. If no residue of Gly or AMPA was detected in a given sample, the value 0 was used for calculating the mean. Pearson correlation and regression linear relationships were determined between the parameters total glyphosate (TotGly=PGly+DGly), TotP, PGly, PP and turbidity for the autumn period (27 September-15 November) and between TotP and turbidity for the spring period (21 March-11 April). Any differences in glyphosate concentrations between the different soil treatments were analysed using Bonferroni post test assuming equal variance and a significance level of <B0.05. Leaching losses from the different plots in the autumn period were calculated by multiplying discharge by measured flow-proportional concentrations in the periods between sample collections. In the spring period, transport of TotGly was estimated from measured values from 14 plots on 28 March. Without any measured values the transport was estimated from TotP transport using the relationship between TotP and TotGly as determined for the 14 samples.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Ulén et al. (2013)

Title: Spatial variation in herbicide leaching from a marine clay soil via subsurface drains
Author: B. M. Ulén, M. Larsbo, J. K. Kreuger and A. Svanbäck
Reference: Published online in Wiley Online Library: 13 June 2013, (wileyonlinelibrary.com) DOI 10.1002/ps.3574
Year: 2013
Results and conclusion: BACKGROUND: Subsurface transport via tile drains can significantly contribute to pesticide contamination of surface waters. The spatial variation in subsurface leaching of nor-

mally applied herbicides was examined together with phosphorus losses in 24 experimental plots with water sampled flow-proportionally. The study site was a flat, tile-drained area with 60% marine clay in the topsoil in southeast Sweden. The objectives were to quantify the leaching of frequently used herbicides from a tile drained cracking clay soil and to evaluate the variation in leaching within the experimental area and relate this to topsoil management practices (tillage method and structure liming).
RESULTS: In summer 2009, 0.14, 0.22 and 1.62%, respectively, of simultaneously applied amounts of MCPA, fluroxypyr and clopyralid were leached by heavy rain five days after spraying. In summer 2011, on average 0.70% of applied bentazone was leached by short bursts of intensive rain 12 days after application. Peak flow concentrations for 50% of the treated area for MCPA and 33% for bentazone exceeded the Swedish no-effect guideline values for aquatic ecosystems. Approximately 0.08% of the glyphosate applied was leached in dissolved form in the winters of 2008/2009 and 2010/2011. Based on measurements of glyphosate in particulate form, total glyphosate losses were twice as high (0.16%) in the second winter. The spatial inter-plot variation was large (72–115%) for all five herbicides studied, despite small variations (25%) in water discharge.
CONCLUSIONS: The study shows the importance of local scale soil transport properties for herbicide leaching in cracking clay soils.
In detail: Concentrations of the herbicides bentazone, clopyralid, fluroxypyr, MCPA and glyphosate were measured in subsurface drain discharge from a clay field during a four-year study. Despite hydrological conditions not representing a worst case scenario for leaching, the relative leaching losses of all herbicides studied were large compared to values reported in the literature. Measured concentrations of bentazone and MCPA exceeded Swedish guideline values based on predicted no effect on aquatic ecosystems for 50 and 33% of the plots for MCPA and bentazone, respectively. All substances studied (except sulphonylure as which were not detected), irrespective of sorption strength, showed similar leaching patterns. These observations clearly demonstrate that preferential transport in macropores is the dominant transport process at this site. The variation in relative leaching losses between plots within the same treatment was greater than that between different substances. Crack stabilisation by gyttja, especially in the deeper subsoil, was suggested as an important explanatory factor for this large spatial variation in pesticide leaching, although it was not possible to investigate differences in gyttja content between plots. Continuous macropores connecting the soil surface to the subsoil may be a factor contributing to the generally large pesticide losses observed after shallow tillage. However, careful studies of soil macropore systems, including topsoil and subsoil properties, are needed to explain the unpredictability in leaching at this site.

Proposed action:
To be considered as additional information.

Type of information (critical, high/low weight, supporting, additional):
Low weight, additional information from a field study

Reliability	
Endpoint	Leaching of pesticides: Bentazone, MCPA, Fluroxypyr, Clopyralid, Glyphosate, Thifensulfuron-methyl and Tribenuron-methyl
Protocol	Not given
Test compound	Bentazone, MCPA, Fluroxypyr, Clopyralid, Glyphosate, Thifensulfuron-methyl and Tribenuron-methyl
Test system and conditions	The field site is located in a flat valley (mean slope less than 0.05‰) with a clay soil of marine origin in eastern Sweden. The experimental field (1.3 ha) with 28 plots (24 m × 20 m) was tile-drained in 2006 to 0.9 m depth (8 m spacing).9 Twenty-four of these plots were used in the experiment. The plots are situated in

	two rows of 14 plots at varying distance from an open ditch that acts as the recipient of drainage water from the surrounding valley. Three management practices were randomly assigned to the plots: Conventional autumn ploughing, shallow autumn tillage and structure-liming (i.e. liming carried out to reduce phosphorus leaching and to improve crop yield by improving soil structure). The leaching of seven different pesticides (Bentazone, MCPA, Fluroxypyr, Clopyralid, Glyphosate, Thifensulfuron-methyl and Tribenuron-methyl) with contrasting properties was studied. Water discharge from each plot was measured with tilting vessels in an underground basement where sampling of drainage water also took place. The water was sampled flow-proportionally, with every subsample representing 0.003 mm discharge in summer and 0.04 mm discharge in the rest of the year. The bulk samples were collected weekly (or for the first flow events following application more frequently). Accordingly, the actual peak concentrations were not captured. Immediately after collection all samples were frozen and sent to the Organic Risk Pollutants Laboratory, Department of Aquatic Sciences and Assessment, SLU, where they were analysed when detectable concentrations of herbicides were expected. The concentration of thifensulfuron-methyl and tribenuronmethyl (in 2008) was determined with solid-phase extraction followed by liquid chromatography and mass spectrometry (LCMS) and the concentration of clopyralid, fluroxypyr and MCPA (in 2009) by the same solid-phase extraction and by derivatisation and gas chromatography/mass spectrometry (GC/MS).14 Fluroxypyr and MCPA(in 2010)and bentazone(in 2011) were analysed by mass spectrometric determination (LC-MS/MS).15 Dissolved glyphosate (DissGly) and its main metabolite AMPA were analysed in winter 2008/2009 and 2010/2011, which involved ion exchange and derivatisation, followed by final identification and quantification by GC/MS. In winter 2010/2011, glyphosate analysis included particulate glyphosate (PartGly), which was trapped using a cellulose acetate filter with pore size 0.45 µm. The median value for limit of detection (LOD; in µg L^{-1}) was: 0.003 for bentazone, 0.005 for clopyralid, 0.01 for fluroxypyr, 0.003 for MCPA, 0.006 for thifensulfuron-methyl and tribenuron-methyl, 0.03 for dissolved glyphosate, 0.1 for particulate glyphosate and 0.2 for AMPA. Measured concentrations were below the LOD values for dissolved glyphosate, particulate glyphosate and AMPA in 20, 22 and 45% of the samples, respectively. Dissolved reactive phosphorus (DRP) and particulate phosphorus (PP) were determined for all samples which were analysed for any pesticides.
Statistical design	Mean, SD, range, maximum
Relevance	
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	No negative evidence.

Vogel and Linard (2011)

Title: AGRICULTURAL HERBICIDE TRANSPORT IN A FIRST- ORDER INTERMITTENT STREAM, NEBRASKA, USA	
Author: J. R. Vogel, J. I. Linard	
Reference: APPLIED ENGINEERING IN AGRICULTURE, Vol. 27(1): 63-74	
Year: 2011	
Results and conclusion: In this study, the transport of 6 herbicides and 12 herbicide degradates was examined during the 2004 growing season in an intermediate- scale agricultural watershed (146 ha) that is drained by a first- order intermittent stream, and the mass load for each herbicide in the stream was estimated. The herbicide load during the first week of storm events after application ranged from 17 % of annual load for trifluralin to 84% of annual load for acetochlor. The maximum weekly herbicide load in the stream was generally within the first 3 weeks after application for those compounds that were applied within the watershed during 2004, and later for herbicides not applied within the watershed during 2004 but still detected in the stream. The apparent dominant mode of herbicide transport in the stream determined by analysis amongst herbicide and conservative ion concentrations at different points in the hydrograph and in base flow samples- - was either overland runoff or shallow subsurface flow, depending on the elapsed time after application and type of herbicide. The load as a percentage of use (LAPU) for the parent compounds in this study was similar to literature values for those compounds applied by the farmer within the watershed, but smaller for those herbicides that had rainfall as their only source within the watershed.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment and the studies were not performed close to standard tests.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as focus was on transport processes in agricultural areas	
Reliability	Medium
Endpoint	Herbicide concentrations in stream base flow, rain, and lysimeter samples; timing of maximum load in the stream, along with the type of transport at that time
Protocol	Non-GLP; field experiments, lysimeter
Test compound	Atrazine, acetochlor, trifluralin, glyphosate, pendimethalin, metolachlor, alachlor, and dimethenamid; herbicide degradates
Test system and conditions	This study was conducted in a small agricultural watershed. The application of test compounds was spread among 14 individual cropped areas within the watershed, with no particular upstream to downstream pattern in their distribution. Sampling intervals varied throughout the experiment, with the shortest intervals (2 min) during the time nearest the peak concentration in the stream at the sampling point. Time of travel was determined by calculating the time lapse from release of the dye to peak concentration at the sampling point.
Statistical design	Calculated by interpolation between times of streamflow and sampling events, (when necessary, modelled results for discharge from SWAT); pre-processing software, Better Assessment Science for Integrating point and Non-point Sources (BASINS) (USEPA,

	2004), was used to create the default parameter files necessary for initial simulations
Relevance	
Environmental relevance	Given; influencing parameters are reported.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

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B.8.11.4 Fate and behaviour in water

B.8.11.4.1 Photochemical degradation

Summary

One article is available on the behaviour of glyphosate when subjected to irradiation in the presence of H_2O_2. Manassero et al. (2010) reported that the reaction rate depends on the initial herbicide concentration and has an optimum plateau of a hydrogen peroxide to glyphosate molar concentration ratio between 7 and 19. The expected non linear dependence on the irradiation rate was observed. Test results are of minor relevance as they are not obtained by a standard test procedure. They can be assessed as additional information only.

Two additional studies reported on the degradation of glyphosate in aqeous medium by photocatalytic mineralization (Chen *et al.*, 2012 and Zhang *et al.*, 2012). The authors analyzed the effect of the presence of UV/TiO_2 or titania nanotubes on degradation, respectively.

Detailed description of open literature on photochemical degradation

Chen et al. (2012)

Title: Photocatalytic mineralization of glyphosate in a small-scale plug flow simulation reactor by UV/TiO_2	
Author: JIAN Q. CHEN, ZHI J. HU and NAN X. WANG	
Reference: Journal of Environmental Science and Health, Part B (2012) 47, 579–588	
Year: 2012	
Results and conclusion: The present work involves the photocatalytic mineralization of glyphosate on a plug flow reactor by UV/TiO_2. The effect of catalyst loading shows an optimal value (0.4 g L^{-1}) which is necessary to mineralize glyphosate. The kinetic rate of glyphosate mineralization decreases with the increasing initial concentration of glyphosate, and the data can be described using the first-order model. An alkaline environment is conducive to glyphosate mineralization. The mineralization efficiency increases with elevated flow rate to 114 mL min^{-1}, which is followed by a decrease with a further increase in flow rate due to the reduction of the residence time. The presence of external oxidants ($K_2S_2O_8$, H_2O_2 and $KBrO_3$) and photosencitizer (humic acid) can significantly enhance glyphosate mineralization. Photocatalysis oxidation ability of the three studied oxidants decrease in the order of: $S_2O_8^{2-}$ –> BrO_3 –> H_2O_2. Finally, the Langmuir–Hinshelwood (L-H) model was used to rationalize the mechanisms of reactions occurring on TiO_2 surfaces and L-H model constants were also determined.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation in aqeous medium by photocatalytic mineralization
Protocol	Non-GLP study
Test compound	98% pure glyphosate (N-phosphonomethylglycine) obtained from Yangnong Chemical Group Co., Ltd, Jiangsu, China (CAS 1071-83-6)
Test system and conditions	A certain amount of TiO_2 catalyst and glyphosate solution were loaded into the quartz jacket and then fitted into the constant temperature water bath (20±1°C), which was fixed on the base of the heater and magnetic stirrer. Prior to an UV irradiation, the suspension was magnetically stirred for 15 min in order to achieve a maximal adsorption of the glyphosate on TiO_2 surface. The suspension was kept stirring during the entire mineralization process. Aliquots of 1.5 mL suspension were sampled from a sample outlet using a syringe at specific time intervals and the supernatant was obtained by high speed centrifugation for 10 min. The supernatantswere then stored at 4◦Cuntil determination of phosphate (one of inorganic products of glyphosate mineralization). Solution pH (3.20–11.11), initial concentration (2.0 × 10−4–8.0 × 10−4 mol L^{-1}), catalyst loading (0–0.8 g L^{-1}), and the presence of oxidants, photosensitizer, organic compounds, inorganic metal cations and anions were investigated for their effects on the photo mineralization efficiency.
Statistical design	No information

Relevance	
Environmental relevance	The relevance is small due to the artificial environment.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard testing.

Manassero et al. (2010)

Title: Glyphosate degradation in water employing the H2O2/UVC process	
Author: A. Manassero, C. Passalia, A.C. Negro, A.E. Cassano, C.S. Zalazar	
Reference: water research 44 (2010) 3875-3882	
Year: 2010	
Results and conclusion: Conversion of glyphosate increases significantly from pH = 3.7. From this value on, the increase becomes much less noticeable. The reaction rate depends on the initial herbicide concentration and has an optimum plateau of a hydrogen peroxide to glyphosate molar concentration ratio between 7 and 19. The expected non linear dependence on the irradiation rate was observed.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Glyphosate degradation in water using H_2O_2 and UV radiation
Protocol	Non-GLP study
Test compound	glyphosate (95% provided by Red Surcos), CAS 1071-83-6 AMPA (>99%, SigmaeAldrich) CAS 1066-51-9
Test system and conditions	The photodegradation of glyphosate was carried out in a cylindrical reactor made of Teflon TM, with two parallel, flat windows made of quartz (Reactor = 110 cm^3). Each window was irradiated with a tubular, germicidal lamp (lambda 253.7 nm) placed at the focal axis of a parabolic reflector made of mirror finished aluminium. Glyphosate was analyzed by ion chromatography with a suppressed conductivity detector.
Statistical design	No information
Relevance	
Environmental relevance	The results are of minor relevance since the experiments do not describe any of the standard endpoints.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared directly with standard tests.

Zhang et al. (2012)

Title: Enhanced photocatalytic performance of titania nanotubes modified with sulfuric acid
Author: Guo-Wen Zhang, Guo-Hua He, Wei-Liang Xue, Xiong-Fa Xu, Dan-Ni Liu, Yue-Hua Xu
Reference: Journal of Molecular Catalysis A: Chemical 363– 364 (2012) 423– 429

Year: 2012	
Results and conclusion: Titania nanotubes modified with sulfuric acid (S-TNTs) were synthesized through hydrothermal treatment and impregnation method, and characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM), photoluminescence spectra (PL), Fourier transform infrared spectroscopy (FT-IR), BET surface area and X-ray photoelectron spectroscopy (XPS). The photocatalytic activities of S-TNTs were investigated by the glyphosate degradation. The lower the PL intensity, the higher the photocatalytic activity. The sulfuric acid modification enhanced the photocatalytic activity of TNTs, and 7%S-TNTs calcined at 400 ◦C showed the highest photocatalytic activity. The photocatalytic performance of as-prepared S-TNTs was strongly related with the sulfuric acid concentration and the degree of crystallinity. The nanotube morphology, the specific surface area, as well as the crystallite size also had important impact on the photocatalytic activity of S-TNTs.	
Proposed action: Consider as additional information.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	
Endpoint	Degradation in aqeous medium by photocatalytic mineralization
Protocol	Non-GLP study
Test compound	Glyphosate (CAS 1071-83-6)
Test system and conditions	Aqueous slurries were prepared by adding 0.1 g photocatalyst to 500 ml of 1.0×10^{-4} mol l^{-1} glyphosate aqueous solution at neutral pH. Irradiations were performed with a 125 W high-pressure mercury lamp, and Fig. 1 shows the wavelength and intensity of this 125 W high-pressure mercury lamp. The high-pressure mercury lamp irradiates lights of many wavelengths including visible lights (400–600 nm). The aqueous slurries were stirred and bubbled with oxygen for 30 min prior to the irradiation. And then, at 10 min intervals, the suspension was extracted and centrifuged to separate the photocatalyst particles. The final oxidation product PO_4^{3-} concentration of the supernatant liquid was analyzed using the Mo–Sb–Ascorbic acid colorimetry
Statistical design	No information
Relevance	
Environmental relevance	The relevance is small due to the artificial environment.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard testing.

References

Chen, J.Q., Hu, Z.J. and Wang, N.X. 2012. Photocatalytic mineralization of glyphosate in a small-scale plug flow simulation reactor by UV/TiO2. Journal of Environmental Science and Health, Part B 47, 579–588

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Zhang et al. 2012. Enhanced photocatalytic performance of titania nanotubes modified with sulfuric acid. Journal of Molecular Catalysis A: Chemical 363– 364 (2012) 423– 429

B.8.11.4.2 Water/sediment study

Summary

Two studies (Degenhardt *et al.*, 2012 and Tsui and Chu, 2008) are available on outdoor freshwater investigations. The results are of no relevance for the glyphosate assessment since the experimental sites were in wetland outside the EU. This information might be used as additional information only.

Another article (Mercurio et al., 2014) was available regarding the degradation of glyphosate in seawater. The results are less relevant for the glyphosate assessment since the authors investigated degradation of glyphosate in natural seawater without any sediment, which does not reflect the conditions of the presence of glyphosate in the aquatic environment.

Detailed description of open literature – Water/sediment study

Degenhardt et al. (2012)

Title: Dissipation of glyphosate and aminomethylphosphonic acid in water and sediment of two Canadian prairie wetlands	
Author: DANI DEGENHARDT, DAVID HUMPHRIES, ALLAN J. CESSNA, PAULMESSING, PASCAL H. BADIOU, RENATA RAINA, ANNEMIEKE FARENHORST and DAN J. PENNOCK	
Reference: Journal of Environmental Science and Health, Part B (2012) 47, 631–639	
Year: 2012	
Results and conclusion: Glyphosate has been detected in a range of surface waters but this is the first study to monitor its fate in prairie wetlands situated in agricultural fields. An ephemeral wetland (E) and a semi-permanent wetland (SP) were each divided into halves using a polyvinyl curtain. One half of each wetland was fortified with glyphosate with the added mass simulating an accidental direct overspray. The results showed that the acute toxic effects of glyphosate contamination will be limited even for a worst-case point-source scenario resulting from direct overspray because glyphosate dissipated very rapidly in the water column of both an ephemeral and semi-permanent wetland (field DT_{50} values of 1.3 and 4.8 d, respectively). Degradation of glyphosate to its major metabolite AMPA in the water-column and sorption of the herbicide to bottom sediment were more important pathways for the dissipation of glyphosate from the water column than movement of the herbicide with infiltrating water. Based upon the maximum concentration of glyphosate detected in the sediment, sorption to bottom sediment accounted for approximately 67% and 10% of the total glyphosate added to the wetland E and wetland SP, respectively.	
Proposed action: Consider as additional information. Recalculation of endpoints on dissipation from water is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Supporting information.	
Reliability	
Endpoint	Dissipation of glyphosate in water and sediment
Protocol	Non-GLP study

Test compound	Glyfos (Cheminova, Denmark), containing 360 g acid equivalent (a.e.)/L glyphosate as its isopropylamine salt (CAS 1071-83-6), unlabelled
Test system and conditions	Two wetlands were selected for the study, they were situated within a cultivated field and consisted of a smaller ephemeral wetland (E) and a larger semi-permanent wetland (SP). Glyfos was applied and water and sediment samples were collected from day 1 to 77.
Statistical design	Not given
Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared to results from reliable water/sediment studies.

Mercurio et al. (2014)

Title: Glyphosate persistence in seawater	
Author: P. Mercurio, F. Flores, J.F. Mueller, S. Carter, A.P. Negri	
Reference: Mar. Pollut. Bull. (2014), http://dx.doi.org/10.1016/j.marpolbul.2014.01.021	
Year: 2014	
Results and conclusion: The biodegradation of glyphosate using standard simulation flask tests with native bacterial populations and coastal seawater from the Great Barrier Reef was quantified. The half-life for glyphosate at 25 °C in low-light was 47 days, extending to 267 days in the dark at 25 °C and 315 days in the dark at 31 °C, which is the longest persistence reported for this herbicide. AMPA, the microbial transformation product of glyphosate, was detected under all conditions, confirming that degradation was mediated by the native microbial community. This study demonstrates glyphosate is moderately persistent in the marine water under low light conditions and is highly persistent in the dark. Little degradation would be expected during flood plumes in the tropics, which could potentially deliver dissolved and sediment-bound glyphosate far from shore.	
Proposed action: Consider as additional information. Furthermore, the experimental site was outside the EU.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	$DegT_{50}$ in water
Protocol	Non-GLP study according to the OECD methods for "simulation tests" (OECD, 2005)
Test compound	The glyphosate standard was obtained from Sigma–Aldrich.
Test system and conditions	The tests were conducted in natural seawater containing a native bacterial community and no addition of nutrients or artificial inoculum to best mimic ecological conditions. The tests were conducted under three scenarios: (1) 25 °C in the dark which corresponds to the mean annual seawater temperature on the GBR (AIMS, 2013); (2) 25 °C in low light conditions and (3) 31 °C in the dark which is a summer maximum temperature for nearshore areas of the mid-northern regions of the GBR. Three temperature-regulated incubator shakers (Thermoline TLM-530) were used in the experiments. A series of 6 × 900 mm LED strips

	(Superlight LED Lighting, Generation 3 High-Output LED Turbostrip) were fitted to one shaker, providing an even light environment of 40 µmol photons m^{-2} s^{-1} over a 12:12 light day cycle. This is equivalent to 1.7 mol photons m^{-2} day^{-1} which is within the range of light environments measured in shallow 3–6 m depths on turbid nearshore reefs of the GBR during the wet season. The position of flasks was randomised after every sampling period and flasks were consistently shaken at 100 rpm. All glassware was washed at 90 °C with laboratory detergent, rinsed and oven dried at 100 °C, acid washed (10% HCl), rinsed × 5 with RO then Milli-Q water until pH neutral, oven dried a second time at 100 °C, baked in a muffle furnace at 350 °C for 30 minutes, and capped with aluminium foil until use. The glyphosate standard was purchased from Sigma–Aldrich, added to 2 mL of the carrier solvent ethanol (to assist in solubility), and made to 5 mg L^{-1} concentration with Milli-Q water. Coastal water was collected from19°16´ (S), 147° 03´ (E) and filtered to 20 µm to introduce the total bacterial diversity from this environment. The seawater was added to 500 mL Erlenmeyer flasks to a final volume of 300 mL and sample treatments were spiked with a final concentration of 10 µg L^{-1}glyphosate. The same volume of carrier was added to control sample flasks and was 0.0004% (v/v). Each flask was stoppered with autoclaved silicone bungs to allow for aerobic conditions. The physical/chemical characteristics of the filtered seawater were measured for: pH, DIC, DOC, DIN, DON, TSS, bacterial counts and particle size distribution. Flow cytometry was used to quantify the microbial populations in the seawater used in the experiment. Samples were fixed with 5% formaldehyde and stored at 4 °C. Sub-samples were stained using Sybr Green, diluted to 1:10,000, and allowed to develop in the dark for 30 min. Samples were run using a BD Accuri C6 cytometer (BD Biosciences, CA, USA) equipped with a red and blue laser (488 nm, 50mWmaximum solid state; 640 nm, 30mWdiode) and standard filter setup. Flow rate was 14 µL min^{-1}, 10-µm core. The natural microbial community populations and their abundances were measured for the initial seawater as well as treatments for the experiment using the Accuri CFlow plus software. For each sampling period, 5 mL control and glyphosate samples were collected and stored at 4 °C. The glyphosate and degradation product concentrations were determined by HPLC-MS/MS using an ABSciex 4000Q Trap mass spectrometer (ABSciex, Concord, Ontario, Canada) equipped with an electrospray (TurboV) interface and coupled to a Shimadzu Prominence HPLC system (Shimadzu Corp., Kyoto, Japan).
Statistical design	Replicates were used. The half-lives (T½) for glyphosate were calculated assuming first order kinetics.
Relevance	
Environmental relevance	Reliable water/sediment studies have shown that, in addition to microbial degradation, a major contributor to the aquatic dissipation of glyphosate is adsorption to the sediment.

	The study of Mercurio et al. (2014) is less relevant since the authors investigated degradation of glyphosate in natural seawater containing a native bacterial community but without any sediment, which does not reflect the conditions of the presence of glyphosate in the aquatic environment. The calculated $DegT_{50}$ values in water cannot be compared to $DissT_{50}$ values in water derived from relibale water/sediment studies.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared to results from reliable water/sediment studies.

Tsui and Chu (2008)

Title: Environmental fate and non-target impact of glyphosate-based herbicide (Roundup) in a subtropical wetland	
Author: M.T.K. Tsui, L.M. Chu	
Reference: Chemosphere 71, 439–446	
Year: 2008	
Results and conclusion: For both ponds, glyphosate concentrations in the water decreased rapidly after 1–3 DPT, but then decreased gradually over time. Both physical adsorption to the bottom sediments and microbial degradation are thought to contribute to these decreases. Interestingly, the persistence of glyphosate in the freshwater pond was longer than in the estuarine system, which is likely due to the considerably higher concentrations of chelating metals (i.e. Cu and Fe) present in the sediment (4.5 and 11-fold higher, respectively) which potentially reduced the bioavailability of glyphosate to the microbial decomposers. Lastly, fishes used in the in situ bioassays (both in applied and unapplied areas) showed similar survival rates, indicating that the use of Roundup at the provided application rate posed no serious hazard.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary. Furthermore, the experimental site was outside the EU.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Analysis of processes involved in microbial glyphosate degradation
Protocol	Non-GLP study
Test compound	Non-labelled glyphosate (CAS 1071-83-6),
Test system and conditions	Glyphosate degradation in pond experiments. The two study sites were estuarine and freshwater ponds in Mai Po. The air temperature recorded for both experiments ranged from 23 to 26 °C.
Statistical design	Two replicates
Relevance	
Environmental relevance	The relevance is less relevant since the authors did not calculate any DT_{50} values.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with reliable studies.

References

Degenhardt et al. 2012. Dissipation of glyphosate and aminomethylphosphonic acid in water and sediment of two Canadian prairie wetlands. Journal of Environmental Science and Health, Part B (2012) 47, 631–639

Mercurio, P., Flores, F., Mueller, J.F., Carter, S., Negri, A.P. 2014. Glyphosate persistence in seawater. Mar. Pollut. Bull. (2014), http://dx.doi.org/10.1016/j.marpolbul.2014.01.021

Tsui, M.T.K, Chu, L.M. 2008. Environmental fate and non-target impact of glyphosate-based herbicide (Roundup) in a subtropical wetland. Chemosphere 71: 439–446.

B.8.11.5 Impact on water treatment procedures

Summary

Several publications also confirm that glyphosate and AMPA can be removed from raw water by treatment processes. In particular, it was shown that glyphosate is readily removed from water by ozonation, chlorination, the modified electro-Fenton-like (EF-like) with Mn^{2+}, UV/TiO_2, the combination of hydrogen peroxide and UV radiation as well as reverse osmosis and subsequent activated carbon filtration. Cl_2 and O_3, are highly effective in degrading glyphosate and AMPA. Removal by coagulation is ineffective as a barrier against contamination in drinking water. Removal or degradation by bank filtration, slow sand filtration and ClO_2 is variable. In addition, there is a potential of ~~bank filtration~~ rapid sand filtration to eliminate glyphosate from water. Other treatment processes, e.g. reverse osmosis exclusively or Granular Activated Carbon (GAC) filtration, are less effective. Ozonation is also a suitable method for the removal of AMPA from water, whereas GAC filtration is not efficient in removing AMPA. The chlorination products of glyphosate are similar to those expected from chlorination of amino acids, proteins, peptides, and many other natural organic matters present in drinking water. Furthermore, it seems that glyphosate degradation by the combination of hydrogen peroxide and UV radiation does not lead to stable toxic end products.

Detailed description of open literature on impact on water treatment procedures

Boucherie et al. (2010)

Title: “Ozone” and “GAC filtration” synergy for removal of emerging micropollutants in a drinking water treatment plant?
Author: C. Boucherie, C. Lecarpentier, N. Fauchon, M. Djafer and V. Heim
Reference: Water Science & Technology: Water Supply—WSTWS | 10.5 | 2010
Year: 2010
Results and conclusion: Ozonation plays an essential role in water disinfection to inactivate viruses, bacteria and some parasites (Giardia). Ozone treatment rates to attain disinfection goals also result in oxidation reactions of emerging pollutants. Pharmaceuticals – except Ciprofloxacin – are very reactive to ozone: they are removed as early as the transfer compartment outlet even at an ozone treatment rate of less than 1 g/m³. Glyphosate, AMPA, Amitrole and Diuron–the four major pesticides in the Seine, Marne and Oise rivers–are reactive to ozone. Twenty-one pesticides are only partially reactive to ozone and an additional “GAC filtration” is needed to remove them. Further investigations have been planned to study the removal of Phthalates, Nonylphenols and Hormones by combining the “Ozone” and “GAC filtration” process units.

Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Low weight; additional information	
Reliability	Medium
Endpoint	PH, temperature, alkalinity (AT), UV254, ozone gas and liquid residual; concentration of micropollutants
Protocol	Non-GLP
Test compound	Bezafibrate, Carbamazepin, Ciproflexacin, Diclofenac, Erythromycin, Fenofibrate, Ketoprofen, Metoprolol, Ofloxacin, Paracetamol, Phenazone, Propanolol, Roxithromycin, Spiramycin, Sulfachloropyridazine, Sulfamerazine Sulfamethoxazole, Tylosine, Acetochlore Alachlore Amitrole AMPA, Atrazine, Azoxystrobine, Bentazone, Bromuconazole, Carbendazime, Carbetamide, Carbofuran, Chloridazone, Chlortoluron, DCPMU, DEA, DEDIA, Deethylterbumeton, DIA, Dichloroprop, Difenoconazole, Dimetachlore, Diuron, Ethofumesate, Fluquinconazole, Flusilazole, Glyphosate, Hydroxyatrazine, Imazamethabenz-methyl, Isoproturon, MCPA, Mecoprop, Metazachlore, Metolachlore, Piclorame, Prochloraze, Propazine
Test system and conditions	The pilot unit consists of an ozonation-deozonation step linked to a GAC filtration column (see Figure 3). The system is continuously fed by SFW from the Neuilly-sur-Marne drinking water plant. Bromide or micropollutants are injected into the feeding line via a static mixer. Pharmaceutical tests:7 tests have been carried out with an ozone treatment level ranging from 0 to 2.1 g/m3 and with the following experimental conditions: 7.3 < pH < 7.4, 17.2 < T(°C) < 17.6, 0.097 < UV254 (cm^{-1}) < 0.130 and AT = 4.0 meq/L. Pesticides tests: 6 tests have been carried out with an ozone treatment level ranging from 0 to 2.3 g/m^3 and with the following experimental conditions: pH = 7.3, 16.9 < T(°C) > 17.7, 0.143 < UV254 (cm^{-1}) < 0.184 and AT = 4.4 meq/L.
Statistical design	2 to 6 measurements
Relevance	
Environmental relevance	Environmental parameter are measured but not reported.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications.

Bozkaya-Schrotter et al. (2009)

Title: Treatment of nanofiltration membrane concentrates: Organic micropollutant and nom removal
Author: B. Bozkaya-Schrotter, C. Daines, A. Brunel, J.-C. Schrotter, P. Breant
Reference: Desalination and Water Treatment 9, 36–42
Year: 2009
Results and conclusion: This study aims to achieve complete elimination of pesticides and the elimination of 60%

of the natural organic matter (NOM) retained during nanofiltration step. The investigation included testing conventional water treatment techniques – adsorption, coagulation, ozonation – and the combination of ozonation and adsorption processes. Eight pesticides detected most commonly in French surface waters were selected as model micropollutants: atrazine, sulcotrione, bentazone, isoproturon, diuron, glyphosate, amitrole and acetochlore. Simultaneous combination of ozonation and powdered activated carbon (PAC) adsorption proved to be an efficient method for the elimination of the polar and ozone resistant pesticides at low carbon and ozone concentrations. This combination also achieved faster NOM removal than PAC adsorption only. It was observed that even with the use of high PAC concentrations, addition of low ozone dosages were necessary to degrade highly polar pesticides together with the NOM. No significant modification of the carbon activity and surface properties was observed at low ozone concentration levels, ca. 3 mg/L.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Low weight; additional information	
Reliability	Medium
Endpoint	Removal %- ; elimination %- figures; no absolute data
Protocol	Standard, Quantitative analysis of pesticides was carried out by a laboratory specialised in environmental analyses
Test compound	Atrazine, sulcotrione, bentazone, diuron, glyphosate, acetochlore, isoproturon
Test system and conditions	1) Adsorption of pesticides from the concentrate: Selected amount of concentrate sample and the adsorbent are placed in a funnel and agitated for contact times between 2 and 30 min. Once the selected contact time is elapsed sample is filtered and analysed; 2) Oxidation by ozone and combination of PAC and O_3: Concentrate sample is placed into a 1 L glass funnel and ozone is introduced in the system. Ozone dosage was varied between 3 and 30 mg/L of concentrate. Funnel is then agitated, concentrate is collected and analyzed. Oxidation by ozone is combined with adsorption process by adding PAC in the system prior to injection of ozone. The amount of PAC varied between 30 and 3000 mg/L.
Statistical design	No data
Relevance	
Environmental relevance	Given; parameter influencing endpoints are measured and partly reported.
Weight of evidence	
“Positive”/”Negative” evidence	No negative evidence.

Brosillon et al. (2006)

Title: Chlorination kinetics of glyphosate and its by-products: Modeling approach
Author: Stephan Brosillon, Dominique Wolbert, Marguerite Lemasle, Pascal Roche, Akbar Mehrsheikh
Reference: WATER RESEARCH 40, 2113 – 2124
Year: 2006
Results and conclusion:

Chlorination reactions of glyphosate, glycine, and sodium cyanate were conducted in well-agitated reactors to generate experimental kinetic measurements for the simulation of chlorination kinetics under the conditions of industrial water purification plants. The contribution of different by-products to the overall degradation of glyphosate during chlorination has been identified. The kinetic rate constants for the chlorination of glyphosate and its main degradation products were either obtained by calculation according to experimental data or taken from published literature. The fit of the kinetic constants with experimental data allowed us to predict consistently the concentration of the majority of the transitory and terminal chlorination products identified in the course of the glyphosate chlorination process. The simulation results conducted at varying aqueous chlorine/glyphosate molar ratios have shown that glyphosate is expected to degrade in fraction of a second under industrial aqueous chlorination conditions. Glyphosate chlorination products are not stable under the conditions of drinking water chlorination and are degraded to small molecules common to the degradation of amino acids and other naturally occurring substances in raw water. The kinetic studies of the chlorination reaction of glyphosate, together with calculations based on kinetic modeling in conditions close to those at real water treatment plants, confirm the reaction mechanism that we have previously suggested for glyphosate chlorination.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Medium weight, additional information	
Reliability	High
Endpoint	Dissipation of glyphosate and the formation of its chlorination products versus time; Chlorination kinetic model
Protocol	Non-GLP
Test compound	Non-labelled glyphosate (96%), (N-phosphonomethyl)glycine and Glycine (99%), 9-Fluorenylmethyl chloroformate (FMOC) (97%)
Test system and conditions	Kinetics experiments: Model solutions of glyphosate (104 M), and glycine (104M), in water buffered at pH 7 or 8 with a borate solution (0.2 M) were placed in a 100mL well-agitated reactor. The samples were chlorinated at HOCl/substrate molar ratios of approximately 1, 4, 20, 50 , 100, and 200 using a 1M solution of NaOCl in water; incubated at room temperature in the dark for 24h. For the kinetic measurements, 104M solutions of glyphosate, glycine, or sodium cyanate were chlorinated in a 1000mL well-agitated reactor using HOCl/substrate molar ratios of approximately 4 and 50; portions of reaction mixture were analyzed at scheduled times.
Statistical design	Chlorination kinetics were simulated, kinetic rate constants for the reaction sequence were either obtained by calculation according to the experimental data or taken from published literature.
Relevance	
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	Positive evidence; results supported by other publications.

Garcia et al. (2013)

Title: The application of microfiltration-reverse osmosis/nanofiltration to trace organics removal for municipal wastewater reuse	
Author: N. Garcia, J. Moreno, E. Cartmell, I. Rodriguez-Roda and S. Judd	
Reference: Environmental Technology, 2013, Vol. 34, No. 24, 3183–3189, http://dx.doi.org/10.1080/09593330.2013.808244	
Year: 2013	
Results and conclusion: The fate of organic micropollutans (MPs) in a membrane system based on microfiltration (MF) and reverse osmosis/nanofiltration (RO/NF) has been investigated for the case of wastewater reuse. Both an operating full-scale water reuse plant and a pilot plant were employed, with 22 individual organic compounds at their ambient concentrations studied for the former and the latter employing two target compounds over a range of feed concentrations. Results revealed removal efficiencies higher than 75% for most compounds in the full-scale plant, though mass flow studies on all streams revealed a significant imbalance of material for some compounds. Rejection efficiencies measured for candidate commercial NF and RO membranes tested at pilot scale challenged with a pharmaceutically active compound (ibuprofen, IBU) and an endocrine disrupting chemical (nonylphenol, NP) exceeded 99%. Permeate concentrations were 0.005–0.14µg/L for IBU and below the limit of detection for NP. A mass balance of the MPs for the full-scale plant across the MF and RO stages revealed a significant imbalance associated with the challenge of accurate determination of low concentrations. Differences in pilot plant and full-scale data were otherwise attributed to the impact of membrane ageing (and specifically hydrolysis) on RO rejection of the MPs examined.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Fate of organic micropollutans (MPs) in a membrane system based on microfiltration (MF) and reverse osmosis/nanofiltration (RO/NF)
Protocol	Non-GLP
Test compound	EDTA, NP, estrone (E1), 17β- oestradiol (E2), 17α-ethynylestradiol (EE2), tributyltin, naphthalene (NAPHT), IBU, ofloxacin (OFLX), oxytetracyc (OXTCY), erythromycin, propranolol, fluoxetine , triclosan, diclofenac (DFC), 2244-tetrabromodiphenyl ether (BDPE47), 22445-pentabromodiphenyl ether (BDPE99), 22446-pentabromodiphenyl ether (BDPE100), 224455-hexabromodiphenyl ether (BDPE153), bis-(2-ethylhexyl) phthalate (DEHP), glyphosate (GLYPH) and mecoprop (MCPP)
Test system and conditions	A full-scale 1200m^3/d capacity UK-based MF-RO plant was used. It is fed with secondary-treated municipal wastewater from the neighbouring wastewater treatment works, and generates desalinated water for industrial reuse. The plant comprises a 150µmscreen for protecting the MF. The hollow fibre (HF) MFoperates with regular backflushing and cleaning in place (CIP) with hypochlorite, acid and alkali for maintenance of permeability. TheMFfiltrate is held in an intermediate storage tank prior

	to treatment by the 2:1 array-configured RO process. Scaling in the RO is ameliorated by upstream dosing with antiscalant and acid. The plant operates at mean recoveries of 86% at the MF stage and 73% for the RO. Performance data for specific commercial RO membrane modules were obtained from an RO pilot plant installed at the Castell-Platja d'Aro WWTP (Catalonia, Spain). The 4.3m^3/d plant treats municipal wastewater, with a MBR fitted upstream to protect the RO. The MBR is assumed to provide biotreated and microfiltered municipal wastewater in a manner analogous to the full-scale reuse plant where classical activated sludge treatment precedes the MF stage. The RO process comprised a pressure vessel housing a single element, fed from an intermediate 200 L holding tank and protected by a cartridge filter. The rig permitted either discharge or recycling of the concentrate, the latter providing increased feedwater or retentate concentrations as encountered through RO staging. Three standard 40–40 in −4 in (or 100 mm) in diameter and 40 in (or 1m) long – commercial membrane modules of differing rejection properties were employed for the study: two RO membranes (HR and LE) and one nanofiltration (NF) membrane (NF270), all provided by the Dow Chemical Company. The membranes were selected so as to provide a range of selectivity. Prior to each experiment, each membrane was conditioned using permeates from the MBR permeate tank for 16–20 h at a pressure of 4 bar for the NF membrane and 9 bar for the two RO membranes. The holding tank was then spiked with 10µg/L of the target compounds of IBU and NP as 50mL aliquots from a 40mg/L standard solution. Trials were undertaken by incrementally increasing the feedwater concentration by passing 50% of the feedwater through the ROprocess and returning the concentrate to the holding tank. This process was repeated seven times for each of the three membranes tested, providing a range of concentration factor values between unity and 4.5. This enabled the overall feedwater to be increased in accordance with retentate concentration across a full-scale RO array. Permeate recoveries of 12–15% were maintained throughout. MP removals at the full-scale installation were determined through sampling of the various streams, and specifically the feed and backwash/reject streams of both the MF and RO processes. Grab samples from this site were taken 2–3 times daily over a three-day period, and contaminants assayed by Anglian Water Laboratories (Huntingdon, UK) according to standards methods based on GC-ICP-MS. 22 MP 'priority' compounds were assayed (see "Test compound").
Statistical design	-
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative"	No negative evidence.

evidence	

Gardner et al. (2013)

Title: Performance of UK wastewater treatment works with respect to trace contaminants	
Author: M. Gardner, V. Jones, S. Comber, M. D. Scrimshaw, T. Coello-Garcia, E. Cartmell, J. Lester, B. Ellor	
Reference: Science of the Total Environment 456-457 (2013) 359–369	
Year: 2013	
Results and conclusion: The study examined the performance of 16 wastewater treatment works to provide an overview of trace substance removal in relation to meeting the objectives of the Water Framework Directive (WFD). Collection and analysis of over 2400 samples including sewage influent, process samples at different stages in the treatment process and final effluent has provided data on the performance of current wastewater treatment processes and made it possible to evaluate the need for improved effluent quality. Results for 55 substances, including metals, industrial chemicals and pharmaceuticals are reported. Data for sanitary parameters are also provided. A wide range of removal efficiencies was observed. Removal was not clearly related to the generic process type, indicating that other operational factors tend to be important. Nonetheless, removals for many substances of current concern were high. Despite this, current proposals for stringent water quality standards mean that further improvements in effluent quality are likely to be required. In detail: The water-soluble regulated and emerging chemicals, such as EDTA, glyphosate and mecoprop, exhibited poor fractional removal (0.3–0.45). The more hydrophobic chemicals such as PAHs and flame retardants were more effectively removed than soluble chemicals such as EDTA, mecoprop, E1 and glyphosate.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Removal efficiencies for 55 substances, including metals, industrial chemicals and pharmaceuticals during waste water treatment
Protocol	Non-GLP
Test compound	55 substances, including metals, industrial chemicals and pharmaceuticals
Test system and conditions	The 16 WwTWs selected represent a cross-section of works types currently in operation in the UK and include AS, TF, membrane bioreactor (MBR) and oxidation ditches (OD) works. These works were a subset of the larger set of 160 works that had been selected for the previous effluent quality study (Gardner et al., 2012) as representative of UK WwTWs. Influent to the works was combined sewage, hence including ‘black’ and ‘grey’ water. The population equivalents for these works ranged from 3424–205, 935 which was representative of the size profile of works present in the UK serving over 70% of the national population (Gardner et al., 2012). The total number of samples taken at any given WwTWs was approximately 150. Therefore, over the 16 WwTWs, approximately

	2400 samples were taken, involving over 150,000 determinations. Spot samples were collected throughout the works to include influent, settled sewage, final effluent and sludge. Where tertiary treatment was present, an additional sample was taken post the secondary stage (secondary sewage). All works were sampled on a monthly basis for a one-year period, throughout 2010/2011. To assess within-day variability, a minimum of two samples were taken from each site over a 12-hour period (08.00 h–20.00 h). Hence, although the exact number of samples differed between works, samples were collected at each sampling point within each plant on approximately 12 occasions, with a minimum of two samples being collected on each sampling occasion. Additionally, at least one sludge process sample was taken on each visit; sludge sampling varied depending on the types of process employed at each works and accessibility issues. Data processing involved taking the average concentration for each sampling day. Mean, median and percentiles were calculated from the daily average values for each WwTWs and determinand. Fractional removal data were calculated from overall median values at each process stage and across the whole works. Prior to principle components analysis (PCA), the dataset was reviewed in order to ensure suitability for assessment using this technique. Initially, compounds with a high proportion of 'less than' (not detected) values were excluded, and subsequently, bivariate correlation between chemicals was also checked (Sharma, 1996). To assess the correlation between variables, both Pearson and Spearman correlation factors were used since some of the variables were not normally distributed. One of the variables in each correlated pair with a correlation factor >0.9 (Field, 2009) was excluded in order to create a dataset without redundant variables. The number of variables (chemicals) included in the PCA analysis was then reduced, as the number of sites in relations to variables resulted in a non-positive definite correlation matrix (Field, 2009). At this point, the criterion for inclusion was based on compounds identified as of interest at a national scale in the UK (Gardner et al., 2012). The statistical analyses were performed with PASW Statistics 18 (free from SPSS) and Scout, 2008 (free from the US EPA).
Statistical design	See "Test system and conditions"
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Gasperi et al. (2010)

Title: Occurrence and removal of priority pollutants by lamella clarification and biofiltration
Author: Johnny Gasperi, Vincent Rocher, Soléne Gilbert, Sam Azimi and Ghassan Cheb-

bo	
Reference: Water Research 44 (2010) 3065 – 3076	
Year: 2010	
Results and conclusion: This study investigates the occurrence of all priority substances (n = 41) listed in the Water Framework Directive and additional substances (n = 47) in raw sewage, as well as the removal performance of lamella clarification and biofiltration techniques. Once the efficiency of both types of techniques has been assessed for typical wastewater parameters, the differences in each technique's ability to remove pollutants becomes obvious; nevertheless, pollutant removal in quantitative terms still depends on the physicochemical properties of the compounds used and operating conditions within the selected facility. For lamella clarification, the removal of organic chemicals was found to be primarily correlated with their sorption potential and, hence, strongly dependent upon log Kow of the compound under study. Compounds with a strong hydrophobic character (log Kow > 4.5) are removed to a significant extent (approx. 85%), while hydrophilic compounds (log Kow < 3.5) are poorly removed (<20%). For biofiltration, the removal of chemicals appears to be compound-dependent, although this outcome involves several mechanisms, namely: i) physical filtration of total suspended solids, ii) volatilisation, iii) sorption, and iv) biotransformation of substances. Even if the complex processes within a biofilter system do not yield an accurate prediction of pollutant removal, two groups of chemicals can still be clearly identified: i) hydrophobic or volatile compounds, for which moderate to high removal rates are observed (from 50% to over 80%); and ii) hydrophilic, non-volatile and refractory compounds for which a low removal rate would be expected (<20%).	
Proposed action: Not to be considered for listing in the summarizing table as raw data are insufficient.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as publication deals with specific targets, namely wastewater treatment at a certain place and time.	
Reliability	High
Endpoint	Glyphosate concentration in final effluent of wastewater treatment
Protocol	No standard protocol; for further details see under test system and conditions
Test compound	Glyphosate (CAS-no: 1071-83-6), AMPA (CAS-no: 1066-51-9), purity not given, monitoring
Test system and conditions	The occurence of 88 substances at 3 sampling points, corresponding to raw sewage (RS), decanted effluents (DE) and final effluents (FE), were analyzed. In 2008, three sampling campaigns were carried out (March, September and December). At each site, 24-h composite samples were collected using automatic refrigerated samplers (at 4°C).
Statistical design	Not reported
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Results are valid for that particular place and time; no negative evidence.

Ghanem et al. (2007)

Title: Concentrations and specific loads of glyphosate, diuron, atrazine, nonylphenol and metabolites thereof in French urban sewage sludge	
Author: Aline Ghanem, Philippe Bados, Arantza Rua Estaun, Luis Felippe de Alencastro, Salima Taibi, Jacques Einhorn, Christian Mougin	
Reference: Chemosphere Volume 69 Issue 9, 1368 - 1373	
Year: 2007	
Results and conclusion: Indirect soil pollution by heavy metals and organics may occur when sewage sludge is used as fertilizer. It is essential to define the nature and amounts of pollutants contained in sewage sludge in order to assess environmental risk. Here, we present results from a one-year monitoring of herbicides (glyphosate, diuron and atrazine) and their major degradates in sewage sludge sampled from three wastewater treatment plants and one composting unit in the vicinity of Versailles, France. The concentrations of these compounds were determined, as well as these of the surfactant nonylphenol. We demonstrated the presence of glyphosate and aminomethylphosphonic acid at the mg/kg (dry matter) level in all samples. Diuron was detected at the µg/kg (d.m.) level, whereas its degradate and triazine compounds were below the limits of quantification. Nonylphenol amounts were higher than the future European limit value of 50 mg/kg (d.m.).	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Medium weight, supporting information of monitoring of sewage sludge	
Reliability	High
Endpoint	Specific load Lsp (load of a specific chemical in the sewage sludge per inhabitant connected per year (mg/cap/y)), Concentration of herbicides and nonylphenol in centrifuged sludge samples
Protocol	Non-GLP
Test compound	Glyphosate (99.5%), diuron (99.0%), atrazine (99.0%), and their respective metabolites; (2-13C, 99%; 15N, 98%)-glyphosate, and (13C, 99%; 15N, 98%; Methylene-D2, 98%)-AMPA; Atrazine-D5 and diuron-D6 in acetone solutions at 100 µg ml^{-1}
Test system and conditions	The concentrations of glyphosate, diuron, atrazine, nonylphenol and their main metabolites have been monitored monthly from July 2004 to June 2005 in sludge samples obtained from the WWTPs (wastewater treatment plants).
Statistical design	The method for glyphosate and AMPA analysis showed mean recoveries of 70% (RSD < 9%) for glyphosate and 63% (RSD < 5%) for AMPA
Relevance	
Environmental relevance	Not reported
Weight of evidence	
“Positive”/”Negative” evidence	The results are supported by other publications.

Ghanem et al. (2006)

Title: Fate of herbicides and nonylphenol in soil–plant–water systems amended with con-

taminated sewage sludge	
Author: Aline Ghanem, Jacqueline Dubroca, Veronique Chaplain, Christian Mougin	
Reference: Environ Chem Lett (2006), DOI 10.1007/s10311-006-0034-5	
Year: 2006	
Results and conclusion: We studied the fate of sludge spiked with ^{14}C-labelled diuron, glyphosate and nonylphenol applied to the soil by the way of contaminated sewage sludge in the soil plant-water system. Here we show that the mineralization of the chemicals in mixture is reduced by 40-80% by comparison with a direct soil contamination. The persistence of the chemicals in soils is increased in the presence of sludge. We showed also that the chemicals present in the sludge are mobile and partly transferred to soil leachates and plant seedlings. These results allow postulating that these compounds may induce an ecotoxicological impact on the soil ecosystem.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information of fate of herbicides contained in sludge, after spreading onto agricultural soil	
Reliability	Medium
Endpoint	Only figures of release of $^{14}CO_2$ (time-dependent mineralization); mass balance: non extractable ^{14}C, ^{14}C extracted by NaOH, ^{14}C extracted by organic solvents, and $^{14}CO_2$; transfer of radioactive chemicals to soil leachates and higher plants in %
Protocol	Non-GLP
Test compound	High purity glyphosate, diuron and nonylphenol, phosphonomethyl-^{14}C glyphosate (2084 MBq $mmol^{-1}$) and ring-^{14}C-U-nonylphenol (1998 MBq $mmol^{-1}$), ring-^{14}C-U-diuron (898 MBq $mmol^{-1}$); silt loam; sludge have been collected in urban WWTPs
Test system and conditions	3 experimental conditions have been retained for incubations in model ecosystems: soil alone and soil amended by sludge at two ratios. 1) model ecosystem = control, 0.7 kg of soil were spiked with labelled glyphosate, diuron and nonylphenol (370 kBq each) and unlabelled chemicals to ensure final amounts of 163, 480 and 203 µg chemical per model ecosystem. 2) model ecosystem = 'worst case' of contamination, sludge (28.5 dry sludge corresponding to 30 T dw ha^{-1}) mixed with soil; spiked with the same amounts of chemicals as the soil alone. 3) model ecosystem = agronomic reality, the soil with 5.7 g dry sludge (equivalent to 6 T dw ha^{-1}) with final amounts of chemicals being 33, 96 and 40 µg per model ecosystem; concentrations of added glyphosate, diuron and nonylphenol were 6, 17 and 7 ppm (dry sludge); spiked samples were 'aged' for 3 days at 4◦C under nitrogen; stream (0.5 l min^{-1}) of wet air, $^{14}CO_2$ trapping in 1 N NaOH solutions; incubated for 91 days at 23°C under 16 h light and 8 h darkness. Leachates were collected after 45, 60 and 90 days of incubation by watering with 190 ml water (equivalent to 20 mm rainwater).Radish and wheat seedlings (sowing after 15 days of incubation), harvested after a further 15- and 45-days-period of growth, and dried.
Statistical design	No information

Relevance	
Environmental relevance	No information about parameters influencing endpoints.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Hanke et al. (2010)

Title: Relevance of urban glyphosate use for surface water quality	
Author: Irene Hanke, Irene Wittmer, Simone Bischofberger, Christian Stamm, Heinz Singer	
Reference: Chemosphere 81, 422–429	
Year: 2010	
Results and conclusion: Relative contributions of agricultural and urban uses to the glyphosate contamination of surface waters were studied in a small catchment (25 km²) in Switzerland. Monitoring in four sub-catchments with differing land use allowed comparing load and input dynamics from different sources. Agricultural as well as urban use was surveyed in all sub-catchments allowing for a detailed interpretation of the monitoring results. Water samples from the river system and from the urban drainage system (combined sewer overflow, storm sewer and outflow of wastewater treatment plant) were investigated. The concentrations at peak discharge during storm events were elevated throughout the year with maximum concentrations of 4.15 µg/L. Glyphosate concentrations mostly exceeded those of other commonly used herbicides such as atrazine or mecoprop. Fast runoff from hard surfaces led to a fast increase of the glyphosate concentration shortly after the beginning of rainfall not coinciding with the concentration peak normally observed from agricultural fields. The comparison of the agricultural application and the seasonal concentration and load pattern in the main creek from March to November revealed that the occurrence of glyphosate cannot be explained by agricultural use only. Extrapolations from agricultural loss rates and from concentrations found in the urban drainage system showed that more than half of the load during selected rain events originates from urban areas. The inputs from the effluent of the wastewater treatment plant, the overflow of the combined sewer system and of the separate sewer system summed up to 60% of the total load.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Low weight; only basic information	
Reliability	High
Endpoint	Lowest base flow concentration; total load; concentration
Protocol	Non-GLP
Test compound	Glyphosate, AMPA
Test system and conditions	Surface water and water from the urban drainage system were sampled by automatic devices at every sampling site except for the WWTP, where daily flow proportional composites were used. Samples were taken at high temporal resolution during 16 out of 35 rain events from March to November 2007.
Statistical design	Three aliquots every 5 min were collected during the first 6 h of an event, followed by a reduced sampling frequency of one composite

	sample per hour (four aliquots every 15 min); The relative standard deviations (RSDs) for the surface water sample were 12% for glyphosate and 14% for AMPA (N = 6). The RSDs of the WWTP samples were 5% for glyphosate and 13% for AMPA (N = 6). The recoveries were in the range of 80–121% for glyphosate and 90 to 118% for AMPA.
Relevance	
Environmental relevance	Given; parameter influencing endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	The results are supported by other publications.

Hedegaard and Albrechtsen (2014)

Title: Microbial pesticide removal in rapid sand filters for drinking water treatment – Potential and kinetics	
Author: M. J. Hedegaard, H.-J. Albrechtsen	
Reference: Water Research 48 (2014) 71-81	
Year: 2014	
Results and conclusion: Filter sand samples, taken from aerobic rapid sand filters used for treating groundwater at three Danish waterworks, were investigated for their pesticide removal potential and to assess the kinetics of the removal process. Microcosms were set up with filter sand, treated water, and the pesticides or metabolites mecoprop (MCPP), bentazone, glyphosate and p-nitrophenol were applied in initial concentrations of 0.03-2.4 mg/L. In all the investigated waterworks the concentration of pesticides in the water decreased – MCPP decreased to 42-85%, bentazone to 15-35%, glyphosate to 7-14% and p-nitrophenol 1-3% – from the initial concentration over a period of 6-13 days. Mineralisation of three out of four investigated pesticides was observed at Sjælsø waterworks Plant II – up to 43% of the initial glyphosate was mineralised within six days. At Sjælsø waterworks Plant II the removal kinetics of bentazone revealed that less than 30 min was needed to remove 50% of the bentazone at all the tested initial concentrations (0.1-2.4 mg/L). Increased oxygen availability led to greater and faster removal of bentazone in the microcosms. After 1 h, bentazone removal (an initial bentazone concentration of 0.1 mg/L) increased from 0.21%/g filter sand to 0.75%/g filter sand, when oxygen availability was increased from 0.28 mg O_2/g filter sand to 1.09 mg O2/g filter sand. Bentazone was initially cleaved in the removal process. A metabolite, which contained the carbonyl group, was removed rapidly from the water phase and slowly mineralised after 24 h, while a metabolite which contained the benzene-ring was still present in the water phase. However, the microbial removal of this metabolite was initiated over seven days.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Medium, additional information	
Reliability	
Endpoint	Removal of mecoprop, bentazone, glyphosate and p-nitrophenol in rapid sand filters
Protocol	Non-GLP
Test compound	^{14}C-labelled pesticides (mecoprop, bentazone, glyphosate and p-

	nitrophenol)
Test system and conditions	Three different experimental laboratory set-ups were used: Degradation potential of filter sand: Filter sand from three Danish waterworks – Islevbro, Sjælsø Plant I and Sjælsø Plant II – was investigated for the removal potential of the pesticides mecoprop (MCPP), bentazone, glyphosate, and the degradation product p-nitrophenol. Removal kinetics: Bentazone removal at different initial concentrations was investigated with filter sand from Sjælsø waterworks Plant II. The removal was investigated intensively over 1 h, which is the residence time of the water in the rapid sand filter, and the experiment lasted for seven days to investigate for mineralisation. Effect of oxygen: Bentazone removal in the filter sand from Sjælsø waterworks Plant II was investigated under enhanced oxygen concentrations. The removal was investigated intensively in the initial phase of the experiment (the first few hours), and the experiment lasted for two days.
Statistical design	Not given in the paper
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Jönsson et al. (2013)

Title: Removal and degradation of glyphosate in water treatment: a review
Author: J. Jönsson, R. Camm and T. Hall
Reference: Journal of Water Supply: Research and Technology – AQUA | 62.7 | 2013
Year: 2013
Results and conclusion: Glyphosate is a broad spectrum, non-selective herbicide, widely used for the post-emergence control of annual and perennial weeds in a variety of applications. Although of low toxicity, its presence in drinking water is undesirable and can cause drinking water compliance failure in the EU if found at concentrations > 0.1 µg L^{-1}. Treatment methods such as ozonation and activated carbon are currently used for pesticide degradation and removal. This article provides a review of the reported efficiency in removal and degradation of glyphosate and aminomethylphosphonic acid (AMPA) by some commonly employed treatment options. Additional experiments have been carried out where knowledge gaps have been identified. Oxidants used in water treatment, particularly Cl_2 and O_3, are highly effective in degrading glyphosate and AMPA. Removal by coagulation and activated carbon is ineffective as a barrier against contamination in drinking water. UV treatment is also ineffective for glyphosate and AMPA degradation but the combination of UV/H_2O_2 provided significant degradation of glyphosate, but not AMPA, under the conditions investigated. UV/TiO_2 treatment can degrade significant amounts of glyphosate but the irradiation time needed is long. Removal or degradation by bank filtration, slow sand filtration, ClO_2 and membranes is variable but can provide significant removal under the right conditions. Summary of removal efficiencies of glyphosate and AMPA:

Treatment process	Glyphosate removal [%]	AMPA removal [%]

Bank and dune filtration	20-50	25-95
Aluminium coagulant and clarification	15-40	20-25
Iron coagulant and clarification	40-70	20-85
Chlorination	24->99	40->95
Chlorine dioxide	17-93	>99
Ozonation	60->99	25-95
Activated carbon adsorption	10-90	20-70
Membrane filtration	>90 (NF/RO), >50 (UF)	>95 (NF/RO)

Proposed action:
To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.

Type of information (critical, high/low weight, supporting, additional):
Medium, additional information

Reliability	
Endpoint	Removal and degradation of glyphosate and AMPA in water treatment
Protocol	Not given
Test compound	Glyphosate and AMPA
Test system and conditions	Batch tests were carried out to investigate the degradation of glyphosate and AMPA by oxidation using Cl_2, ClO_2, O_3, O_3/H_2O_2, and by adsorption using PAC (powdered activated carbon). Furthermore, a review of water treatment removal and degradation by bank filtration, chemical coagulation, clarification/filtration, slow sand filtration, chlorination, degradation of glyphosate by chlorine dioxide, ozone, UV, AOPs, activated carbon, pressure driven membrane process and air stripping was performed.
Statistical design	Not given
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	The results are supported by other publications.

Mehrsheikh et al. (2006)

Title: Investigation of the mechanism of chlorination of glyphosate and glycine in water

Author: Akbar Mehrsheikh, Marian Bleeke, Stephan Brosillon, Alain Laplanche, Pascal Roche

Reference: WATER RESEARCH 40, 3003 – 3014

Year: 2006

Results and conclusion:
The chlorination reactions of glyphosate and glycine in water were thoroughly studied. Utilizing isotopically enriched (^{13}C and ^{15}N) samples of glycine and glyphosate and 1H, ^{13}C, ^{31}P, and ^{15}N NMR spectroscopy we were able to identify all significant terminal chlo-

rination products of glycine and glyphosate, and show that glyphosate degradation closely parallels that of glycine. We have determined that the C1 carboxylic acid carbon of glycine/glyphosate is quantitatively converted to CO_2 upon chlorination. The C2 methylene carbon of glycine/glyphosate is converted to CO_2 and methanediol. The relative abundance of these two products is a function of the pH of the chlorination reactions. Under near neutral to basic reaction conditions (pH 6–9), CO_2 is the predominant product, whereas, under acidic reaction conditions (pH<6) the formation of methanediol is favoured. The C3 phosphonomethylene carbon of glyphosate is quantitatively converted to methanediol under all conditions tested. The nitrogen atom of glycine/glyphosate is transformed into nitrogen gas and nitrate, and the phosphorus moiety of glyphosate produces phosphoric acid upon chlorination. In addition to these terminal chlorination products, a number of labile intermediates were also identified including N-chloromethanimine, N-chloroaminomethanol, and cyanogen chloride. The chlorination products identified in this study are not unique to glyphosate and are similar to those expected from chlorination of amino acids, proteins, peptides, and many other natural organic matters present in drinking water.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Medium weight, additional information about the mechanism of chlorination of glyphosate	
Reliability	High
Endpoint	Proposed mechanism of glycine chlorination and of glyphosate chlorination
Protocol	Non-GLP
Test compound	Isotopically labelled glyphosate and glycine
Test system and conditions	Isotopically enriched (^{13}C and ^{15}N) glyphosate and glycine were utilized to investigate the various chlorination products formed from these compounds using ^{13}C, ^{15}N, ^{31}P, and 1H NMR spectroscopy. Chlorination was conducted in un-buffered D_2O at initial pHs of 8, 7, and 5. Additionally, the chlorination reactions were carried out in a 0.48M borate buffer in D_2O at pH 8 and 9. Chlorination products of glycine and glyphosate were monitored by HPLC using the corresponding ^{14}C-labeled test materials in unbuffered water at initial pHs of 9, 8, 7, 6, and 5 with aqueous chlorine at chlorine to substrate molar ratio of 100:1. Additionally, the chlorination reactions were carried out in a 0.05M borate buffer at pH 8 and 9 or a 0.05M phosphate buffer at pH 7, 6, and 5 in separate experiments.
Statistical design	No data
Relevance	
Environmental relevance	Supplementary data associated with this article can be found in the online version at doi:10.1016/j.watres.2006.06.027.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Nowack (2002)

Title: Aminopolyphosphonate removal during wastewater treatment	
Author: Bernd Nowack	

Reference: Water Research 36, 4636–4642	
Year: 2002	
Results and conclusion: Phosphonates are not biodegraded during wastewater treatment but are removed by adsorption processes. Field measurements from different wastewater treatment plants affirm that they are removed almost completely during wastewater treatment. Adsorption of nitrilotrismethylenephosphonic acid onto activated sludge, amorphous iron oxide and humic acids (HAs) was studied under controlled conditions. The adsorption onto HAs decreases sharply with increasing pH with negligible adsorption at pH above 6.5. Adsorption onto amorphous iron oxide follows a Langmuir behaviour. The presence of 1mM Ca doubles the maximum surface capacity at pH 7. Adsorption onto activated sludge is not very pH sensitive and is explained to a large extent by adsorption onto amorphous iron oxides, but the contribution of organic matter or other mineral phases cannot be ruled out.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Low weight; experiments were not done with glyphosate	
Reliability	Medium
Endpoint	Not applicable
Protocol	Not applicable
Test compound	The phosphonates, nitrilotrismethylenephosphonic acid (NTMP), ethylenediaminetetramethylenephosphonic acid (EDTMP) and diethylenetriaminepentamethylenephosphonic acid (DTPMP)
Test system and conditions	1) Samples were taken from the WWTP of Weil, Germany. This WWTP receives wastewater from several textile factories that use phosphonates in their dyeing and bleaching processes and operates with chemical phosphate precipitation/flocculation. 24 hours flow proportional samples from the influent and effluent were taken; 2) A field experiment was carried out in the WWTP Ikast, Denmark. DTPMP was added at a rate of 7.6 kg/d to the wastewater stream from textile industry (1000-3000m³/d), which is treated separately from the municipal wastewater. Daily water samples were taken by the personnel of the WWTP; 3) Adsorption experiments with hydrous ferric oxide: Increasing concentrations of NTMP were added to samples of the HFO-suspension, stirred for 2 h; filtered; analyzed for dissolved NTMP.; 4) Adsorption experiments with HA-SiO_2: addition of NTMP or metal-NTMP complexes; adsorption isotherms, pH was adjusted to 3.6, 4.8 and 6.5 and NTMP was added at concentrations between 0.1 and 15 mM
Statistical design	Not applicable
Relevance	
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	No negative evidence

Olofsson et al. (2013)

Title: Comprehensive mass flow analysis of Swedish sludge contaminants	
Author: U. Olofsson, E. Brorström-Lundén, H. Kylin, P. Haglund	
Reference: Chemosphere 90 (2013) 28–35	
Year: 2013	
Results and conclusion: A screening of metals, persistent organic pollutants, pharmaceuticals and personal care products (PPCPs), and other organic contaminants in sludge from seven Swedish sewage treatment plants (STPs) was performed in this study. This extensive screening provides information on mass flows of 282 compounds used in the Swedish society to sewage sludge. It reveals constant relative contaminant concentrations (ng mg kg^{-1} d.w.), except for some pesticides and perfluorinated compounds, indicating that these originate from broad usage and diffuse dispersion rather than (industrial) point sources. There was a five order of magnitude difference in the sum concentrations of the most and least abundant species (metals and polychlorinated dibenzo-p-dioxins and -furans, respectively). Lower total concentrations were found in sludge from STPs processing primarily food industry or household sewage. Proportions of the amounts used (in Sweden) found in sludge were lower for compounds that are present in consumer goods or are diffusely dispersed into the environment (0.01-1% recovered in sludge) than for compounds used as detergents or PPCPs (17–63%). In some cases, the recovery seemed to be affected by evaporation (e.g. octamethylcyclotetrasiloxane) or biotransformation (e.g. adipates) losses, while polychlorinated alkanes and brominated diphenyl ethers were recovered to disproportionately high degree (ca. 4%); likely due to incomplete statistics for imported goods. Concentration of glyphosate in sewage sludge: 0.6 µg g^{-1}, n.d., <2 µg g^{-1}, 0.1 µg g^{-1}, <2 µg g^{-1}, n.q., 0.7 µg g^{-1}	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Concentration metals, persistent organic pollutants, pharmaceuticals and personal care products (PPCPs), and other organic contaminants in sludge
Protocol	The analyses were performed by several qualified laboratories, each following strict quality guidelines. Generally, internal standard quantification was used to compensate for losses during clean-up and analysis. For non-accredited analyses the extraction efficiencies were checked (e.g. using re-extraction) and found to be sufficient (better than 95%).
Test compound	Metals, persistent organic pollutants, pharmaceuticals and personal care products (PPCPs), and other organic contaminants
Test system and conditions	A screening of metals, persistent organic pollutants, pharmaceuticals and personal care products (PPCPs), and other organic contaminants in sludge from seven Swedish sewage treatment plants (STPs) was performed.
Statistical design	Not given
Relevance	

Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Saitúa et al. (2012)

Title: Drinking water obtaining by nanofiltration from waters contaminated with glyphosate formulations: Process evaluation by means of toxicity tests and studies on operating parameters	
Author: Hugo Saitúa, Fernando Giannini and Antonio Perez Padilla	
Reference: Journal of Hazardous Materials 227– 228 (2012) 204– 210	
Year: 2012	
Results and conclusion: Glyphosate formulations toxicity depends on all its components but commercial products only specify the active principle in their label. To treat contaminated waters and to verify if the unknown components which add toxicity have been removed represent a challenge. Nanofiltration and permeate analysis by toxicity tests with fish are an interesting alternative to evaluate the process. Permeates of solutions with concentrations five times above the lethal doses (48 mg/l) did not present toxicity, pointing that all toxic compounds were removed at the same time. Glyphosate rejection over an 80% despite its molecular weight is lower than membrane MWCO, this could be associated to a predominant Donnan exclusion mechanism, combined with dielectric exclusion due to the solute high charge density. Glyphosate concentration did not show any effect over rejection. It increased when pressure was incremented from 2.5 to 4 bar and then remained constant in a 4–10 bar range. Because of dissociation of the glyphosate and the surface charged of the membrane depend on pH value, the rejection increase from 72.5 to 92.5% when pH increase from 4 to 8.5. Studies with river water showed the same behavior with a slight decrease in rejection.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information	
Reliability	Medium
Endpoint	No fate related endpoint
Protocol	Non GLP
Test compound	glyphosate acid (CAS 1071-83-6) and glyphosate isopropylamine (IPA, CAS 38641-94-0)
Test system and conditions	This work consisted in treatment of synthetic and natural waters contaminated with glyphosate commercial formulations, using a NF pilot plant. Process efficiency was evaluated analyzing the permeate by acute toxicity tests with fish. It was studied feed concentration, pressure, pH and ionic strength influence on glyphosate rejection in synthetic water and also in river water. The latter aspect was further studied by considering the changes in the relevant surface water characteristics, such as the pH, the concentration of dissolved organic compounds and the conductivity.
Statistical design	No information
Relevance	

Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Schoonenberg et al. (2010)

Title: Reverse osmosis followed by activated carbon filtration for efficient removal of organic micropollutants from river bank filtrate	
Author: F. Schoonenberg, Kegel, B. M. Rietman and A. R. D. Verliefde	
Reference: Water Science & Technology – WST | 61.10 |	
Year: 2010	
Results and conclusion: The aim of this research was to assess the robustness of a drinking water treatment plant equipped with reverse osmosis and subsequent activated carbon filtration for the removal of these pollutants. The total removal efficiency of 47 organic micropollutants was investigated. Results indicated that removal of most organic micropollutants was high for all membranes tested. Some selected micropollutants were less efficiently removed (e.g. the small and polar NDMA and glyphosate, and the more hydrophobic ethylbenzene and napthalene). Very high removal efficiencies for almost all organic micropollutants by the subsequent activated carbon, fed with the permeate stream of the RO element were observed except for the very small and polar NDMA and 1,4-dioxane. RO and subsequent activated carbon filtration are complementary and their combined application results in the removal of a large part of these emerging organic micropollutants. Based on these experiments it can be concluded that the robustness of a proposed treatment scheme for the drinking water treatment plant Engelse Werk is sufficiently guaranteed.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information	
Reliability	Medium
Endpoint	The results for solute removal by RO were compared to calculations obtained using a predictive model, based on solute structure (QSAR (quantitative structure-activity relationship))
Protocol	Non GLP
Test compound	NDMA, 1,4-dioxane, NMOR, Diglyme, Glyphosate, Triglyme, Caffeine, TBA, MTBE, Phenazon, Metamitron, Terbutaline, Sulfamethoxazol, Sotalol, Pentoxifylline, ETBE, TAME, 2,4-dinitrophenol, Carbendazim, Monuron, Metribuzin, Metoxuron, Pirimicarb, Bisphenol-S, Metoprolol, TCEP, Benzene, Isoproturon, Chlorotoluron, Atrazine, Diethylphthalate, Diuron, Carbamazepine, Bentazon, Metobromuron, Dimethenamid, Ethylbenzene, Naphthalene, 2-MIB, Ibuprofen, Mecoprop (MCPP), Bisphenol-A, Linuron, Estrone, Dibutylphthalate, Diclofenac, Bezafibrate
Test system and conditions	1) the total removal efficiency of a wide selection of organic micropollutants by the combination RO-ACF was investigated. Rejection experiments were carried out on 4 different commercial 4-inch spiral wound reverse osmosis membranes. 2) the same selec-

	tion of organic micropollutants was also spiked in the feed of an activated carbon column, which was fed with the permeate of the best performing membrane.
Statistical design	No information
Relevance	
Environmental relevance	Information on the analytical protocol can be found in Sacher et al. (2001).
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Song et al. (2013)

Title: Composite hollow fiber nanofiltration membranes for recovery of glyphosate from saline wastewater	
Author: J. Song, X.-M. Li, A. Figoli, H. Huang, C. Pan, T. He, B. Jiang	
Reference: Water Research 47 (2013) 2065-2074	
Year: 2013	
Results and conclusion: A high performance versatile composite hollow fiber nanofiltration (NF) membrane is reported for the separation of glyphosate from saline waste streams. Preparation of SPEEK based on an amorphous poly (ether ether ketone, PEEK) was investigated. The membrane was prepared by coating sulfonated polyether ether ketone (SPEEK) onto a polyethersulfone (PES) ultrafiltration (UF) hollow fiber membrane. The composite membrane was characterized by water permeability, scanning electron microscopy, and rejection toward sodium sulfate (Na_2SO_4), sodium chloride (NaCl), and calcium chloride ($CaCl_2$). About 90% rejection toward sulfate anions and only 10% rejection for calcium cations were obtained. A water permeability around 10-13 LMHBar and 90% rejection for polyethylene glycol (PEG) with a molecular weight of 4000-6000 Da were observed. In the separation of glyphosate from saline wastewater, the membrane rejected less than 20% of NaCl and higher than 90% of glyphosate at an operating pressure of 5 bars and pH = 11.0. An economic analysis indicated that the cost for recovery of glyphosate was comparably low to the value gained by an increase in the productivity. The results may lead to a new promising low energy solution for the environmental problem faced by the herbicide industry.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Recovery of glyphosate by a high performance versatile composite hollow fiber nanofiltration (NF) membrane
Protocol	Not given
Test compound	Glyphosate
Test system and conditions	A composite hollow fiber nanofiltration membrane was prepared by coating commercial ultrafiltration membrane with sulfonated amorphous PEEK for the separation of glyphosate from highly saline wastewater. Optimization of the membrane preparation parameters was carried out. Membranes with satisfactory properties were obtained by coating SPEEK of

	1.5 wt. % solution onto a commercial UF membrane. Glyphosate concentration was measured according to the Chinese Standard Regulation (GB12686-2004) by UV-Vis spectrophotometry.
Statistical design	Repeated NF tests
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Suhadolc et al. (2010)

Title: Single application of sewage sludge – Impact on the quality of an alluvial agricultural soil	
Author: Metka Suhadolc, Reiner Schroll, Alexandra Hagn, Ulrike Dörfler, Michael Schloter, Franc Lobnik	
Reference: Chemosphere in press; doi:10.1016/j.chemosphere.2010.08.024	
Year: 2010	
Results and conclusion: The effects of sewage sludge on soil quality with regard to its nutrient and heavy metal content, microbial community structure and ability to maintain specific soil function (degradation of herbicide glyphosate) were investigated in a three months study using an alluvial soil (Eutric Fluvisol). Dehydrated sewage sludge significantly increased soil organic matter (up to 20.6% of initial content), total and available forms of N (up to 33% and 220% of initial amount, respectively), as well as total and plant available forms of P (up to 11% and 170% of initial amount, respectively) and K (up to 70% and 47% of initial amount, respectively) in the upper 2 cm soil layer. The increase of organic matter was most prominent 3 d after the application of sewage sludge, after 3 months it was no longer significant. Contents of nutrients kept to be significantly higher in the sewage sludge treated soil till the end of experiment. Contents of some heavy metals (Zn, Cu, Pb) increased as well. The highest increase was found for Zn (up to 53% of initial amount), however it was strongly bound to soil particles and its total content was kept below the maximum permissible limit for agricultural soil. Based on molecular fingerprinting of bacterial 16S rRNA gene and fungal ITS fragment on 3rd day and 3rd month after sewage sludge amendment, significant short term effects on bacterial and fungal communities were shown due to the sewage sludge. The effects were more pronounced and more long-term for bacterial than fungal communities. The mineralization of ^{14}Cglyphosate in the sewage sludge soil was 55.6% higher than in the control which can be linked to (i) a higher glyphosate bioavailability in sewage sludge soil, which was triggered by the pre-sorption of phosphate originating from the sewage sludge and/or (ii) beneficial alterations of the sewage sludge to the physical–chemical characteristics of the soil.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Medium weight, additional information that sewage sludge amendment to soil has significantly increased the mineralization of ^{14}C-glyphosate	
Reliability	High
Endpoint	Total heavy metal content (mg/kg); daily degradation rates of ^{14}C-

	Glyphosate; amount of evolved $^{14}CO_2$; mineralization kinetics (a two component first-order kinetic model)
Protocol	Non-GLP
Test compound	^{14}C-glyphosate, ^{14}C-labelled on the phosphonomethyl group (purity >98.0%), mixed with the commercial available product ‘‘Round-up ready” (Monsanto, USA); Zn, Pb, Ni, Cr, Cu, Cd
Test system and conditions	Microcosm leaching experiment in columns: 1) Soil nutrient change by sewage sludge application, 2) Heavy metals contents, availability and mobility in soil, 3) Microbial community structure: Soil samples were taken from four depths at the 3rd day, the 3rd week and the 3rd month after sewage sludge application for further analysis. All treatments were performed in four replicates resulting in 24 columns for the whole experiment.; 4) Glyphosate mineralization: Biodegradation of ^{14}C-glyphosate was studied in a discontinuously aerated laboratory system; ^{14}C-glyphosate, ^{14}C-labelled on the phosphonomethyl group (purity >98.0%), mixed with the commercial available product ‘‘Round-up ready” (final specific radioactivity of 0.2 MBq mg-1) and mixed with soil to a final glyphosate concentration of 7.3 µg/g, which corresponds to the recommended field concentration of 1.1 kg/ha; in the dark at 20 ± 1°C; $^{14}CO_2$ from glyphosate mineralization was fixed in 0.1 M NaOH solution
Statistical design	4 replicates
Relevance	
Environmental relevance	Given; parameter influencing endpoints are measured and reported.
Weight of evidence	
“Positive”/”Negative” evidence	The results are supported by other studies. No negative evidence.

Yang et al. (2009)

Title: Real-time contaminant detection and classification in a drinking water pipe using conventional water quality sensors: Techniques and experimental results
Author: Y. Jeffrey Yang, Roy C. Haught, James A. Goodrich
Reference: Journal of Environmental Management 2009 Jun;90(8):2494-506
Year: 2009
Results and conclusion: Accurate detection and identification of natural or intentional contamination events in a drinking water pipe is critical to drinking water supply security and health risk management. To use conventional water quality sensors for the purpose, we have explored a real-time event adaptive detection, identification and warning (READiw) methodology and examined it using pilot-scale pipe flow experiments of 11 chemical and biological contaminants each at three concentration levels. The tested contaminants include pesticide and herbicides (aldicarb, glyphosate and dicamba), alkaloids (nicotine and colchicine), E. coli in terrific broth, biological growth media (nutrient broth, terrific broth, tryptic soy broth), and inorganic chemical compounds (mercuric chloride and potassium ferricyanide). First, through adaptive transformation of the sensor outputs, contaminant signals were enhanced and background noise was reduced in time-series plots leading to detection and identification of all simulated contamination events. The improved sensor detection threshold was 0.1% of the background for pH and oxidation–reduction potential (ORP), 0.9% for free

chlorine, 1.6% for total chlorine, and 0.9% for chloride. Second, the relative changes calculated from adaptively transformed residual chlorine measurements were quantitatively related to contaminant-chlorine reactivity in drinking water. We have shown that based on these kinetic and chemical differences, the tested contaminants were distinguishable in forensic discrimination diagrams made of adaptively transformed sensor measurements.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Low weight	
Reliability	
Endpoint	Concentrations, kinetics
Protocol	No standard protocol followed, none GLP-study
Test compound	Glyphosate (Roundup solution (Monsanto Corp., St. Louis) contained 18% glyphosate), CAS-no: 1071-83-6
Test system and conditions	Pilot-scale pipe flow experiment
Statistical design	Not given in the publication
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Comparable studies are not known. Publication is plausible, and thus no negative evidence occurs.

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B.8.11.6 Predicted environmental concentrations in surface water and in groundwater (PEC_{SW}, PEC_{GW})

B.8.11.6.1 PEC in groundwater

Summary

~~One article (de Paz and Rubio, 2006) was available on PEC_{GW} determination. It deals with the use of GIS-AF/RF system to compile herbicide pollution-risk maps for a citrus-growing area in eastern Spain. The article can be considered as additional information. No modification of the PEC_{GW} calculation is needed.~~

Two articles are available on PEC_{GW} determination. The articles can be considered as additional information, as the approaches cannot be compared to standard FOCUS modeling. No modification of the PEC_{GW} calculation is needed.

Detailed description of open literature – PEC in groundwater

de Paz and Rubio (2006)

Title Application of a GIS–AF/RF model to assess the risk of herbicide leaching in a citrus-growing area of the Valencia Community, Spain	
Author: José M. de Paz, José L. Rubio	
Reference: Science of the Total Environment (article in press)	
Year: 2006	
Results and conclusion: The GIS–AF/RF system developed in the present study was used to compile herbicide pollution-risk maps for a citrus-growing area in eastern Spain. The GIS capabilities of the system enable it to identify areas of potential risk in terms of herbicide leaching. A ranking of the potential leaching risk of herbicides from the highest risk (terbumeton) to the lowest risk (diquat) was done. Sandy soils such as Arenosols were identified as having the highest herbicide leaching risk. The obtained ranking of the leaching potential of analysed herbicides were as follows, from highest to lowest risk: terbumeton > bromacil > simazine > terbuthylazine > diuron > linuron > glyphosate > diquat.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	PEC_{GW}
Protocol	Modelling study
Test compound	No test compounds used in the study
Test system and conditions	A one-dimensional model was developed to estimate the potential leaching of pesticides through the soil profile. The RF index is a measure of the time taken by a pesticide to leach throughout the root zone compared to the time taken by a non-adsorbed tracer. It is less complex than the standard FOCUS leaching models.
Statistical design	No information
Relevance	
Environmental relevance	The relevance is rather small since the Spanish conditions are different from conditions in the central zone. Furthermore the model was rather simple.

Weight of evidence	
"Positive"/"Negative" evidence	The trend is in line with results of standard FOCUS leaching models.

Lindahl and Bockstaller (2012)

Title: An indicator of pesticide leaching risk to groundwater	
Author: Anna M.L. Lindahl, Christian Bockstaller	
Reference: Ecological Indicators 23 (2012) 95–108	
Year: 2012	
Results and conclusion: The objective of this study is (i) to develop a new groundwater sub indicator for an existing indicator, I-Phy (former Ipest), that explicitly takes preferential flow into account, and (ii) to test the possibility of developing an indicator by means of data-mining methods using simulations of a mechanistic model. The groundwater sub indicator developed is in the form of decision trees based on fuzzy inference systems. It was derived through neuro-adaptive learning on data sets from simulations running the process-based MACRO model. Unlike the previous version, the new indicator considers preferential flow, climatic differences and differences in soil texture with depth. Other benefits are less dependency on expert knowledge and the possibility to integrate a broad range of conditions.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Not applicable
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

References

de Paz J.M., Rubio, J.L. 2006. Application of a GIS–AF/RF model to assess the risk of herbicide leaching in a citrus-growing area of the Valencia Community, Spain. Science of the Total Environment (article in press)

Lindahl and Bockstaller 2012. An indicator of pesticide leaching risk to groundwater. Ecological Indicators 23 (2012) 95–108

B.8.11.6.2 PEC in surface water

Summary

Two articles are available on PEC_{SW} determination and the calculation of indicators for surface water contamination. The articles can be considered as additional information, as the approaches cannot be compared to standard FOCUS modeling. No modification of the PEC_{SW} calculation is needed.

Detailed description of open literature – PEC in surface water

Malaguerra et al. (2013)

Title: Assessment of the contamination of drinking water supply wells by pesticides from surface water resources using a finite element reactive transport model and global sensitivity analysis techniques	
Author: Flavio Malaguerra, Hans-Jørgen Albrechtsen, Philip John Binning	
Reference: Journal of Hydrology 476 (2013) 321–331	
Year: 2013	
Results and conclusion: A reactive transport model is employed to evaluate the potential for contamination of drinking water wells by surface water pollution. The model considers various geologic settings, includes sorption and degradation processes and is tested by comparison with data from a tracer experiment where fluorescein dye injected in a river is monitored at nearby drinking water wells. Three compounds were considered: an older pesticide MCPP (Mecoprop) which is mobile and relatively persistent, glyphosate (Roundup), a newer biodegradable and strongly sorbing pesticide, and its degradation product AMPA. Global sensitivity analysis using the Morris method is employed to identify the dominant model parameters. Results show that the characteristics of clay aquitards (degree of fracturing and thickness), pollutant properties and well depths are crucial factors when evaluating the risk of drinking water well contamination from surface water. This study suggests that it is unlikely that glyphosate and AMPA in streams can pose a threat to drinking water wells, while MCPP in surface water can represent a risk: MCPP concentration at the drinking water well can be up to 7% of surface water concentration in confined aquifers and up to 10% in unconfined aquifers. Thus, the presence of confining clay aquitards may not prevent contamination of drinking water wells by persistent compounds in surface water. Results are consistent with data on pesticide occurrence in Denmark where pesticides are found at higher concentrations at shallow depths and close to streams.	
Proposed action: Consider as additional information. The results of the modelling exercise are in line with the standard models.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Quantification of the amount of pesticides that can leach from a stream into drinking water during water abstraction in a primary aquifer
Protocol	Modelling study
Test compound	No test compounds used in the study
Test system and conditions	In order to study the link between surface water and a nearby drinking water well, a generic model of contaminant transport from surface water into groundwater is established. The model is

	designed to quantify the amount of pesticides that can leach from a stream into drinking water during water abstraction in a primary aquifer. The conceptual model is illustrated in Fig. 2. A pumping well is placed at a distance d (m) from a stream and pumps water at a constant pumping rate Q (m3/d) from a depth D (m). The geology is simplified to be a 3-layer system: a hyporheic layer separates the stream from an underlying sandy aquifer, below which a clay aquitard overlies a chalk aquifer; Ds, Dcl and Dch, respectively, are the thicknesses of the three layers, and Kcl is the hydraulic conductivity of the fractured clay till. The natural flow in the aquifer is driven by a regional groundwater gradient i (m/m) and to simplify the system, the hydraulic gradient is assumed to be the same in both aquifers. During pumping the well modifies the natural water flow, lowering the water head in the aquifer, so that surface water from the stream can seep into the groundwater and reach the pumping well. Pollutants in the stream may be retarded by sorption and degraded by microorganisms during their travel to the well. Both the sandy and chalk aquifer are considered to be strictly anaerobic, while the hyporheic zone can be aerobic.
Statistical design	Modelling study
Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	The results for glyphosate and AMPA confirm the risk assesmant according to standard approaches. No negative evidence.

Rousseau et al. (2012)

Title: A Hydrological Modeling Framework for Defining Achievable Performance Standards for Pesticides
Author: Alain N. Rousseau, Pierre Lafrance, Martin-Pierre Lavigne, Stéphane Savary, Brou Konan, Renaud Quilbé, Paul Jiapizian and Mohamed Amrani
Reference: J. Environ. Qual. 41:52–63 (2012)
Year: 2012
Results and conclusion: This paper proposes a hydrological modeling framework to define achievable performance standards (APSs) for pesticides that could be attained after implementation of recommended management actions, agricultural practices, and available technologies (i.e., beneficial management practices [BMPs]). An integrated hydrological modeling system, Gestion Intégrée des Bassins versants à l'aide d'un Système Informatisé, was used to quantify APSs for six Canadian watersheds for eight pesticides: atrazine, carbofuran, dicamba, glyphosate, MCPB, MCPA, metolachlor, and 2,4-D. Outputs from simulation runs to predict pesticide concentration under current conditions and in response to implementation of two types of benefi cial management practices (reduced pesticide application rate and 1- to 10-m-wide edge of- field and/or riparian buff er strips, implemented singly or in combination) showed that APS values for scenarios with BMPs were less than those for current conditions. Moreover, APS values at the outlet of watersheds were usually less than ecological thresholds of good condition, when available. Upstream river reaches were at greater risk of having concentrations above a given ecological thresholds because of limited stream fl ows and overland loads of pesticides. Our integrated approach of "hydrological modeling–APS

estimation–ecotoxicological significance" provides the most effective interpretation possible, for management and education purposes, of the potential biological impact of predicted pesticide concentrations in rivers.	
Proposed action: Consider as additional information.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Not applicable, surface water entries are modelled to analyze the impact of different management practices
Protocol	Modelling study
Test compound	No test compounds used in the study
Test system and conditions	Development of a hydrological modeling framework for defi ning nonregulatory, watershedscale, agro-environmental APSs for pesticides for six Canadian watersheds. Using an integrated modeling system consisting of hydrological, erosion, pesticide fi eld transport, and water quality models, APSs for a specifi c pesticide in a given stream reach were defi ned as a statistical value of the cumulative frequency curve of simulated in-stream concentrations during the period of interest (e.g., summer) and simulation interval (e.g., 30 yr). For each watershed, simulations were run to predict pesticide concentration under current concentrations ("reference" scenario) and in response to implementation of two types of BMPs, applied singly or in combination: (i) reduced rate of pesticide application and (ii) implementation of an edge-of-fi eld or riparian buff er strips (1–10 m wide, depending on the feasibility and the necessity for each watershed). Th e resulting APS for each pesticide and stream reach was then compared with ETs derived using dose–response curves or other approaches for determining aquatic life protection criteria.
Statistical design	Modelling study
Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

References

Malaguerra et al. 2013. Assessment of the contamination of drinking water supply wells by pesticides from surface water resources using a finite element reactive transport model and global sensitivity analysis techniques. Journal of Hydrology 476 (2013) 321–331

Rousseau et al. 2012. A Hydrological Modeling Framework for Defining Achievable Performance Standards for Pesticides. J. Environ. Qual. 41:52–63 (2012)

B.8.11.6.3 Monitoring data

Publications regarding off-site movement and surface water monitoring

Summary

Publications which are available in open literature mostly deal with glyphosate concentrations in runoff water rather than are derived from comprehensive monitoring programs of surface waters.

Out of the screened open literature, ~~12~~ 19 citations deal with glyphosate and AMPA concentrations in runoff water within the EU, ~~7~~ 11 are related to non-EU sites. They include analyses of storm water, urban runoff, roof and railways runoff as well as runoff from specific agri-culturally used sites such as vineyards. Concentrations in rainwater, information on bulk atmospheric deposition (wet and dry) are published as well. Some of the publications present results obtained from field studies and thus do not present results from monitoring campaigns in their common sense.

~~9~~ 20 citations show results on European surface water (e.g. streams, rivers, small creeks) monitoring, ~~8~~ 13 give results on monitoring outside ~~the EU~~ Europe. The design and also the presentation of the results obtained from monitoring campaigns are quite heterogeneous. Information is rather incomplete. Also, 2 different publications might deal with the same campaign (Ludvigsen and Ode, 2001; Ludvigsen and Ode, 2002) but publish slightly different information. Furthermore, a few references focus on modeling aspects, PEC determination, risk assessment using the monitoring data published elsewhere and risk mitigation. In one case, glyphosate is discussed only but without publication of decent data. In one case information was extracted from a presentation, and graphs only were given. Exact data were not obtained from the graphs.

Maximum glyphosate and AMPA concentrations in European surface waters as measured in comprehensive monitoring campaigns (Horth, 2012; see point 8.6.3) range between 1.3 - 370 µg/L and 0.22 - > 200 µg/L for glyphosate and AMPA, respectively. Compared to these findings, the maximum concentrations which were published in open literature are rather low, namely in the range of ~~0.4 - 1.37~~ 0.21 - 7.2 µg/L and 0.2 - 13 µg/L for glyphosate and AMA, respectively. Therefore, information published in open literature does not really modify the already existing assessment of glyphosate and AMPA occurrence in surface water (see B.8.6.3).

For reasons of completeness results extracted from the publications on off-site movement and surface water monitoring are listed in the following tables though information might be lacking.

Table B.8.11–2: Results of the publications on off-site movement

Country/ Substance	Description of sample	Date	No. Sites	No. Samples	Detected (samples)		Samples ≥ 0.1 µg/L		Max. conc.	LOQ (LOD)
					No.	%	No.	%	µg/L	µg/L
France										
Glyphosate	Urban stormwater	2008-09	3	15	14	93	-	-	232	0.03
AMPA	Urban stormwater	2008-09	3	15	14	93	-	-	9.37	-
Glyphosate	Urban stormwater	2007-08	2	20	-	-	-	-	71	-

Country/ Substance	Description of sample	Date	No. Sites	No. Samples	Detected (samples)		Samples ≥ 0.1 µg/L		Max. conc.	LOQ (LOD)
					No.	%	No.	%	µg/L	µg/L
AMPA	Urban stormwater	2007-08	2	20	-	-	-	-	1.5	-
Glyphosate	Wet atmos-pheric dep-osition	2008-09	1	-	-	-	-	-	0.4	-
AMPA	Wet atmos-pheric dep-osition	2008-09	1	-	-	-	-	-	0.7	-
Glyphosate	Vineyard runoff	2003-06	1	303	303	100	-	100	86	0.1
AMPA	Vineyard runoff	2003-06	1	303	303	100	-	100	44	0.1
Glyphosate	Vineyard runoff	2009-10	1	48	-	-	-	-	3.9	0.1 (0.03)
AMPA	Vineyard runoff	2009-10	1	48	-	-	-	-	1.8	0.1 (0.03)
Glyphosate	Roof runoff (rural site)	2009-10	-	-	-	-	-	-	6	0.1
Glyphosate	Wet atmos-pheric dep-osition	2008-09	1	-	-	-	-	-	150	-
AMPA	Wet atmos-pheric dep-osition	2008-09	1	-	-	-	-	-	19	-
UK										
Glyphosate	road run-off	1997	1	2	-	-	-	-	51.8[1)]	0.05 (0.01)
Glyphosate	Railway runoff	1999-2000	1	3	-	-	0	0	< 0.1	0.05 (0.01)
Glyphosate	surface water drains (storm drains)	2009	-	-	-	-	-	-	8.99	0.007 (0.002)
AMPA	surface water drains (storm drains)	2009	-	-	-	-	-	-	1.15	0.01 (0.003)
Belgium										
Glyphosate	rainwater	1997-2001	8	ca. 870 analyses	113	13	-	-	1.2	-
Glyphosate	storm drainage outflow	2013	-	-	-	-	-	-	6.1	-
AMPA	storm drainage outflow	2013	-	-	-	-	-	-	5.8	-
Denmark										
Glyphosate	Stormwater runoff	2008-09	5	10	10	100	-	-	9.0	-
AMPA	Stormwater runoff	2008-09	5	10	10	100	-	-	1.0	-
Glyphosate	Landfill leachate	2003	10	-	2	20	-	-	27	-
AMPA	Landfill leachate	2003	10	-	2	20	-	-	4.3	-

[1)] Predicted - = no information

Table B.8.11–3: Results of the publications on surface water monitoring

Country/ Substance	Descrip-tion of sample	Date	No. Sites	No. Sam-ples	Detected (samples)		Samples ≥ 0.1 µg/L		Max. conc.	LOQ (LOD)
					No.	%	No.	%	µg/L	µg/L
Belgium										
Glyphosate	rainwater	1997-2001	8	ca. 870 analyses	-	13	-	-	1.2	-
Hungary										
Glyphosate	Rivers	2010-2011	13	24	9	-	6	-	0.68	0.05 to 0.12
Luxembourg										
Glyphosate	River	2008	1	14	-	-	-	-	6.220	0.001
AMPA	River	2008	1	14	-	-	-	-	1.118	0.001
France										
Glyphosate	rivers	2007	3	104	-	-	-	-	1.0	0.1
AMPA	rivers	2007	3	104	-	-	-	-	1.0	0.1
Glyphosate	rivers	2007	1	5	-	-	-	-	0.12	-
AMPA	rivers	2007	1	5	-	-	-	-	0.65	-
Norway										
Glyphosate	Streams, rivers	1996-2000	12	57	52	91	-	-	-	(0.01)
Glyphosate[2)]	Streams, rivers	1995-1999	12	49	42	86	-	-	0.92	(0.01)
AMPA[2)]	Streams, rivers	1995-1999	12	49	43	87	-	-	0.2	(0.01)
Netherlands										
Glyphosate	river	-	-	-	-	-	-	-	0.21	-
AMPA	river	-	-	-	-	-	-	-	2.28	-
Switzerland										
Glyphosate	rivers	2005-2012	565	-	-		-	-	7.2	-
Germany										
Glyphosate	Local creek	1999	1	3	-	-	-	-	0.9	(0.04)
AMPA	Local creek	1999	1	3	-	-	-	-	0.4	(0.04)
Glyphosate	Rivers in Hessen	1995-2005	2	-	-	-	-	-	0.4	(0.05)
Glyphosate	Surface water in MV	2008	60	180	105	58	40	22	1.37	0.02
AMPA	Surface water in MV	2008	60	180	147	82	83	46	5.58	0.01
Glyphosate	Surface water in NRW	1996-2012	-	1899	-	-	225	12	0.93	-
AMPA	Surface water in NRW	1996-2012	-	1903	-	-	1377	72	13	-

[2)] Same campaign as first entry for Norway, other publication with partly differing numbers
- = no information

It has to be noted that there are a few results available from studies investigating the behaviour of glyphosate in railway systems in Switzerland (Brauchli-Theotokis, 2004). These investigations show that after the application of glyphosate in railway systems, concentrations up to 100 µg/L glyphosate are detected in the drainage water of the experimental set-ups. In general, it was shown that the higher the rainfall quantity, the higher the cumulative amount of glyphosate that was washed-off. During normal operation, glyphosate concentrations along

railway tracks reached values up to 10 g/L in the drainage water. Similar concentrations were also detected in drainage ditches alongside railway lines in England. There, about 1800 g /ha glyphosate were applied experimentally and 12 µg/L glyphosate was measured in run-off water (Heather et al., 1999). According to the representative GAP applications on railway facilities are not indented and therefore, not addressed in the risk assessment. Generally, the RMS considers that additional specific information in order to assess the potential contamination of surface water by runoff as well as the potential contamination of groundwater via run-off in surface water with subsequent bank filtration are required, if applications of glyphosate on railway facilities are intended at the national level.

Detailled description of open literature on off-site movement

Augustin and Seibel (2002)

Title: Herbicide treatment of urban areas – a possible source of surface water contamination	
Author: Bernd Augustin and Helmut Seibel	
Reference: GESUNDE PFLANZEN, 54. Jahrg., Heft 7	
Year: 2002	
Results and conclusion: A rough concrete surface with an inclination of 1-2 % for rainwater elimination was treated with Roundup Ultra® (Glyphosat), Basta® (Glufosinate) and Vorox G® (Glyphosat-Diuron). Run-off-water was collected after artificial rain (2 mm) given in different periods after herbicide application (1 and 24 h; 10 days). Chemical analysis showed that the run-off-water contained considerable quantities of Glyphosate and Glufosinate even 10 days after herbicide treatment and 17 mm of artificial and natural rainfall. The results are discussed considering recent detection of Glyphosate contamination of surface water in Germany.	
Proposed action: Not to be considered in the summarizing table of the off-site movement paragraph as an artificial scenario (runoff from a concrete surface) was designed. Obtained data are not comparable to those given in the summarizing table.	
Type of information (critical, high/low weight, supporting, additional): Low weight, may be additional information to already existing	
Reliability	Low
Endpoint	Concentration in leachate
Protocol	For chemical analysis: DFG-method 405, non-GLP. General set-up without specific protocol but research project.
Test compound	Roundup Ultra® (Glyphosat), Vorox G® (Glyphosat-Diuron), CAS-no.: 1071-83-6
Test system and conditions	A rough concrete surface with an inclination of 1-2 % for rainwater elimination was treated with Roundup Ultra® (Glyphosat), Basta® (Glufosinate) and Vorox G® (Glyphosat-Diuron). Run-off-water was collected after artificial rain (2 mm) given in different periods after herbicide application (1 and 24 h; 10 days).
Statistical design	Not given
Relevance	
Environmental relevance	Relevant
Weight of evidence	
“Positive”/”Negative” evidence	The results support teh monitoring data. However, monitoring studies and campaigns are of more reliability and relevance.

	No negative evidence.

Augustin (2003)

Title: Urban areas - sources of pesticide-contamination of surface water?	
Author: Augustin B.	
Reference: Presentation at: Second International Symposium Plant Health in Urban Horticulture, Berlin, August 27-29, 2003	
Year: 2003	
Results and conclusion: In Rhineland-Palatinate numerous (14-days-mix) samples of surface water (Mosel, Nahe, Selz) were repeatedly controlled for pesticide pollution between 1997 and 1999. Investigations focused on 35 different active ingredients. Regularly present were Bentazon, Diuron, Dichlorprop, Ethofumesat, Glyphosat, IPU, MCPA, Mecoprop, Tebuconazol and Simazin. Especially GIyphosate and Bentazon were detected in all water sources and partly all over the year. An additional investigation of a sewage disposal plant ("Hahnheim"), which drains into river Selz clearly showed, that waste water was polluted by the same active ingredients. Pesticide concentration was about ten times as high as in the river water. Detectable pesticides mostly formed distinct peaks during time of investigation indicating a direct dependence on application period. This was not the case for Glyphosate. Up to now there are no indications for the presence of Glyphosate in drain-water of agricultural areas. Since the herbicide was detectable during the entire year, it is unlikely that it derived from application of farmland, vineyards or orchards. The fact that larger quantities are used on urban areas, let to the presumption, that there might also be runoff from sealed areas. In-depth worst-case investigations on Glyphosate concentrations in urban runoff (concrete runoff) showed concentrations between 0.03 - 17.9 mg/L depending on precipitation period and quantity.	
Proposed action: Not to be considered as the publication is of low weight because of low reliability of data. No raw data are published.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information to already existing, summary from an oral presentation with several figures but without reliable results on concentrations.	
Reliability	Low
Endpoint	Monitoring, concentrations in surface waters, sewage disposal plant and urban runoff
Protocol	No standard protocol, no further information on monitoring given
Test compound	Glyphosate (monitored, purity cannot be given, CAS-no: 1071-83-6)
Test system and conditions	Monitoring programme described briefly only without presentation of design of the campaigns, no LOD or LOQ given
Statistical design	Not known
Relevance	
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	Supporting information by other monitoring programmes. No negative evidence.

Birch et al. (2011)

Title: Micropollutants in stormwater runoff and combined sewer overflow in the Copenhagen area, Denmark	
Author: H. Birch, P. S. Mikkelsen, J. K. Jensen and H.-C. Holten Lützhøft	
Reference: Water Science & Technology; doi: 10.2166/wst.2011.687	
Year: 2011	
Results and conclusion: Stormwater runoff contains a broad range of micropollutants. In Europe a number of these substances are regulated through the Water Framework Directive, which establishes Environmental Quality Standards (EQSs) for surface waters. Results from a screening campaign including more than 50 substances at four stormwater discharge locations and one combined sewer overflow (CSO) in Copenhagen are reported here. Glyphosate was found in all samples. The results give a valuable background for designing further monitoring programs focusing on the chemical status of surface waters in urban areas.	
Proposed action: To be considered in the summarizing table and discussion.	
Type of information (critical, high/low weight, supporting, additional): High weight, additional information to already existing as the screening campaign was meant for designing further monitoring programs focusing on the chemical status of surface waters in urban areas. No data on Glyphosate concentrations published.	
Reliability	Low
Endpoint	Monitoring, concentrations in surface waters
Protocol	Monitoring, for details see under test system and conditions.
Test compound	Glyphosate (CAS-no: 1071-83-6) and AMPA (CAS-no.: 1066-51-9, monitored, purity cannot be given)
Test system and conditions	Substances for analysis primarily selected from the WFD list, but earlier Danish runoff studies and a risk assessment for one of the catchment areas were also considered. Sampling sites and regimes described. Total concentrations in the samples measured. No further information on analytical procedures, statistical treatment, quality assurance.
Statistical design	Not reported
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supporting information by other monitoring programmes. No negative evidence.

Botta et al. (2009)

Title: Transfer of glyphosate and its degradate AMPA to surface waters through urban sewerage systems
Author: Fabrizio Botta, Gwenaëlle Lavison, Guillaume Couturier, Fabrice Alliot, Elodie Moreau-Guigon, Nils Fauchon, Bénédicte Guery, Marc Chevreuil, Hélène Blanchoud
Reference: Chemosphere 77(1): 133-139 doi: 10.1016/j.chemosphere.2009.05.008
Year: 2009
Results and conclusion: A study of glyphosate and aminomethyl phosphonic acid (AMPA) transfer in the Orge watershed (France) was carried out during 2007 and 2008. Water samples were collected in

surface water, wastewater sewer, storm sewer and wastewater treatment plant (WWTP). These two molecules appeared to be the most frequently detected ones in the rivers and usually exceeded the European quality standard concentrations of 0.1 µg L^{-1} for drinking water. The annual glyphosate estimated load was 1.9 kg year1 upstream (agricultural zone) and 179.5 kg year1 at the catchment outlet (urban zone). This result suggests that the contamination of this basin by glyphosate is essentially from urban origin (road and railway applications). Glyphosate reached surface water prevalently through storm sewer during rainfall event. Maximum concentrations were detected in storm sewer just after a rainfall event (75–90 µg L^{-1}). High concentrations of glyphosate in surface water during rainfall events reflected urban runoff impact. AMPA was always detected in the sewerage system. This molecule reached surface water mainly via WWTP effluent and also through storm sewer. Variations in concentrations of AMPA during hydrological episodes were minor compared to glyphosate variations. Our study highlights that AMPA and glyphosate origins in urban area are different. During dry period, detergent degradation seemed to be the major AMPA source in wastewater.	
Proposed action: To be considered in the summarizing table and discussion.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information about non-agricultural application of glyphosate and the relevant contribution on the glyphosate annual load.	
Reliability	Medium
Endpoint	Concentration of glyphosate and AMPA
Protocol	For glyphosate and AMPA the analytical method was the DIN 38407-22 and international Norm NF ISO 21548
Test compound	Glyphosate, CAS-no.: 1071-83-6; AMPA, CAS-no.: 1066-51-9
Test system and conditions	Monitoring network: Sample campaigns were organized to gather data according four different levels: 1) at the basin scale to calculate the budget of glyphosate load in the Orge River, 2) at the urban area scale to verify the impact of sewage network on the river contamination, 3) at the network scale to study the transfer of glyphosate and its degradate by runoff in urban areas and 4) at the waste water treatment plant scale to verify the potential impact of urban wastes on surface waters.
Statistical design	No information
Relevance	
Environmental relevance	No reported data of environmental parameters.
Weight of evidence	
“Positive”/”Negative” evidence	Results supported by other publications; no negative evidence.

Bressy et al. (2012)

Title: Towards the determination of an optimal scale for stormwater quality management: Micropollutants in a small residential catchment	
Author: A. Bressy, M.-C. Gromaire, C. Lorgeoux, M. Saad, F. Leroy, G. Chebbo	
Reference: Water Research 46(20): 6799-6810. doi:10.1016/j.watres.2011.12.017	
Year: 2012	
Results and conclusion: Stormwater and atmospheric deposits were collected on a small residential urban catchment (0.8 ha) near Paris in order to determine the levels of certain micropollutants (using a preliminary scan of 69 contaminants, followed by a more detailed quantification of PAHs, PCBs, alkylphenols and metals). Atmospheric inputs accounted for only 10% to 38% of the stormwater contamination (except for PCBs), thus indicating substantial release within the catchment. On this small upstream catchment however, stormwater contamination is significantly lower than that observed downstream in storm sewers on larger adjacent urban catchments with similar land uses. These results likely stem from cross-contamination activity during transfers inside the sewer system and underscore the advantages of runoff management strategies at the source for controlling stormwater pollutant loads. Moreover, it has been shown that both contamination levels and contaminant speciation evolve with the scale of the catchment, in correlation with a large fraction of dissolved contaminants in upstream runoff, which differs from what has been traditionally assumed for stormwater. Consequently, the choice of treatment device/protocol must be adapted to the management scale as well as to the targeted type of contaminant.	
Proposed action: Not to be considered. The publication gives an overview of the stormwater and atmospheric deposits of 66 micropollutants, but not all raw data needed for the evaluation of a monitoring campaign are reported.	
Type of information (critical, high/low weight, supporting, additional): Low weight	
Reliability	Medium
Endpoint	Total mass of contaminants in proportion to exposure time; flux
Protocol	Analyses were performed in a laboratory certified by a French Environment Ministry committee, i.e. COFRAC (French Accreditation Committee), in accordance with French (AFNOR) or International (ISO) standard methods to the extent of their availability.
Test compound	Glyphosate one of the compounds analyzed (CAS-no.: 1071-83-6)
Test system and conditions	1) the micropollutants present in runoff water were identified and quantified by encompassing a wide spectrum of substances over 3 rainfall events; 2) for a greater number of rain events over a one year period, the fluxes being conveyed were quantified for a selection of parameters
Statistical design	No information, no raw data
Relevance	
Environmental relevance	Environmental parameter are not reported.
Weight of evidence	
“Positive”/”Negative” evidence	Results are supported by other publications.

Baun et al. (2004)

Title: Xenobiotic organic compounds in leachates from ten Danish MSW landfills—chemical analysis and toxicity tests	
Author: A. Baun, A. Ledin, L.A. Reitzel, P.L. Bjerg, T.H. Christensen	
Reference: Water Research 38, 3845–3858	
Year: 2004	
Results and conclusion: Glyphosate and AMPA were found in the leachate of 2 (out of 10) Danish landfills at concentrations of 1.7 and 27 µg/L (Glyphosate) and 4.3 and 3.8 µg/L (AMPA). It furthermore was concluded to include degradation products in future monitoring programs as this may provide additional insight in the fate of chemicals in landfills. The present study provided several examples of concomitant presence of parent compounds and degradation products (Glyphosate/ AMPA).	
Proposed action: Supporting information which is presented in the summarising table and discussion.	
Type of information (critical, high/low weight, supporting, additional): High weight, supporting information to already existing.	
Reliability	Medium
Endpoint	Monitoring, concentration in landfill leachate.
Protocol	No standard protocol
Test compound	Glyphosate (CAS-no.: 1071-83-6) and AMPA (CAS-no: 1066-51-9) (monitored, purity cannot be given)
Test system and conditions	10 landfills tested comprehensive monitoring campaign for pesticides and several other organic compounds.
Statistical design	Not known
Relevance	
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	Supporting information by other studies. No negative evidence.

Eriksson et al. (2007)

Title: Risk assessment of xenobiotics in stormwater discharged to Harrestrup Å, Denmark
Author: Eva Eriksson, Anders Baun, Peter Steen Mikkelsen, Anna Ledin
Reference: Desalination 215, 187–197
Year: 2007
Results and conclusion: Surface waters are highly manipulated in many cities in Europe, and the flow is largely impacted by discharges of stormwater and combined sewer overflow. Toxicity tests shown adverse effects in some of these recipients due to the presence of xenobiotic organic carbons (XOCs). Harrestrup Å, situated in the City of Copenhagen, is one of these recipients, where biotest using algae showed measurable toxicity in eight samples taken in 2003. Twenty-five different XOCs were quantified in the same samples. The present study aimed at identifying the most relevant XOCs out of these 25 to be selected for further analysis with respect to potential source control options. Fourteen XOCs (56%) were identified to constitute a potential hazard based on the RICH evaluation (Ranking and Identification of Chemical Hazards), while 9 XOCs (36%) were found to constitute a hazard towards the aquatic ecosystem based on an environmental-concentration/predicted-no-effect-

concentration-quotient. The quantified levels did, however, fulfil the Danish and European surface water quality criteria (QC) and environmental quality standards (ESQ). Thus, although the QC and ESQ are met there is an actual risk due to stormwater-related pollutants. This clearly illustrates that there is a need for monitoring the stormwater quality in order to protect the ecosystems. It also shows that actions are needed to implement source control options and emission barriers. Twelve XOCs were selected for further evaluation of possible source control option to be implemented in order to improve the water quality. These are five pesticides (diuron, glyphosate, isoproturon, MCPA, Terbutylazin), 4 PAHs (acenaphthene, fluoranthene, fluorene, pyrene), 3 others (LAS, nonylphenol and dinitro-o-cresol.	
Proposed action: Not to be considered as publication deals with hazard ranking based on monitoring and effect data and does not comprehensively report monitoring data.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Low
Endpoint	Hazard ranking
Protocol	No protocol, no experimental design
Test compound	Glyphosate (CAS-no: 1071-83-6), no experimental design
Test system and conditions	Hazard ranking procedure, no experimental design but monitoring data cited from other sources
Statistical design	Not applicable
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	Similar ranking approaches support the results. No negative evidence.

Eriksson et al. (2007)

Title: Selected stormwater priority pollutants – a European perspective
Author: E. Eriksson, A. Baun, L. Scholes, A. Ledin, S. Ahlman, M. Revitt, C. Noutsopoulos, P.S. Mikkelsen
Reference: Science of the Total Environment 383, 41–51
Year: 2007
Results and conclusion: The chemical characteristics of stormwater are dependent on the nature of surfaces (roads, roofs etc.) with which it comes into contact during the runoff process as well as natural processes and anthropogenic activities in the catchments. The different types of pollutants may cause problems during utilisation, detention or discharge of stormwater to the environment and may pose specific demands to decentralised treatment. This paper proposes a scientifically justifiable list of selected stormwater priority pollutants (SSPP) to be used, e.g., for evaluation of the chemical risks occurring in different handling strategies. The SSPP-list consists of 25 pollutant parameters including eight of the priority pollutants currently identified in the European Water Framework Directive. It contains general water quality parameters (organic and suspended matter, nutrients and pH); metals (Cod, Cr, Cu, Ni, BP, Pt and Zn); PAH (naphthalene, preen and benzoic[a]preen); herbicides (pendimethalin, phenmedipham, glyphosate and terbutylazine); and other representative industrially derived compounds (nonylphenol ethoxylates, pentachlorophenol, di(2-ethylhexyl)phthalate, PCB-28 and methyl tert-butyl ether). Tools for flux modelling, ena-

bling calculation of predicted environmental concentrations (PECs), and for ranking the susceptibility of a pollutant to removal within a range of structural stormwater treatment systems or best management practices (BMPs) have been developed, but further work is required to allow all SSPPs to be addressed in the development of future stormwater pollution control measures. In addition, the identified SSPPs should be considered for inclusion in stormwater related monitoring campaigns.	
Proposed action: Not to be considered as publication deals with hazard ranking and priority setting and does not comprehensively and in detail report monitoring data.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Low
Endpoint	Hazard ranking and priority list
Protocol	No protocol, no experimental design
Test compound	Glyphosate (CAS-no: 1071-83-6), no experimental design
Test system and conditions	Hazard ranking procedure, no experimental design but monitoring data cited from other sources
Statistical design	Not applicable
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	Similar ranking approaches support the results. No negative evidence.

Gregoire et al. (2010)

Title: Use and fate of 17 pesticides applied on a vineyard catchment	
Author: Caroline Gregoire, Sylvain Payraudeau and Nicolas Domange	
Reference: Intern. J. Environ. Anal. Chem. Vol. 90, Nos. 3–6, 15, 406–420	
Year: 2010	
Results and conclusion: Flux of 17 pesticides from a small agricultural catchment was monitored. Some 78% of the total pesticide applications in the catchment were herbicides and glyphosate was the most used herbicide with annual application ranging from 18 to 61 kg. The run-off coefficient was low (less than 2%), but the frequency of determination was high for some pesticides such as the fungicide dimetomorph (72%) and the herbicides diuron (98%) and glyphosate (100%). The pesticide export coefficients for Glyphosate ranged between 0.009 – 0.033 %. Every water sample exceeded the EU drinking water limit of 0.1 µg L^{-1}. In detail, concentrations were for Glyphosate: 7.5 µgL^{-1} (mean) and 86 mgL^{-1} (max), and for AMPA 2.9 µgL^{-1} (mean) and 44 mgL^{-1} (max).	
Proposed action: To be considered in the summarizing table and discussion.	
Type of information (critical, high/low weight, supporting, additional): High weight, supporting information.	
Reliability	High
Endpoint	Glyphosate concentration in runoff water
Protocol	No standard protocol; for further details see under test system and conditions
Test compound	Glyphosate (CAS-no: 1071-83-6), AMPA (CAS-no: 1066-51-9), purity not given, monitoring

Test system and conditions	The flux of 17 pesticides from a small (42.7 ha) agricultural (vineyard) catchment in the Alsatian piemont (France) was systematically monitored over 4 years (2003–2006) from June to September. A metrological station is located within the catchment area and run-off of 58 run-off events was monitored throughout. A water sample for pesticide analyses was collected every 8m^3 of run-off. Chemical analysis described. Calculations described
Statistical design	Frequency of determination calculated
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Huang et al. (2004)

Title: Herbicide Runoff along Highways. 1. Field Observations
Author: Xinjiang Huang , Theresa Pedersen, Michael Fischer, Richard White, and Thomas M. Young
Reference: Environ. Sci. Technol., 38, 3263-3271
Year: 2004
Results and conclusion: To determine whether herbicide runoff along highways threatens water quality, a field study was conducted at two sites in northern California for three rainy seasons. The herbicides oryzalin, isoxaben, diuron, glyphosate, and clopyralid were selected for study to include compounds with significant variation in physical/chemical properties. Concentrations of herbicides in runoff were monitored for up to 11 storms following herbicide application, and 24 samples were collected per storm, providing unprecedented temporal detail. Flow-weighted event mean concentrations were calculated for each herbicide in each storm and ranged from below detection limits to 43.13 µg/L for oryzalin. The least soluble compounds, isoxaben and oryzalin, were detected in all storms monitored while the more soluble compounds, diuron and clopyralid, declined to levels below detection limits before monitoring was concluded. Very small amounts of glyphosate were mobilized, but its transformation product aminomethylphosphonic acid was detected at higher concentrations, in more storm events, and at greater depth in the soil profile. A first order model successfully described the declining herbicide concentrations in spray zone soil and in surface runoff for all sites and herbicides. Fitted first-order coefficients were always higher for runoff than for soil, indicating that the herbicide that persists in the source zone becomes less available for runoff as the time since application increases. The percentage of the applied herbicide that was detected in surface runoff over a season ranged from 0.05% to 43.5%, and the most critical variables in controlling the variation were the solubility of the herbicide and the runoff volume. For a given herbicide and site, the most critical factors in determining seasonal herbicide loss to surface water were the timing and intensity of the first storm following application, affecting total seasonal runoff by up to 2 orders of magnitude. Minimizing runoff of herbicides along highways will thus require careful attention to the intrinsic mobility of the compound and the timing of its application.
Proposed action: Not to be considered as the study deals with a site outside the EU (USA)
Type of information (critical, high/low weight, supporting, additional): Low weight

Reliability	Medium
Endpoint	Variation in herbicide concentration over time, herbicide mass loading and runoff concentration; first-order Dissipation coefficients (k), pre-exponential factors (a), and fitting criteria (R 2) estimated from runoff, event mean concentration and herbicide concentrations
Protocol	Non-GLP
Test compound	Oryzalin, isoxaben, diuron, glyphosate (CAS-no.: 1071-83-6), clopyralid and AMPA (CAS-no.: 1066-51-9)
Test system and conditions	The herbicides were applied to a 1.23 or 1.83 m wide strip along the highway shoulder using a truck sprayer. Application typically occurred after the first fall storm. Exact application rates were determined by analyzing herbicides recovered from deposit collection plates constructed of glass fibersample pad on corrugated cardboard that were located within the spray zone during herbicide application. At the monitoring location runoff flow rates were determined using a flume and an automatic sampling station, which included a rain gauge and a bubbler flow module, began taking samples when 0.01 mm of rain fell in 30 min and the flow level exceeded 3.0 mm. Each sampling event included up to 24 samples collected at intervals of between 20 and 120 min. Sampling times, rainfall volumes, and runoff flow rates were recorded by the automatic sampling system
Statistical design	-
Relevance	
Environmental relevance	Tables giving herbicide application rates and rainfall and runoff comparison at the two sampling sites and figures showing event first-flush analysis and vertical distribution of glyphosate and AMPA content within soil. This material is available free of charge via the Internet at http://pubs.acs.org.
Weight of evidence	
“Positive”/”Negative” evidence	No negative evidence.

Huang et al. (2004)

Title: Herbicide Runoff along Highways. 2. Sorption Control
Author: Xinjiang Huang , Theresa Pedersen, Michael Fischer, Richard White, and Thomas M. Young
Reference: Environ. Sci. Technol., 38, 3272-3278
Year: 2004
Results and conclusion: This study examines the sorption and desorption of five herbicides with a wide range of properties (isoxaben, oryzalin, diuron, clopyralid, and glyphosate) on soil samples from two roadsides in northern California and uses the results to examine field runoff data from multiple rainy seasons. Non-ideal sorption processes do not appear to be significant in determining herbicide runoff at the field sites because (i) sorption isotherms were linear or slightly nonlinear for all compounds but glyphosate, (ii) field runoff concentration ratios between isoxaben and oryzalin were consistent with linear partitioning predictions, (iii) runoff leaving the site appeared to be in equilibrium with local soil concentrations, and (iv) desorption distribution coefficients for aged herbicides on soil samples collected from the

field site did not differ substantially from those obtained in short term laboratory adsorption experiments. Collectively, these findings indicate that linear equilibrium models are adequate for predicting the concentration of herbicides in runoff in these field settings and that more complicated non-ideal models do not need to be invoked. Vegetated slopes effectively reduced the herbicide loads, with average removals of 35-80% occurring as runoff traversed a 3-m segment 1 m from the edge of the spray zone.	
Proposed action: Not to be considered as the study deals with a site outside the EU (USA).	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information. This research has demonstrated that, although the herbicides display some evidence of non-ideal (i.e., nonlinear and incompletely reversible) sorption behaviours in the laboratory, ideal sorption models (i.e., linear isotherms and completely reversible) are likely to be sufficient for describing the sorption component of this process in the field.	
Reliability	High
Endpoint	Sorption isotherms; carbon-normalized distribution coefficients (K_{oc}), and regression R^2 values, K_d, K_f soil concentration
Protocol	Similar to OECD 106, non-GLP
Test compound	Oryzalin, isoxaben, diuron, glyphosate (CAS-no.: 1071-83-6), clopyralid and AMPA (CAS-no.: 1066-51-9)
Test system and conditions	1) To measure herbicide attenuation as runoff moved down the grassy slope, six runoff collectors were installed at the sampling site at defined distances; vegetation samples along the slope were also collected and analyzed for herbicides; 2) Adsorption isotherms were determined for the five target herbicides and AMPA on surface soils from the field sites. To obtain greater than 50% adsorption and final concentrations above method quantitation limits, soil: water ratios (g/mL) were determined. The time required to attain an apparent equilibrium was also determined. Batch adsorption experiments were conducted by combining herbicide solution in a 0.005 M $CaCl_2$ matrix with soil at the predetermined ratios in 45-mL Teflon centrifuge tubes. Six samples with varying initial herbicide concentrations (0-1000 µg/L) were mixed in triplicate at 23 +/- 1°C; 3) Combined herbicide adsorption and desorption study with isoxaben and oryzalin; 4) Desorption of herbicides from spray zone soils collected from the Tolay Creek field site at different times after herbicide application was determined.
Statistical design	Measurements in triplicate, Sorption isotherms: linear model and the Freundlich model
Relevance	
Environmental relevance	Given as influence by environmental parameter was tested.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Imfeld et al. (2012)

Title: Transport and attenuation of dissolved glyphosate and AMPA in a stormwater wetland

Author: Gwenaël Imfeld, Marie Lefrancq, Elodie Maillard and Sylvain Payraudeau	
Reference: Chemosphere 90 (2013) 1333–1339	
Year: 2012	
Results and conclusion: Here we show that transport and attenuation of runoff-associated glyphosate and AMPA in a stormwater wetland differ and largely vary over time. Dissolved concentrations and loads of glyphosate and AMPA in a wetland receiving runoff from a vineyard catchment were assessed during three consecutive seasons of glyphosate use (March to June 2009, 2010 and 2011). The load removal of glyphosate and AMPA by the wetland gradually varied yearly from 75% to 99%. However, glyphosate and AMPA were not detected in the wetland sediment, which emphasises that sorption on the wetland vegetation, which increased over time, and biodegradation were prevailing attenuation processes. The relative load of AMPA as a percentage of total glyphosate increased in the wetland and ranged from 0% to 100%, which indicates the variability of glyphosate degradation via the AMPA pathway. Our results demonstrate that transport and degradation of glyphosate in stormwater wetlands can largely change over time, mainly depending on the characteristics of the runoff event and the wetland vegetation. We anticipate our results to be a starting point for considering degradation products of runoff-associated pesticides during their transfer in wetlands, in particular when using stormwater wetlands as a management practice targeting pesticide attenuation. Max. concentrations detected were 150 µg L^{-1} (glaphosate) and 19 µg L^{-1} (AMPA).	
Proposed action: To be considered in the summarizing table and discussion.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as publication deals with specific targets, namely stormwater and wastewater at a certain place and time.	
Reliability	Low
Endpoint	Monitoring, concentrations in surface waters (stormwater wetlands)
Protocol	Monitoring, for details see test system and conditions.
Test compound	Glyphosate (CAS-no: 1071-83-6) and AMPA (CAS-no.: 1066-51-9, monitored, purity cannot be given)
Test system and conditions	Runoff discharges entering and outflowing the wetland were continuously monitored from March 23 to June 30, 2009, 2010 and 2011.
Statistical design	Not reported
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Lamprea and Ruban (2010)

Title: Characterization of atmospheric deposition and runoff water in a small suburban catchment	
Author: Katerine Lamprea and Véronique Ruban	
Reference: Environmental Technology, Vol. 32, No. 10, 1141–1149	
Year: 2010	
Results and conclusion: A study has been carried out with the objective of characterizing atmospheric deposition, roof runoff and street runoff in a small (31 ha) suburban catchment in Nantes equipped with a separate sewer system. Street runoff: Glyphosate was not detected in winter, yet the summer glyphosate and AMPA concentrations were found to lie between 60 and 470 ng/L for glyphosate, and 50 and 770 ng/L for AMPA. Deposition: To the best of our knowledge, glyphosate and AMPA have rarely been identified in atmospheric deposition. Though their volatility remains low, these molecules are present in the atmosphere from having been transported via vaporization when applied to the catchment and in the neighbourhood. Glyphosate and AMPA were detected in roughly 10% of samples. Roof-runoff: Glyphosate was detected in samples collected in September 2007 campaigns. The concentrations range from 50 to 980 ng/L. AMPA was detected in samples collected from slate roof in the June 2008 campaign, displaying a concentration of 120 ng/L. It is very likely, that atmospheric deposition constitutes the glyphosate and AMPA contributor to roof runoff.	
Proposed action: To be considered in the summarizing table and discussion.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as publication deals with specific targets, namely street runoff, roof-runoff and atmospheric deposition at a certain place and time.	
Reliability	Medium
Endpoint	Glyphosate concentrations in street-runoff, roof-runoff and atmospheric deposition.
Protocol	For details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), purity not given, monitoring
Test system and conditions	This study was conducted on the Pin Sec catchment in the eastern part of the French city of Nantes between the Loire and Erdre rivers. Sampling campaigns were performed on bulk atmospheric (both dry and wet) deposition, roof runoff and street runoff. Samples were analyzed for Glyphosate and AMPA, LOQ = 0.05 µg/L.
Statistical design	Mean values, standard deviations, Whisker blots.
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Lamprea and Ruban (2011)

Title: Pollutant concentrations and fluxes in both stormwater and wastewater at the outlet of two urban watersheds in Nantes (France)
Author: Katerine Lamprea and Véronique Ruban
Reference: Urban Water Journal ,Vol. 8, No. 4, 219–231

Year: 2011	
Results and conclusion: A two-year study of pollutants in both the stormwater and wastewater of urban watersheds has been conducted in Nantes (France). The present paper discusses the characteristics of pollutants transported by stormwater and wastewater collection networks in two urban watersheds. A physicochemical characterisation of the effluents was performed, along with an estimation of pollutant fluxes discharged into the Gohards River. Concentrations in stormwater: Glyphosate = 0.23 - 3.27 µg/L; AMPA = < 0.1 - 0.46 µg/L. concentrations in waste water during wet weather season: Glyphosate and AMPA = 0.3 - 49 µg/L.	
Proposed action: To be considered in the summarizing table and discussion.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as publication deals with specific targets, namely stormwater and wastewater at a certain place and time.	
Reliability	Medium
Endpoint	Glyphosate concentrations in stormwater and waste water.
Protocol	For details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), purity not given, monitoring
Test system and conditions	So as to characterize quality and pollutant substances transported by stormwater, dry and wet weather conditions were studied in both stormwater and wastewater networks. The campaigns were carried out from September 2007 to October 2008 for stormwater, and from April 2007 to December 2008 for wastewater. Samples were analyzed for Glyphosate and AMPA, LOQ = 0.05 µg/L.
Statistical design	Mean values, standard deviations, Whisker blots
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Maillard et al. (2011)

Title: Removal of pesticide mixtures in a stormwater wetland collecting runoff from a vineyard catchment
Author: Elodie Maillard, Sylvain Payraudeau, Etienne Faivre, Caroline Grégoire, Sophie Gangloff, Gwenaël Imfeld
Reference: Science of the Total Environment 409, 2317–2324
Year: 2011
Results and conclusion: Here we show that stormwater wetlands can efficiently remove pesticides in runoff from vineyard catchments during the period of pesticide application, although flow and hydrochemical conditions of the wetland largely vary over time. During the entire agricultural season, the inflowing load of nine fungicides, six herbicides, one insecticide and four degradation products was 8.039 g whereas the outflowing load was 2.181 g. Removal rates of dissolved loads by the wetland ranged from 39% (simazine) to 100% (cymoxanil, gluphosinate, kresoxim methyl and terbuthylazine). Dimethomorph, diuron, glyphosate, metalaxyl and tetraconazole were more efficiently removed in spring than in summer. More than 88% of the input mass of suspended solids was retained, underscoring the capability of

the wetland to trap pesticide-laden particles via sedimentation. Only the insecticide flufenoxuron was frequently detected in the wetland sediments. Our results demonstrate that stormwater wetlands can efficiently remove pesticide mixtures in agricultural runoff during critical periods of pesticide application, although fluctuations in the runoff regime and hydrochemical characteristics can affect the removal rates of individual pesticides.	
Proposed action: Considered by listing in the summarizing table and discussion.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information: stormwater wetlands have the potential to serve as a tool for urban and agricultural stormwater management practices, thus contributing to the improvement of water quality for receiving aquatic ecosystems.	
Reliability	High
Endpoint	Reduction of pesticide concentration, RC (%), was calculated for each runoff event as the reduction of mean concentrations at the outlet relatively to the mean concentrations at the inlet of the wetland. Removal rates of pesticide load RL (%) were calculated for each runoff event as the reduction of the load at the outlet relatively to the load at the inlet of the wetland.
Protocol	Pesticide analysis was performed according to the NF XPT 90–210 French standards at the Pasteur Institute of Lille (France), which is a service of pesticide residues analysis accredited by the French National Accreditation Authority (COFRAC).
Test compound	Azoxystrobin, Cymoxanil, Cyprodinil, Carbendazim, Dimethomorph, Diuron, DCPU, DCPMU, 3.4-dichloroaniline, Flufenoxuron, Gluphosinate, Glyphosate (CAS-no. 1071-83-6), AMPA (CAS-no.: 1066-51-9), Isoxaben, Kresoxim methyl, Metalaxyl, Pyrimethanil, Simazine, Terbuthylazine, Tetraconazole
Test system and conditions	The main objective of the present study was to assess the ability of a stormwater wetland to remove pesticides in runoff from a vineyard catchment during an entire period of pesticide application. Nine fungicides, six herbicides, one insecticide and four degradation products were selected for the present study. Samples were collected from the inlet, the sediment deposition zone, the gravel filter, and the outlet of the wetland from April 01 through September 29 2009, corresponding to the period of pesticide application. Runoff discharges were continuously monitored by measurements of water depth. Series of discrete water samples taken over a runoff event were combined in a single composite sample. In parallel, 10 sampling campaigns were performed every two weeks during quiescent period (i.e. in the period between two runoff events) to collect water and sediment samples within the wetland.
Statistical design	Detection and quantification limits, relative standard deviation (RSD) and recovery efficiencies
Relevance	
Environmental relevance	Supplementary data to this article can be found online at doi:10.1016/j.scitotenv.2011.01.057.
Weight of evidence	
“Positive”/”Negative” evidence	No negative evidence

Maillard et al. (2011)

Title: Removal of dissolved pesticide mixtures by a stormwater wetland receiving runoff from a vineyard catchment: an inter-annual comparison	
Author: Elodie Maillard, Sylvain Payraudeau, Floro Ortiz and Gwenael Imfeld	
Reference: Intern. J. Environ. Anal. Chem., 1–16,	
Year: 2011	
Results and conclusion: We show here that stormwater wetlands that primarily serve for flood protection can also be effective tools for reducing concentrations and removing loads of runoff-related pesticides and some of their degradation products into downstream aquatic ecosystems. Dissolved concentrations and loads of seven fungicides, six herbicides and four degradation products in runoff from a vineyard catchment were continuously recorded at the inlet and the outlet of the stormwater wetland during two successive periods of pesticide application (April to June). Reduction of pesticide concentrations by the wetland ranged from 50% (simazine) to 100% (azoxystrobin, cymoxanil, cyprodinil, gluphosinate, terbuthylazine and tetraconazole). Removal rates of dissolved load ranged from 26% for aminomethylphosphonic acid (AMPA) to 100% (azoxystrobin, cymoxanil, cyprodinil, diuron, 1-(3,4-dichlorophenyl)-3-methylurea (DCPMU), gluphosinate, kresoxym methyl, terbuthylazine and tetraconazole). More than 77% of the input mass of total suspended solids was retained, underscoring the capability of the wetland to trap pesticide-laden particles via sedimentation. Inter-annual change in the removal of AMPA, isoxaben, kresoxim methyl and simazine was mainly linked to the larger vegetal cover in 2010. Our results demonstrate that stormwater wetlands can remove pesticide mixtures in agricultural runoff, although removal of individual pesticides can vary over time, depending on the characteristics of runoff events and the vegetation cover.	
Proposed action: Considered by listing in the summarizing table and discussion.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information: Stormwater wetlands that primarily serve for flood protection can also be effective tools for reducing concentrations and removing loads of a wide range of runoff-related pesticides and some of their degradation products into downstream aquatic ecosystems	
Reliability	Medium
Endpoint	Reduction of pesticide concentration (RC (%)) was calculated as the relative decrease of mean concentration at the outlet with respect to that at the inlet. Removal rate of pesticide mass loading RL (%) was calculated as the relative decrease of mass loading at the outlet with respect to that at the inlet for each runoff event.
Protocol	Pesticide analysis was performed according to the NF XPT 90–210 French standards at the Pasteur Institute of Lille (France), which is a service of pesticide residues analysis accredited by the French National Accreditation Authority (COFRAC).
Test compound	Azoxystrobin, cymoxanil, cyprodinil, dimethomorph, diuron, gluphosinate, glyphosate (CAS-no.: 1071-83-6), isoxaben, kresoxim methyl, metalaxyl, simazine, terbuthylazine, tetraconazole, 3,4-dichlorophenyl urea (DCPU), DCPMU, 3,4-dichloroaniline and AMPA (CAS-no.: 1066-51-9)
Test system and conditions	Runoff is collected at the outlet of the vineyard catchment by a stormwater wetland and represents the main entry route of pesticides into the wetland. Runoff discharges entering and outflowing the wetland were continuously monitored by measuring water

	depth using bubbler flow modules. Water samples (300 mL) were collected in glass jars, stored in the dark at 4°C after each runoff event. A series of discrete flow proportional water samples taken over a runoff event were combined in a single composite sample prior to analysis.
Statistical design	Paired nonparametric Wilcoxon Signed Rank test
Relevance	
Environmental relevance	
Weight of evidence	
"Positive"/"Negative" evidence	The results are supported by other publications.

Majewski et al. (2014)

Title: PESTICIDES IN MISSISSIPPI AIR AND RAIN: A COMPARISON BETWEEN 1995 AND 2007	
Author: M. S. MAJEWSKI, R. H. COUPE, W. T. FOREMAN and P. D. CAPEL	
Reference: Environmental Toxicology and Chemistry, Vol. 33, No. 6, pp. 1283–1293, 2014	
Year: 2014	
Results and conclusion: A variety of current-use pesticides were determined in weekly composite air and rain samples collected during the 1995 and 2007 growing seasons in the Mississippi Delta (MS, USA) agricultural region. Similar sampling and analytical methods allowed for direct comparison of results. Decreased overall pesticide use in 2007 relative to 1995 generally resulted in decreased detection frequencies in air and rain; observed concentration ranges were similar between years, however, even though the 1995 sampling site was 500m from active fields whereas the 2007 sampling site was within 3 m of a field. Mean concentrations of detections were sometimes greater in 2007 than in 1995, but the median values were often lower. Seven compounds in 1995 and 5 in 2007 were detected in ≥50% of both air and rain samples. Atrazine, metolachlor, and propanil were detected in ≥50% of the air and rain samples in both years. Glyphosate and its degradation product, aminomethyl-phosphonic acid (AMPA), were detected in ≥75% of air and rain samples in 2007 but were not measured in 1995. The 1995 seasonal wet depositional flux was dominated by methyl parathion (88%) and was >4.5 times the 2007 flux. Total herbicide flux in 2007 was slightly greater than in 1995 and was dominated by glyphosate. Malathion, methyl parathion, and degradation products made up most of the 2007 nonherbicide flux.	
Proposed action: Not to be considered as publication deals with air and rain monitoring outside EU.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as the articles deals with air and rain monitoring outside EU.	
Reliability	
Endpoint	Concentration of pesticides in air and rain samples
Protocol	Not given
Test compound	Pesticides (Glyphosate and AMPA (amongst others))
Test system and conditions	Weekly composite air and rainfall samples were collected during the growing season (March through September) in 1995 and 2007. The sampling sites were located in the Delta area of the lower Mississippi River watershed in west-central Mississippi. The 1995

	sampling site was located at the center of a catfish farm pond complex near the town of Rolling Fork, Mississippi, and was approximately 500m from the nearest agricultural fields. This site was selected to minimize the influence of pesticide applications on nearby cotton, corn, alfalfa, and soybean fields. The 2007 sampling site was located near Pace, Mississippi, approximately 100 km north of the 1995 site and within approximately 3 m of a soybean field in an area surrounded by both soybean and rice fields. The analytical methods used in 1995 and 2007 were very similar overall, but there were a few differences, primarily in the number of target analytes and in the applied reporting levels.
Statistical design	Not given
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Martin et al. (2011)

Title: Seasonal Changes of Macroinvertebrate Communities in a Stormwater Wetland Collecting Pesticide Runoff From a Vineyard Catchment (Alsace, France)
Author: Sylvain Martin, Aurélie Bertaux, Florence Le Ber, Elodie Maillard, Gwenael Imfeld
Reference: Arch Environ Contam Toxicol (2012) 62:29–41
Year: 2011
Results and conclusion: Agricultural land use may influence macroinvertebrate communities by way of pesticide contamination associated with agricultural runoff. However, information about the relation between runoff-related pesticides and communities of benthic macroinvertebrates in stormwater wetland that receive agricultural runoff does not currently exist. Here we show changes in macroinvertebrates communities of a stormwater wetland that collects pesticidecontaminated runoff from a vineyard catchment. Sixteen runoff-associated pesticides, including the insecticide flufenoxuron, were continuously quantified at the inlet of the stormwater wetland from April to September (period of pesticide application). In parallel, benthic macroinvertebrate communities, pesticide concentrations, and physicochemical parameters in the wetland were assessed twice a month. Twenty-eight contaminated runoffs ranging from 1.1 to 114 m3 entered the wetland during the study period. Flufenoxuron concentrations in runoff-suspended solids ranged from 1.5 to 18.5 lg kg-1 and reached 6 lg kg-1 in the wetland sediments. However, flufenoxuron could not be detected in water. The density, diversity, and abundance of macroinvertebrates largely varied over time. Redundancy and formal concept analyses showed that concentrations of flufenoxuron, vegetation cover, and flow conditions significantly determine the community structures of stormwater wetland macroinvertebrates. This study shows that flow conditions, vegetation cover, and runoff-related pesticides jointly affect communities of benthic macroinvertebrates in stormwater wetlands.
Proposed action: Not to be considered for listing in the summarizing table as pesticide concentrations are presented in graphs only.
Type of information (critical, high/low weight, supporting, additional):

Low weight, additional information	
Reliability	Medium
Endpoint	Pesticide inputs into stormwater wetlands
Protocol	Pesticide analysis was performed according to the NF XPT 90–210 French standards at the Pasteur Institute of Lille (France), which is a service of pesticide residues analysis accredited by the French National Accreditation Authority (COFRAC).
Test compound	Azoxystrobin, cymoxanil, cyprodinil, dimethomorph, diuron, gluphosinate, glyphosate (CAS-no.: 1071-83-6), isoxaben, kresoxim methyl, metalaxyl, simazine, terbuthylazine, tetraconazole, 3,4-dichlorophenyl urea (DCPU), DCPMU, 3,4-dichloroaniline and AMPA (CAS-no.: 1066-51-9)
Test system and conditions	The stormwater wetland has a surface area of 319 m^2 and a total volume of 1500 m3 and was constructed in 2002 to control flood in the downstream urban area. Water samples for hydrochemical and pesticide analyses were collected at the inlet and in the sediment-deposition zone of the wetland from April 6 through September 29, 2009. Runoff discharges were continuously monitored by water-depth measurements using bubbler flow modules (Hydrologic; Sainte-Foy, Que´bec, Canada) combined to a Venturi channel. Water samples were collected every 6 m^3 at the inlet of the wetland using a 6712FR ISCO Teledyne automatic sampler (ISCO, Lincoln, NE, USA). Water samples (100 mL) were collected in jars and were stored in the dark at 4————————————C. The water samples systematically were collected after each runoff event and placed on ice during transportation to the laboratory.
Statistical design	Pesticide loads at the inlet of the wetland that correspond to a single runoff event were obtained by multiplying mean pesticide concentrations by the corresponding runoff volume. Loads at the inlet of the wetland were calculated from the integral sum of all event loads between two sampling campaigns.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	The results are supported by other publications.

Messing et al. (2011)

Title: Predicting wetland contamination from atmospheric deposition measurements of pesticides in the Canadian Prairie Pothole region
Author: Paul G. Messing, Annemieke Farenhorst, Don T. Waite, D.A. Ross McQueen, James F. Sproull, David A. Humphries, Laura L. Thompson
Reference: Atmospheric Environment 45, 7227-7234
Year: 2011
Results and conclusion: This is the first field study to compare the masses of pesticides entering wetlands by atmospheric deposition with those concentrations of pesticides detected in the water-column of prairie wetlands. Weekly air and bulk deposition samples were collected from May 26th

to Sept. 15th, 2008 at the Manitoba Zero Tillage Research Association (MZTRA) Farm, Brandon, Manitoba, with four on-site wetlands (approximate sizes 0.15-0.45 ha) monitored every second week. Twelve pesticides were detected in the air, with MCPA (one of the three pesticides applied on the farm in 2008 in addition to clopyralid and glyphosate), trial late, and g-HCH being detected every week. Calculations were performed to predict wetland pesticide concentrations based on bulk deposits alone for those pesticides that had detectable concentrations in the bulk deposition samples (in order of the highest total seasonal deposition mass to the lowest): MCPA, glyphosate, 2,4-D, clopyralid, bromoxynil, atrazine, dicamba, metolachlor, and mecoprop. The estimated concentrations were closest to actual concentrations for MCPA (Pearson correlation coefficient's ¼ 0.91 to 0.98; p-values < 0.001) and predictions were also reasonable for a range of other herbicides, but a source other than atmospheric deposition was clearly relevant to detections of clopyralid in the wetland water-column. Although the types and levels of pesticides detected in the wetlands of the current study suggest that regional pesticide applications can contribute to pesticide surface water contamination following atmospheric transport and deposition, the greater frequency and concentrations of clopyralid, MCPA, and glyphosate detections in wetlands confirm that on-farm pesticide applications have a greater impact on on-site water quality. Beneficial management practices that reduce application drift, as well as rainfall or snowmelt runoff, will be important measures in reducing pesticide loading into wetlands situated in agricultural fields of the Prairie Pothole Region of North America.	
Proposed action: Not to be considered for listing in the summarizing table as raw data are insufficient.	
Type of information (critical, high/low weight, supporting, additional): Medium weight; additional information	
Reliability	High
Endpoint	Maximum weekly averaged atmospheric concentrations of herbicides; maximum weekly bulk (wet + dry) deposition; maximum wetland grab sample concentrations
Protocol	QA/QC protocols were followed including using field blanks, surrogate solutions as a check on extraction efficiency, matrix spikes, and blanks
Test compound	Glyphosate (CAS-no.: 1071-83-6), MCPA, glufosinate, Bromoxynil, 2,4-D, Ethalfluralin, Trifluralin, Dicamba, Clopyralid, Atrazine, Triallate, Mecoprop, Metolachlor, AMPA (CAS-no.: 1066-51-9), a-HCH, g-HCH
Test system and conditions	Air was sampled continuously; water samples were taken (grab samples) from the four wetlands every second week for a total of 9 sampling events.
Statistical design	No information
Relevance	
Environmental relevance	Given in supplementary material at doi:doi:10.1016/j.atmosenv.2011.08.074.
Weight of evidence	
"Positive"/"Negative" evidence	The results are supported by other publications.

Queiroz et al. (2011)

Title: GLYPHOSATE TRANSPORT IN RUNOFF AND LEACHING WATERS IN AGRICULTURAL SOIL
Author: Gabriela Marina Pompeo Queiroz, Marcos Rivail da Silva, Renata Joaquim Ferraz

Bianco, Adilson Pinheiro, Vander Kaufmann	
Reference: Quim. Nova, Vol. 34, No. 2, 190-195	
Year: 2011	
Results and conclusion: Glyphosate was determined in runoff and leaching waters in agricultural soil that received an application of active ingredient and was exposed to simulated intensive rain conditions. The concentrations decreased during the simulation period and the concentrations of the runoff were higher than those achieved in the samples of leaching waters. The concentrations were lower than the pattern in the Brazilian Regulation MS N. 518/2004 for drinking water. The transported load of the applied active ingredient by the leaching was of 15.4% (w/w) and for the runoff was of 1.7% (w/w).	
Proposed action: Not to be considered as the data are obtained from a site outside the EU (Brasil).	
Type of information (critical, high/low weight, supporting, additional): Low weight, additonal information on Glyphosate concentrations in runoff and leachate waters in Brasil .	
Reliability	Low
Endpoint	Concentrations in leaching and runoff waters after application of Glyphosate
Protocol	No standard protocol, non-GLP
Test compound	Glyphosate (CAS-no.: 1071-83-6; purity 99.7 %)
Test system and conditions	Outdoor trials, 1m^2 plots, lysimeters
Statistical design	? (english summary only, text Spanish)
Relevance	
Environmental relevance	? (english summary only, text Spanish)
Weight of evidence	
"Positive"/"Negative" evidence	? (english summary only, text Spanish)

Ramwell, C.T. et al. (2014)

Title: Contribution of household herbicide usage to glyphosate and its degradate aminomethylphosphonic acid in surface water drains
Author: C.T. Ramwell, M. Kah, P. D. Johnson
Reference: Pest Manag Sci (2014), Published online in Wiley Online Library: (wileyonlinelibrary.com) DOI 10.1002/ps.3724
Year: 2014
Results and conclusion: The aim of this study was to quantify the widely used herbicide glyphosate and its degradation product aminomethylphosphonic acid (AMPA) in surface water drains (storm drains) that could be attributed to amateur, non-professional usage alone. Results: Maximum glyphosate and AMPA concentrations in surface water drains were 8.99 and 1.15 µL^{-1} respectively after the first rain event following the main application period, but concentrations rapidly declined to < 1.5 and < 0.5 µL^{-1}. The AMPA:glyphosate ratio was typically 0.35. Less than 1% of the applied glyphosate was recovered in drain water. In detail: Of 148 houses in the catchment, 82 separate households were interviewed and, of these, 34 agreed to participate in the study. The study area was typical of a middle-class housing estate in the United Kingdom. The majority of applications occurred within the first 2 weeks of the study, with a notable

53 g of glyphosate being applied on a single day. More than half of this application could be attributed to a single person who applied 5 L (and therefore 36 g of Roundup) over a period of 2 days primarily to an area of ~10m^2 that had a high weed infestation rate of >50% for weeds that were ~10 cm high. It clearly states on the label that 5 L of Round-up can treat an area of 150m^2; thus, even when accounting for errors in the estimation of the area, over application was considerable. A total of 76.5 g of glyphosate was applied within the catchment during a period of approximately 1 month.
The first rain event after the main application period occurred on 3 July 2009 (2 weeks after the first recorded application), and three further events were monitored. The highest concentrations of glyphosate (8.99 µgL^{-1}) and AMPA (1.15 µgL^{-1}) occurred during this first rain event, although the concentrations rapidly declined within the first hour to <2 µg L^{-1}, with the final sample taken containing <1 µg L^{-1}. A short rain event on the following day (4 July 2009) generated further samples (after a further 0.79 g of glyphosate had been applied in the catchment), with peak concentrations of 2.08 µg L^{-1} of glyphosate and 0.66 µg L^{-1} of AMPA. Glyphosate concentrations in the last monitored rain event were <1 µg L^{-1}, in spite of more than 4 g of glyphosate being applied in the intervening dry period between sampling events. AMPA concentrations ranged from 0.17 to 0.54 µg L^{-1} in this last event. These concentrations are the same order of magnitude as the initial 'background' samples. It should be noted that the glyphosate and AMPA concentrations reported here are those measured in the surface water drains, where there is relatively low discharge and therefore low dilution, and they are not representative of concentrations in surface water, where it would be expected that significant dilution would occur.
Although over 71 g of glyphosate was applied prior to the first monitored post-application rain event, less than 0.5% of this glyphosate was detected in surface water drain flow, even when accounting for both the glyphosate +AMPA. Samples collected on the next day, the second rain event after application, added very little glyphosate and AMPA to the total loss, such that the accumulated loss as a percentage of amount applied was still <0.5%. Between 0.56 and 0.81% (for the measured and extrapolated data respectively) of the applied glyphosate had been recovered in drain flow by the end of the sampling period. These findings highlight that only a very small percentage of the applied glyphosate is recovered in surface water drains, and it is assumed that the majority of the applied glyphosate is retained in the catchment and/or degraded. Glyphosate has previously been detected in the subsoil beneath and in the sand/soil in-between bricks after application, 15 confirming infiltration as a retention mechanism of glyphosate for bricked surfaces. The presence of glyphosate in the background sample indicated that more glyphosate was likely to have been applied than was accounted for, which means that the quantity of glyphosate recovered as a percentage of that applied (up to 0.81%) would be lower in reality. Extrapolating the known usage from the households surveyed (76.5 g glyphosate used by 34 out of 82 households) to the total number of households in the catchment (n=148) would give a total of 138 g of glyphosate applied. The quantity of glyphosate detected in the drains would then equate to 0.31 or 0.45% of the amount applied using the measured and extrapolated sampling data respectively.
Conclusion: Glyphosate and AMPA losses from urban areas that arise solely from amateur usage have been quantified. In spite of overdosing occurring, glyphosate concentrations in drain flow were lower than concentrations reported elsewhere from professional use in urban areas.

Proposed action:
Consider as additional information.

Type of information (critical, high/low weight, supporting, additional):
Additional information due to the fact that the representativeness of the study for other EU Member States conditions is called into question.

Reliability	
Endpoint	Glyphosate and AMPA concentrations in surface water drains, % glyphosate recovered in drain flow, AMPA:glyphosate ratio
Protocol	For details see under test system and conditions, non-GLP
Test compound	Plant protection products containing glyphosate (e.g. Fast Action Roundup Ready-To-Use weedkiller and Pathclear)
Test system and conditions	**Study site** A small, residential catchment (5.16 ha) where the houses had separate foul sewers and surface water drains was identified in York, England. There were 148 houses, detached or semi-detached, in the catchment. All the houses had front and back gardens which were, typically, very well maintained and neat. The surface water drains fed into a single collection point, enabling monitoring of the entire catchment. The areas of different land use ('soft' and 'hard' surfaces) were measured from aerial maps and groundtruthing. These were compared with existing data to ascertain the representativeness of the catchment. **Water sampling** Two ISCO 6172 automatic water samplers were installed to sample water (120 mL) from the final drain every 5min, with the water from three consecutive samples being directed to a single bottle, giving one composite sample (360 mL) every 15 min. One sampler was triggered when rainfall exceeded 0.4mm within 2 h; the other was triggered when the water level in the drain was >0.01 m. This approach was taken to minimise missing a sampling event because of equipment failure. Rainfall was monitored using a tipping-bucket rain gauge (resolution 0.1 mm) sited on top of one of the boxes used to house the water samplers. Discharge was measured using an ISCO 750 area/velocity flow module. The study was undertaken in early summer (June–July 2009) when herbicide applications in private gardens are common in response to the favourable weather conditions for weed growth. Samples were taken during the first rain event (15 June 2009) after the equipment was installed (22 May 2009) and prior to the survey of the residents in order to monitor any 'background' levels of glyphosate. After that, samples were collected in response to all rain events until the end of July 2009. Samples were collected within 24 h. Samples were decanted from the glass collection bottles into high-density polyethylene (HDPE) bottles on return to the laboratory and stored in the freezer until dispatched for analysis. **Glyphosate usage in the catchment** The inputs of glyphosate into the catchment were established by means of a questionnaire. All houses in the catchment were approached by door-to-door visits over a period of 5 days during the day, in the evening and at the weekends. Houses were approached 4 times before they were excluded from the study owing to lack of contact. The aim of the study was explained to the participants in more detail, and they were asked questions regarding the types of pesticide used, timing and frequency of use and application method. The participants were requested to keep a note of pesticide

usage on a pro forma, recording details of when and where a product was used, the nature of the surface type and the level of weed infestation as defined by a picture card with examples of weed infestation classes. No other instructions were given. When the pro formas were collected, participants were then questioned with regard to storage, disposal, safety precautions and the ease of use of the products. Fast Action Roundup Ready-To-Use (RTU) weedkiller (glyphosate 7.2 g L^{-1} MAPP 14481) in either a 1 L trigger sprayer or a 5 L 'pump and spray' container was supplied to those participants who requested it, or participants used products that they already had (n=2; Tesco's own-brand glyphosate and Pathclear – containing glyphosate, oxadiazon + diflufenican). The 1 L bottles were weighed before and after use in order to quantify the amount used. This was not possible with the 5 L RTUs as these were too heavy for field-portable scales. For the 5 L containers and where other products were used, the glyphosate usage was calculated from knowledge of the weed density, the size of the area treated and the intended rate of application (i.e. 33 mL treats 1 square metre). It was necessary to estimate the amounts applied for 39% of the residents. Similarly, only the total quantity of glyphosate used per household was known, so the amount used per application was calculated from knowledge of the weed density and area treated, as indicated on their pro forma, in order to distribute the total amount of glyphosate spray solution used between each application date. The local authority was contacted in order to ascertain the nature of weed control on the roads within the catchment, which was ordinarily a subcontractor using glyphosate. The subcontractors postponed any treatment until the study was completed.

Data analysis

Measurements of concentration and discharge were used to calculate the total mass of glyphosate leaving the catchment. Discharge measurements were collected every minute, whereas bulk drain water samples were collected every 15 min. It was therefore necessary to extrapolate the chemical data. It was assumed that there was a linear increase or decrease in concentration between successive samples, enabling a concentration per minute to be estimated. In addition, two total masses per rainfall event were calculated. The first was the total load between the first and last measured concentration. However, as this was not always the very first or very last sample generated, because some samples had insufficient volume for analysis, a second calculation was made where a concentration of zero was assumed as soon as the water sampler was triggered, and concentrations up to the first analysed sample were calculated by linear extrapolation as described above. Similarly, towards the end of the sampling event, a concentration of zero was assumed when the discharge became constant. It is recognised that this approach has limitations as it is probable that, while glyphosate concentrations are likely to decrease, they will not be zero. This second extrapolated calculation of total mass is to account for the absence of samples at either end of the event and to avoid miss-

	ing high discharges, with a potentially substantial influence on load, at the start of an event. These total masses are referred to as 'measured' and 'extrapolated' respectively. The final total glyphosate loss per event was calculated from the sum of the loads for glyphosate + AMPA, where the final mass of AMPA was calculated from initial mass of AMPA × (molecular weight of glyphosate/molecular weight of AMPA). **Calculation of predicted stream concentrations from measured loads** The volume of water (L) in a stream available for dilution during a rain event is calculated from [Root square of catchment area (m) × water depth × (0.3 m) × 1000] + volume of run-off (L). This formula was used to estimate a glyphosate concentration in an urban stream by dividing the measured loads by the calculated stream volume (full details in HardSPEC model).
Statistical design	See under test system and conditions
Relevance	
Environmental relevance	Given.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Ruel et al. (2010)

Title: On-site evaluation of the efficiency of conventional and advanced secondary processes for the removal of 60 organic micropollutants
Author: S. Martin Ruel, M. Esperanza, J.-M. Choubert, I. Valor, H. Budzinski and M. Coquery
Reference: Water Science & Technology, 2.12/2010
Year: 2010
Results and conclusion: The next challenge of wastewater treatment is to reliably remove micropollutants at the microgram per litre range in order to reduce the discharge for priority substances and to meet the environmental quality standards set by the European Water Framework Directive. The present work assessed the occurrence of 60 organic substances (priority substances and other relevant pollutants) in municipal wastewater and sludge. Their fate in the treatment processes and their removal efficiencies were quantified. Thorough on-site mass balances were carried out at 8 municipal wastewater treatment plants chosen among conventional and advanced secondary processes. It was found that 70% of the substances were quantified in raw wastewater and 50% in effluent, with a transfer without a limited degradation for most of them. Low loaded activated sludge (AS) process reduced the emission of more than half of micropollutants. At low sludge retention time (AS under high load), lower removal efficiencies were measured compared to low loaded AS. No influence of temperature of the biological reactor was shown. The membrane bioreactor process increased the removal efficiencies for one third of the substances that were partially removed with AS. Still, five substances were measured at concentrations exceeding the environmental quality standards at the outlet of the studied plants. In addition to efforts for source-reduction, complementary treatments need to be set-up. Glyphosate was quantified in more than 70% of the samples. Three substances with concentration higher than 0.1 mg/L in raw wastewater were removed to less than 30% (glypho-

sate, AMPA, diuron), thus passing through the process almost unaffected.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Medium weight, additional information	
Reliability	Medium
Endpoint	No endpoint was determined
Protocol	Non-GLP
Test compound	Monitoring
Test system and conditions	Eight WWTP were studied, representative of various sizes, types of sewer networks (rural vs. urban, combined vs. separate) and types of treatment processes. They included 7 activated sludge lines with different operating conditions (F/M ratio, sludge retention time, temperature) and 1 membrane bioreactor (MBR) process (Ultrafor) equipped with 4 Zenon ZW500d modules (hollow-fiber) for a total membrane surface of 10,000m2. Daily average composite samples were collected on the influent (inlet), effluent (outlet), waste sludge and return of sludge dewatering during 2 or 3 successive 24 hperiod and under dry weather flow conditions.
Statistical design	Mass balances were performed to determine the fluxes of micropollutants at the inlet and outlet of the WWTP to calculate their removal efficiencies. Calculation is complex due to the variability of concentrations in raw wastewater, to the different WWTP that are considered and to analytical uncertainties. In order to obtain robust data, the following rules were elaborated: 1. Two confidence levels (high/low) were defined for each substance and each type of matrix (raw wastewater, treated water and sludge) with respect to the LoQ. Low confidence level was for concentrations between LoQ and 5–10 times the LoQ. High confidence level was for concentrations higher than 5–10 times the LoQ. From analytical practice, at low confidence level a 50–100% analytical uncertainty is a regular value for most substances whereas an analytical uncertainty below 30% is usual for high confidence level; 2. When both inlet and outlet concentrations were included within the low level, or if they were lower than the LoQ, the removal efficiency value was not calculated to address the fact that the analyticaluncertainty gets higher for values close to the limit of quantification; 3. When a concentrationamong inletoroutlet concentrations was lower than the LoQ, a value equal to half of the LoQ was adopted and the removal efficiency was calculated. The quantification frequency (QF) of each substance, defined as number of times that a substance is quantified divided by the number of samples, was calculated for raw wastewater, treated effluent and treated sludge.
Relevance	
Environmental relevance	Given

Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence. The results are supported by other publications.

Ruel et al. (2011)

Title: On-site evaluation of the removal of 100 micro-pollutants through advanced wastewater treatment processes for reuse applications	
Author: S. Martin Ruel, J. M. Choubert, M. Esperanza, C. Miège, P. Navalón Madrigal, H. Budzinski, K. Le Ménach, V. Lazarova and M. Coquery	
Reference: Water Science & Technology, 63.11/2011	
Year: 2011	
Results and conclusion: The next challenge of wastewater treatment is to reliably remove micro-pollutants at the microgram per litre range in order to meet reuse applications and contribute to reach the good status of the water bodies. A hundred priority and relevant emerging substances were measured to evaluate at full-scale the removal efficiencies of seven advanced treatment lines (one membrane bioreactor process and six tertiary treatment lines) that were designed for reuse applications. To reliably compare the processes, specific procedures for micro-pollutants were applied for sampling, analysis and calculation of removal efficiencies. The membrane bioreactor process allowed to upgrade the removal efficiencies of about 20% of the substances measured, especially those that were partially degraded during conventional processes. Conventional tertiary processes like high rate clarification, sand filtration and polishing pond achieved significant removal for some micro-pollutants, especially for adsorbable substances. Advanced tertiary processes, like ozonation, activated carbon and reverse osmosis were all very efficient to complete the removal of polar pesticides and pharmaceuticals; metals and less polar substances were better retained by reverse osmosis.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Medium weight, additional information	
Reliability	Medium
Endpoint	No endpoint was determined
Protocol	Non-GLP
Test compound	Monitoring
Test system and conditions	Seven WWTP of various sizes were studied, which included various types of treatment: one full-scale MBR; five full-scale conventional tertiary treatments, including high rate clarification, sand filtration or polishing pond; two advanced tertiary treatments at full-scale (ozonation and micro-filtration (MF) þ reverse osmosis (RO)) and two advanced tertiary treatments at pilot-scale (activated carbon filtration and silex filtration þ ultrafiltration þ RO). The upstream treatment stages achieved both carbon and nitrogen removal to meet regulatory requirements. Influent and effluents of the studied processes were collected under dry weather flow conditions during two successive 24 h or 2 h periods
Statistical design	Mass balances were performed based on wastewater flow and micro-pollutant concentration data at the inlet and at the outlet of the studied processes. The removal efficiencies (R) were calculated

	with the following rules to obtain robust information: – High and low levels of concentration were defined for each substance with respect to the LoQ. Low confidence level was for concentrations between LoQ and 2.5–5 times the LoQ (depending on the substance). High confidence level was for concentrations higher than 2.5–5 times the LoQ, depending on the substance. From analytical practice, at low confidence level, an analytical uncertainty in the range of 50–100% is a regular value for most substances whereas an analytical uncertainty below 30% is usual a high confidence level. – When both inlet and outlet concentrations were lower than the LoQ or within the low level, the removal efficiency value was not calculated. – When only one concentration, either inlet or outlet concentration, was lower than the LoQ, a value equal to half of the LoQ was adopted and the removal efficiency was calculated. In addition to these criteria, removal efficiency data was displayed as a removal range (<30%, 30–70% and >70%), since the analytical uncertainty and the variability of the concentrations related to micro-pollutants in wastewater do not allow to certify precise values.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence. The results are supported by other publications.

Ruel et al. (2012)

Title: Occurrence and fate of relevant substances in wastewater treatment plants regarding Water Framework Directive and future legislations
Author: S. Martin Ruel, J.-M. Choubert, H. Budzinski, C. Miège, M. Esperanza and M. Coquery
Reference: Water Science & Technology, 65.7/2012
Year: 2012
Results and conclusion: The next challenge of wastewater treatment is to reliably remove micropollutants at the microgram per litre range. During the present work more than 100 substances were analysed through on-site mass balances over 19 municipal wastewater treatment lines. The most relevant substances according to their occurrence in raw wastewater, in treated wastewater and in sludge were identified, and their fate in wastewater treatment processes was assessed. About half of priority substances of WFD were found at concentrations higher than 0.1 µg/L in wastewater. For 26 substances, potential non-compliance with Environmental Quality Standard of Water Framework Directive has been identified in treated wastewater, depending on river flow. Main concerns are for Cd, DEHP, diuron, alkylphenols, and chloroform. Emerging substances of particular concern are by-products, organic chemicals (e.g. triclosan, benzothiazole) and pharmaceuticals (e.g. ketoprofen, diclofenac, sulfamethoxazole, carbamazepine). About 80% of the load of micropollutants was removed by conventional activated sludge plants, but about two-thirds of removed substances were mainly transferred to sludge.

Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Medium weight, additional information	
Reliability	Medium
Endpoint	No endpoint was determined
Protocol	Non-GLP
Test compound	Monitoring
Test system and conditions	Overall, 19 WWTP treatment lines were studied, chosen as representative of various sizes (100 to 1,000,000 PE and of various types of treatment processes. Sampling was performed in the influent and effluent during two or three successive 24 h-periods under dry weather flow conditions, with refrigerated samplers equipped with Teflon pipes and glass containers. Grab samples were collected for treated sludge. Strict procedures of cleaning, sampling, and field blanks were carried out.
Statistical design	The results were described using: • The frequency of quantification (Fq) and total concentration in influents, effluents and sludges. • The specific daily average load received at WWTP (g/d/PE), calculated for each substance. • The removal rate for different processes, with some calculation rules to take into account the variability of concentrations in raw wastewater and the analytical uncertainties associated with lowconcentrations of substances in complex matrices. If inlet concentration was not higher than 10 times the limit of quantification, removal efficiency was not calculated. Additionally, resultswere displayed as a removal efficiency range : 0–30%, 30–70% or 70–100%.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence. The results are supported by other publications.

Screpanti et al. (2005)

Title: Glyphosate and glufosinate-ammonium runoff from a corn-growing area in Italy
Author: Claudio Screpanti, Cesare Accinelli, Alberto Vicari, Pietro Catizone
Reference: Agron. Sustain. Dev. 25, 407-412
Year: 2005
Results and conclusion: The main objective of this experiment was to estimate field-scale runoff losses of glyphosate and glufosinate-ammonium under natural rainfall conditions. Glyphosate and glufosinate-ammonium were applied as pre-emergence herbicides on 350 m² field plots characterized by a uniform slope of 15%. Field plots were cultivated with corn. The persistence and sorption isotherms of the two herbicides were also determined. During the 3-year experimental period low runoff volumes were observed. More specifically, annual runoff volumes did not exceed 4.7 mm. Glyphosate and glufosinate-ammonium concentrations in

collected runoff samples rapidly declined with time. The highest glyphosate and glufosinate-ammonium concentrations were 16 and 24µg/L, respectively. These peaks were observed in a runoff event occurring 1 day after herbicide treatment. The total maximum amounts of glyphosate and glufosinate-ammonium losses were 0.031 and 0.064‰ of the applied active ingredients, respectively. On the basis of the obtained results, both glyphosate and glufosinate-ammonium showed low potential to contaminate surface water resources. These results were supported by their estimated short persistence and strong sorption in soil. The half-lives of glyphosate and glufosinate-ammonium were 17.5 and 6.4 days, respectively, and their distribution coefficients (K_d) were 746.6 and 23.4 mL/g, respectively.	
Proposed action: Not to be considered in the summarizing table and discussion as results are shown in a figure only without listing exact data on concentrations, i.e. results are not sufficiently precisely reported.	
Type of information (critical, high/low weight, supporting, additional): Medium weight; additional information of degradation and sorption isotherms	
Reliability	Low
Endpoint	Outflow coefficient, herbicide half-life; distribution coefficient K_d, r^2
Protocol	Non-GLP; similar to OECD 106
Test compound	Roundup Bioflow (isopropylamine salt of glyphosate, 360 g a.i./L formulation; CAS-no.: 40465-66-5) Basta (ammonium salt of glufosinate-ammonium, 120 g a.i./L formulation)
Test system and conditions	1) Field plot management: 8 plots were cultivated with corn. Glyphosate and glufosinate-ammonium were applied shortly after seeding. 2) Laboratory investigations: a) Herbicide persistence: 5 g of soil were treated with glyphosate or glufosinate-ammonium (10µg a.i./g soil) and were incubated at 25±0.5°C for 21 days. Triplicate samples were removed at 0, 1, 7, 14 and 21 days. b) Sorption isotherms: Sorption isotherms were determined using triplicate samples at 5 initial herbicide concentrations (0.05 to 10µg/mL). Samples were shaken for 12 hours.
Statistical design	3 - 4 replicates, first-order kinetics, regression analyses (Statitica ver. 6.1); analysis of variance
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence; results supported by other publications

Shipitalo et al. (2008)

Title: Impact of Glyphosate-Tolerant Soybean and Glufosinate-Tolerant Corn Production on Herbicide Losses in Surface Runoff
Author: Martin J. Shipitalo, Robert W. Malone, and Lloyd B. Owens
Reference: Journal of Environmental Quality 37:401–408
Year: 2008
Results and conclusion: Residual herbicides used in the production of soybean and corn are often detected in surface runoff at concentrations exceeding their maximum contaminant levels (MCL) or

health advisory levels (HAL). With the advent of transgenic, glyphosate-tolerant soybean and glufosinate-tolerant corn this concern might be reduced by replacing some of the residual herbicides with short half-life, strongly sorbed, contact herbicides. We applied both herbicide types to two chiseled and two no-till watersheds in a 2-yr corn-soybean rotation and at half rates to three disked watersheds in a 3-yr corn/soybean/wheat -red clover rotation and monitored herbicide losses in runoff water for four crop years. In soybean years, average glyphosate loss (0.07%) was ~1/7 that of metribuzin (0.48%) and about one-half that of alachlor (0.12%), residual herbicides it can replace. Maximum, annual, flow-weighted concentration of glyphosate (9.2 µg/L) was well below its 700 µg/L MCL and metribuzin (9.5 µg/L) was well below its 200 µg/L HAL, whereas alachlor (44.5 µg/L) was well above its 2 µg/L MCL. In corn years, average glufosinate loss (0.10%) was similar to losses of alachlor (0.07%) and linuron (0.15%), but about one-fourth that of atrazine (0.37%). Maximum, annual, flow-weighted concentration of glufosinate (no MCL) was 3.5 µg/L, whereas atrazine (31.5 µg/L) and alachlor (9.8 µg/L) substantially exceeded their MCLs of 3 and 2 µg/L, respectively. Regardless of tillage system, flow-weighted atrazine and alachlor concentrations exceeded their MCLs in at least one crop year. The glyphosate and glufosinate concentrations never exceeded their established or proposed standards either on an annual flow-weighted basis or for an individual event, even when runoff occurred within 1 d after application. Thus, by growing transgenic, glyphosate-tolerant soybean and glufosinate-tolerant corn and by completely or partially replacing these residual herbicides with glyphosate or glufosinate the environmental impact of herbicide losses in runoff resulting from production of these crops should be reduced.	
Proposed action: Not to be considered as study deals with a site outside the EU (USA).	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information: that by growing transgenic, glyphosate-tolerant soybean and glufosinate-tolerant corn and by completely or partially replacing these residual herbicides with glyphosate or glufosinate the environmental impact of herbicide losses in runoff resulting from production of these crops should be reduced.	
Reliability	Medium
Endpoint	Flow-weighted average concentrations for each runoff event were computed using the concentrations measured in individual samples and runoff volumes obtained from the hydrographs. These values were then used to determine annual flow weighted concentrations.
Protocol	Non-GLP
Test compound	Alachlor, Atrazine, Atrazine, DEA, and DIA, Linuron, Metribuzin, Glyphosate (CAS-no.: 1071-83-6), AMPA (1066-51-9), Glufosinate
Test system and conditions	The transport of contact and residual herbicides was investigated for 4 crop years using seven small watersheds that were instrumented to automatically measure and sample surface runoff. Four watersheds were cropped in a 2-yr corn–soybean rotation, two receiving no tillage and the other two were chisel plowed each year. The three remaining watersheds were farmed in a 3-yr, corn/soybean/wheat-clover rotation with disking as the primary tillage operation during corn and soybean years. During runoff the samplers collected discrete samples (~300 mL) every 10 min for the first 100 min, every 20 min for the next 200 min, and every 60 min thereafter until the capacity of the samplers was reached or runoff ceased.
Statistical design	-

Relevance	
Environmental relevance	Environmental parameter partly reported and partly reported in other studies
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence

Shipitalo and Owens (2011)

Title: Comparative Losses of Glyphosate and Selected Residual Herbicides in Surface Runoff from Conservation-tilled Watersheds Planted with Corn or Soybean	
Author: Martin J. Shipitalo and Lloyd B. Owens	
Reference: J. Environ. Qual. 40:1281–1289	
Year: 2011	
Results and conclusion: Residual herbicides regularly used in conjunction with conservation tillage to produce corn and soybean are often detected in surface water at concentrations that exceed their U.S. maximum contaminant levels (MCL) and ecological standards. These risks might be reduced by planting glyphosate-tolerant varieties of these crops and totally or partially replacing the residual herbicides alachlor, atrazine, linuron, and metribuzin with glyphosate, a contact herbicide that has a short half-life and is strongly sorbed to soil. Therefore, we applied both herbicide types at typical rates and times to two chisel-plowed and two no-till watersheds in a 2-yr corn/ soybean rotation and at half rates to three disked watersheds in a 3-yr corn/soybean/wheat–red clover rotation and monitored herbicide losses in surface runoff for three crop years. Average dissolved glyphosate loss for all tillage practices, as a percentage of the amount applied, was significantly less ($P \leq 0.05$) than the losses of atrazine (21.4x), alachlor (3.5x), and linuron (8.7x) in corn-crop years. Annual, flow-weighted, concentration of atrazine was as high as 41.3 µg/L, much greater than its 3 µg/L MCL. Likewise, annual, flow weighted alachlor concentration (MCL = 2 µg/L) was as high as 11.2 and 4.9 µg/L in corn- and soybean-crop years, respectively. In only one runoff event during the 18 watershed-years it was applied did glyphosate concentration exceed its 700 µg/L MCL and the highest, annual, flow-weighted concentration was 3.9 µg/L. Planting glyphosate-tolerant corn and soybean and using glyphosate in lieu of some residual herbicides should reduce the impact of the production of these crops on surface water quality.	
Proposed action: Not to be considered as study deals with a site outside the EU (USA).	
Type of information (critical, high/low weight, supporting, additional): Medium weight; additional information: Annual, flow-weighted glyphosate concentrations were much less than its drinking water standard in each of the 18 watershed years it was applied. It is critical to maintain a diversity of weed management practices in the face of the evolution of glyphosate-resistant weeds.	
Reliability	Medium
Endpoint	Concentrations in individual samples were used to calculate flow-weighted average concentrations for each runoff event and annual flow weighted concentrations
Protocol	Non-GLP
Test compound	Alachlor, Atrazine, Atrazine, DEA, and DIA, Linuron, Metribuzin, Glyphosate (CAS-no.: 1071-83-6), AMPA (1066-51-9)
Test system and conditions	Losses of glyphosate in surface runoff were compared with losses of commonly used residual herbicides for three crop years, using seven small watersheds used to grow glyphosate-tolerant corn and

	soybean. Two watersheds were in 2-yr, no-till corn/soybean rotation and two watersheds were in the same rotation but were chisel plowed before planting. Cereal rye was drilled into these watersheds following soybean harvest and served as a cover crop. Three watersheds were in a 3-yr, corn/soybean/ wheat–clover rotation and were disked in the spring just before corn and soybean planting. Approximately 300-mL discrete samples for each event every 10 min for the first 100 min, every 20 min for the next 200 min, and then every 60 min until all bottles were full or runoff ended were collected.
Statistical design	1015 runoff events were sampled for the seven watersheds during the 3-yr period
Relevance	
Environmental relevance	Environmental parameter reported in other studies.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Starrett and Klein (2008)

Title: Glyphosate Runoff When Applied to Zoysiagrass under Golf Course Fairway Conditions
Author: Steven K. Starrett and Jamie Klein
Reference: Chapter 14, pp 237-253, in: The Fate of Nutrients and Pesticides in the Urban Environment, Nett MT et al. (eds). ACS Symposium Series, Volume 997. DOI: 10.1021/bk-2008-0997.ch014
Year: 2008
Results and conclusion: Glyphosate is the primary ingredient of Roundup®, which is the most widely used herbicide by volume in the United States. Glyphosate runoff is therefore an important subject for both existing and newly constructed courses. The objectives of the current research were: (1) to measure glyphosate runoff from zoysiagrass fairways on a golf course following the application of Roundup herbicide, (2) to determine glyphosate runoff concentrations and their resulting effect on the environment, and (3) to provide up-to-date data of research findings on pesticide transport when applied to turfgrass. Previous research on glyphosate runoff from turfgrass has been done on test plots and not full-scale watersheds. In addition, Roundup applications for this study were made by the Colbert Hills Golf Course staff and not prescribed specifically for research purposes. Water quality and quantity monitoring systems were set up on the 115-acre study watershed, which contains a 3-acre detention and irrigation pond. Over 600 water samples were taken from fairway drains, the inlet and outlet of the pond, and the pond itself throughout a three-year study period. Ten of the twenty-three tested samples contained detectable concentrations (>0.10 µg/L) of glyphosate. The maximum observed glyphosate concentration was 5.18 µg/L, which is well below the United States Environmental Protection Agency (USEPA) Drinking Water Maximum Contaminant Level (MCL) of 700 µg/L. These results suggest that Roundup applications made to turfgrass fairways do not cause hazardous levels of glyphosate in downstream surface water.
Proposed action: Not to be considered. Publication deals with situation outside the EU (USA).
Type of information (critical, high/low weight, supporting, additional):

Low weight. Glyphosate concentrations found in tested runoff samples from the 115 acre study watershed, following annual applications of Roundup, were much lower than associated health standards.	
Reliability	Low
Endpoint	Concentration
Protocol	Non-GLP
Test compound	Glyphosate (CAS-no.: 1071-83-6), AMPA (1066-51-9)
Test system and conditions	The objectives of this study were: 1.) To measure glyphosate runoff from zoysiagrass fairways on a golf course following the application of Roundup herbicide. 2.) To determine glyphosate runoff concentrations and the resulting effect on the environment. 3.) To provide up-to-date data of research findings on pesticide fate and transport when applied to turfgrass.
Statistical design	211 runoff samples from the inlet, and 125 samples from the outlet; 61 samples taken from three separate fairway drains; 208 samples were taken from different locations and depths of the pond; no statistics
Relevance	
Environmental relevance	The influencing endpoints are not reported.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Tang., T. et al. (2014)

Title: Losses of glyphosate and AMPA via drainflow in a typical Belgian residential area
Author: T. Tang, W. Boënne, A. v. Griensven, P. Seuntjens, J. Bronders, N. Desmet
Reference: Geophysical Research Abstracts, Vol. 16, EGU2014-3627, 2014, EGU General Assembly 2014
Year: 2014
Results and conclusion: To obtain concurrent high-resolution data for a detailed investigation on the losses of pesticide runoff from hard surfaces, a monitoring campaign was performed in a typical Belgian residential area (9.5 ha) between 7 May and 7 August, 2013. The campaign yielded a concurrent dataset of rainfall (1-mm rainfall interval), discharge (1-min interval), glyphosate application by the residents and the occurrences of glyphosate and its major degradation product aminomethylphosphonic acid (AMPA) in the separated storm drainage outflow during 12 rainfall events. In addition, detailed information was obtained on the spatial characteristics of the study area. The resulting dataset allows us to investigate the relevance of catchment hydrology, urban surface properties and pesticide application to the transport and losses of glyphosate in a residential environment. During the campaign, glyphosate was only applied by local residents, mainly on their private driveways. As a result of their continuous use, both glyphosate and AMPA were detected in all analysed outflow samples, with maximum concentrations of 6.1 µg/L and 5.8 µg/L, respectively. Overall, the storm drainage system collected 0.43% of the applied amount of glyphosate. However, this loss rate varied considerably among rainfall events, ranging from 0.04% to 23.36%. According to statistical analysis of the 12 rainfall events, the loss rate was significantly correlated with three factors: the application amount prior to a rainfall event ($p < 0.005$), rainfall amount during the event ($p < 0.02$) and the weighted

lag time between glyphosate application and the start of the rainfall event (negatively, $p < 0.05$). A regression analysis showed that these three factors can explain more than 85% of the variation in the loss rate of glyphosate. Furthermore, three types of glyphosate runoff were classified by a clustering analysis based on these factors: events dominated by runoff availability (runoff-limited), dominated by glyphosate availability (pesticide-limited) and controlled by both runoff and glyphosate availability. To sum up, proper management of the amount and timing of glyphosate application can greatly help to control its losses from urban impervious surfaces.	
Proposed action: Consider as additional information.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Losses of glyphosate and AMPA from hard surfaces
Protocol	No protocol
Test compound	Glyphosate
Test system and conditions	No exact description of the test system and condition is given, since this the publication by Tang et al. is only an abstract.
Statistical design	No information provided in the abstract
Relevance	
Environmental relevance	Not assessable, since e.g. parameters influencing the losses of glyphosate and AMPA from hard surfaces are not described in detail.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Tournebize et al. (2012)

Title: Co-Design of Constructed Wetlands to Mitigate Pesticide Pollution in a Drained Catch-Basin: A Solution to Improve Groundwater Quality
Author: JULIEN TOURNEBIZE, CHRISTELLE GRAMAGLIA, FRANCOIS BIRMANT, SAMI BOUARFA, CEDRIC CHAUMONT AND BERNARD VINCENT
Reference: Irrig. and Drain. 61 (Suppl. 1): 75–86 (2012)
Year: 2012
Results and conclusion: Numerous situations exist in France in which groundwater is imperfectly protected by a shallow impervious layer in the topsoil, meaning that sinkholes may connect the surface water and relay pollution directly to the aquifer. These impervious layers induce subsurface drainage demand and construction so that the effluent can be collected at outfalls of drainage systems, and be processed in constructed wetlands before falling down the sinkholes, thus decreasing net pollution. The Champigny aquifer corresponds to this description, and has been shown to be highly vulnerable. The mission of AQUI'Brie, a non-profit organisation, is to protect this groundwater, which is one of the water resources of Paris. Although well aware of the benefits of buffer zones, but at this time without incentives to implement them, AQUI'Brie started a co-construction process of constructed wetlands, involving all the stakeholders of a small catchment located upstream from a sinkhole. This paper describes the co-construction from the first meeting to the final structures in a context of high land use pressure. It shows that the number and area of constructed wetlands has diminished beyond the threshold for which the performance of depollution process may

fail. In 2011, a performance assessment programme was set up to determine if the co-constructed wetlands comply with the new current regulations.	
Proposed action: Not to be considered for listing in the summarizing table as raw data are insufficient.	
Type of information (critical, high/low weight, supporting, additional): Medium weight, additional information.	
Reliability	High
Endpoint	Concentration at catchment outlet
Protocol	Non-GLP
Test compound	Monitoring
Test system and conditions	The experimental site is representative of the infiltration of drainage water through sinkholes down into the Champigny aquifer. The catchment is located in the city of Rampillon (0303′37.300 E, 4832′16.700 N, 70 km south-east of Paris, France) and the total drained area is 355 ha (according to drainage maps). The average annual air temperature is 10.5 C, the annual mean rainfall is 689mm and the annual mean potential evaporation is 679 mm. Most of the basin, covered with tableland loess up to 10m thick, is relatively flat and sub-horizontal. The soil of the catchment is mainly Luvisol. A measurement station was installed upstream from the sinkhole at the basin's outfall. Fifteenminute time-step discharge measurements were taken using a wooden controlled section adapted to natural ditch cross section (Birgand et al., 2005) and a height and velocity Doppler sensor. Quality was measured using an automated water sampler set up at a 14-h constant time step. This provides both accurate monitoring during peak flows and a basic weekly restored flow-weight sample. Water samples were measured using LC-MS-MS or GC-MS methods providing a quantification threshold of 0.01 mg l————————————1 for the majority of compounds tested. Continuous monitoring was carried out from September 2007 to September 2009. In 2006–2007, 11 compounds were analysed, 6 out of the 72 compounds applied were detected. In 2007–2008, 68 compounds were applied, 49 analysed and 24 detected. Weather data were provided by a National Weather Broadcast network station, located 6 km from the experimental site.
Statistical design	Not reported
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	The results are supported by other studies. No negative evidence.

Vialle et al. (2013)

Title: Pesticides in roof runoff: Study of a rural site and a suburban site
Author: C. Vialle, C. Sablayrolles, J. Silvestre, L. Monier, S. Jacob, M.-C. Huau, M. Montrejaud-Vignoles
Reference: Journal of Environmental Management 120 (2013) 48-54

Year: 2013	
Results and conclusion: The quality of stored roof runoff in terms of pesticide pollution was assessed over a one-year period. Two tanks, located at a rural and suburban site, respectively, were sampled monthly. The two studied collection surface were respectively a tile slope roof and a bituminous flat roof. Four hundred and five compounds and metabolites were screened using liquid and gas chromatography coupled with various detection systems. Principal Component Analysis was applied to the data sets to elucidate patterns. At the rural site, two groups of compounds associated with two different types of agriculture, vineyard and crops, were distinguished. The most frequently detected compound was glyphosate (83%) which is the most commonly used herbicide in French vineyards. At the suburban site, quantified compounds were linked to agriculture rather than urban practices. In addition, all samples were contaminated with mecoprop which is a roof-protecting agent. Its presence was attributed to the nature of roofing material used for rainwater collection. For both sites, the highest number and concentrations of compounds and metabolites were recorded at the end of spring and through summer. These results are consistent with treatment periods and higher temperatures. In detail: At the rural site, the most frequently detected compounds were glyphosate (83%), DNOC (75%), AMPA (58%), metolachlor (58%), carbendazim (50%), and 2,4-MCPA (50%). Analysis revealed that the highest concentrations measured were for glyphosate (6 µg L^{-1}). In addition, concentrations of several hundreds of ng L^{-1} were measured for AMPA, metolachlor, DNOC and metaldehyde in order of decreasing concentrations. As a reference, limit values in potable water are 0.1 mg L^{-1} per pesticide and 0.5 mg L^{-1} for the sum, according to French regulation.	
Proposed action: To be considered in the summarizing table.	
Type of information (critical, high/low weight, supporting, additional): High weight, additional information to already existing.	
Reliability	
Endpoint	Pesticide concentrations (e.g. glyphosate, AMPA) measured in a roof runoff tank
Protocol	Not given
Test compound	Four hundred and five compounds (e.g. glyphosate, AMPA)
Test system and conditions	Two sites in south-western France were selected to install commercially available domestic rainwater collection systems (Sotralentz Habitat). Rainwater is first collected from the roof area and then channelled via gutters through pipes to an underground PEHD storage tank in order to be reused later. Prior to entering the tank, the water is passed through a screen rake. The first site was a private house surrounded by cultivated fields. This site was located near a rural village 40 km north-west of Toulouse. The annual average rainfall in this region is 760 mm, and the average temperatures range from 7.9 to 18.3 ºC. Agriculture in this area is characterised by the vineyards of Gaillac and crops such as wheat, maize and colza. The second site was the research building of an engineering school located in the suburban area of Toulouse, which has an urban population of around 860 000 inhabitants. This site is 12 km from the city centre. The annual average rainfall is 668 mm, with average temperature ranging from 8.6 to 18.1 ºC. The area is near a well-travelled road and 70 ha of experimental cultivation fields. Stored roof runoff sampling was carried out monthly from

	January 2009 to December 2009 for site 1 and between November 2009 and October 2010 for site 2. Analysis was performed by La Drôme Laboratoires. Water samples were screened for 405 compounds. Liquide-liquid extractions with a dichloromethane/ethyl acetate mix (80/20, v:v) at various pH levels were conducted for each sample. Extracts were simultaneously analysed by liquid chromatography (HPLC) and gas chromatography (GC) with systematic multi-detection: with diode array detector (HPLC-DAD), coupled with tandem mass spectrometry (HPLC-MS-MS), with an electron capture detector and a nitrogen phosphorus detector (GC-ECD-NPD), or coupled with mass spectrometry (GC-MS). Other sample aliquots were analysed by HPLC after a derivation, or by head-space with GC-MS. Some compounds were quantified by direct injection and analysis by HPLC-MS-MS. Glyphosate and AMPA were analysed by FMOCCl derivation + HPLC fluorescence (LOQ = 0.100 µg L^{-1}). PCA was performed using the commercial software XL stat. A data matrix, with columns representing the different samplings (12 observations per site) and rows corresponding to the measured compounds (variables), was constructed for analysis with PCA. For standardization each variable was replaced by its value minus the average of the variable and dividing by the standard deviation of the variable. Values less than the quantification limit were considered to be half of the quantification limit, and values less than the detection limit were considered to be zero. Pearson's correlations between different compounds were first obtained. Then components were determined, and the two first components corresponding to the greatest part of the total variance of the data set were retained.
Statistical design	See "Test system and conditions"
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Yang et al. (2013a)

Title: Field evaluation of a new biphasic rain garden for stormwater flow management and pollutant removal
Author: H. Yang, W. A. Dick, E. L. McCoy, P. L. Phelan, P. S. Grewal
Reference: Ecological Engineering 54 (2013) 22-31
Year: 2013
Results and conclusion: Field-scale rain gardens were constructed using a novel biphasic concept, involving water movement from a saturated to an unsaturated zone in sequence, for increasing retention time of runoff and improving bioremediation. Hydraulic performance and removal efficiencies of the biphasic rain gardens were evaluated in natural and simulated runoff events. Influent and effluent of two replicate biphasic rain gardens from natural runoff events were monitored during a 2-yr study. Three agricultural runoff events

with high concentrations of nutrients (i.e. nitrate and phosphate) and the herbicide, atrazine (6-chloro- N-ethyl-N`-(1-methylethyl)-1,3,5-triazine-2,4-diamine), were undertaken during the summer of 2008. Five urban runoff events with spiked concentrations of nutrients (i.e. nitrate and phosphate) and herbicides, glyphosate (N-(phosphonomethyl)glycine), dicamba (3,6-dichloro-2-methoxybenzoic acid), and 2,4-d (dichlorophenoxyacetic acid) were applied to the rain gardens during the summer of 2009. Both peak flow and runoff volume were reduced by holding runoff in the rain gardens (mainly in the water saturated zone) until the next runoff event. The created biphasic rain gardens in our study were highly effective in removing nitrate (~91%), phosphate (~99%), atrazine (~90%), dicamba (~92%), glyphosate (~99%), and 2,4-d (~90%) under high levels of pollution loading with simulated runoff events. Increased retention time of runoff pollutants and water-saturated conditions, as determined by design configuration and rainfall size, intensity, and interval, were found to significantly affect overall nutrient and herbicide removal in the biphasic rain gardens.	
Proposed action: Consider as additional information.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Removal of pollutants (glyphosate amongst others) in biphasic rain gardens
Protocol	Not given
Test compound	Glyphosate (amongst other herbicides)
Test system and conditions	Biphasic rain gardens: Two replicate biphasic rain gardens were constructed in the spring of 2008 at The Ohio State University's Wooster campus. The biphasic rain garden is a new type of design that consists of a biphasic (water saturated to unsaturated) sequence and a recharge zone. In these biphasic rain gardens, runoff is first directed through the water saturated (anaerobic) zone and then through the water unsaturated (aerobic) zone. The saturated zone is created by placing an impervious liner to capture the first flush of runoff and to increase retention time for more sustained water saturated conditions. Sediments are filtered and adsorption and/or biological treatment of pollutants occurs in the saturated zone. U-shaped reverse drainage pipes have only perforated portions at the bottom. This configuration maximizes the retention time of the first flush runoff in the saturated zone and allows only the treated overflow water to exit into the unsaturated zone. Effluent rate of the treated overflow water through the reverse drainage pipes is mainly controlled by the total head difference. The unsaturated zone is designed using an under drainage configuration to further retard flow for subsequent aerobic treatment. Impervious liner is placed at the bottom of the unsaturated zone to collect and discharge the treated water through a final discharge pipe. Finally, the treated water from the unsaturated zone is discharged into the recharge zone located at the bottom of the rain garden. The recharge zone filled with pea gravel is designed to facilitate groundwater recharge. Each rain garden was sized to handle 44.7 mm (1.76 in.) of runoff from a drainage area consisting of a 99% impervious concrete surface pad with surface area of 69.5 m2. This value was the precipi-

	tation of a 1-h intense rainfall with 10-year return frequency in Wooster, Ohio estimated by the National Oceanic and Atmospheric Administration Atlas 14 Model. The target water quality volume generated from 44.7 mm rainfall was estimated to be 3.1 m3 based on the drainage area. The required size and infiltration rate of each zone in the rain garden was designed based on expected water volumes using the rational and mass continuity methods (Davis and McCuen, 2005). The surface area of the saturated zone was 6.8 m^2. The depth was 1.2 m made up of fine gravel (3.2–12.7 mm diam.; 0.15 m deep), soil medium (0.85 m deep), and 0.2 m free board space above the rain garden surface to permit ponding of water during runoff events. The saturated zone provides a storage volume of approximately 1.58 m^3 and a holding capacity of 25.4 mm runoff from the concrete surface pad. The storage volume in the unsaturated zone was 1.34 m^3 with a depth of 0.5 m (i.e. fine gravel of 0.15 m depth and soil medium of 0.35 m depth) subsequently. The soil medium used in this study was a mixture of sand, topsoil, and compost in a 6:2:2 volume ratio obtained from Kurtz Bros. Inc., Cleveland, OH. The soil medium consists of 90.6% sand, 6.9% silt, and 2.5% clay with 0.7% organic matter, 3.1 meq/100 g of cation exchange capacity (CEC), 12.0 cm/h of saturated conductivity, and pH of 7.2. Six native plant species were planted in 0.2-m2 (2.25-ft2) spacing intervals. These intervals were selected based on the growing habit of the selected plants. The saturated zone included boneset (Eupatorium perfoliatum), spiderwort (Tradescantia ohiensis), and culver's root (Veronicastrum virginicum), while purple love grass (Eragrostis spectabilis), Indian grass (Sorghastrum nutans), and purple coneflower (Echinacea purpurea) were planted in the unsaturated zone. Design, construction, soil medium properties, and hydraulic characteristics of the biphasic rain gardens have been described in detail previously (Yang et al., 2009). Conditions: The experiments were performed under natural runoff conditions, simulated agricultural runoff conditions and simulated urban runoff conditions. Analytic method (given only for glyphosate): Agilent 6890 gas chromatography mass spectrometry (Limit of detection were <1.0 µg/L)
Statistical design	To examine changes in pollutant removal efficiency over the simulated urban runoff events, regression analysis was performed on mean removal efficiency of each pollutant using MINITAB v.15 (Minitab, Inc., State College, PA). Mean removal efficiency of target pollutants taken at the two replicate rain gardens was considered as a responding variable and the number of runoff events as a predictor. Regression slope was considered as an indicator of increase (positive slope) or decrease (negative slope) of removal efficiency over the events. When p-value for the regression slope was below 0.05, such changes were considered significant (regression slope is significantly different from zero).
Relevance	

Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Detailled description of open literature on surface water monitoring

Aparicio et al. (2013)

Title: Environmental fate of glyphosate and aminomethylphosphonic acid in surface waters and soil of agricultural basins	
Author: Virginia C. Aparicio, Eduardo De Gerónimo, Damián Marino, Jezabel Primost, Pedro Carriquiriborde and José L. Costa	
Reference: Chemosphere 93 (2013) 1866–1873	
Year: 2013	
Results and conclusion: The aim of this work is to study the environmental fate of glyphosate and its major degradation product, aminomethylphosphonic acid (AMPA), in surface water and soil of agricultural basins. In cultivated soils, glyphosate was detected in concentrations between 35 and 1502 lg g^{-1}, while AMPA concentration ranged from 299 to 2256 µg kg^{-1}. In the surface water studied, the presence of glyphosate and AMPA was detected in about 15% and 12% of the samples analyzed, respectively. In suspended particulate matter, glyphosate was found in 67% while AMPA was present in 20% of the samples. In streams sediment glyphosate and AMPA were also detected in 66% and 88.5% of the samples respectively. In the present study, it was demonstrated that glyphosate and AMPA are present in soils under agricultural activity. It was also found that in stream samples the presence of glyphosate and AMPA is relatively more frequent in suspended particulate matter and sediment than in water.	
Proposed action: Not to be considered. Monitoring data for a site outside the EU (Argentina) are reported.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information, concentrations in a non-EU country measured and compared to a developed model.	
Reliability	Medium
Endpoint	Concentrations in surface water, soil and sediment
Protocol	Not applicable, see also under test system, non-GLP
Test compound	Monitoring
Test system and conditions	Sixteen agricultural sites and forty-four streams in the agricultural basins were sampled three times during 2012. The samples were analyzed by UPLC-MS/MS ESI(+/————————————).
Statistical design	Not reported
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supporting information by other monitoring programmes. No negative evidence.

Bachor et al. (2008)

Title: Special report on detection of pesticides and pharmaceuticals in surface water and ground water in Mecklenburg-Vorpommern (Germany) in spring 2008	
Author: Alexander Bachor, Gabriele Lemke, André Schumann	
Reference: Report of the State Agency for the Environment, Nature Conservation and Geology Mecklenburg-Vorpommern (LUNG)	
Year: 2008	
Results and conclusion: During April 1st and 25.06.2008 samples from 60 surface water (river) and 83 groundwater measuring points in Mecklenburg-Vorpommern were analysed for different pesticides and pharmaceuticals. In 40 of 180 surface water samples glyphosate was found with concentrations > 0.1 µg/L and in 4 of 180 samples with > 1.0 µg/L. The maximum values for glyphosate were found in Sauter Bach/Wiepkenhagen with 1.37 µg/L. AMPA was found in 83 of 180 samples with concentrations > 0.1 µg/L and in 16 of 180 samples with values > 1.0 µg/L (maximum 5.58 µg/L). Potential contamination pathway of surface water could be municipal sewage plants, because also pharmaceuticals were detected here in higher concentrations. Beside the intensive use of glyphosate in regional agriculture other origins could be washing/ cleaning agents and freezing agents.	
Proposed action: To be considered in the summarizing table of surface water monitoring in spite of the short duration time. Obtained data are comparable to those given in the summarizing table.	
Type of information (critical, high/low weight, supporting, additional): Low weight, may be used as additional information	
Reliability	Low
Endpoint	Concentration in surface water and groundwater
Protocol	No information in the report on analysis methods
Test compound	Glyphosate, (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), purity not given
Test system and conditions	No information in the report, only overview of results
Statistical design	Not given
Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	The results support the long time monitoring data. However, monitoring studies and campaigns over more years and with more detailed information are of more reliability and relevance. No negative evidence (no clarification of contamination pathway).

Battaglin et al. (2014)

Title: Glyphosate and Its Degradation Product AMPA Occur Frequently and Widely in U.S. Soils, Surface Water, Groundwater, and Precipitation
Author: W.A. Battaglin, M.T. Meyer, K.M. Kuivila, and J.E. Dietze
Reference: JOURNAL OF THE AMERICAN WATER RESOURCES ASSOCIATION, Vol. 50, No. 2
Year: 2014
Results and conclusion:

Glyphosate use in the United States increased from less than 5,000 to more than 80,000 metric tons/yr between 1987 and 2007. Glyphosate is popular due to its ease of use on soybean, cotton, and corn crops that are genetically modified to tolerate it, utility in no-till farming practices, utility in urban areas, and the perception that it has low toxicity and little mobility in the environment. This compilation is the largest and most comprehensive assessment of the environmental occurrence of glyphosate and aminomethylphosphonic acid (AMPA) in the United States conducted to date, summarizing the results of 3,732 water and sediment and 1,018 quality assurance samples collected between 2001 and 2010 from 38 states. Results indicate that glyphosate and AMPA are usually detected together, mobile, and occur widely in the environment. Glyphosate was detected without AMPA in only 2.3% of samples, whereas AMPA was detected without glyphosate in 17.9% of samples. Glyphosate and AMPA were detected frequently in soils and sediment, ditches and drains, precipitation, rivers, and streams; and less frequently in lakes, ponds, and wetlands; soil water; and groundwater. Concentrations of glyphosate were below the levels of concern for humans or wildlife; however, pesticides are often detected in mixtures. Ecosystem effects of chronic low-level exposures to pesticide mixtures are uncertain. The environmental health risk of lowlevel detections of glyphosate, AMPA, and associated adjuvants and mixtures remain to be determined.	
Proposed action: Not to be considered as monitoring data outside the EU are reported.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information to already existing, as monitoring was in USA, i.e. outside the EU	
Reliability	High
Endpoint	Monitoring, concentrations in surface waters, soils, groundwater- and precipitation
Protocol	Monitoring, for details see under test system and conditions, quality assurance programme
Test compound	Glyphosate (CAS-no: 1071-83-6) and AMPA(CAS-no: 1066-51-9), (monitored, purity cannot be given)
Test system and conditions	Monitoring campaign exactly described, i.e.: sampling sites and time, sampling procedures, analytical methods, quality assurance, laboratory reporting level (LRL)
Statistical design	Not known
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supporting information by other monitoring programmes. No negative evidence.

Battaglin et al. (2009)

Title: The occurrence of glyphosate, atrazine, and other pesticides in vernal pools and adjacent streams in Washington, DC, Maryland, Iowa, and Wyoming, 2005–2006
Author: William A. Battaglin, Karen C. Rice, Michael J. Focazio, Sue Salmons, Robert X. Barry
Reference: Environ Monit Assess, 155:281–307
Year: 2009
Results and conclusion: In this study, we investigated the occurrence of glyphosate, its primary degradation product

aminomethyl-phosphonic acid (AMPA), and additional pesticides in vernal pools and adjacent flowing waters. Most sampling sites were chosen to be in areas where glyphosate was being used either in production agriculture or for non-indigenous plant control. The four site locations were in otherwise protected areas (e.g. in a National Park). When possible, water samples were collected both before and after glyphosate application in 2005 and 2006. Twenty-eight pesticides or pesticide degradation products were detected in the study, and as many as 11 were identified in individual samples. Glyphosate was measured at the highest concentration (328 µg/l) in a sample from Riley Spring Pond in Rock Creek National Park. This concentration exceeded the freshwater aquatic life standard for glyphosate of 65 µg/l. Aminomethylphosphonic acid (AMPA) was detected at concentrations greater than 3.0 µg/l.	
Proposed action: Not to be considered as monitoring data outside the EU are reported.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information to already existing, as monitoring was in USA, i.e. outside the EU	
Reliability	High
Endpoint	Monitoring, concentrations in surface waters
Protocol	Monitoring, for details see under test system and conditions, quality assurance programme
Test compound	Glyphosate (CAS-no: 1071-83-6) and AMPA(CAS-no: 1066-51-9), (monitored, purity cannot be given)
Test system and conditions	Monitoring campaign exactly described, i.e.: sampling sites and time, sampling procedures, analytical methods, quality assurance, laboratory reporting level (LRL)
Statistical design	Not known
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supporting information by other monitoring programmes. No negative evidence.

Battaglin et al. (2005)

Title: GLYPHOSATE, OTHER HERBICIDES, AND TRANSFORMATION PRODUCTS IN MIDWESTERN STREAMS, 2002
Author: William A. Battaglin, Dana W. Kolpin, Elizabeth A. Scribner, Kathryn M. Kuivila, and Mark W. Sandstrom
Reference: Journal of the American Water Resources Association (JAWRA), 323 - 332
Year: 2005
Results and conclusion: The objective of this study was to document the occurrence of glyphosate and the transformation product aminomethylphosphonic acid (AMPA) in Midwestern streams and to compare their occurrence with that of more commonly measured herbicides such as acetochlor, atrazine, and metolachlor. The frequency of glyphosate and AMPA detection, range of concentrations in runoff samples, and ratios of AMPA to glyphosate concentrations did not vary throughout the growing season as substantially as for other herbicides like atrazine, probably because of different seasonal use patterns. Glyphosate was detected at or above 0.1 µg/l in 35 percent of pre-emergence, 40 percent of post-emergence, and 31 percent of harvest season samples, with a maximum concentration of 8.7 µg/l. AMPA was

detected at or above 0.1 µg/l in 53 percent of pre-emergence, 83 percent of post-emergence, and 73 percent of harvest season samples, with a maximum concentration of 3.6 µg/l. Glyphosate was not detected at a concentration at or above the U.S. Environmental Protection Agency's maximum contamination level (MCL) of 700 µg/l in any sample.	
Proposed action: Not to be considered as monitoring data outside the EU are reported.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information to already existing as monitoring was in USA, i.e. outside the EU	
Reliability	High
Endpoint	Monitoring, concentrations in surface waters
Protocol	Monitoring, for details see under test system and conditions, quality assurance programme
Test compound	Glyphosate (CAS-no: 1071-83-6) and AMPA(CAS-no: 1066-51-9), (monitored, purity cannot be given)
Test system and conditions	Water samples were collected at sites on 51 streams in nine Midwestern states in 2002 during three runoff events: after the application of pre-emergence herbicides, after the application of post-emergence herbicides, and during harvest season. All samples were analyzed for glyphosate and 20 other herbicides using gas chromatography/mass spectrometry or high performance liquid chromatography/mass spectrometry. Quality assurance given, laboratory reporting level (LRL) given.
Statistical design	For all figures and statistics given in this report, non-detects were treated as zero. Box plots are used on some figures to show concentration distributions. Box plots are truncated at the MRL. The nonparametric Kruskal-Wallis test is used to test for differences in the distributions of either herbicide concentrations or transformation product to source herbicide concentration ratios from the three sample collection periods.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supporting information by other monitoring programmes. No negative evidence.

Bruchet et al (2012)

Title: Natural attenuation of priority and emerging contaminants during river bank filtration and artificial recharge
Author: Auguste BRUCHET, Samuel ROBERT, Mar ESPERANZA, Marie-Laure JANEX-HABIBI, Cécile MIÈGE, Marina COQUERY, He´le`ne BUDZINSKI and Karine LEMENACH
Reference: Eur. j. water qual. 42 (2011) 123–133
Year: 2012
Results and conclusion: The only pesticide or degradate found at a level exceeding 0.1 µg/L in the Seine river is glyphosate (on one occasion) and its degradate AMPA (systematically in the range 0.25–0.65 µg/L). AMPA can also be present as a wastewater contaminant, from household detergent use. These two compounds are totally removed by bank filtration, in accordance with

previous observations (Reemtsma and Jekel, 2002) and do not reappear in the aquifer.	
Proposed action: To be considered in the summarizing tables of surface water monitoring. Obtained data are comparable to those given in the summarizing table.	
Type of information (critical, high/low weight, supporting, additional): No details are provided in the report about the finding localities and special causes. The information should be considered as additional.	
Reliability	
Endpoint	Concentrations of glyphosate and AMPA in surface water and groundwater
Protocol	Glyphosate and AMPA were determined by FMOC derivatization-HPLC-fluorescence.
Test compound	Glyphosate and AMPA
Test system and conditions	The fate of various emerging contaminants as well as priority pollutants from the European UnionWater Framework directive-was examined along a complex combination of natural and engineered processes used to produce drinking water downstream of a major metropolitan area. The sampling points examined omprised Seine riverwater downstream of the Paris area, water from a primary well after bank filtration, water from a secondary well influenced by an artificial recharge process and water from the mixture of secondary wells after drinkingwater treatment. More than 80 organic contaminants including drugs, polycyclic aromatic hydrocarbons, pesticides, oestrogenic hormones, polybrominated diphenyl ethers, chlorophenols, nonylphenols, were monitored during five campaigns. River bank filtration and to a lesser extent artificial recharge clearly decreased the variety of contaminants, in particular a variety of drugs detected in the river. On the other hand riverbank filtration was found to increase nonylphenols by anaerobic degradation of nonylphenolpolyethoxylate precursors. Traces of aspirin, nonylphenols and stimulants were occasionally detected in the finished drinking water above 0.1 µg/L.
Statistical design	Not given
Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Busch and Reupert (LANUV) 2013

Title: Belastungsentwicklung von Oberflächengewässern und Grundwasser in NRW mit Glyphosat und AMPA
Author: Dieter Busch, Rolf Reupert (LANUV)
Reference: Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (LANUV)
Year: 2013
Results and conclusion: Results from a federal monitoring programme in North Rhine-Westphalia, Germany regarding concentrations of glyphosate and AMPA in surface water and groundwater are pre-

sented. The results are summarised in the following.

2001-2012:
Concentration of glyphosate in surface water:
Ruhr, 3 sites: max. 0.10 - 0.29 µg/L
Concentration of AMPA in surface water:
Ruhr, 3 sites: max. 0.86 - 2.02 µg/L

1996-2012:
Concentration of glyphosate in surface water:
NRW: 1899 samples, 225 samples > 0.1 µg/L (maximum 0.93 µg/L)

1996-2012:
Concentration of AMPA in surface water:
NRW: 1903 samples, 1377 samples > 0.1 µg/L (maximum 13 µg/L)

2006-2012:
Concentration of glyphosate in groundwater:
NRW: 245 samples, 0 samples > 0.1 µg/L (maximum 0.08 µg/L)

2006-2012:
Concentration of AMPA in groundwater:
NRW: 260 samples, 7 samples > 0.1 µg/L (maximum 0.45 µg/L)

Proposed action:
To be considered in the summarizing tables of surface water and groundwater monitoring. Obtained data are comparable to those given in the summarizing table.

Type of information (critical, high/low weight, supporting, additional):
No details are provided in the report about the finding localities and special causes. The information should be considered as additional.

Reliability	
Endpoint	Concentrations of glyphosate and AMPA in surface water and groundwater
Protocol	In-house standard according to ISO 21458; DIN 38407-22
Test compound	Glyphosate and AMPA
Test system and conditions	Monitoring programme
Statistical design	Not given
Relevance	
Environmental relevance	Relevant
Weight of evidence	
“Positive”/”Negative” evidence	No negative evidence.

Busetto and Frattini (2010)

Title: Surveys of herbicide glyphosate and degradation product aminomethyl phosphonic acid in waterways of Monza-Brionza province

Author: Busetto, M., Frattini, V.

Reference: iL boLLettino 2010/4

Year: 2010	
Results and conclusion: During the period 2006-2009 ARPA (the Lombardy Regional Environmental Agency) has been collecting analytical data concerning the presence and concentration of glyphosate and its metabolite aminomethylphosphonic acid (AMPA) in the water of Lambro, Seveso and Terrò rivers in the Brianza region. River flow-rate, COD, BOD5 and conductivity have also been measured in each sample. Both AMPA and glyphosate have been found in every sample, with AMPA concentration always higher than the glyphosate concentration. Larger amounts of herbicide have been observed in the water sampled in the autumnal season, while in the following months concentration decreases. Our data are in accordance with the available information about use and release of herbicide during the year.	
Proposed action: Not to be considered as the publication is written in Italian language.	
Type of information (critical, high/low weight, supporting, additional): Additional information.	
Reliability	
Endpoint	Concentrations of glyphosate and AMPA in surface water
Protocol	Not available (as the publication is written in Italian language)
Test compound	Glyphosate and AMPA
Test system and conditions	Monitoring programme
Statistical design	Not available (as the publication is written in Italian language)
Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Byer et al. (2008)

Title: Low Cost Monitoring of Glyphosate in Surface Waters Using the ELISA Method: An Evaluation
Author: Byer J., Struger J., Klawunn P., Todd A. and Sverko E.
Reference: Environ. Sci. Technol, 42, 6052–6057
Year: 2008
Results and conclusion: The goal of our study was to evaluate a cost effective method to measure glyphosate concentrations in surface waters. The reliability of enzyme-linked immune-sorbent assay (ELISA) results was evaluated against liquid chromatography tandem mass spectrometry, and linear regression results for 30 water samples from urban watersheds revealed a strong relationship (R^2 = 0.88). These results suggest that ELISA methods, used in conjunction with traditional methods, represent a cost-effective approach to enhance the spatial and temporal resolution of a water quality monitoring study. Additionally, we measured a total of 739 surface water samples from over 150 sampling locations throughout Ontario using ELISA from April to October 2007. Concentrations exceeded the method detection limit of 0.1 µg/L in 33% of the samples, with a maximum concentration of 12.0 µg/L. Glyphosate showed a bimodal temporal distribution with peak concentrations occurring in late spring/early summer and fall, and did not exceed the Canadian Council of Ministers of the Environment (CCME) guideline for the protection of aquatic life (65 µg/L) in any of the samples.

Proposed action: Not to be considered as publication deals with method development and monitoring data outside EU (Canada).	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information to already existing as the articles deals with method development and monitoring data outside EU (Canada).	
Reliability	Medium
Endpoint	Monitoring, concentrations in surface waters
Protocol	Monitoring, for details see under test system and conditions.
Test compound	Glyphosate (CAS-no: 1071-83-6, monitored, purity cannot be given)
Test system and conditions	Sampling methodology, analytical methods described in detail. 30 surface water samples from three urban creeks in southern Ontario were analyzed via LC/MS/MS and ELISA. Quality control measures given, instrument variability considered.
Statistical design	Correlation between LC/MS/MS and ELISA-results by regression analysis
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supporting information by other monitoring programmes. No negative evidence.

Comoretto et al. (2007)

Title: Pesticides in the Rhône river delta (France): Basic data for a field-based exposure assessment	
Author: Laetitia Comoretto, Bruno Arfib, Serge Chiron	
Reference: Science of the Total Environment 380, 124–132	
Year: 2007	
Results and conclusion: The pesticide concentration levels flowing into paddy fields and surrounding lagoons of the Rhône river delta were investigated over a period of 6 months in 2004. The mean load of Glyphosate is assessed to be around 8 tons per year. However, Glyphosate was not found in any of the samples.	
Proposed action: Not to be considered as raw data are not sufficiently precisely reported.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Medium
Endpoint	Monitoring, concentrations in surface water
Protocol	Monitoring, for details see under test system and conditions.
Test compound	Glyphosate (CAS-no: 1071-83-6) monitored, purity cannot be given
Test system and conditions	Pesticides were selected according to their occurrence in the Rhône river waters or their usage in rice farming. Water samples were collected at the outlets of the major ditches and in the lagoons in order to study the seasonal variation in pesticide concentrations and the spatial contamination profile. Twenty four pesticides were monitored, mainly herbicides and insecticides. Sampling sites de-

	scribed, sample preparation and chemical analysis described.
Statistical design	Not given
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Similar monitoring programmes not known. No negative evidence.

Coupe et al. (2012)

Title: Fate and transport of glyphosate and aminomethylphosphonic acid in surface waters of agricultural basins	
Author: Richard H Coupe, Stephen J Kalkhoff, Paul D Capel and Caroline Gregoire	
Reference: Pest Management Science 68(1): 16-30. DOI : 10.1002/ps.2212	
Year: 2012	
Results and conclusion: Results: Glyphosate and AMPA were frequently detected in the surface waters of four agricultural basins. The frequency and magnitude of detections varied across basins, and the load, as a percentage of use, ranged from 0.009 to 0.86% and could be related to three general characteristics: source strength, rainfall runoff and flow route. Median concentrations were for Glyphosate: 0.1 - 380 µg/L, for AMPA: 0.1 - 26 µg/L. Conclusions: Glyphosate use in a watershed results in some occurrence in surface water; however, the watersheds most at risk for the offsite transport of glyphosate are those with high application rates, rainfall that results in overland runoff and a flow route that does not include transport through the soil.	
Proposed action: Not to be considered as a) highest reported median concentration of 380 µg/L were found in USA, and thus data are related to sites outside the EU b) data monitored in France are not sufficiently comprehensively reported.	
Type of information (critical, high/low weight, supporting, additional): Low weight, supporting information	
Reliability	High
Endpoint	Surface water concentrations, monitoring.
Protocol	Glyphosate and AMPA (USA): Water sample collection and processing followed USGS protocols. Online solid-phase extraction and analysis by HPLC/MS. Both compounds had a reporting level of 0.02 µg L^{-1}. Glyphosate and AMPA (France): filtered and analyzed using similar methods, with a reporting level of 0.1 µg L^{-1}.
Test compound	Glyphosate (CAS-no.: 1071-83-6), AMPA (CAS-no: 1066-51-9)
Test system and conditions	Surface water monitoring in 4 agricultural water basins in USA and France. The study conducted by the NAWQA Program of the USGS included four basins: the South Fork Iowa River, Iowa; Sugar Creek, Indiana; Bogue Phalia, Mississippi; Rouffach, France
Statistical design	6 field blanks, 11 replicates
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative"	Positive evidence, no negative evidence.

evidence	

Daouk et al. (2013)

Title: Dynamics and environmental risk assessment of the herbicide glyphosate and its metabolite AMPA in a small vineyard river of the Lake Geneva catchment	
Author: SILWAN DAOUK, PIERRE-JEAN COPIN, LUCA ROSSI, NATHALIE CHÈVRE and HANS-RUDOLF PFEIFER	
Reference: Environmental Toxicology and Chemistry, Vol. 32, No. 9, pp. 2035–2044, 2013	
Year: 2013	
Results and conclusion: The use of pesticides may lead to environmental problems, such as surface water pollution, with a risk for aquatic organisms. In the present study, a typical vineyard river of western Switzerland was first monitored to measure discharged loads, identify sources, and assess the dynamic of the herbicide glyphosate and its metabolite aminomethylphosphonic acid (AMPA). Second, based on river concentrations, an associated environmental risk was calculated using laboratory tests and ecotoxicity data from the literature. Measured concentrations confirmed the mobility of these molecules with elevated peaks during flood events, up to 4970 ng/L. From April 2011 to September 2011, a total load of 7.1 kg was calculated, with 85% coming from vineyards and minor urban sources and 15% from arable crops. Compared with the existing literature, this load represents an important fraction (6–12%) of the estimated amount applied because of the steep vineyard slopes (10%). The associated risk of these compounds toward aquatic species was found to be negligible in the present study, as well as for other rivers in Switzerland. A growth stimulation was nevertheless observed for the algae Scenedesmus vacuolatus with low concentrations of glyphosate, which could indicate a risk of perturbation in aquatic ecosystems, such as eutrophication. The combination of field and ecotoxicity data allowed the performance of a realistic risk assessment for glyphosate and AMPA, which should be applied to other pesticide molecules.	
Proposed action: Obtained data are comparable to those given in the summarizing table. Not be considered in the summarizing tables of surface water monitoring, as only the max. concentrations in the surface waters are reported.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Medium
Endpoint	Monitoring, concentrations of glyphosate and AMPA in surface water
Protocol	Monitoring
Test compound	Glyphosate and AMPA, purity cannot be given, monitoring
Test system and conditions	The Lutrive River was sampled during the year 2011 upstream and downstream of the vineyard area. The automatic samplers were programmed for 2 types of sampling: a regular and an event-based sampling. One-third of the bottles were dedicated to the regular sampling and the other two-thirds to the event-based sampling. The first was done every 4 d and the second when the water level reached a predefined level, indicating the start of a rain event, and then every 2 h. Fifty samples for the downstream site and 20 for the upstream site were selected from April 2011 to October 2011 and analyzed. Twenty of them—15 downstream and 5 upstream—

	were regularly collected during dry periods to estimate background levels of glyphosate and AMPA. The herbicide glyphosate and its metabolite AMPA were quantified by ultraperformance liquid chromatography coupled to tandem mass spectrometry after their derivatization with 9-fluorenylmethyl chloroformate followed by solid-phase extraction.
Statistical design	Not given
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	The results support the long time monitoring data. However, monitoring studies and campaigns over more years and with more detailed information are of more reliability and relevance. No negative evidence.

Daouk et al. (2013a)

Title: The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, western Switzerland: Proof of widespread export to surface waters. Part I: Method validation in different water matrices
Author: S. DAOUK, D. GRANDJEAN, N. CHEVRE, L. F. DE ALENCASTRO and H.-R.F. PFEIFER
Reference: Journal of Environmental Science and Health, Part B (2013) 48, 717-724
Year: 2013
Results and conclusion: An analytical method for the quantification of the widely used herbicide, glyphosate, its main by-product, aminomethylphosphonic acid (AMPA) and the herbicide glufosinate at trace level was developed and tested in different aqueous matrices. Their derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) was done prior to their concentration and purification by solid phase extraction. The concentrated derivates were then analyzed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Spiking tests at three different concentrations were realized in several water matrices: ultrapure water, Evian© mineral water, river water, soil solution and runoff water of a vineyard. Except for AMPA in runoff water, obtained regression curves for all matrices of interest showed no statistical differences of their slopes and intercepts, validating the method for the matrix effect correction in relevant environmental samples. The limits of detection and quantification of the method were as low as 5 and 10 ng/l respectively for the three compounds. Spiked Evian© and river water samples at two different concentrations (30 and 130 ng/l) showed mean recoveries between 86 and 109%, and between 90 and 133% respectively. Calibration curves established in spiked Evian© water samples between 10 and 1000 ng/l showed r^2 values above 0.989. Monitoring of a typical vineyard river showed peaks of pollution by glyphosate and AMPA during main rain events, sometimes above the legal threshold of 100 ng/l, suggesting the diffuse export of these compounds by surface runoff. The depth profile sampled in the adjacent lake near a waste water treatment plant outlet showed a concentration peak of AMPA at 25m depth, indicating its release with treated urban wastewater. In detail: The validation of the method to quantify the herbicide glyphosate, its metabolite AMPA and the herbicide glufosinate at trace level in several types of natural waters was successful and allows following these potential hazardous molecules in the environment. Further investigations to better understand their behavior in soils after their application and

their transport to surface water will be possible. Preliminary results of field studies show that river water samples exhibit a frequent pollution by the studied herbicides, which finally end up in Lake Geneva. Several samples showed concentrations above the legal threshold of 100 ng/l. This highlights the importance of monitoring these substances in the aquatic system.	
Proposed action: Consider as additional information as only method validation in different water matrices is described.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Glyphosate and AMPA export to surface waters, method validation in different water matrices
Protocol	Not given
Test compound	Glyphosate (PESTANALR, 99.7%), glufosinate-ammonium (PESTANALR, 99.2%) and AMPA (99%)
Test system and conditions	Environmental sampling: The Lutrive is a local river in the east of the city of Lausanne, at the western limit of the Lavaux vineyard area. Its small watershed (6.4 km^2) is characterized by different land uses: agricultural fields (45%), of which 4.1% are vineyards, urban and impervious surfaces (31%) and forests (24%). Grab samples were collected in the vineyard area during the growing season of 2010 and during both dry- and wet-weather conditions. Daily precipitations data of the meteorological station of Pully, located at 2 km west of the Lutrive River, were provided by MeteoSwiss. Lake Geneva was sampled during dry weather on the 1st of July 2010, in the Vidy Bay near the waste water treatment plant (WWTP)outlet at nine different depths:−2,−5,−10,−15, −18.5, −21, −23, −25 and −29 m. Corresponding real-time temperature and electrical conductivity data were obtained from Bonvin et al.[
Statistical design	Mean and maximum values, standard deviations
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Daouk et al. (2013b)

Title: The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, western Switzerland: Proof of widespread export to surface waters. Part II: The role of infiltration and surface runoff
Author: S. DAOUK, L. F. DE ALENCASTRO and H.-R. PFEIFER
Reference: Journal of Environmental Science and Health, Part B (2013) 48, 725-736
Year: 2013
Results and conclusion: Two parcels of the Lavaux vineyard area, western Switzerland, were studied to assess to which extent the widely used herbicide, glyphosate, and its metabolite aminomethylphosphonic acid (AMPA) were retained in the soil or exported to surface waters. They were equipped at their bottom with porous ceramic cups and runoff col-

lectors, which allowed retrieving water samples for the growing seasons 2010 and 2011. The role of slope, soil properties and rainfall regime in their export was examined and the surface runoff/throughflows ratio was determined with a mass balance. The results revealed elevated glyphosate and AMPA concentrations at 60 and 80 cm depth at parcel bottoms, suggesting their infiltration in the upper parts of the parcels and the presence of preferential flows in the studied parcels. Indeed, the succession of rainy days induced the gradual saturation of the soil porosity, leading to rapid infiltration through macropores, as well as surface runoff formation. Furthermore, the presence of more impervious weathered marls at 100 cm depth induced throughflows, the importance of which in the lateral transport of the herbicide molecules was determined by the slope steepness. Mobility of glyphosate and AMPA into the unsaturated zone was thus likely driven by precipitation regime and soil characteristics, such as slope, porosity structure and layer permeability discrepancy. Important rainfall events (>10 mm/day) were clearly exporting molecules from the soil top layer, as indicated by important concentrations in runoff samples. The mass balance showed that total loss (10–20%) mainly occurred through surface runoff (96%) and, to a minor extent, by throughflows in soils (4%), with subsequent exfiltration to surface waters. Results in detail: The total amount of glyphosate and AMPA retrieved in both type of samples from parcel 2 (surface = 845 m^2), and likely to be exported from it, was 4.3 g in 2010 and 9.1 g in 2011. This represents respectively 10 and 20% of the initial amount, which, despite the uncertainty of such kind of calculations, is in agreement with previous studies. The 80–90% remaining were either retained, and possibly as bound residues after some time, or degraded in the soil, as volatilization is not likely to happen due to their properties. The relative contribution of throughflows in the unsaturated zone versus surface runoff was 3–5% versus 95–97%.	
Proposed action: To be considered as additional information as it is pointed out that total loss (10–20%) mainly occurred through surface runoff (96%) and, to a minor extent, by throughflows in soils (4%).	
Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Export of glyphosate and AMPA to surface waters, role of infiltration and surface runoff
Protocol	Not given
Test compound	Glyphosate
Test system and conditions	Study area and soil features: The Lavaux is a vineyard area located in western Switzerland of 830 ha including about 10,000 terraces on 40 km between Montreux and Lutry to the east of Lausanne. Based on a previous work dealing with risk assessment of pesticide transfer from vineyards to surface waters, two risky parcels were chosen close to the Lutrive River. The general character of the soils was obtained through drilling with an auger and digging two pits of a depth up to two meters at their bottom. Textures, colours and the presence of carbonates were determined in the field, whereas soil pH was measured in the laboratory on water extractions (Soil: H_2O = 1:2.5). More precise grain size analyses were obtained by laser diffractometry (Mastersizer 2000, Malvern,Worcestershire, UK) for samples of various depths at the bottom of both parcels, treated with HCl beforehand to remove carbonates. Soils of both parcels are colluvial calcosols, according to the French classification, with anthropogenic influences.

	The dominant colour, referring to the Munsell colour system, was medium to light brown (10YR4/3) with a grey tendency (2.5Y5/3) in the deepest horizons. Both soils showed a silt loam texture and light differences were observed between plots and depths. HCl reactivity at all depths revealed the carbonate nature of soils, which was confirmed by pH-values between 8 and 9. Organic carbon contents varied from the surface layer to deeper horizons between 1.7 and 0.7% in parcel 1 and 2.2 and 0.8% in parcel 2. Copper concentrations in top soils (0–30 cm) varied between 300 and 500 mg/kg in parcel 1 and 100 and 170 mg/kg in parcel 2. Sampling and analytical methods: In both parcels, the herbicide glyphosate was applied the same day and only under the rows, leaving a grass band in between them. It is mainly applied in spring time to avoid a nutrient and water competition between grapevines and weeds during the growing season. Application data were obtained from winegrowers. In previous years, the same amounts had been applied, but the authors assumed that all glyphosate and AMPA degrade from year to year according to their properties. Precipitation data were obtained from the closest meteorological station (Source: MeteoSwiss), located in Pully at 1.8 km from the two parcels. In order to sample the soil solution, both parcels were equipped at their bottom with porous ceramic suction cups (SPS200, Ø63 mm, porosity = 1 µm, SDEC, Reignac sur Indre, France) at four different depths: 20, 40, 60 and 80 cm (Fig. 1c). The applied tension was 0.6 bars and the recovery of samples was done every week or more frequently during intense rainfall periods. As the slope of parcel 2 is more representative of the Lavaux region, it was equipped with three runoff collectors. They were built inspired from previous studies and placed at the end of grapevine rows according to observed erosion paths. They comprised a bottle buried in the soil, a funnel with a sieve of 1 mm size to avoid macro fauna or large particles, a PVC conducting ramp placed just under the root zone and ending above the funnel, and a roof to avoid direct rain inputs. Samples were collected in 250 mL high density polyethylene bottles and transported to the laboratory in a cool box. They were placed in a freezer until their analysis, for which they were then gently de-frozen. Electrical conductivity and pH were determined in the field. The herbicide glyphosate and its metabolite AMPA were quantified by LC-MS/MS with a previously developed method, based on their pre-column derivatization with FMOC-Cl and their enrichment by solid phase extraction. The limit of quantification was 10 ng/L and it was tested successfully for the matrix effect that could occur by analyzing soil solution and runoff samples. Dissolved organic and inorganic carbon (DOC/DIC) concentrations were measured with a C-analyzer (LiquiTOC, Elementar, Hanau, Germany). Ion concentrations were obtained with an ion chromatography system (ICS-1100/2100, Dionex-Thermo Fischer, Olten, Switzerland). Copper analyzes were done by ICP-MS (ELANR® 6100 DRC, Perkin Elmer, Waltham, MA, USA) in some soil water samples, previously diluted, acidified with HNO_3 and treated with H_2O_2 to avoid

	possible interference with organic matter. A principal component analysis (PCA) was performed on the soil water samples using the R software to help interpreting all the analyses and discriminating the observations made in the two different parcels. Prior to it, each of the parameters was normalized to zero mean and unit variance, by subtracting the mean value of the variable and by dividing by the standard variation, allowing them to have the same influence in the PCA. In order to determine the surface runoff/throughflows ratio, a mass balance was done for both surface runoff and soil solution samples of parcel 2 (surface = 845 m^2). As glyphosate was applied only under the grapevine rows, the initial quantities correspond to half of the surface. The mass of glyphosate and AMPA were obtained by multiplying the concentrations with cumulated precipitations that fell on the parcel surface between two sampling events.
Statistical design	See "Test system and conditions"
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

de Armas et al. (2007)

Title: SPATIAL-TEMPORAL DIAGNOSTIC OF HERBICIDE OCCURRENCE IN SURFACE WATERS AND SEDIMENTS OF CORUMBATAÍ RIVER AND MAIN AFFLUENTS	
Author: Eduardo Dutra de Armas, Regina Teresa Rosim Monteiro, Paula Munhoz Antunes, Maria Alice Penna Firme dos Santos e Plinio Barbosa de Camargo	
Reference: Quim. Nova, Vol. 30, No. 5, 1119-1127	
Year: 2007	
Results and conclusion: Residues of herbicides from sugarcane were monitored in waters and sediments of Corumbataí River and tributaries. Ametryne, atrazine, simazine, hexazinone, glyphosate, and clomazone were detected in water samples, with negligible levels of ametryne and glyphosate in sediment samples. The area of recharge of the Guarani aquifer presented the highest triazine and clomazone levels. The triazines were detected at higher levels, with atrazine above Brazil's potability and quality standards. Total herbicide levels at some sampling points were 13 times higher than the European Community potability limit.	
Proposed action: Not to be considered as monitoring outside the EU (Brazil).	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Low
Endpoint	Monitoring, concentrations in surface water and sediment
Protocol	Monitoring
Test compound	Glyphosate (CAS-no: 1071-83-6), purity cannot be given, monitoring
Test system and conditions	Sampling regime, analytical procedure, LOD given (in Spanish language)

Statistical design	Not given
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Similar monitoring programmes not known. No negative evidence.

Fooken and Stachel (2000)

Title: Selected organic trace impurities in the river Elbe and its tributaries during 1994 - 1999	
Author: Cornelia Fooken, Burkhard Stachel,	
Reference: Report of the Working Group for Prevention of ELBE Pollution (ARGE Elbe)	
Year: Oktober 2000	
Results and conclusion: 2 - 7 samples per year from 3 - 10 measuring points per year of Elbe and its tributaries were investigated. Glyphosate was detected in the range of 0.05 - 0.09 µg/l, AMPA with 0.2 - 1.0 µg/L. In the summer samples the concentrations of AMPA were 2 - 3 times higher than in winter samples.	
Proposed action: Not to be considered in the summarizing table of surface water monitoring. Obtained data are comparable to those given in the summarizing table.	
Type of information (critical, high/low weight, supporting, additional): Low weight, may be used as additional information	
Reliability	Low
Endpoint	Concentration in surface water (Elbe)
Protocol	Analysis: • solid phase enrichment with cation exchange column, elution with hydrochloric acid • two step derivatisation with hypochlorit and o-phthaldialdehyd • HPLC/FLD
Test compound	Glyphosate, (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), purity not given
Test system and conditions	Both compounds were detected in surface water samples in 1998.
Statistical design	Not given
Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	The results support the long time monitoring data. However, monitoring studies and campaigns over more years and with more detailed information are of more reliability and relevance. No negative evidence.

Freire et al. (2012)

Title: Monitoring of toxic chemical in the basin of Maringá stream
Author: Rosane Freire, Roselene Maria Schneider, Fabrício Hernandes de Freitas, Cássia

Maria Bonifácio and Célia Regina Granhen Tavares	
Reference: Acta Scientiarum. Technology Maringá, v. 34, n. 3, p. 295-302, July-Sept., 2012	
Year: 2012	
Results and conclusion: This study aimed to track the spatial and temporal variations of toxic chemical compounds, such as the metals Al, Cd, Pb, Cu, Cr, Mn, Zn and the pesticide glyphosate, in Maringá stream and in a stretch of Pirapó river. The results pointed out that, in the case of metals, one of the possible sources of these elements is associated to agricultural activities. For glyphosate, were not found concentrations above those established by the Brazilian Water Quality Legislation (CONAMA 357/2005). Concerning this, we emphasized that the impact caused by the agrochemical on water quality should be evaluated considering the adverse effects to the environment caused by its degradation, that produces recalcitrant and surfactant compounds that may be even more toxic for humans and aquatic environment.	
Proposed action: Not to be considered as publication deals with monitoring outside the EU (Brazil).	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Medium
Endpoint	Glyphosate concentrations in stream samples
Protocol	For details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6), purity not given, monitoring
Test system and conditions	Samples were collected at 9 sites distributed throughout the basin of the Maringá stream (monthly from July to December 2009). The concentration was determined by ion chromatography with suppressed conductivity detection. The climatological data relative to the study period were furnished by the Main Weather Station of the State University of Maringá.
Statistical design	Not reported
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Glozier et al. (2011)

Title: Occurrence of glyphosate and acidic herbicides in select urban rivers and streams in Canada, 2007
Author: Nancy E. Glozier, John Struger, Allan J. Cessna, Melissa Gledhill, Myriam Rondeau, William R. Ernst, Mark A. Sekela, Steve J. Cagampan, Ed Sverko, Clair Murphy, Janine L. Murray, David B. Donald
Reference: Environ Sci Pollut Res (2012) 19:821–834
Year: 2012
Results and conclusion: A national survey was designed to monitor eight commonly used herbicides in urban rivers and streams across Canada. The herbicides 2,4-D, mecoprop, dicamba, glyphosate and its major metabolite aminomethylphosphonic acid (AMPA) were most frequently detected. Maximum concentrations of glyphosate: 11,800 ng/L (glyphosate).
Proposed action:

Not to be considered as publication deals with a specific situation outside the EU (Canada).	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Medium
Endpoint	Glyphosate concentrations in surface water
Protocol	For details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6), AMPA (CAS-no: 1066-51-9), purity not given, monitoring
Test system and conditions	Samples were collected monthly on one of two predetermined dates from April to September, 2007 from 19 sites within 16 watersheds, including 15 sites downstream of urban lands and two reference sites. Water samples were also collected approximately three times from each watershed during or after precipitation events. All samples were collected using a common sampling protocol and all were analyzed using the same analytical laboratories.
Statistical design	Nonparametric statistical tests were used. All statistical analyses (t tests, Mann–Whitney tests, twoway analysis of variance (ANOVA) analyses, regressions) were performed with Systat Version 11.
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Grunewald et al. (2001)

Title: Behaviour of Glyphosate and aminomethylphosphonic acid (AMPA) in soils and water of reservoir Radeburg II catchment (Saxony/Germany)	
Author: Grunewald K., Schmidt W., Unger C., Hanschmann G.	
Reference: J. Plant Nutr. Soil Sci, 164, 65-70	
Year: 2001	
Results and conclusion: The behaviour of Glyphosate and AMPA was investigated in soils and water in a well defined catchment of the reservoir Radeburg II near Dresden. Half-life of Glyphosate in soil ranged from 11 to 17 days. Glyphosate and AMPA completely disappeared from the soil after 5 months following application of "Roundup Ultra" and "Touchdown". The aquatic system in the test areas (surface water, soil solution, groundwater) was not significantly affected by the direct application of the compound. In general, there was a clear indication of strong sorption of the substances by soil particles. Settlement areas were recognized as possible sources of glyphosate and AMPA intake in aquatic systems.	
Proposed action: Considered by listing in the summarizing table and discussion.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Medium
Endpoint	Glyphosate concentrations in surface water, soil solution, groundwater and soil
Protocol	For details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6), AMPA (CAS-no: 1066-51-9), purity not given, monitoring

Test system and conditions	Study area located in the north/north-east sub-urban district of the town Radeburg near Dresden. Landscape has an undulating surface between 145 – 250 m a.s.l. Climatic conditions described. Soil sampling, groundwater sampling, runoff- sampling and reservoir sampling described in detail. Glyphosate and AMPA were determined according to reference method No. 105 of Deutsche Forschungsgemeinschaft, 1991. LOD in soil and water is given.
Statistical design	Not given
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Houtman et al. (2013)

Title: A MULTICOMPONENT SNAPSHOT OF PHARMACEUTICALS AND PESTICIDES IN THE RIVER MEUSE BASIN
Author: C. J. HOUTMAN, R. T. BROEK, K. DE JONG, B. PIETERSE and J. KROESBERGEN
Reference: Environmental Toxicology and Chemistry, Vol. 32, No. 11, pp. 2449–2459, 2013
Year: 2013
Results and conclusion: The river Meuse serves as a drinking-water source for more than 6 million people in France, Belgium, and The Netherlands. Pharmaceuticals and pesticides, both designed to be biologically active, are important classes of contaminants present in this river. The variation in the presence of pharmaceuticals in time and space in the Dutch part of the Meuse was studied using a multicomponent analytical method for pharmaceuticals combined with univariate and multivariate statistical analyses of the results. Trends and variation in time in the presence of pharmaceuticals were investigated in a dead-end side stream of the Meuse that serves as an intake point for the production of drinking water, and 93% of the selected compounds were detected. Highest concentrations were found for the antidiabetic metformin. Furthermore, a spatial snapshot of the presence of pharmaceuticals and pesticides was made along the river Meuse. Principal component analysis was successfully applied to reveal that wastewater-treatment plant effluent and water composition at the Belgian border were the main factors determining which compounds are found at different locations. The Dutch part of the river basin appeared responsible for approximately one-half of the loads of pharmaceuticals and pesticides discharged by the Meuse into the North Sea. The present study showed that multicomponent monitoring in combination with principal component analysis is a powerful tool to provide insight into contamination patterns in surface waters. In detail: Concentrations along the Meuse River basin: Nineteen detected pesticides belong to the class of herbicides. Among them were glyphosate and aminomethylphosphonic acid (its degradation product). They are notorious contaminants in the river Meuse. The main emission pathways to the Dutch part of the Meuse are runoff from pavements. Glyphosate is not well degraded in WWTPs. Degradation to aminomethylphosphonic acid takes place mainly in the environment. Glyphosate and aminomethylphosphonic acid were the only pesticides found in all samples. Concentrations detected in the 'snapshot' study:

Glyphosate: max 0.21 µg/L AMPA: max 2.28 µg/L	
Proposed action: To be considered in the summarizing tables of surface water monitoring. Obtained data are comparable to those given in the summarizing table.	
Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Concentrations of pharmaceuticals and pesticides (amongst others: glyphosate and AMPA) along the Meuse River basin
Protocol	Not given
Test compound	Glyphosate and AMPA (amongst others)
Test system and conditions	- Analysis of pharmaceuticals with the ultra-HPLC/MS-MS multicomponent method - Spatial snapshot of pharmaceuticals along the Meuse - Analysis of general water-quality parameters - Multicomponent analysis of pharmaceuticals and pesticides - Statistical analysis - Calculation of the loads discharged into the North Sea
Statistical design	The presence of pharmaceuticals in the Dutch part of the river Meuse was studied in time and space using a multicomponent analytical method combined with univariate and multivariate statistical analyses of the results. Box plot figures representing minimum, first quartile, median, third quartile, and maximum concentrations were made in Excel for pharmaceuticals that were detected in at least 5 samples (20% of the samples). Concentrations less than the minimum reporting limit were artificially set at 25% of the individual minimum reporting limit. The significance of longterm time trends and seasonal variation was tested using the statistical software package Trendanalist (AMO-Icastat). For this purpose, the obtained data set was complemented with archived monitoring results for those pharmaceuticals that had also been monitored with enough sensitivity with LC/MS and gas chromatography (GC)/MS methods at the same location from 2005 to 2010 (the test requires results of a period of at least 4.5 yr). Long-term time trends were tested with linear regression (in case of normally distributed data), and the Mann-Kendall test corrected for seasonal effects (if data were not normally distributed). Seasonal variation was tested with Kruskal-Wallis tests.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Kaiser (2011)

Title: Preliminary Study of Pesticide Drift into the Maya Mountain Protected Areas of Belize
Author: Kristine Kaiser

Reference: Bull. Environ. Contam. Toxicol. 86, 56–59	
Year: 2011	
Results and conclusion: In Belize, Central America, many farms surrounding the Protected Areas of the Maya Mountains rely heavily on the application of agrochemicals. The purpose of this study was to test whether orographic drift of glyphosate and organophosphates into the nearby Maya Mountain Protected Areas occurred by collecting phytotelmic water from seven sites over 3 years. Regardless of location within the Maya Mountain Protected Areas, glyphosate was present; organophosphates were more common at ridge sites. Although glyphosate concentrations were low (0.22 – 1.71 µg/L), due to the number of threatened species and the human use of stream water outside the Maya Mountain Protected Areas, better understanding of these effects is warranted.	
Proposed action: Not to be considered as publication deals with a specific situation outside the EU (Belize).	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Medium
Endpoint	Glyphosate concentrations in groundwater, wells and waterworks
Protocol	for details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6), purity not given, monitoring
Test system and conditions	A total of seven sites across the protected areas of the Maya Mountains were sampled during June–August, 2006–2007 the rainiest months of the year in this region. Based on self-reported use of agrochemicals glyphosate and common organophosphates and carbamate (OP/C) tests were implemented. Analyses by chemical specific test kits. Glyphosate ELISA-method applied.
Statistical design	Five samples were collected per site per year. Samples for all tests were run in duplicate. Statistical analysis was carried out using StataIC 10.0.
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Kolpin et al. (2006)

Title: Urban contributions of glyphosate and its degradate AMPA to streams in the United States
Author: Dana W. Kolpin, E. Michael Thurman, Edward A. Lee, Michael T. Meyer, Edward T. Furlong, Susan T. Glassmeyer
Reference: Science of the Total Environment 354, 191– 197
Year: 2006
Results and conclusion: In 2002, treated effluent samples were collected from 10 wastewater treatment plants (WWTPs) to study the occurrence of glyphosate and AMPA. Concentrations were generally low, although nine detections of AMPA (maximum concentration = 3.9 µg/L) and three detections of glyphosate (maximum concentration = 2.2 µg/L) exceeded 1 µg/L. The results document the apparent contribution of WWTP effluent to stream concentrations of glyphosate and AMPA, with roughly a two-fold increase in their frequencies of detection between

stream samples collected upstream and those collected downstream of the WWTPs. Thus, urban use of glyphosate contributes to glyphosate and AMPA concentrations in streams in the United States. Overall, AMPA was detected much more frequently (67.5%) compared to glyphosate (17.5%).	
Proposed action: Not to be considered as publication deals with monitoring outside the EU (USA).	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Medium
Endpoint	Glyphosate concentrations in stream samples upstream and downstream of waste water treatment plants
Protocol	For details see under test system and conditions, non-GLP, all samples were sampled by the same personnel and according to special, consistent protocols.
Test compound	Glyphosate (CAS-no: 1071-83-6), AMPA (CAS-no: 1066-51-9), purity not given, monitoring
Test system and conditions	Stream samples were collected upstream and downstream of the 10 WWTPs. The network consisted of 40 sampling sites: eight upstream samples, 11 WWTP effluents, 19 downstream samples. Two reference sites were sampled in areas anticipated to have little glyphosate use because of limited human activity. The 10 WWTP locations represent a variety of climatic conditions, population densities, stream sizes, and treatment practices. Sampling reported, analytical methods including reporting limit reported,
Statistical design	Significances for differences in concentrations tested by Kruskal–Wallis test.
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Munz et al. (2012)

Title: Pestizidmessungen in Fliessgewässern - Schweizweite Auswertung
Author: N. Munz, C. Leu, I. Wittmer
Reference: AQUA & GAS No 11 | 2012
Year: 2012
Results and conclusion: A variety of current-use pesticides were analysed in river waters samples collected during the 2005 and 2012 at 565 sites in Switzerland. The results for glyphosate are summarised in the following: Max. Concentration: 7.2 µg/L 95. percentile: 0.6 µg/L Amounts above the limit of determination (LOD): 42 % Number of sites above LOD: 81 %
Proposed action: To be considered in the summarizing tables of surface water monitoring. Obtained data are comparable to those given in the summarizing table.
Type of information (critical, high/low weight, supporting, additional): Supporting information

Reliability	
Endpoint	Glyphosate concentrations in river waters
Protocol	Not given
Test compound	Pesticides (Glyphosate (amongst others))
Test system and conditions	- Sample taking (in river waters in Switzerland and determination of pesticides concentrations - Calculation of 95. percentiles, amounts above the limit of determination (LOD) and number of sites above LOD
Statistical design	Not given
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Ludvigsen and Lode (2001)

Title: RESULTS FROM "JOVÅ" THE AGRICULTURAL AND ENVIRONMENTAL MONITORING PROGRAM OF PESTICIDES IN NORWAY 1995 – 1999	
Author: Gro Hege Ludvigsen and Olav Lode	
Reference: Fresenius Environment Bulletin, Vol. 10 (5)	
Year: 2001	
Results and conclusion: In the monitoring program called "JOVÅ" we have the following types of investigations: streams and rivers, drainage water, groundwater, sediments and precipitations. In this paper only the results from 12 locations concerning streams and rivers are presented from the years 1995 – 1999. Results for glyphosate: 86 % positive findings, maximum concentration = 0.93 µg/L, average = 0.13 µg/L. Results for AMPA: 87 % positive findings, maximum concentration = 0.2 µg/L, average = 0.06 µg/L.	
Proposed action: Data are presented in the summarizing table and discussed in the summarizing paragraph.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as publication deals with specific objective, namely surface water concentrations at a certain place and time.	
Reliability	Medium
Endpoint	Glyphosate concentrations in surface waters
Protocol	For details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), purity not given, monitoring
Test system and conditions	In six of the drainage basins data on the use of pesticides have been collected. 851 samples have been collected during this period. Sampling and analyses described, LOD = 0.01 µg/L
Statistical design	Number analyzed: 49, average values, maximum values given
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Ludvigsen and Lode (2002)

Title: TRENDS OF PESTICIDES IN NORWEGIAN STREAMS AND RIVERS (1996–2000)	
Author: Gro Hege Ludvigsen and Olav Lode	
Reference: Intern. J. Environ. Anal. Chem., Vol. 82, No. 8–9, pp. 631–643	
Year: 2002	
Results and conclusion: In Norway twelve streams and medium size rivers have been monitored for pesticides in a four to six years period. Trend analyses have been done on the years 1996–2000 to gain information on whether there have been reductions in the retrieval of the pesticides. The situation in these streams has not changed much during this period, but there are indications towards a slight positive development. Trend analyses might therefore be useful together with careful interpretation. Results for glyphosate: 91 % positive findings, no further information given.	
Proposed action: Data are presented in the summarizing table and discussed in the summarizing paragraph.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as publication gives no explicit values.	
Reliability	Low
Endpoint	Glyphosate concentrations in surface waters
Protocol	For details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6), purity not given, monitoring
Test system and conditions	The basis for the monitoring programme is six rather small catchments that have continuous discharge measurements and water proportional samplings monitored 1995 till 2000. The catchments vary in size from 50 to 680 hectares and the total number of farms varies from 5 to 30. Sampling and analyses described, LOD = 0.01 µg/L.
Statistical design	Number analyzed: 57, no detailed values reported
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Major et al. (2003)

Title: Concentrations of Glyphosate and AMPA in Sediment Following Operational Applications of Rodeo® to Control Smooth Cordgrass in Willapa Bay, Washington, USA
Author: W. W. Major III, C. E. Grue, S. C. Gardner, J. M. Grassley
Reference: Bull. Environ. Contam. Toxicol. 71, 912 - 918
Year: 2003
Results and conclusion: Previous studies in the Bay have examined the fate and persistence of glyphosate and AMPA following an aerial spray of Rodeo® to Spartina clones at half the recommended application rate or repeated band applications. Here, we report concentrations of glyphosate and AMPA in sediment following operational hand and aerial applications of Rodeo® to Spartina in the Bay at maximum allowable rates. Sediment concentrations were for Glyphosate: 0.3 - 16.2 ppm (0 days after application, hand sprayed), < 1.8 ppm (360 days after appl., hand sprayed); 0.04 - 2.5 ppm (0 days after

application, aerial spraying), < 1.8 ppm (360 days after appl., aerial spraying). For AMPA: 0.02 - 1.7 ppm (0 days after application, hand sprayed), < 0.5 ppm (360 days after appl., hand sprayed); 0.02 - 1.7 ppm (0 days after application, aerial spraying), < 0.5 ppm (360 days after appl., aerial spraying).	
Proposed action: Not to be considered. Publication deals with a specific event outside EU.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as publication gives information on concentrations in US sediments.	
Reliability	Low
Endpoint	Glyphosate concentrations in sediments
Protocol	For details see under test system and conditions, non-GLP
Test compound	Glyphosate, isopropyl salt (CAS-no: 38641-94-0) and AMPA (CAS-no: 1066-51-9), purity not given, monitoring
Test system and conditions	Sites being representative for hand spraying of clones were selected. Additionally, aerial spraying was performed. Aerial deposition (on filter paper) measured, sediment cores sampled and analyzed,
Statistical design	Recoveries from filter paper and sediment determined, relatively low recovery (86.7 % for Glyphosate, 78.5 % for AMPA) from sediment taken into account
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Results are valid for that particular place and time; no negative evidence.

Meyer et al. (2011)

Title: Concentrations of dissolved herbicides and pharmaceuticals in a small river in Luxembourg
Author: Berenike Meyer, Jean-Yannick Pailler, Cédric Guignard, Lucien Hoffmann and Andreas Krein
Reference: Environ Monit Assess (2011) 180:127–146
Year: 2011
Results and conclusion: The pollution is derived from leaching by subsurface flow, as well as wash-off and erosion caused by surface runoff. In the Luxembourgish Mess River catchment, the pharmaceutical and pesticide concentrations are comparable with those detected by other authors in different river systems worldwide. Some investigated pesticide concentrations infringe current regulations. Glyphosate (6,220 ng l−1) and AMPA (1,118 ng l−1) were among the pesticides found in the highest concentrations during flood events in the Mess River. The load of dissolved pesticides reaching the stream gauge is primarily determined by the amount applied to the surfaces within the catchment area. Storm water runoff from urban areas causes short-lived but high-pollutant concentrations and moderate loads, whereas moderate concentrations and high loads are representative for agricultural inputs to the drainage system. Glyphosate and AMPA were found in higher concentrations in urban basins, whereas terbutylazine, metolachlor, atrazine and DEA were prominent in rural zones.
Proposed action: Data are presented in the summarizing table and discussed in the summarizing paragraph.

Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Medium
Endpoint	Concentrations in surface water
Protocol	Not applicable, see also under test system, non-GLP
Test compound	Monitoring study
Test system and conditions	glyphosate and its main metabolite AMPA were analysed by LC-MS/MS.
Statistical design	Not reported
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Peruzzo et al. (2008)

Title: Levels of glyphosate in surface waters, sediments and soils associated with direct sowing soybean cultivation in north pampasic region of Argentina	
Author: Pablo J. Peruzzo, Atilio A. Porta, Alicia E. Ronco	
Reference: Environmental Pollution 156, 61 - 66	
Year: 2008	
Results and conclusion: Levels of glyphosate were determined in water, soil and sediment samples from a transgenic soybean cultivation area located near to tributaries streams of the Pergamino-Arrecifes system in the north of the Province of Buenos Aires, Argentina. In the field, levels of glyphosate in waters ranged from 0.10 to 0.70 mg/L, while in sediments and soils values were between 0.5 and 5.0 mg/Kg. Temporal variation of glyphosate levels depended directly on the time of application and the rain events. The results obtained from the application of the model are in accordance with the values found in the field.	
Proposed action: Not to be considered. Monitoring data for a site outside the EU (Argentina) are reported.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information, concentrations in a non-EU country measured and compared to a developed model.	
Reliability	Medium
Endpoint	Concentrations in surface water, soil and sediment; model output
Protocol	Not applicable, see also under test system, non-GLP
Test compound	Glyphosate, (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), purity not given ("high purity")
Test system and conditions	a) Field work took into account both the pesticide application and the rains occurring after applications. The pesticide was analysed by HPLC-UV detection. b) In addition, SoilFug multimedia model was used to analyse the environmental distribution of the pesticides (suggested for the calculation of predicted environmental concentrations in water, since it generally produces acceptable results from a relatively small set of input data). This method assesses the degradation, evaporation, leaching and runoff of a pesticide applied to a surface soil and consequently its potential impact on nearby water bodies considering

	the properties of the system in study regarding soil, pesticide and characteristics of the application events (number of events, time of application, dose and rains). The model was loaded with adjusted parameters from runoff and leaching tests at laboratory scale conducted during the development of the research project. The scaling applied to the real situation and the rain and application events recorded specially for this case were considered for the application of this model to the field situation.
Statistical design	See under test system and conditions
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable, model development; no negative evidence.

Peschka et al. (2005)

Title: Trends in Pesticide Transport into the River Rhine	
Author: M. Peschka, J.Müller, T. P. Knepper and P. Seel	
Reference: Hdb Env Chem Vol. 5, Part L (2006): 155–175; DOI 10.1007/698_5_016	
Year: 2005	
Results and conclusion: The occurrence of relevant pesticides in the River Rhine and two of its tributaries is presented over a period of ten years. Trace determinations of 66 target pesticides and their metabolites in water from the River Main and the River Nidda were performed on continuously sampled wastewater and surface water utilizing different solid phase extraction protocols and detection by gas chromatography mass spectrometry, directly or after derivatization. The transport rates of pesticides in municipal wastewater treatment plant (WWTP) effluents and surface waters were determined from data obtained in 1994, and these show that WWTPs contribute significantly to the pesticide pollution in the surface water. A trial education program providing improved methodology, spraying equipment and support to farmers living close to a single WWTP lead to a drastic reduction (more than 90%) in the total pesticide transport caused by this WWTP. During two extensive sampling campaigns in 1999 and 2000, mixed samples from a total of 106 (for 1999) and 35 (for 2000) WWTPs in agricultural used areas from Hesse (Germany) were investigated for selected priority pesticides and metabolites. In this case, the mitigation measures mentioned above were found to be unsuccessful overall, which is most likely attributable to less interaction with the pesticide users as compared to projects in small villages with high public attention.	
Proposed action: To be considered by listing in the summarizing table and discussion.	
Type of information (critical, high/low weight, supporting, additional): Medium weight; additional information of degradation and sorption isotherms	
Reliability	Medium
Endpoint	Concentration in surface water
Protocol	Not given
Test compound	Glyphosate (CAS no.:1071-83-6) and AMPA (CAS-no.: 1066-51-9)
Test system and conditions	Sampling and analyses in surface water
Statistical design	Not given

Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence; results supported by other publications.

Robles-Molina et al. (2014)

Title: Monitoring of selected priority and emerging contaminants in the Guadalquivir River and other related surface waters in the province of Jaén, South East Spain	
Author: J. Robles-Molina, B. Gilbert-López, J. F. García-Reyes, A. Molina-Díaz	
Reference: Science of the Total Environment 479–480 (2014) 247–257	
Year: 2014	
Results and conclusion: The aim of this survey is to monitor a total number of 373 compounds belonging to different families (pesticides, PAHs, nitrosamines, drugs of abuse, pharmaceuticals and life-style compounds) in surface waters located at different points of the province of Jaén (Spain). Among these compounds some priority organic substances (regulated by the EU Directive 2008/105/EC) and pollutants of emerging concern (not regulated yet) can be found. A liquid chromatography electrospray time-off light mass spectrometry (LC–TOFMS) method covering 340 compounds was developed and applied, together with a gas chromatography triple–quadrupole mass spectrometry (GC–MS/MS) method which enabled the analysis of 63 organic contaminants (30 of these compounds are analyzed by LC–TOFMS as well). From April 2009 to November 2010 a total of 83 surface water samples were collected (rivers, reservoirs and wetlands). In this period numerous organic contaminants were detected, most of them at the ng L^{-1} level. The most frequently priority substances found were chlorpyrifos ethyl, diuron and hexachlorobenzene. Within the other groups, the most frequently detected compounds were: terbuthylazine, oxyfluorfen, desethyl terbuthylazine, diphenylamine (pesticide family); fluorene, phenanthrene, pyrene (PAHs group), codeine, paracetamol (pharmaceuticals compounds) and caffeine, nicotine (life-style compounds). As is could be expected, the total concentration of emerging contaminants is distinctly larger than that of priority pollutants, highlighting the importance of continuing with the study of their presence, fate and effects in aquatic environments. However, concentration levels (at the ng per liter level) are low in general for both kinds of contaminants which minimizes the possible harmful effect on the environment. Glyphosate concentrations in surface water are not provided in the study.	
Proposed action: To be considered as additional information.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Concentration of pesticides, PAHs, nitrosamines, drugs of abuse, pharmaceuticals and life-style compounds in surface waters
Protocol	Not given
Test compound	373 compounds belonging to different families (pesticides, PAHs, nitrosamines, drugs of abuse, pharmaceuticals and life-style compounds)
Test system and conditions	A total of 19 sampling points were selected in surface waters located at different points of the province of Jaén (Spain). Representative samples of each point were collected in amber glass bot-

	tles with Teflon caps (1 L), and then were transported to the laboratory where they were stored at 4 °C for a maximum of 48 h before extraction. During a period of 20 months, 83 surface water samples were collected comprising 3 rivers, 5 reservoirs and 11wetlandswithin the province of Jaén. The analysis of the samples was carried out by using two different analytical methods depending on the vast array of different physicochemical features of the pollutants tested.
Statistical design	Not given
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Stewart et al. (2014)

Title: A survey of emerging contaminants in the estuarine receiving environment around Auckland, New Zealand
Author: M. Stewart, G. Olsen, C. W. Hickey, B. Ferreira, A. Jelić, M. Petrović, D. Barcelo
Reference: Science of the Total Environment 468–469 (2014) 202–210
Year: 2014
Results and conclusion: Increasing urbanisation in the future will put mounting stresses on the receiving environments around those urban centres due to increased sedimentation and contaminant runoff. Emerging contaminants (ECs) are an extensive array of chemicals and many are not under regulatory action. Within New Zealand likely future pressures from ECs will be in both urban centres and rural areas due to intensive agriculture, although at present there is a lack of information on the state of the environment in both sectors. This study was initiated to gauge the distribution of ECs in the urban environment by measuring concentrations of flame retardants, plasticisers, alkylphenols, herbicides and pesticides, steroid oestrogens, pharmaceuticals and heavy metals in sediment from 13 estuarine sites around Auckland, New Zealand's biggest city. Total polybrominated diphenyl ether (PBDE) flame retardant concentrations (7ΣPBDE) ranged from 0.55 to 573 ng/g (dw). The phthalate plasticiser di(2-ethylhexyl)phthalate (DEHP) was measured at up to 11,500 ng/g from one site. Nonylphenol (NP) was found at up to 32,000 ng/g at one site adjacent to the city's major wastewater treatment plant (WWTP). However, median concentrations of NP were 153 ng/g, suggesting this site was not representative of the region. Nonylphenol mono- and di-ethoxylates (NPEO1,2) had highest concentrations (1600 ng/g) at a marina. Highest glyphosate concentrations (up to 950 ng/g) were observed at residential sites. Steroid oestrogens were detected at extremely low concentrations (maximum 2.2 ng/g), while all other pesticides or herbicides were not detected at any sites. Multi-residue analysis of 46 pharmaceuticals showed presence of 21 compounds at one or more sites, with average concentrations ranging from 0.16 to 7.66 ng/g. Generally, environmental concentrations of ECs were similar to those reported world-wide. However, comparisons for pharmaceuticals were problematic, due to very few studies on pharmaceutical concentrations in estuarine sediments, with most focussed on sewage and stream water phases. Glyphosate and AMPA in estuarine sediments from Auckland, New Zealand: Glyphosate was detected at 8 of the 13 sites, ranging from 58 to 950 ng/g with a median value of 120 ng/g. AMPA was detected at 2 sites; Puketutu Island at 345 ng/g and Meola at 215 ng/g. Glufosinate was not detected at any site, with a LOQ of 20 ng/g. The sites with

the highest glyphosate concentrations: Meola (950 ng/g); Whau (315 ng/g); and Motions (235 ng/g) are all established residential areas. Other potential inputs to these catchments include industrial (Whau) and sewage & landfill (Meola & Motions). These sites are not unique in their land-use and so it is difficult to assign land-use patterns to potential glyphosate sources. Other sites are residential areas and/or have industrial, sewage and landfill inputs but have much reduced glyphosate concentrations. Due to the high polarity and water solubility of glyphosate and AMPA, their environmental concentrations have been largely restricted to stream water samples (Botta et al., 2009; Glozier et al., 2012; Kolpin et al., 2006; Scribner et al., 2003), while those that do include sediment or soils are centred around the use of glyphosate resistant crops (Mamy et al., 2010; Peruzzo et al., 2008). Furthermore, little data appear to exist on the environmental occurrence of glyphosate and AMPA derived from the extensive urban use of glyphosate (Kolpin et al., 2006). As such, it is difficult to make comparisons between the concentrations found in this study and those observed elsewhere.	
Proposed action: Not to be considered as monitoring outside the EU (New Zealand).	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	
Endpoint	Concentrations of emerging contaminants in sediment from 13 estuarine sites around Auckland, New Zealand
Protocol	Not given
Test compound	Glyphosate, glufosinate and AMPA amongst other emerging contaminants
Test system and conditions	The study was conducted in Auckland region, New Zealand. Thirteen sites were selected for sampling around the greater Auckland region. The contaminants chosen for analysis were based on information from a review on ECs of potential environmental concern in Auckland (Ahrens, 2008) and the logistics in finding analytical laboratories that provided robust analytical measurements of these contaminants. All analyses were undertaken between April and October 2008, with the exception of pharmaceuticals which were undertaken in May 2010. Sediments were analysed for 3 herbicides – glyphosate, aminomethylphosphonic acid (AMPA; primary breakdown product of glyphosate) and glufosinate amongst other emerging contaminants.
Statistical design	Replicate analyses were undertaken for glyphosate. A relative contamination ranking for each site was calculated based on contaminant concentrations.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Similar monitoring programmes not known. No negative evidence.

Publications regarding groundwater monitoring

Summary

Groundwater monitoring data were obtained for ~~4~~ 6 EU-countries (Spain, Denmark, Norway, Hungary, Sweden and Germany), whereas ~~one~~ two citations ~~was~~ were found for ~~a~~ non-EU sites (Canada). As for surface water, information is rather heterogeneous. Maximum glyphosate and AMPA concentrations in either groundwater or drinking water well in the 4 EU-countries named above are in the range of 0.02 - 2.56 µg/L and 0.02 - 0.45 µg/L, respectively, whereas maximum glyphosate and AMPA concentrations as obtained from comprehensive monitoring programs (Horth, 2012; see point 8.6.3) are in the range of 0.01 -24 µg/L and 0.02 - 19 µg/L, respectively. Therefore, information published in open literature does not really modify the already existing assessment of glyphosate occurrence in groundwater (see B.8.6.3).

The results of the groundwater monitoring studies are listed in the following table though some information is lacking.

Table B.8.11–4: Results of the publications on groundwater monitoring

Country/ Substance	Description of sample	Date	No. Sites	No. Samples	Detected (samples)		Samples ≥ 0.1 µg/L		Max. conc.	LOQ (LOD)
					No.	%	No.	%	µg/L	µg/L
Spain										
Glyphosate	Ground-water	2007-10	69	139	61	47.3	-	-	2.56	0.01 (0.003)
Hungary										
Glyphosate	Ground water	2010-2011	6	36	36	100	36	100	0.98	0.05 to 0.12
Denmark										
Glyphosate	Drinking water wells	-	289	-	-	-	27[1)]	9.3	-	-
AMPA	Drinking water wells	-	286	-	-	-	24[1)]	8.4	-	-
Glyphosate	Ground-water monitoring	1999-2010	1	223	-	-	0	0	n.d.	-
AMPA	Ground-water monitoring	1999-2010	1	223	-	-	0	0	0.022	-
Glyphosate	Ground-water monitoring	1999-2010	1	236	-	-	0	0	0.031	-
AMPA	Ground-water monitoring	1999-2010	1	236	-	-	0	0	0.08	-
Glyphosate	Ground-water monitoring	1999-2010	1	551	-	-	3	0.5	0.67	-
AMPA	Ground-water monitoring	1999-2010	1	553	-	-	0	0	0.07	-
Glyphosate	Ground-water monitoring	1999-2010	1	284	-	-	0	0	0.017	-
AMPA	Ground-water monitoring	1999-2010	1	284	-	-	0	0	0.029	
Norway										
Glyphosate	Shallow ground-water, farm wells, public water words	1995-2004	64	3	1	33	-	-	0.1	(0.01)
AMPA	Shallow ground-water, farm wells, public water words	1995-2004	64	2	1	50	-	-	0.02	(0.01)
Germany										
Glyphosate	Shallow ground water wells	2008	83	83	0	0	0	0	-	0.02
AMPA	Shallow ground water wells	2008	83	83	1	1	1	1	0.123	0.01
Glyphosate	Ground-	2006-	-	245	-	-	0	0	0.08	-

Country/ Substance	Description of sample	Date	No. Sites	No. Samples	Detected (samples)		Samples ≥ 0.1 µg/L		Max. conc.	LOQ (LOD)
					No.	%	No.	%	µg/L	µg/L
	water NRW	2012								
AMPA	Ground-water NRW	2006-2012	-	260	-	-	7	3	0.45	-

[1)] Above threshold of 0.01 µg/L

Detailed description of open literature on groundwater monitoring

Bachor et al. (2008)

Title: Special report on detection of pesticides and pharmaceuticals in surface water and ground water in Mecklenburg-Vorpommern (Germany) in spring 2008	
Author: Alexander Bachor, Gabriele Lemke, André Schumann	
Reference: Report of the State Agency for the Environment, Nature Conservation and Geology Mecklenburg-Vorpommern (LUNG)	
Year: 2008	
Results and conclusion: During April 1st and 25.06.2008 samples from 60 surface water (river) and 83 groundwater measuring points in Mecklenburg-Vorpommern were analysed for different pesticides and pharmaceuticals. In 0 of 83 groundwater samples glyphosate was found with concentrations > 0.1 µg/L. AMPA was found in only 1 of 83 samples with concentrations > 0.1 µg/L (0.123 µg/L).	
Proposed action: To be considered in the summarizing table of groundwater monitoring in spite of the small time window. Obtained data are comparable to those given in the summarizing table.	
Type of information (critical, high/low weight, supporting, additional): Low weight (only small time window), may be used as additional information	
Reliability	Low
Endpoint	Concentration in groundwater
Protocol	No information in the report on analysis methods
Test compound	Glyphosate, (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), purity not given
Test system and conditions	No information in the report, only overview of findings
Statistical design	Not given
Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	The results support the long time monitoring data. However, monitoring studies and campaigns over more years and with more detailed information are of more reliability and relevance. No negative evidence.

Busch and Reupert (LANUV) 2013

Title: Belastungsentwicklung von Oberflächengewässern und Grundwasser in NRW mit Glyphosat und AMPA
Author: Dieter Busch, Rolf Reupert (LANUV)
Reference: Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen

(LANUV)	
Year: 2013	
Results and conclusion: Results from a federal monitoring programme in North Rhine-Westphalia, Germany regarding concentrations of glyphosate and AMPA in surface water and groundwater are presented. The results are summarised in the following. 2001-2012: Concentration of glyphosate in surface water: Ruhr, 3 sites: max. 0.10 - 0.29 µg/L Concentration of AMPA in surface water: Ruhr, 3 sites: max. 0.86 - 2.02 µg/L 1996-2012: Concentration of glyphosate in surface water: NRW: 1899 samples, 225 samples > 0.1 µg/L (maximum 0.93 µg/L) 1996-2012: Concentration of AMPA in surface water: NRW: 1903 samples, 1377 samples > 0.1 µg/L (maximum 13 µg/L) 2006-2012: Concentration of glyphosate in groundwater: NRW: 245 samples, 0 samples > 0.1 µg/L (maximum 0.08 µg/L) 2006-2012: Concentration of AMPA in groundwater: NRW: 260 samples, 7 samples > 0.1 µg/L (maximum 0.45 µg/L)	
Proposed action: To be considered in the summarizing tables of surface water and groundwater monitoring. Obtained data are comparable to those given in the summarizing table.	
Type of information (critical, high/low weight, supporting, additional): No details are provided in the report about the finding localities and special causes. The information should be considered as additional.	
Reliability	
Endpoint	Concentrations of glyphosate and AMPA in surface water and groundwater
Protocol	In-house standard according to ISO 21458; DIN 38407-22
Test compound	Glyphosate and AMPA
Test system and conditions	Monitoring programme
Statistical design	Not given
Relevance	
Environmental relevance	Relevant
Weight of evidence	
“Positive”/”Negative” evidence	No negative evidence.

BVL (2010)

Title: Proceedings of the 25th meeting of the consulting committee of natural environment of BVL, 24./25.February 2010	
Author: Consulting committee of natural environment of BVL (Germany)	
Reference: Proceedings of the 25th meeting of the consulting committee of natural environment of BVL, 24./25.February 2010	
Year: 2010	
Results and conclusion: Discussion about glyphosate: • Groundwater monitoring in the context of implementation of water framework directive (WRRL) requires improved cooperation with plant protection service. • Harmonisation of the assessment approaches of different federal states in Germany regarding glyphosate findings in groundwater is preferable • Considering latest research results regarding the pathway bank filtration of glyphosate a new runoff-mitigation measure (minimum: 5 m bare buffer zone) will be required for pesticides containing glyphosate. The discussion is ongoing.	
Proposed action: Not to be considered in the summarizing table of groundwater monitoring. The protocol only contains national management proposals.	
Type of information (critical, high/low weight, supporting, additional): Low weight (German approach), may be used as additional information	
Reliability	Low
Endpoint	None (management measures)
Protocol	Not relevant (proceedings)
Test compound	Not relevant (proceedings)
Test system and conditions	Not relevant (proceedings)
Statistical design	Not relevant (proceedings)
Relevance	
Environmental relevance	Relevant only for German authorities
Weight of evidence	
“Positive”/”Negative” evidence	Relevant only for German authorities

Crowe et al. (2011)

Title: Application of a glyphosate-based herbicide to *Phragmites australis*: Impact on groundwater and near-shore lake water at a beach on Georgian Bay
Author: Allan S. Crowe, Natalie Leclerc, John Struger, Susan Brown
Reference: Journal of Great Lakes Research 37, 616–624
Year: 2011
Results and conclusion: Groundwater and lake water were tested to determine if glyphosate enters the groundwater and lake at the beach and how long glyphosate will persist. Two days after application, the geometric mean concentration of glyphosate in the groundwater below the Phragmites was 0.060 µg/L with a maximum of 12.50 µg/L. Concentrations rapidly declined over the next two to three weeks to below minimum detection limits (0.020 µg/L). Glyphosate was also detected in the nearshore lake water with concentrations peaking at a geometric mean of 0.14 µg/L one week after application, and declining to 0.039 µg/L four weeks after applica-

tion. An approximate half-life for the dissipation of glyphosate by degradation and dilution/flushing as groundwater flows toward the lake, assuming a first order kinetic reaction, yielded a half-life of 3.5 d during the 4 weeks after the herbicide was applied. The application of Roundup® resulted in an 90% reduction in the size of the stand of Phragmites.	
Proposed action: Not to be considered as the monitoring was outside the EU (Canada).	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Medium
Endpoint	Monitoring, concentrations in surface water and groundwater
Protocol	Monitoring, for details see under test system and conditions.
Test compound	Glyphosate (CAS-no: 1071-83-6) AMPA (CAS-no: 1066-51-9) purity cannot be given, monitoring
Test system and conditions	Sampling campaign described, also clean-up, analytical methods, LOD
Statistical design	Not given
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	~~Similar monitoring programs not known.~~ No negative evidence.

Haarstad and Ludvigsen (2007)

Title: Ten Years of Pesticide Monitoring in Norwegian Ground Water	
Author: K. Haarstad and G. H. Ludvigsen.	
Reference: Ground Water Monitoring & Remediation 27, no. 3, pp. 75–89	
Year: 2007	
Results and conclusion: Pesticides in Norwegian groundwater have been monitored since 1995. Here, we report data including 2004. The monitoring has focused on shallow groundwater near agricultural fields (4 locations), on farm wells (22 locations), and on public waterworks (38 locations). 450 samples were analyzed for a total of 62 pesticide compounds and metabolites, and the result was 514 detections of single compounds. Though glyphosate has highest use in Norway (ca. 215 tons in 2004) it was detected in only one sample from waterworks, while the metabolite AMPA was detected in one farm well. Low detection is probably due to high adsorption in soils. Glyphosate have frequently been detected in streams in Norway.	
Proposed action: Considered by listing in the summarizing table and discussion. To be used as additional information.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information	
Reliability	High
Endpoint	Glyphosate concentrations in groundwater, wells and waterworks
Protocol	for details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6), AMPA (CAS-no: 1066-51-9), purity not given, monitoring
Test system and conditions	Four different locations have been monitored, two of them for 10 years (S1 and S2). S1 and S2 sampling sites are intensively used for crop production and thus are prone for leaching. The mean

	groundwater depth at S1 is 1.0 m, with extreme values varying from 0.18 to 1.98 m below the surface, and the mean depth at S2 was 0.5 to 0.9 m, with extreme values varying from 0.15 to 1.7 m below the surface. A total of 22 farm wells were selected among volunteers after public advertising. Locations were chosen to represent different geology (rock and soil), well types, and depths, and to include wells with and without influence from point sources. Some wells were sampled only for a short period, while those with frequent detections have been followed over many years to evaluate trends. A total of 144 samples were taken. A total of 38 waterworks were sampled, located in most parts of Norway as far north as the county Nordland.
Statistical design	A Spearman's Rho nonparametric pair wise correlation analysis was carried out, between total pesticide concentrations and the well depths. The analysis included 19 farm wells and 5 wells sampling shallow groundwater. Only locations with at least five detections of pesticides were used, with a level of significance of 5%. A linear regression trend analysis was also carried out on selected locations (Excel), testing if the angle of the straight regression line is significantly different from zero.
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Kjaer et al. (2009)

Title: The Danish Pesticide Leaching Assessment Programme
Author: Jeanne Kjær, Annette E. Rosenbom, Walter Brüsch, René K. Juhler, Lasse Gudmundsson, Finn Plauborg, Ruth Grant and Preben Olsen
Reference: Geological Survey of Denmark and Greenland
Year: 2011
Results and conclusion: In 1998, the Danish Parliament initiated the Pesticide Leaching Assessment Programme (PLAP), an intensive monitoring programme aimed at evaluating the leaching risk of pesticides under field conditions. The objective of the PLAP is to improve the scientific foundation for decision-making in the Danish regulation of pesticides. The specific aim is to analyse whether pesticides applied in accordance with current regulations leach to groundwater in unacceptable concentrations. The programme currently evaluates the leaching risk of 42 pesticides and 41 degradation products at five agricultural sites ranging in size from 1.1 to 2.4 ha. The evaluation is based upon monitoring results representing detections in 1 meters depth (water collected via drains and suction cups) and detections in groundwater monitoring screens (1.5-4.5 meter below ground surface, hereafter m b.g.s.). This report presents the results for the entire monitoring period May 1999-June 2010. The results of the entire monitoring period 1999-2010 covering 42 pesticides, show that: The monitoring data indicate pronounced leaching of 14 of the applied pesticides and/or their degradation products. Glyphosate and its degradation product AMPA leached through the soil entering drains and suction cups (placed 1 m b.g.s) in average concentrations exceeding 0.1 µg/l. For glyphosate and AMPA, pronounced leaching is mainly confined to the depth of 1 meter, where

pesticides were frequently found in samples collected from drains and suction cups, while a limited number of detections exceeding 0.1 µg/l were found in groundwater monitoring wells. In the following the maximum concentration (µg/l) from the groundwater monitoring screens are given: **Glyphosate** Tylstrup: - Jyndevad: n.d. Silstrup: 0.031 µg/l Estrup: 0.67 µg/l Faardrup: 0.017 µg/l **AMPA** Tylstrup: - Jyndevad: 0.022 µg/l Silstrup: 0.08 µg/l Estrup: 0.07 µg/l Faardrup: 0.029 µg/l Numbers of glyphosate detections exceeding 0.1 µg/l in groundwater monitoring wells are very limited (only 3 samples).
Proposed action: To be considered in the summarizing table of groundwater monitoring. Obtained data are comparable to those given in the summarizing table.
Type of information (critical, high/low weight, supporting, additional): To be used as supporting information.

Reliability	
Endpoint	Concentration of glyphosate and AMPA in groundwater at agricultural sites
Protocol	No information
Test compound	Glyphosate and AMPA
Test system and conditions	Monitoring programme aimed at evaluating the leaching risk of pesticides under field conditions
Statistical design	Statistical analysis of the internal QA data: The statistical tool used is an analysis of variance (ANOVA) and encompasses all duplicate pesticide analyses, single analyses being excluded. The analysis can be divided into three stages: 1) Normality, 2) Between-day contribution, 3) Calculating standard deviations
Relevance	
Environmental relevance	Relevant
Weight of evidence	
“Positive”/”Negative” evidence	No negative evidence.

Krause et al. (2009)

Title: Organic Trace Substances Relevant for Drinking Water – Assessing their Elimination through Bank Filtration
Author: Björn Krause, Astrid Weigert, Stefan Heise, Norbert Litz
Reference: in: Report of the 2nd experimental phase of the TRACE-project; Copyright 2009 by the Kompetenz Zentrum Wasser Berlin gGmbH.
Year: 2009
Results and conclusion:

To estimate the occurrence of glyphosate and its main metabolite AMPA in the surroundings of Berlin samples from 22 surface water sites were analysed within this study. In 5 samples the glyphosate concentration was above the European threshold for herbicides of 0.1 µg/L in drinking water. Up to 70 % of Berlin's drinking water is produced via bank filtration and aquifer recharge characterized by comparatively low flow velocities (< 1 m/d), long contact times (3-6 months) and mainly anoxic redox conditions. Results of enclosures show that the breakthrough of glyphosate was retarded remarkably despite of the initially postulated low adsorption potential of the sandy filter substrate. Also a significant reduction, probably due to degradation was observed. However, adsorption and degradation parameters obtained in the laboratory and semi-technical experiments vary significantly due to the difficulty to imitate natural conditions in the laboratory.	
Proposed action: Not to be considered as publication deals with a specific situation. It furthermore addresses the laboratory to field extrapolation problems.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information	
Reliability	High
Endpoint	Glyphosate concentrations in surface water around Berlin, in enclosure outlets, K_f-values, DT_{50}-values, break-through curves
Protocol	For details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6), purity 98.7 %
Test system and conditions	a) surface water sampling around Berlin and analysis b) evaluation of the potential of bank filtration to protect the drinking water from glyphosate contaminations: Three enclosures dosed with three different concentration levels (average concentration: 0.7, 3.5 and 11.6 µg/L) over a time period of 14 days. The effluent was sampled daily for 34 days. Glyphosate and AMPA were analysed applying the HPLC method according to the German Standard DIN 38407-22/2001. c) laboratory column leaching (OECD 312), sorption (batch, OECD 106) and degradation (in sediment, similar to OECD 307, 8°C) studies
Statistical design	Freundlich isotherms, 1. order degradation kinetics, break-through curves,
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Lindqvist et al. (2007)

Title: Om förekomst av bekämpningsmedelsrester i grundvatten: Erfarenheter från Simrishamn kommun 2002-2007 (Monitoring of pesticides in rawwater wells in Simrishamn)
Author: Bengt-Olov Lindqvist, Jan-Bertil Hansson, Christina Jönsson and Kenneth M. Persson
Reference: Vatten 63:159–163. Lund 2007
Year: 2007
Results and conclusion: In January 2007, all municipal water wells of Simrishamn, south Sweden, were sampled

and analyzed for the presence of pesticide residuals. In total 34 wells were analyzed. The samples were analyzed at an accredited laboratory with respect to 77 different parameters of pesticides which either are used or have been used in the recharge area of the wells. The results from the investigation 2007 show that residuals of pesticides were detected in 12 different water wells distributed across the municipality. For virtually all analyses, the concentrations reported are very low, in the order of 0,01 µg/l, and only one or occasionally two parameters were found in each sample. The same investigation was undertaken in 2002. Compared with those results, an important finding is that more parameters were found in 2007 compared with 2002. The overall concentration of residuals has not changed since 2002. The concentrations are not higher but not lower either. The fundamental conclusion is that the work with water protection areas and the control of pesticide spread is still necessary and inevitable. Maximum glyphosate concentration reported: 0.08 µg L^{-1}	
Proposed action: Not considered as the text written in Swedish language, thus the study cannot be fully evaluated. The results seem to be in the same range as other similar monitoring studies.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information	
Reliability	High
Endpoint	Glyphosate concentrations in groundwater, wells and waterworks
Protocol	for details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6), AMPA (CAS-no: 1066-51-9), purity not given, monitoring
Test system and conditions	? (text in Swedish language)
Statistical design	? (text in Swedish language)
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Text written in Swedish language, thus the study cannot be fully evaluated. The results seem to be in the same range as other similar monitoring studies; no negative evidence.

Malaguerra et al. (2010)

Title: CONTAMINATION OF DRINKING WATER SUPPLY WELLS BY PESTICIDES FROM SURFACE WATER RESOURCES
Author: Flavio Malaguerra, Hans-Jørgen Albrechtsen and Philip J. Binning
Reference: Presentation at: XVIII International Conference on Water Resources, CMWR 2010, J. Carrera (Ed), ® CIMNE, Barcelona
Year: 2010
Results and conclusion: A reactive transport model is developed to evaluate the potential of contamination of drinking water wells by surface water pollution. The model is validated using data of a tracer experiment. The fate of MCPP, glyphosate and its degradation product AMPA is investigated. Global sensitivity analysis using the Morris method is used to identify model dominant parameters. Results show that the existence of a clay aquifer, pollutant properties and the well depth are the crucial factors when evaluating the risk of drinking water well contamination from surface water.
Proposed action: Considered by listing in the summarizing table and discussion. To be used as additional

information.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information, model development	
Reliability	Not applicable
Endpoint	Model development, no experimental study
Protocol	Not applicable, see also under test system, non-GLP
Test compound	Glyphosate, (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), purity not given, model development using published data
Test system and conditions	A conceptual model has been developed and applied using published experimental data. The sensitivity analysis is performed using Morris method. The Morris method is a global sensitivity analysis method, determining the sensitivity over the whole parameter space. The method determines elementary effects for each input. Parameters are varied one at a time, and for every change the model is evaluated: the elementary effect is then defined to be the output change divided by the input change. The distribution of elementary effects is evaluated for the parameter space and the mean and the standard deviation of the elementary effects are used as sensitivity measures.
Statistical design	See under test system and conditions
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable, model development; no negative evidence.

Malaguerra et al. (2012)

Title: Pesticides in water supply wells in Zealand, Denmark: A statistical analysis
Author: Flavio Malaguerra, Hans-Jørgen Albrechtsen, Lærke Thorling and Philip J. Binning
Reference: Science of The Total Environment 414: 433-444. doi:10.1016/j.scitotenv.2011.09.071
Year: 2012
Results and conclusion: Data from the Danish National Borehole Database are used to predict drinking water well vulnerability to contamination by pesticides, and to identify the dominant mechanisms leading to well pollution in Zealand, Denmark. The frequency of detection and concentrations of 4 herbicides and 3 herbicide metabolites are related to factors accounting for geology (thicknesses of sand, clay and chalk layers), geographical location (distance to surface water and distance to contaminated sites), redox conditions and well depth using logistic regression, the binomial test and Spearman correlation techniques. Parameters accounting for the hydraulic connection between the well and the surface (well depth and thickness of the clay confining layer) are often strongly related to well vulnerability. Results also show that wells close to surface water are more vulnerable to contamination, and that sandy layers provide better protection against the leaching of oxidizable pesticides than clay aquitards, because they are more likely to be aerobic The field data are used to create a set of probabilistic models to predict well vulnerability to contamination by pesticides.
Proposed action: Considered by listing in the summarizing table and discussion. To be used as additional

information.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information, predictive model development	
Reliability	Not applicable
Endpoint	Predictive model development, no experimental study
Protocol	Not applicable, see also under test system, non-GLP
Test compound	Glyphosate, (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), purity not given, model development using published data
Test system and conditions	The data base for Glyphosate was: 289 wells, 707 samples (Zealand), 44 wells, 108 samples (Jutland); for AMPA: 286 wells, 691 samples (Zealand), 44 wells, 108 samples (Jutland).
Statistical design	Spearman rank correlation was used to delineate trends between the parameters (D, Ds, Dcl, Dch, dsw, dcs) and pesticide concentrations. Spearman correlation was selected in order to consider non-linear responses and because the data were not normally distributed. Correlations were calculated for two separate datasets: to all drinking water wells and to the drinking water wells where the amount of pesticides detected was above the detection limit. This last dataset was used to avoid the bias caused by the points where the pesticide was detected but could not be quantified.
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable, model development; no negative evidence.

Mörtl et al. (2013)

Title: Determination of glyphosate residues in Hungarian water samples by immunoassay
Author: Mária Mörtl, Gyöngyi Németh, Judit Juracsek, Béla Darvas, Lisa Kamp, Fernando Rubio and András Székács
Reference: Microchemical Journal 107 (2013) 143–151
Year: 2013
Results and conclusion: An enzyme-linked immunosorbent assay (ELISA) for the detection of glyphosate was investigated for assay performance characteristics and was applied for determination of glyphosate contamination levels in selected surface and ground water resources in Hungary in 2010 and 2011. The method was applied for the analysis of 42 surface and ground water samples collected from Békés county in Hungary at 14 sampling sites in 2010 and 18 surface water samples collected from the Danube River and Lake Velencei in Hungary at 12 sampling sites in 2011. Exceedingly high glyphosate levels (nearly 1 ng/ml) were measured in 5 samples, and significant concentrations were determined in 16 cases (0.54–0.76 ng/ml) in 2010,while practically no contamination was found in 2011. The great contrast between the two sampling regimes is explained by differing agricultural locations, natural precipitation and, to a greater extent, catchment area characteristics, resulting in varying leaching or run-off of glyphosate to surface waters.
Proposed action: Considered by listing in the summarizing table and discussion. To be used as additional information.
Type of information (critical, high/low weight, supporting, additional):

Low weight, additional information, model development	
Reliability	Medium
Endpoint	Glyphosate concentrations in groundwater and surface water
Protocol	Not applicable, see also under test system, non-GLP
Test compound	Monitoring study
Test system and conditions	In the scope of a national environmental survey, 42 water samples (6 surface water and 36 ground water samples) were obtained on September 7–8, 2010, from14 sampling sites in Békés county, Hungary. In addition, 18 surface water samples were collected on October 1, 2011, from 11 sampling sites along the Danube River and one site at Lake Velencei, Hungary.
Statistical design	See under test system and conditions
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar studies. However, results are valid for that particular place and time; no negative evidence.

Riedl et al. (2005)

Title: Sickerwasserversuche an der Forschungsstation Wagna zur Untersuchung der Verlagerung des Herbizids Glyphosate in der ungesättigten Bodenzone	
Author: Hans-Erik Riedl and Heimo Stadlbauer	
Reference: Amt der Steiermärkischen Landesregierung Fachabteilung 17C - Technische Umweltkontrolle und Sicherheitswesen, Dokumentation zum Thema Gewässerschutz	
Year: 2005	
Results and conclusion: (study is written in German language) Die Versuche beim Lysimeter in Wagna zeigten, dass unter den hydrometeorologischen Rahmenbedingungen, die dem langjährigen Mittel entsprechen (Sättigung des Bodens über die Wintermonate; intensive Frühjahrsniederschläge in April und Mai), eine noch raschere Verlagerung von Glyphosate und AMPA in höheren Konzentrationen bis in den Kiesbereich nicht ausgeschlossen werden kann. Dies vor allem dann, wenn der Wirkstoff kurz nach – oder noch schlechter, weil nicht vorhersehbar, kurz vor einem intensiven Niederschlagsereignis ausgebracht wird.	
Proposed action: Not considered for listing in the summarizing table and discussion. To be used as additional information.	
Type of information (critical, high/low weight, supporting, additional): High weight, additional information	
Reliability	Medium
Endpoint	Glyphosate concentrations in groundwater
Protocol	Non-GLP
Test compound	Roundup-Ultra
Test system and conditions	Die Verlagerung des Wirkstoffes Glyphosate bzw. dessen Metabolit AMPA wurde unter ortsüblichen Bewirtschaftungsweisen bei den hier herrschenden meteorologischen Rahmenbedingungen und den existenten Boden- und Fruchtfolgebedingungen detailliert untersucht und schlüssige Aussagen hinsichtlich einer potentiellen Gefährdung des Grundwassers der quartären Talfüllungen des Murtales durch den Einsatz des angeführten Herbizids abgeleitet.

Statistical design	Not reported
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar studies. However, results are valid for that particular place and time; no negative evidence.

Van Stempvoort et al. (2013)

Title: Residues of the herbicide glyphosate in riparian groundwater in urban catchments
Author: D.R. Van Stempvoort, J.W. Roy, S.J. Brown, G. Bickerton
Reference: Chemosphere 95 (2014) 455–463
Year: 2013
Results and conclusion: The herbicide glyphosate and its putative metabolite aminomethylphosphonic acid (AMPA) have been found in urban streams, but limited information is available on their presence in urban riparian groundwater. Information is also lacking regarding the source of AMPA in these urban settings (glyphosate metabolite or wastewater), and whether, if present, glyphosate residues in urban riparian groundwater contribute significantly to urban streams. Glyphosate and AMPA were detected in shallow riparian groundwater at 4 of 5 stream sites in urban catchments in Canada and each were found in approximately 1 in 10 of the samples overall. Frequency of observations of glyphosate and AMPA varied substantially between sites, from no observations in a National Park near the Town of Jasper Alberta, to observations of both glyphosate and AMPA in more than half of the samples along two short reaches of streams in Burlington, Ontario. In these two catchments, AMPA was correlated with glyphosate, rather than the artificial sweetener acesulfame, suggesting that the AMPA is derived mainly from glyphosate degradation rather than from wastewater sources. Land use, localized dosage history, depth below ground and other factors likely control the occurrence of detectable glyphosate residues in groundwater. Detections of glyphosate and AMPA in samples of riparian groundwater (2009): All data:

Substance	Number of samples	With detections (%)	Maximum concentration [ngL^{-1}]
Glyphosate	281	37 (13.2 %)	42
AMPA		33 (11.7 %)	2870

Proposed action: Not to be considered as the monitoring was outside the EU (Canada).
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.

Reliability	
Endpoint	Glyphosate and AMPA concentrations in samples of riparian groundwater
Protocol	Not given
Test compound	Glyphosate and AMPA
Test system and conditions	Study sites: At Burlington there are many small streams that drain through urban areas into Lake Ontario. Groundwater was sampled in June 2009 along two of these streams: Tuck Creek and Shoreacres

	Creek. Groundwater samples were also collected in the catchments of two streams in the City of Barrie that drain into Lake Simcoe: along Dyment's Creek in September 2009 and along Hewitt's Creek in October 2009. Furthermore, groundwater was collected along two reaches of the Athabasca River in Jasper National Park in August 2009. Sampling and analysis: All of the groundwater samples from this study were collected at shallow depths within 2 m of the edge of the streams, usually within the streambed but occasionally on the shore. Groundwater samples were collected from depths of generally 0.25–1.0 m below the ground or streambed surface using a drive-point miniprofiler connected to a peristaltic pump. An ion chromatography electrospray ionization triple quadruple mass spectrometry (IC/MS/MS) was used to analyze glyphosate and aminomethylphosphonic acid (AMPA). The artificial sweetener acesulfame (as a wastewater indicator) was analyzed using a IC/MS/MS method.
Statistical design	In the study, the authors encountered many non-detections of both glyphosate and AMPA. For this reason, the statistical analyses were restricted to the Burlington datasets where the paired analytes were both present in the majority of the samples. For correlation analyses of these compounds, the authors used Minitab 16 (Minitab Inc., State College, PA, USA) to calculate Spearman rank correlation coefficients (q). The standard approach could be used because for each test, each analyte had a single detection (reporting) limit: all non-detections were given the same rank, and all non-quantifiable trace detections were ranked together immediately above the non-detections.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

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B.8.11.7 Other studies

Summary

~~83~~ 113 articles obtained from open literature can be subsumed under the heading “other studies”. They either focus on other topics than those given in the points B.8.11.2 to B.8.11.6 or present reviews on fate and behaviour in the environment.

~~25~~ 33 articles draw conclusions on the interactions of glyphosate with micronutrients, nutrient uptake and metabolism. In particular, the ability of glyphosate to chelate micronutrients such as manganese, iron and phosphate and the resulting effects in plants are addressed.

~~24~~ 32 articles discuss these effects for glyphosate resistant plants, for example resistant soybeans. Work has focused on effects in both, conventional and glyphosate resistant crops, and from direct application to the crop, drift from adjacent fields or uptake from soil. Additionally, effects of genetically modified crop cultivation on soil and water quality are addressed.

All other publications deal with miscellaneous topics, which are:

- reviews on fate of glyphosate in the environment,
- good agricultural practice,
- risk mitigation,
- ecosystem management,
- phytotoxicity,
- bioremediation,
- cost-/efficiency assessment,
- bioavailability.

The listed articles are valuable and might be used as additional information. However, they are not taken into account for any endpoint- or PEC-calculation. Consequently, they are not evaluated following the approach as it has been done for the studies from point B.8.11.2 to B.8.11.6 and the criteria "relevance" and "evidence" are characterised as "not applicable". The proposed action is: "not to be considered as publication does not focus on an environmental fate-related endpoint".

Detailed description of open literature

Aisha et al. (2009)

Title: Kinetics of Reduction of Colloidal MnO_2 by Glyphosate in Aqueous and Micellar Media	
Author: Aisha, U., Qamruzzaman, Rafiquee, M.Z.A.	
Reference: International Journal of Inorganic Chemistry, Volume 2011	
Year: 2011	
Results and conclusion: The kinetics of the reduction of colloidal MnO_2 by glyphosate has been investigated spectrophotometrically in an aqueous and micellar (cetyltrimethylammonium bromide, sodium lauryl sulfate) media. The reaction follows first-order kinetics with respect to colloidal MnO_2 in both the aqueous and micellar media. The rate of oxidation increases with increase in [glyphosate] in the lower concentration range but becomes independent at its higher concentrations. The addition of both the anionic (NaLS) and cationic (CTAB) micelles increased the rate of reduction of colloidal MnO_2 by glyphosate while the nonionic TX-100 micelles did not influence the rate of reaction. In both aqueous and micellar media, the oxidation of glyphosate occurs through its adsorption over colloidal MnO_2 surface. The reaction in micellar media was treated by considering the pseudophase model. The values of reaction rates and binding constants in the presence of micelles were determined.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Non-Labelled glyphosate (CAS 38641-94-0)
Test system and conditions	Not applicable
Statistical design	Not applicable

Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Akamatsu et al. (2014)

Title: Evaluation of glyphosate application in regulating the reproduction of riparian black locust (Robinia pseudoacacia L.) after clear-cutting, and the possibility of leaching into soil	
Author: F. Akamatsu, M. Makishima, Y. Taya, S. Nakanishi, J. Miwa	
Reference: Landscape Ecol Eng (2014) 10:47–54, DOI 10.1007/s11355-013-0215-x	
Year: 2014	
Results and conclusion: Black locust (Robinia pseudoacacia L.)—an invasive alien species in riparian forests—is becoming more prevalent in many rivers of eastern Japan. Riparian black locust forests are typically cut down to maintain river-flow capacity. However, such forests often reproduce rapidly by stump sprouting and root suckering and regenerate by germination. Thus, more effective riparian forest management approaches are required. To regulate the reproduction of black locust forests after clear-cutting, we examined the regrowth-inhibiting effects of glyphosate herbicide application to stumps, in accordance with current river management protocol (i.e., winter logging operation). Further, we investigated the concentrations of glyphosate leaching into the soil at a depth of 30 cm in a riparian area of the Tenryu River. Our results showed that glyphosate application to stumps completely inhibited stump sprouting but not root suckering or seedling germination. The glyphosate concentration leaching into the soil reached a maximum (2.6 ± 0.7 mg kg^{-1}, mean ± standard error) on day 1 after the application, and subsequently declined to below the detection limit on day 2. Thus, the rapid degradation of glyphosate was confirmed, despite the fact that the herbicide leached into the soil after application to the stumps. The glyphosate application has limited effectiveness against root suckering and germination of riparian black locust forests after clear-cutting in winter, in accordance with the current river management protocol.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Potassium salt of glyphosate (Roundup Maxload, Nissan Chemical Industries Ltd., Tokyo, Japan)
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Albrecht et al. (2012)

Title: RR soybean seed quality after application of glyphosate in different stages of crop development	
Author: Leandro Paiola Albrecht, André Prechlak Barbosa, André Felipe Moreira Silva, Matheus Akiyama Mendes, Alfredo Júnior Paiola Albrecht, Marizangela Rizzatti Ávila	
Reference: Revista Brasileira de Sementes (2012) Vol. 34 (3): 373 - 381	
Year: 2012	
Results and conclusion: This study was aimed at assessing physiological quality, as well as the seed health quality, of the transgenic soybean, cv. CD 219RR, produced under the use of glyphosate applied in different phenological stages of the soybean crop. The authors stated that the herbicide glyphosate can negatively affect the physiological quality of RR soybean seeds, cultivar 219RRCD, when applied in doses ranging from 1,440 to 2,880 g acid equivalent per hectare during the stage of vegetative development V6 and reproductive stage R2 of the soybean crop.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Alletto et al. (2009)

Title: Tillage management effects on pesticide fate in soils. A review
Author: Lionel Alletto, Yves Coquet, Pierre Benoit, Djilali Heddadj, Enrique Barriuso
Reference: Agron. Sustain. Dev. (2009) 10.1051/agro/2009018
Year: 2009
Results and conclusion: Major findings of the review were: 1) Pesticide interception is enhanced under conservation tillage practices. 2) Pesticide retention, which is generally positively correlated with organic carbon content, is increased in the topsoil layer under conservation tillage. 3) Transport of pesticides is affected by tillage management and by its interactions with climatic conditions
Proposed action: Consider as additional information. PEC_{GW} given as % of applied dose is reported but no

comprehensive raw data are presented. Recalculation of endpoints on degradation or sorption is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	PEC_{GW}, but given as % of applied dose
Protocol	Review article
Test compound	Non-Labelled glyphosate (CAS 38641-94-0) and AMPA (CAS: 74341-63-2)
Test system and conditions	No single test system
Statistical design	Not reported
Relevance	
Environmental relevance	Low relevance (concentrations are not reported)
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with other studies as the endpoints were not calculated adequately.

Aslam et al. (2013)

Title: Adsorption and desorption behavior of selected pesticides as influenced by decomposition of maize mulch
Author: Sohaib Aslam, Patricia Garnier, Cornelia Rumpel, Serge E.Parent and Pierre Benoit
Reference: Chemosphere 91 (2013) 1447–1455
Year: 2013
Results and conclusion: Assessing pesticide fate in conservation agricultural systems requires adetailed understanding of their interaction with decomposing surface crop residues (mulch). Adsorption and desorption behavior of glyphosate, s-metolachlor and epoxiconazole was investigated on maize mulch residues decomposed under laboratory and field conditions. Our conceptual approach included characterization of chemical composition and hydrophobicity of mulch residues in order to generate parameters to predict sorption behavior. Adsorption of s-metolachlor and epoxiconazole greatly increased with mulch decomposition,whereas glyphosate adsorption was less affected but its desorption was increased. Mulch characteristics including aromaticity, hydrophobicity and polarity indices were strongly correlated to Koc of the non-ionic pesticides. A predictive model based on compositional data (CoDa)analysis revealed that the sorption capacity of decomposing mulch can be predicted from descriptors such as aromatic and alkyl corresponding respectively to lignin and NDF biochemical fractions.The decomposition degree of mulch residues should be taken into account while predicting the fate of pesticides. Adsorption isotherms on mulch residues (0, 150 and 300 d) were described by the Freundlich model with $R^2 \geq 0.998$ for s-metolachlor and epoxiconazole and $R^2 \geq 0.989$ for glyphosate. All s-metolachlor and epoxiconazole isotherms were almost linear ($n \geq 0.9$) whereas glyphosate isotherms for initial mulch and mulch sampled after 150 dof field exposure were non-linear ($n < 0.9$). Adsorption coefficients (Kf/oc) were significantly different ($P < 0.01$) for the three molecules. Glyphosate was least adsorbed while epoxiconazole was most strongly adsorbed followed by S-metolachlor. Linear isotherms allowed estimating adsorption coefficients (Kd and Koc) at single concentr ation of 0.75 mg L_1 for all mulch residues (Table 2). We did not observe a significant effect of mulch decomposition degree on the adsorption behavior of glyphosate and almost similar adsorption coefficients were recorded for all mulch residues ranging from 24 to 23 and 30 L kg^{-1} for maize residues decomposed under laboratory and

field conditions respectively.	
Proposed action: Not to be considered for the endpoint sorption and mobility. Raw data on mass balances and test item concentrations in the aqueous and solid phases are not reported. Though the study is plausible, the validity cannot be proven. Moreover, the adsorption on mulch is not a parameter considered relevant for the environmental risk assessment.	
Type of information (critical, high/low weight, supporting, additional): Low weight, supportive information	
Reliability	Low
Endpoint	K_f and K_{foc}
Protocol	Modified OECD 106, non-GLP
Test compound	^{14}C-labeled Glyphosate (purity not given)
Test system and conditions	Individual solutions of each pesticide were prepared in 0.01 M $CaCl_2$. All solutions were prepared by using both ^{14}C-labeled and unlabeled molecules to achieve the desired radioactivity . Adsorption isotherms with mulch decomposed for 0, 150 and 300 d were conducted with concentrations of 0.2, 0.4, 0.75, 1.5 and 3 mg L^{-1} for each molecule whereas intermediate concentration of 0.75 mg L^{-1} was selected to study adsorption on all other mulch samples. Centrifuge tubes with sorbents and pesticide solutions were rotated during 24 h with an end-over-head shaker and then centrifuged at 6000 g (Sorvall Evolution RC, Kendro) for 15 min. Radioactivity in the supernatants was measured by scintillation counting.
Statistical design	Not reported
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence, no negative evidence.

Bai et al. (2014)

Title: Dissolved organic phosphorus use by the invasive freshwater diazotroph cyanobacterium, *Cylindrospermopsis raciborskii*
Author: Fang Bai, Rui Liu, Yanjun Yang, Xiaofei Ran, Junqiong Shi, Zhongxing Wu
Reference: Harmful Algae 39 (2014) 112–120
Year: 2014
Results and conclusion: This study examines the physiological responses of the diazotrophic cyanobacteria, *Cylindrospermopsis raciborskii*, to different dissolved organic phosphorus (DOP) compounds to explore mechanisms of environmental acclimation in this invasive species. Our results show that the specific growth rates of *C. raciborskii* cells in media treated with b-glycerol phosphate, D-glucose-6-phosphate, and (2-aminoethyl)-phosphinic acid were significantly higher than those of cells grown in phosphorus free media. We observed that maximal net photosynthesis was highest when cells were cultured with D-glucose-6-phosphate and lowest when cells were cultured with glyphosate. Similarly, rates of photosynthetic activity (maximum quantum yield, maximum electron transport rate, and photosynthetic efficiency) were observed to be highest in media treated with D-glucose-6-phosphate. We report that rates of alkaline phosphatase activity to the different organophosphates tested changed markedly in response to the concentration of dissolved inorganic phosphorus (DIP); a result

supported by the amount of green fluorescent products revealed by ELF197 phosphate dye (ELFP) and gene up-regulation for alkaline phosphatase (phoA). Our results indicate that *C. raciborskii* is able to use different organic phosphorus to support its growth when phosphorus is limited. In addition, we show that C. raciborskii has a higher availability to phosphate (C–O–P) than phosphonate (C–P). The results suggest that the strategic flexibility to environmental phosphorus might play an important role in the domination of *C. raciborskii*.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate purchased from Sigma–Aldrich (USA) (>99%)
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Bailey et al. (2002)

Title: Glyphosate Interactions with Manganese
Author: WILLIAM A. BAILEY, DANIEL H. POSTON, HENRY P. WILSON, and THOMAS E. HINES
Reference: Weed Technology. Volume 16:792–799
Year: 2002
Results and conclusion: Field experiments were conducted on the Eastern Shore of Virginia from 1999 to 2001 to evaluate the effects of tank mixture applications of isopropylamine or trimethylsulfonium salts of glyphosate with two liquid formulations of manganese (Mn lignin or Mn chelate) on spray solution pH and weed control in glyphosate-resistant soybean. Additions of manganese to herbicide solutions resulted in a reduction in the acidifying effects of the herbicides as well as in the control of common lambsquarters, large crabgrass, morningglory spp., and smooth pigweed. Reduced control caused by manganese could be overcome with higher rates of the herbicides on some species, but reduced control of common lambsquarters was seen when manganese was included with any herbicide application rate. For most species, Mn chelate caused a greater reduction in control than did Mn lignin. Although manganese caused significant decreases in weed control, soybean yield was not influenced by glyphosate salt, application rate, or manganese. Reduced weed control caused by the addition of manganese to herbicide solutions may be due to the complexing of the herbicide formulations, which could result in the formation of insoluble salt complexes that are not readily absorbed through the plant cuticle, resulting in decreased glyphosate phytotoxicity.
Proposed action:

Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate (isopropylamine salt) was Roundup Ultra®, marketed by Monsanto Company, 800 N; CAS-no.: 38641-94-0. Trimethyl-sulfonium salt of glyphosate was Touchdown 5®, marketed by Zeneca Ag. Products, 1200 S.Glyphosate, CAS-no.: 81591-81-3
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Baker et al. (2014)

Title: THE DIRECT AND INDIRECT EFFECTS OF A GLYPHOSATE-BASED HERBICIDE AND NUTRIENTS ON CHIRONOMIDAE (DIPTERA) EMERGING FROM SMALL WETLANDS
Author: L. F. BAKER, J. F. MUDGE, J. E. HOULAHAN, D. G. THOMPSON and K. A. KIDD
Reference: Environmental Toxicology and Chemistry, Vol. 33, No. 9, pp. 2076–2085, 2014
Year: 2014
Results and conclusion: Laboratory and mesocosm experiments have demonstrated that some glyphosate-based herbicides can have negative effects on benthic invertebrate species. Although these herbicides are among the most widely used in agriculture, there have been few multiple-stressor, natural system–based investigations of the impacts of glyphosate-based herbicides in combination with fertilizers on the emergence patterns of chironomids from wetlands. Using a replicated, split-wetland experiment, the authors examined the effects of 2 nominal concentrations (2.88 mg acid equivalents/L and 0.21 mg acid equivalents/L) of the glyphosate herbicide Roundup WeatherMax, alone or in combination with nutrient additions, on the emergence of Chironomidae (Diptera) before and after herbicide-induced damage to macrophytes. There were no direct effects of treatment on the structure of the Chironomidae community or on the overall emergence rates. However, after macrophyte cover declined as a result of herbicide application, there were statistically significant increases in emergence in all but the highest herbicide treatment, which had also received no nutrients. There was a negative relationship between chironomid abundance and macrophyte cover on the treated sides of wetlands. Fertilizer application did not appear to compound the effects of the herbicide treatments. Although direct toxicity of Roundup WeatherMax was not apparent, the authors observed longer-term impacts, suggesting that the indirect effects of this herbicide deserve more consideration when assessing the ecological risk of using herbicides in proximity to wetlands.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-

point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Roundup WeatherMax (glyphosate)
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Balci (2009)

Title: Decontamination of Aqueous Glyphosate, (Aminomethyl) phosphonic Acid, and Glufosinate Solutions by Electro-Fenton-like Process with Mn^{2+} as the Catalyst	
Author: BEYTUL BALCI, MEHMET A. OTURAN, NIHAL OTURAN, AND IGNASI SIRÉS	
Reference: J. Agric. Food Chem. 2009, 57, 4888–4894	
Year: 2009	
Results and conclusion: The ability of the modified electro-Fenton-like (EF-like) process to degrade aqueous solutions of glyphosate, which is the most widely used herbicide in the world, has been assessed with Mn^{2+} and other metal ions as catalysts to overcome the problems posed by some stable metal ion complexes of phosphonate herbicides. Bulk electrolyses with a carbon-felt cathode and Pt anode were performed in an undivided cell under galvanostatic conditions to study the effect of the applied current as well as Mn^{2+} and glyphosate concentrations. The herbicide was completely destroyed in all cases following a pseudo first-order kinetics, and the second-order rate constant for its reaction with •OH was determined. The decay trends obtained by high-performance liquid chromatography-fluorometric detection (HPLC-FL) and ion chromatography analysis were similar. AMPA [(aminomethyl)phosphonic acid] was the major reaction intermediate and showed slower pseudo first-order destruction kinetics. The high mineralization degree obtained for glyphosate solutions confirmed the great performance of the EF-like process with Mn^{2+}, which promotes the C-N cleavage by •OH attack as the first oxidation step and the C-P cleavage in a further step. High-level decontamination achieved for AMPA and glufosinate solutions corroborated the benefits of this oxidation process.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable

Test compound	Glyphosate, CAS-no.: 1071-83-6; glufosinate-ammonium, CAS-no.: 77182-82-2; AMPA, CAS-no.: 1066-51-9
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Balthazor and Hallas (1986)

Title: Glyphosate-Degrading Microorganisms from Industrial Activated Sludge	
Author: Balthazor, T.M. and Hallas, L.E.	
Reference: Applied and Environmental microbiology, Feb. 1986, Vol. 51, No. 2, 432-434	
Year: 1986	
Results and conclusion: A plating medium was developed to isolate N-phosphonomethylglycine (glyphosate)-degrading microorganisms, with glyphosate as the sole phosphorus source. Two industrial biosystems treating glyphosate wastes contained elevated microbial counts on the medium. One purified isolate metabolized glyphosate to aminomethylphosphonic acid, mineralizing this accumulating intermediate during log growth. This microorganism has been identified as a *Flavobacterium* species.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Basso et al. (2011)

Title: Foliar application of manganese in transgenic soybean tolerant to glyphosate
Author: Claudir José Basso, Antônio Luis Santi, Fabiane Pinto Lamego, Eduardo Girotto
Reference: Ciência Rural, v.41, n.10, p.1726-173
Year: 2011
Results and conclusion:

The yellowing of Roundup Ready soybean after glyphosate application can be associated to a momentary manganese deficiency. Because of that, with the hypothesis that glyphosate tolerant soybean would need supplementary addition of manganese; the objective of this research was to evaluate different managements in the foliar application of manganese in some soybean parameters. It was developed two experiments, one at Taquaruçú do Sul and other at Boa Vista das Missões, RS in the year of 2009/2010. It was tested the following treatments: 1) without glyphosate application with manual weed control and without manganese foliar application (untreated check); 2) without glyphosate application with manual weed control and one manganese foliar application at 7 days after this manual weed control; 3) with glyphosate application and without manganese foliar application; 4) glyphosate application in mixture with manganese; 5) glyphosate application added of one manganese foliar application at 7 days after glyphosate application; 6) glyphosate application added of manganese foliar application split in two times, at 7 and 14 days after glyphosate application; 7) glyphosate application and one of manganese foliar application at 14 days after glyphosate application. The glyphosate application was realized in the V5 soybean stage, using 720g/L i.e., while the used dose of Mn was 2.0L/ha of a formulation with 14% (m/v) of Mn. There were no significant difference among the treatments to plant height and height insertion of the first legume. The glyphosate application did not affect the absorption and the foliar amount of manganese and nitrogen in soybean crop. Even with the increase in foliar manganese amount, there was no increasing in soybean productivity. This shows that in soils with Mn levels above of the sufficient, it is not necessary foliar manganese addition in genetically modified soybean tolerant to glyphosate.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Bellaloui et al. (2009)

Title: Effect of Glyphosate-Boron Application on Seed Composition and Nitrogen Metabolism in Glyphosate-Resistant Soybean
Author: NACER BELLALOUI, HAMED K. ABBAS, ANNE M. GILLEN, AND CRAIG A. ABEL
Reference: J. Agric. Food Chem. 57, 9050–9056
Year: 2009
Results and conclusion: The objective of this study was to evaluate the effects of foliar application of glyphosate

(Gly) alone, boron (B) alone, and Gly-B combined on seed composition and nitrogen metabolism in glyphosate-resistant soybean (Glycine max (L.) Merr.). No Gly and no B application plants were used as control (C). Results showed that Gly, Gly-B, or B applications increased protein, oleic acid, and total amino acid concentrations in seed. However, oil and linolenic acid concentrations decreased under those treatments compared with the nontreated control. Gly-B combined or B treatments increased B concentration in leaves and seed, nitrate reductase activity (NRA), and nitrogenase activity and resulted in a significant positive correlation between B concentration in leaves and NRA (r = 0.54; P < 0.0001) and B concentration in leaves and nitrogenase activity (r = 0.35; P = 0.005). The results suggest that Gly-B tank mixing may not antagonize B uptake and translocation to leaves and seeds, and the inhibitory effect of Gly on nutrient uptake and translocation may depend on the ion species and form of the nutrient mixed with Gly. These results demonstrate that Gly-B application alters seed composition, nitrogen metabolism, and B status in leaves and seed.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; Flumetsulam, CAS-no.: 98967-40-9; metolachlor, CAS-no.: 51218-45-2; and paraquat CAS-no.: 1910-42-5
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Bellaloui et al. (2009)

Title: Effects of Glyphosate Application on Seed Iron and Root Ferric (III) Reductase in Soybean Cultivars
Author: NACER BELLALOUI, KRISHNA N. REDDY, ROBERT M. ZABLOTOWICZ, HAMED K. ABBAS, AND CRAIG A. ABEL
Reference: J. Agric. Food Chem. 57, 9569–9574
Year: 2009
Results and conclusion: Previous research demonstrated that plant nutrient assimilation was reduced by glyphosate (Gly). A 2 year field experiment investigated the effects of Gly at drift rate (12.5% of commercial use rate) on Fe concentrations in leaves and seeds of Gly-sensitive (GS) soybean, and a greenhouse experiment evaluated Gly effects on Fe assimilation using root in vivo ferric reductase activity (FRA) in two GS and one Gly-resistant (GR) soybean cultivars. Field studies showed that Gly drift rates resulted in a significant decrease in the Fe concentration in seeds and leaves compared to the non-treated plants. In greenhouse studies, leaf Fe and FRA were inhibited in GS cultivars Hutcheson and DP 5110 and the GR

cultivar AG 4604RR and leaf Fe was positively correlated with root FRA (p <0.0001). These results indicate that Gly can interfere with Fe assimilation in both GS and GR soybean. Understanding the implication of Gly on Fe nutrition in soybean seed would help soybean agronomists and breeders seeking to improve seed mineral nutrition qualities.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; Flumetsulam, CAS-no.: 98967-40-9; metolachlor, CAS-no.: 51218-45-2; and paraquat CAS-no.: 1910-42-5
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Bellaloui et al. (2006)

Title: Simulated Glyphosate Drift Influences Nitrate Assimilation and Nitrogen Fixation in Non-glyphosate-Resistant Soybean
Author: NACER BELLALOUI, KRISHNA N. REDDY, ROBERT M. ZABLOTOWICZ, ANDALEMU MENGISTU
Reference: J. Agric. Food Chem. 54, 3357-3364
Year: 2006
Results and conclusion: Non-target injury from glyphosate drift is a concern among growers using non-glyphosate-resistant (non-GR) cultivars. The effects of glyphosate drift on nitrate assimilation and nitrogen fixation potential, nodule mass, and yield of non-GR soybean were assessed in a field trial at Stoneville, MS. A non-GR soybean cultivar ‘Delta Pine 4748S’ was treated with glyphosate at 12.5% of use rate of 0.84 kg of active ingredient/ha at 3 (V2), 6 (V7), and 8 (R2, full bloom) weeks after planting (WAP) soybean to simulate glyphosate drift. Untreated soybean was used as a control. Soybeans were sampled weekly for 2 weeks after each glyphosate treatment to assess nitrate assimilation and N2 fixation potential. Nitrate assimilation was assessed using in vivo nitrate reductase assay in leaves, stems, roots, and nodules. Nitrogen fixation potential was assessed by measuring nitrogenase activity using the acetylene reduction assay (ARA). Nitrogen content of leaves, shoots, and seed and soybean yield were also determined. In the first sampling date (4 WAP); glyphosate drift caused a significant decrease in NRA in leaves (60%), stems (77%), and nodules (50%), with no decrease in roots. At later growth stages, NRA in leaves was more sensitive to glyphosate drift than stems and roots. Nitrogenase activity was reduced 36-58% by glyphosate treatment at 3 or 6 WAP. However, glyphosate treatment at 8 WAP had no effect on nitrogenase activity. Nitrogen content was affected by glyphosate application only in

shoots after the first application. No yield, seed nitrogen, protein, or oil concentration differences were detected. These results suggest that nitrate assimilation and nitrogen fixation potential were significantly reduced by glyphosate drift, with the greatest sensitivity early in vegetative growth. Soybean has the ability to recover from the physiological stress caused by glyphosate drift.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; Flumetsulam, CAS-no.: 98967-40-9; Metolachlor, CAS-no.: 51218-45-2; and Paraquat CAS-no.: 1910-42-5
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Bellaloui et al. (2008)

Title: Nitrogen Metabolism and Seed Composition As Influenced by Glyphosate Application in Glyphosate-Resistant Soybean
Author: NACER BELLALOUI, ROBERT M. ZABLOTOWICZ, KRISHNA N. REDDY, AND CRAIG A. ABEL
Reference: J. Agric. Food Chem. 56, 2765–2772
Year: 2008
Results and conclusion: Previous research has demonstrated that glyphosate can affect nitrogen fixation or nitrogen assimilation in soybean. This 2-year field study investigated the effects of glyphosate application of 1.12 and 3.36 kg of ae ha^{-1} on nitrogen metabolism and seed composition in glyphosate-resistant (GR) soybean. There was no effect of glyphosate application on nitrogen fixation as measured by acetylene reduction assay, soybean yield, or seed nitrogen content. However, there were significant effects of glyphosate application on nitrogen assimilation, as measured by in vivo nitrate reductase activity (NRA) in leaves, roots, and nodules, especially at high rate. Transiently lower leaf nitrogen or 15N natural abundance in high glyphosate application soybean supports the inhibition of NRA. With the higher glyphosate application level protein was significantly higher (10.3%) in treated soybean compared to untreated soybean. Inversely, total oil and linolenic acid were lowest at the high glyphosate application rate, but oleic acid was greatest (22%) in treated soybean. These results suggest that nitrate assimilation in GR soybean was more affected than nitrogen fixation by glyphosate application and that glyphosate application may alter nitrogen and carbon metabolism.
Proposed action:

Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; Metolachlor, CAS-no.: 51218-45-2; and Paraquat CAS-no.: 1910-42-5
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Beltrao et al. (2013)

Title: Changes in Soluble Manganese and Iron Concentrations of Tropical Wetland Soils as Influenced by Glyphosate Dosage	
Author: DANIELLE S. BELTRÃO, ALFREDO B. DE-CAMPOS, DANILLO B. MOURA AND RICARDO F. SOUSA	
Reference: Communications in Soil Science and Plant Analysis, 44:1092–1096, 2013	
Year: 2013	
Results and conclusion: Glyphosate is largely used to control weeds in wetland soils of Brazil. We investigated changes in the chemistry of soluble manganese (Mn) and iron (Fe) in these soils as affected by glyphosate dosage. Triplicate samples of the A horizon of wetland soils with different organic-matter contents were incubated with deionized water (1:2) for 1, 3, and 30 days under flooding. Three different glyphosate doses (0, 0.048, and 0.096 g L−1 m−2) were spiked on the flooded water at the beginning of the incubation periods. After incubation, pH was measured and samples of the supernatant were collected for determination of Mn/Fe concentrations by atomic absorption. Glyphosate application impacted Mn but had no effect on pH and Fe. Soluble Mn concentrations decreased as glyphosate dosage increased for the high organic-matter soil after 3 days of incubation. It indicated that glyphosate application can change the chemistry of soil metals. The intensity of these changes depends on the glyphosate dosage, evolved metal, incubation time, and soil properties.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): No weight, not relevant for endpoints related to environmental fate.	
Reliability	
Endpoint	Dependence of Mn and Fe Concentrations from glyphosate dosage
Protocol	Not applicable
Test compound	Roundup-Nortox made by Monsanto Co., St. Louis, Mo
Test system and conditions	Not applicable

Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Bernards et al. (2005)

Title: Glyphosate interaction with manganese in tank mixtures and its effect on glyphosate absorption and translocation	
Author: Mark L. Bernards, Kurt D. Thelen, Donald Penner, Rajendra B. Muthukumaran, John L. McCracken	
Reference: Weed Science, 53:787–794	
Year: 2005	
Results and conclusion: It was hypothesized that Mn complexed with glyphosate in a similar manner to Ca^{2+}-forming salts that were not readily absorbed and, thereby, reducing glyphosate efficacy. This study was conducted to confirm the interaction of Mn^{2+} and glyphosate and to measure the effect of Mn^{2+} on glyphosate absorption and translocation in velvetleaf.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): No weight, not relevant for endpoints related to environmental fate.	
Reliability	
Endpoint	Glyphosate adsorption to Mn2+ in tank solution followed by adsorption and translocation in velvetleaf.
Protocol	Not applicable
Test compound	^{14}C-Glyphosate
Test system and conditions	Growth chamber bioassays were conducted to measure absorption and translocation of ^{14}C-labeled Glyphosate.
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Bois et al. (2013)

Title: Herbicide mitigation in microcosms simulating stormwater basins subject to polluted water inputs
Author: P. Bois, D. Huguenot, K. Je´ze´quel, M. Lollier, J.Y. Cornu and d, T. Lebeau
Reference: Waterresearch 47 (2013) 1123-1135
Year: 2013
Results and conclusion: Non-point source pollution as a result of wine-growing activity is of high concern. Stormwater basins (SWB) found downstream of vineyard watersheds could show a potential for

the mitigation of runoff water containing herbicides. In this study, mitigation of vinery-used herbicides was studied in microcosms with a very similar functioning to that recorded in SWB. Mitigation efficiency of glyphosate, diuron and 3,4-dichloroaniline (3,4-DCA) was investigated by taking into account hydraulic flow rate, mitigation duration, bioaugmentation and plant addition. Mitigation efficiency measured in water ranged from 63.0% for diuron to 84.2% for 3,4-DCA and to 99.8% for glyphosate. Water-storage duration in the SWB and time between water supplies were shown to be the most influential factors on the mitigation efficiency. Six hours water-storage duration allowed an efficient sorption of herbicides and their degradation by indigenous microorganisms in 5 weeks. Neither bioaugmentation nor plant addition had a significant effect on herbicide mitigation. Our results show that this type of SWB are potentially relevant for the mitigation of these herbicides stemming from wine-growing activity, providing a long enough hydraulic retention time.	
Proposed action: Not to be considered; does not affect the fate relevant endpoints of the monograph.	
Type of information (critical, high/low weight, supporting, additional): Low weight	
Reliability	
Endpoint	Dissipation efficiency (in microcosms)
Protocol	No standard protocol, non-GLP
Test compound	Glyphosate
Test system and conditions	This work aimed at studying the effects of bioaugmentation by themixed bacterial culture ‘106’, plants (Phragmites australis) and hydraulic regime on pollutant dissipation, in order to enhance glyphosate, diuronand 3,4-DCAremoval inbothrunoff water (transiting through SWB) and sediment (accumulating into the basins). As pesticides are rarely applied alone, Cu was added to the mixture of glyphosate, diuron and 3,4-DCA supplied to the microcosms. Cu is indeed applied in vineyards until 120 years as Copper Bordeaux mixture to control powdery mildew. The studywas performed insmall-scale devices. These microcosmswere by aspects (hydraulic regime, sandesediment mix) close to the aforementioned vineyard SWB.
Statistical design	Not applicable
Relevance	
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	No negative evidence

Bott et al. (2011)

Title: Phytotoxicity of glyphosate soil residues re-mobilised by phosphate fertilisation
Author: Sebastian Bott, Tsehaye Tesfamariam, Angelika Kania, Birceyudum Eman, Nergiz Aslan, Volker Römheld, Günter Neumann
Reference: Plant Soil, 342:249–263
Year: 2011
Results and conclusion: The results suggest that remobilization of glyphosate may represent an additional transfer pathway for glyphosate to non-target plants which is strongly influenced by soil character-

istics such as P fixation potential, content of plant-available iron, pH, cation exchange capacity, sand content and soil organic matter.	
Proposed action: Not to be considered and included in the monograph; does not affect the fate relevant endpoints of the monograph.	
Type of information (critical, high/low weight, supporting, additional): Low weight; may be additional information on re-mobilisation	
Reliability	
Endpoint	Visual symptoms of glyphosate toxicity, plant biomass, intracellular shikimate accumulation as physiological indicator for glyphosate toxicity and the plant nutritional status were determined.
Protocol	No standard protocol, non-GLP
Test compound	glyphosate formulations (applied as Roundup Ultra-Max®)
Test system and conditions	In model experiments under greenhouse conditions, the potential for glyphosate re-mobilisation by P-fertiliser application was evaluated by bio-indication with soybean cultivated on five contrasting soils with or without glyphosate application at 10–35 days before sowing. Different levels of P-fertilisation (0, 20, 40, 80, 240 mg P kg^{-1} soil) were supplied at the date of sowing.
Statistical design	Experiments were conducted in a randomized block design with four replicates for each treatment. Analysis of variance and the Tukey test for detection of significant differences were performed using the SigmaStatsoftware.
Relevance	
Environmental relevance	Given, influence of environmental parameter investigated
Weight of evidence	
“Positive”/”Negative” evidence	No negative evidence

Bott et al. (2009)

Title: Evidence for glyphosate damage of winter wheat depending on waiting-times after pre-crop glyphosate application and density of desiccated weed plants under field and experimental conditions
Author: Bott, Sebastian, Lebender, Ulrike, Yoon, Duck-Joong, Tesfamariam, Tsehaye, Römheld, Volker, Neumann, Günter,
Reference: The Proceedings of the International Plant Nutrition Colloquium XVI, http://escholarship.org/uc/item/25v599pr
Year: 2009
Results and conclusion: Previous model experiments under greenhouse conditions identified high weed density and short waiting times for sowing after glyphosate desiccation as potential risk factors, mediating glyphosate phytotoxicity to non-target crops. To evaluate these factors under field conditions, a set of three field trials with different waiting times after pre-crop glyphosate application was conducted in non-tillage winter wheat cropping systems in Southwest Germany. Additionally, model experiments with short waiting time (2 d) and a high density of target weeds were performed, using a track-spraying device to simulate conditions for field application. Both, in model experiments and under field conditions, short waiting times after pre-crop glyphosate application resulted in lower germination, delayed or arrested plant development, reduced shoot biomass production, partly impaired micronutrient acqui-

sition as well as intracellular accumulation of shikimate as physiological indicator of glyphosate toxicity. Thus, it can be concluded that short waiting times and high density of target plants can be considered as relevant risk factors for phytotoxicity of glyphosate to non-target crops No-tillage cropping systems seem to be associated with a particularly high sensitivity to glyphosate-induced damage of crop plants. Recommendations of waiting times appropriate to the cropping system should be considered as promising strategy to avoid harvest losses due to phytotoxicity, impaired growth and micronutrient deficiency. Further elucidation of environmental risk factors promoting the expression of crop damage is necessary.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate as Roundup Ultra Max®,
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Bott et all. (2008)

Title: Glyphosate-induced impairment of plant growth and micronutrient status in glyphosate-resistant soybean (Glycine max L.)
Author: Sebastian Bott & Tsehaye Tesfamariam & Hande Candan & Ismail Cakmak & Volker Römheld & Günter Neumann
Reference: Plant Soil 312:185–194
Year: 2008
Results and conclusion: This investigation demonstrated potential detrimental side effects of glyphosate on plant growth and micronutrient (Mn, Zn) status of a glyphosate-resistant (GR) soybean variety (Glycine max cv. Valiosa), which were found to be highly dependent on the selected growth conditions. In hydroponic experiments with sufficient Mn supply [0.5 µM]; the GR cv. Valiosa produced similar plant biomass, root length and number of lateral roots in the control treatment without glyphosate as compared to its non-GR parental line cv. Conquista. However, this was associated with 50% lower Mn shoot concentrations in cv. Conquista, suggesting a higher Mn demand of the transgenic cv. Valiosa under the selected growth conditions. Glyphosate application significantly inhibited root biomass production, root elongation, and lateral root formation of the GR line, associated with a 50% reduction of Mn shoot concentrations. Interestingly, no comparable effects were detectable at low Mn supply [0.1 µM]. This may indicate Mn-dependent differences in the intracellular transformation of glyphosate to the toxic metabolite aminomethylphosphonic acid (AMPA) in the two isolines. In soil culture experiments conducted on a calcareous loess sub-soil of a Luvi-

sol (pH 7.6) and a highly weathered Arenosol (pH 4.5), shoot biomass production and Zn leaf concentrations of the GR-variety were affected by glyphosate applications on the Arenosol but not on the calcareous Loess sub-soil. Analysis of micronutrient levels in high and low molecular weight (LMW) fractions (80% ethanol extracts) of young leaves revealed no indications for internal immobilization of micronutrients (Mn, Zn, Fe) by excessive complexation with glyphosate in the LMW phase.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate formulation Roundup® UltraMax (Monsanto Agrar, Düsseldorf, Germany) containing N-[phosphonomethyl]glycine isopropylamine salt, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Cakmak et al. (2009)

Title: Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean
Author: Ismail Cakmak, Atilla Yazici, Yusuf Tutus, Levent Ozturk
Reference: Europ. J. Agronomy 31, 114–119
Year: 2009
Results and conclusion: Greenhouse experiments were conducted to study the effects of glyphosate drift on plant growth and concentrations of mineral nutrients in leaves and seeds of non-glyphosate resistant soybean plants (Glycine max, L.). Glyphosate was sprayed on plant shoots at increasing rates between 0.06 and 1.2% of the recommended application rate forweed control. In an experiment with 3-week-old plants, increasing application of glyphosate on shoots significantly reduced chlorophyll concentration of the young leaves and shoots dry weight, particularly the young parts of plants. Concentration of shikimate due to increasing glyphosate rates was nearly 2-fold for older leaves and 16-fold for younger leaves compared to the control plants without glyphosate spray. Among the mineral nutrients analyzed, the leaf concentrations of potassium (K), phosphorus (P), copper (Cu) and zinc (Zn) were not affected, or even increased significantly in case of P and Cu in young leaves by glyphosate, while the concentrations of calcium (Ca), manganese (Mn) and magnesium (Mg) were reduced, particularly in young leaves. In the case of Fe, leaf concentrations showed a tendency to be reduced by glyphosate. In the second experiment harvested at the grain maturation, glyphosate application did not reduce the seed concentrations of nitrogen (N), K, P, Zn and Cu. Even, at the highest application rate of glyphosate, seed concentra-

tions of N, K, Zn and Cu were increased by glyphosate. By contrast, the seed concentrations of Ca, Mg, Fe and Mn were significantly reduced by glyphosate. These results suggested that glyphosate may interfere with uptake and re-translocation of Ca, Mg, Fe and Mn, most probably by binding and thus immobilizing them. The decreases in seed concentration of Fe, Mn, Ca and Mg by glyphosate are very specific, and may affect seed quality.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Isopropylamine salt of glyphosate (Roundup Ultra Herbicide, Monsanto Ltd., Adana, Turkey), CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Cavalieri et al. (2012)

Title: Nutrient and Shoot Dry Matter Accumulation of Two GR Soybean Cultivars under the Effect of Glyphosate Formulations
Author: Cavalieri, S.D., Velini, E.D., Silva, F.M.L., Sao Jose, A.R. and Andrade, G.J.M.
Reference: Planta Daninha, Viçosa-MG, vol. 30 (2): 349-358
Year: 2012
Results and conclusion: The study of selectivity and secondary effects of herbicides on crops is extremely important to successful agriculture. This research aimed to evaluate the effect of glyphosate formulations on nutrient accumulation and dry matter production on the shoot of two glyphosateresistant (GR) soybean cultivars. The assay was carried out in a greenhouse and arranged in a randomized complete block design, replicated six times. The treatments were in a factorial arrangement including six glyphosate formulations (Roundup Original®, Roundup Ready®, Roundup Transorb®, Roundup WG®, Roundup Ultra® and Zapp Qi®), plus a control treatment, and two soybean cultivars (CD 225 RR and V Max RR). The herbicide applications were performed when the plants were at the V3 growth stage, using a dose of 960 g a.e. ha-1. The macronutrient and micronutrient accumulation and dry matter production in the shoot of the soybean plants were greater in V Max RR cultivar than in CD 225 RR cultivar. The formulations Roundup Ready® and Roundup Ultra® did not promote nutrient accumulation reduction in the shoot of the cultivars. In addition, the formulations Roundup Original®, Roundup Transorb® and Roundup WG® caused the greatest damage to nutrient accumulation and dry matter production. It was concluded that nutrient accumulation and dry matter production in the shoots of the soybean plants are affected by glyphosate application, even for GR cultivars.

Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Cerdeira and Duke (2006)

Title: The Current Status and Environmental Impacts of Glyphosate-Resistant Crops: A Review
Author: Antonio L. Cerdeira and Stephen O. Duke
Reference: J. Environ. Qual. 35:1633–1658
Year: 2006
Results and conclusion: Glyphosate [N-(phosphonomethyl) glycine]-resistant crops (GRCs), canola (Brassica napus L.), cotton (Gossypium hirsutum L.), maize (Zea mays L.), and soybean [Glycine max (L.) Merr.] have been commercialized and grown extensively in the Western Hemisphere and, to a lesser extent, elsewhere. Glyphosate-resistant cotton and soybean have become dominant in those countries where their planting is permitted. Effects of glyphosate on contamination of soil, water, and air are minimal, compared to some of the herbicides that they replace. No risks have been found with food or feed safety or nutritional value in products from currently available GRCs. Glyphosate-resistant crops have promoted the adoption of reduced- or no-tillage agriculture in the USA and Argentina, providing a substantial environmental benefit. Weed species in GRC fields have shifted to those that can more successfully withstand glyphosate and to those that avoid the time of its application. Three weed species have evolved resistance to glyphosate in GRCs. Glyphosate-resistant crops have greater potential to become problems as volunteer crops than do conventional crops. Glyphosate resistance transgenes have been found in fields of canola that are supposed to be non-transgenic. Under some circumstances, the largest risk of GRCs may be transgene flow (introgression) from GRCs to related species that might become problems in natural ecosystems. Glyphosate resistance transgenes themselves are highly unlikely to be a risk in wild plant populations, but when linked to transgenes that may impart fitness benefits outside of agriculture (e.g., insect resistance), natural ecosystems could be affected. The development and use of failsafe introgression barriers in crops with such linked genes is needed.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.

Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Review article: Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Cerdeira and Duke (2010)

Title: Effects of glyphosate-resistant crop cultivation on soil and water quality	
Author: Antonio L. Cerdeira and Stephen O. Duke	
Reference: GM Crops 1:1, 1-9; January/February 2010; © 2010 Landes Bioscience	
Year: 2010	
Results and conclusion: Review article: Transgenic glyphosate-resistant crops (GRCs) have been commercialized and grown extensively in the western Hemisphere and, to a lesser extent, elsewhere. GRCs have generally become dominant in those countries where they have been approved for growing. Potential effects of glyphosate on soil and water are minimal, compared to the effects of the herbicides that are replaced when GRCs are adopted. Perhaps the most important indirect effect is that GRCs crops promote the adoption of reduced- or no-tillage agriculture, resulting in a significant reduction in soil erosion and water contamination. Glyphosate and its degradation product, aminomethylphosphonate (AMPA), residues are not usually detected in high levels in ground or surface water in areas where glyphosate is used extensively. Furthermore, both glyphosate and AMPA are considered to be much more toxicologically and environmentally benign than most of the herbicides replaced by glyphosate.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Review article: Glyphosate, CAS-no.: 1071-83-6; AMPA, CAS-no.: 1066-51-9
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable

Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Cerdeira et al. (2007)

Title: Review of potential environmental impacts of transgenic glyphosate-resistant soybean in Brazil	
Author: ANTONIO L. CERDEIRA, DIONSIO L. P. GAZZIERO, STEPHEN O. DUKE, MARCUS B. MATALLO and CLAUDIO A. SPADOTTO	
Reference: Journal of Environmental Science and Health Part B 42, 539–549	
Year: 2007	
Results and conclusion: Transgenic glyphosate-resistant soybeans (GRS) have been commercialized and grown extensively in the Western Hemisphere, including Brazil. Worldwide, several studies have shown that previous and potential effects of glyphosate on contamination of soil, water, and air are minimal, compared to those caused by the herbicides that they replace when GRS are adopted. In the USA and Argentina, the advent of glyphosate-resistant soybeans resulted in a significant shift to reduced- and no-tillage practices, thereby significantly reducing environmental degradation by agriculture. Similar shifts in tillage practiced with GRS might be expected in Brazil. Transgenes encoding glyphosate resistance in soybeans are highly unlikely to be a risk to wild plant species in Brazil. Soybean is almost completely self-pollinated and is a non-native species in Brazil, without wild relatives, making introgression of transgenes from GRS virtually impossible. Probably the highest agricultural risk in adopting GRS in Brazil is related to weed resistance. Weed species in GRS fields have shifted in Brazil to those that can more successfully withstand glyphosate or to those that avoid the time of its application. These include Chamaesyce hirta (erva-de-Santa-Luzia), Commelina benghalensis (trapoeraba), Spermacoce latifolia (erva-quente), Richardia brasiliensis (poaia-branca), and Ipomoea spp. (corda-de-viola). Four weed species, Conyza bonariensis, Conyza Canadensis (buva), Lolium multiflorum (azevem), and Euphorbia heterophylla (amendoim bravo), have evolved resistance to glyphosate in GRS in Brazil and have great potential to become problems.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Review, no study
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Clua et al. (2012)

Title: The Effects of Glyphosate on the Growth of Birdsfoot Trefoil (Lotus corniculatus) and Its Interaction with Different Phosphorus Contents in Soil	
Author: Clua, A., Conti, M. And Beltrano, J.	
Reference: Journal of Agricultural Science; Vol. 4 (7): 208-218	
Year: 2012	
Results and conclusion: Glyphosate residues from applications or exuded by roots of treated crops and by senescing weeds could be absorbed by new crops. The aim of this work was to study the effect of glyphosate in soil on the growth of Lotus corniculatus and its interaction with phosphorus. A completely randomized 3 x 4 factorial design was used for the experiment, with 3 levels of phosphorus (0, 100, and 200 ppm) and 4 of glyphosate (0; 0.5; 1.0, and 2.0 times the recommended dosage, 4 L. ha^{-1}), amended to soil. Glyphosate residues decreased growth parameters, chlorophyll and protein contents, and membrane stability. Glyphosate effect was increased by the greater availability of phosphorus, so there was a significant interaction between glyphosate and phosphorus. The findings of this study provide evidence of the detrimental effect of glyphosate present in soil as well as its remobilization through the presence of additional phosphorus in soil.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Review, no study
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Chang et al. (2011)

Title: OCCURRENCE AND FATE OF THE HERBICIDE GLYPHOSATE AND ITS DEGRADATE AMINOMETHYLPHOSPHONIC ACID IN THE ATMOSPHERE	
Author: FENG-CHIH CHANG, MATT F. SIMCIK, and PAUL D. CAPEL	
Reference: Environmental Toxicology and Chemistry, Vol. 30, No. 3, pp. 548–555	
Year: 2011	
Results and conclusion: This is the first report on the ambient levels of glyphosate, the most widely used herbicide in the United States, and its major degradation product, aminomethylphosphonic acid (AMPA), in air and rain. The frequency of glyphosate detection ranged from 60 to 100% in both air and rain. The concentrations of glyphosate ranged from <0.01 to 9.1 ng/m^3 and from <0.1 to 2.5mg/L in air and rain samples, respectively. The frequency of detection and	

median and maximum concentrations of glyphosate in air were similar or greater to those of the other high-use herbicides observed in the Mississippi River basin, whereas its concentration in rain was greater than the other herbicides. It is not known what percentage of the applied glyphosate is introduced into the air, but it was estimated that up to 0.7% of application is removed from the air in rainfall. Glyphosate is efficiently removed from the air; it is estimated that an average of 97% of the glyphosate in the air is removed by a weekly rainfall >30 mm..	
Proposed action: Not to be considered as publication deals with atmospheric concentrations outside EU.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as the articles deals with ambient air monitoring outside EU.	
Reliability	Medium
Endpoint	Monitoring, concentrations in air and rain
Protocol	Monitoring, for details see under test system and conditions.
Test compound	Glyphosate (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), monitored, purity cannot be given
Test system and conditions	Concurrent, weekly integrated air particle and rain samples were collected during two growing seasons in agricultural areas in Mississippi and Iowa. Rain was also collected in Indiana in a preliminary phase of the study. Description of sampling sites, of field sampling, of analytical methods and quality assurance
Statistical design	Calculation of median, maximum, minimum, standard deviation, %D
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	Similar monitoring programmes not known. No negative evidence.

Cornish and Burgin (2005)

Title: Residual Effects of Glyphosate Herbicide in Ecological Restoration
Author: P. S. Cornish and S. Burgin
Reference: Restoration Ecology Vol. 13, No. 4, pp. 695–702
Year: 2005
Results and conclusion: This study assesses the risks in ecological restoration arising from transplanting into soil containing glyphosate residues. Four Australian restoration species were grown for 60 days in non-adsorbing media treated continuously with glyphosate to establish threshold concentrations for damage. Visual signs of injury were observed in three species and severe effects on root growth in all species, at solution concentrations as low as 18 mg/L. Only the perennial grass Themeda sp. died at this concentration, with other species surviving at concentrations in the range 36–360 mg/L, beyond which all plants died. Fourteen days exposure followed by removal of glyphosate from root media produced similar effects. Field and glasshouse experiments with the relatively tolerant tree species Angophora costata showed that application rates in the range 10–50 L/ha of herbicide product (360 g/L) would be needed to sustain damage to young plants transplanted into soil typical of local restoration sites. The volume of spray delivered using a hand operated sprayer varied between operators by 5- and 10-fold to complete the same tasks, at the high end presenting a poten-

tial risk to the most tolerant species under field conditions, even when spray concentrations follow label instructions. For all but the most sensitive species, the risk of glyphosate residues in ecological restoration should be minimized by training operators of unregulated applicators to deliver controlled volumes of herbicide when spot spraying prior to transplanting.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Roundup (Monsanto Australia Ltd., Melbourne, Australia), Nufarm Glyphosate 360 (Nufarm Ltd., Melbourne, Australia), Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Correia and Durigan (2009)

Title: Glyphosate and Foliar Fertilization Using Manganese in Transgenic Soybean Crop
Author: CORREIA, N.M., DURIGAN, J.C.
Reference: Planta Daninha, Viçosa-MG, v. 27, n. 4, p. 721-72
Year: 2009
Results and conclusion: Based on the hypothesis that glyphosate-tolerant transgenic soybean would need a manganese complementation due to alterations in the absorption and metabolism of this element by the plants, this work aimed to evaluate the interaction of transgenic soybean sprayed with glyphosate and manganese foliar fertilization. The experiment was carried out under field conditions in the agricultural year 2007/2008 on the UNESP Campus Teaching, Research and Production Farm in Jaboticabal, São Paulo, Brazil. An experiment was arranged in a randomized block design, in a factorial scheme (4 x 4), with four replications. Four weed controls [glyphosate (c.p. Roundup Ready) at 0.72 and 1.20 kg/ha of equivalent acid; fluazifop-p-butyl plus fomesafen (c.p. Fusiflex) at 0.25 plus 0.25 kg/ha and under mechanical control, without herbicide] and four manganese rates (0, 42, 84 and 126 g/ha) were applied on the soybean leaf. The treatments did not significantly affect grain yield, manganese concentration in the soil, height and dry matter of the soybean plants. Only the mixture fluazifop-p-butyl plus fomesafen caused visible injuries in the plants. However, the symptoms were restricted to the leaves that intercepted spraying. The herbicide treatments did not differ from the control for 100 grain mass, although the plants treated with glyphosate 0.72 kg/ha presented less grain mass. Manganese application did not influence element concentration in the plant treated with glyphosate and under mechanical control. Therefore, glyphosate did not impair manganese absorption or metabolism by the plant. Growth and

development of the herbicide-treated plants were statistically similar to those of the plants not treated with herbicides.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate (Roundup Ready), CAS-no.: 1071-83-6; fluazifop-p-butyl CAS-no.: 79241-46-6 plus fomesafen, CAS-no.: 72178-02-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Coutinho and Mazo (2005)

Title: METALLIC COMPLEXES WITH GLYPHOSATE: A REVIEW	
Author: Cláudia F. B. Coutinho e Luiz Henrique Mazo	
Reference: Quim. Nova, Vol. 28, No. 6, 1038-1045	
Year: 2005	
Results and conclusion: We present studies involving metallic ions and the herbicide glyphosate. The metallic complexes of Cu(II), Zn(II), Mn(II), Ni(II), Cd(II), Pb(II), Cr(III), Fe(III), Co(III), ammonium, sodium, Ag(I), alkaline earth metals and of some lanthanides ions are described. The complexes are discussed in terms of their synthesis, identification, stability and structural properties, based on data from the current literature.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	

"Positive"/"Negative" evidence	Not applicable

Davis et al. (2011)

Title: Environmental impacts of irrigated sugarcane production: Herbicide run-off dynamics from farms and associated drainage systems	
Author: A.M. Davis, P.J. Thorburn, S.E. Lewis, Z.T. Bainbridge, S.J. Attard, R. Milla, J.E. Brodie	
Reference: Agric. Ecosyst. Environ. (2011), doi:10.1016/j.agee.2011.06.019	
Year: 2011	
Results and conclusion: This study determined the dynamics of off-site paddock-scale pesticide movement and subsequent concentrations in local receiving environments in fully irrigated sugarcane farming systems of the lower Burdekin floodplain region, the largest sugar producing area in Australia. Chemical movement (both mass and concentration) in paddock surface run-off followed a similar pattern across sites in the region for several of the commonly applied herbicides such as diuron, atrazine and ametryn. Highest losses (loads and event concentrations) occurred in the first irrigation run-off events following application, with subsequent irrigation losses tailing off rapidly. Significant losses could also occur during wet season rainfall run-off events from paddocks with recent pesticide applications. There was a strong seasonal signal evident in catchment monitoring results. Pesticide concentrations in nearby receiving creek systems were invariably an order of magnitude or more lower than values collected at paddock-scale, highlighting the considerable dilution that takes place over relatively short distances. While the concentrations found in receiving creek systems were considerably lower than direct paddock run-off, they regularly exceeded some ecological guidelines and results of pesticide risk modelling suggested concentrations, particularly under dry season conditions, posed considerable ecological risk to aquatic ecosystems.	
Proposed action: Not to be considered as modelling is presented but without data relevant for Glyphosate. Furthermore, the publication is related to a site outside the EU (Australia).	
Type of information (critical, high/low weight, supporting, additional): Low weight; modelling, no relevant data of glyphosate	
Reliability	Medium
Endpoint	Herbicide loads (active ingredient); box plots summarising herbicide concentration during wet-season flood events (B, D, and F) and low flow conditions
Protocol	Water samples were analyzed at the National Association of Testing Authorities accredited QHFSS laboratory; QHFSS method number 16315, QHFSS method number 16631
Test compound	Diuron, Atrazine, Ametryn, Hexazinone, 2,4-D, Glyphosate (CAS-no.: 1071-83-6), Paraquat
Test system and conditions	An emerging approach to predict ecosystem risk of a mixture of herbicides (as measured in monitoring) to freshwater ecosystems will be applied to lower Burdekin sub-catchment and catchment scale water quality monitoring results. Paddock scale data were collected from seven farms distributed across the lower Burdekin floodplain, spanning a wide range of soils, applied herbicides and application dates. The majority of paddock run-off monitoring effort focused on the initial irrigation events following herbicide application. At several instrumented sites, paddock run-off vol-

	umes were measured. To provide additional data on herbicide concentrations in irrigation tail water run-off, multiple discrete herbicide samples were also manually collected during irrigation events at a number of less intensively monitored sites lacking discharge monitoring capacity. A total of nine sampling sites were monitored. Grab samples were collected manually at all sites through the use of a sampling pole. A total of 275 samples were collected over the monitoring period, including 205 high flow event samples and 70 samples collected during low flow, dry season conditions. Modelling: dry season herbicide concentration data were analyzed with the Predict the Ecological Risk of Pesticides in freshwater ecosystems (PERPEST; Version 3.0).
Statistical design	BROLGA program; SPSS software package (SPSS 2007)
Relevance	
Environmental relevance	Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.agee.2011.06.019.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

de Andrade and Rosolem (2011)

Title: UPTAKE OF MANGANESE IN RR SOYBEAN UNDER GLIFOSATE APPLICATION	
Author: Gabriel José Massoni de Andrade & Ciro Antonio Rosolem	
Reference: R. Bras. Ci. Solo, 35:961-968, 2011	
Year: 2011	
Results and conclusion: It was hypothesized that Mn uptake efficiency and transport by transgenic, glifosate-resistant soybean would be affected by application of the herbicide. Two experiments were carried out to study manganese uptake, long-distance transport and absorption kinetics of genetically modified soybean as affected by glifosate application. Experiment 1: The treatments consisted of two near-isogenic soybean cultivars grown in nutrient solution (Conquista and Valiosa RR with or without application of glifosate). The Mn levels in the nutrient solution were 0, 0.085, 0.125, 0.250, 0.500 mg/L. Twenty-five days after emergence, part of the total transgenic soybean plants were sprayed with herbicide. Experiment 2: Plants were sprayed with glifosate on the 26th day of cultivation at rates of 0 (zero), 15 and 960 g/ha to study the Mn absorption kinetics of cultivar Valiosa RR. It was found that genetic resistance to glifosate did not affect manganese nutrition in soybean cultivar Valiosa RR. Despite reducing the root dry matter, glifosate does not hamper Mn absorption and transport in transgenic soybean plants. The Mn absorption kinetic parameters of Valiosa RR, *Km, Vmax and Cmin* are not altered by glifosate applied to leaves.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate isopropylammonium salt, CAS-no.: 38641-94-0

Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

De Souza et al. (2013)

Title: Degradation of the Commercial Herbicide Glyphosate by Photo-Fenton Process: Evaluation of Kinetic Parameters and Toxicity	
Author: D. R. de Souza, A. G. Trovó, N. R. A. Filho, M. A. A. Silvac, A. E. H. Machadob	
Reference: J. Braz. Chem. Soc., Vol. 24, No. 9, 1451-1460, 2013	
Year: 2013	
Results and conclusion: The aim of the present work was to evaluate at laboratory scale the influence of some parameters (use of Fe^{2+} and combination of Fe^{2+} and Fe^{3+} at a 1:1 molar ratio (Fe^{2+}/Fe^{3+}), addition of oxalate, concentration of H_2O_2 and oxalate) on the kinetics of mineralization and release of phosphate ion during the degradation of commercial glyphosate induced by photo-Fenton process. It was also monitored the degradation of glyphosate on a large scale under optimal experimental conditions, using a pilot plant and solar radiation in order to assess the possible commercial use of this technology. Parameters that influence the efficiency of the degradation of glyphosate (addition of Fe^{2+}, simultaneous addition of Fe^{2+} and Fe^{3+} at a 1:1 (Fe^{2+}/Fe^{3+}) molar ratio, addition of oxalate and of H_2O_2) were evaluated at lab-scale. Synergic effects on its degradation and release of phosphate were observed using Fe^{2+}/Fe^{3+}, as well as adding oxalate. On the other hand, the concentration increase of Fe^{2+}/Fe^{3+}, oxalate and H_2O_2 did not promote a linear increase of glyphosate mineralization and release of phosphate. Using high concentrations of these species, the efficiency of glyphosate mineralization and release of phosphate was constant or even decreased. Under optimized conditions (0.27 mmol L^{-1} of Fe^{2+}/Fe^{3+}; 1.13 mmol L^{-1} of oxalate and 10.3 mmol L^{-1} of H_2O_2), close results for mineralization and release of phosphate were obtained in lab-scale and using a solar pilot plant. A direct ratio between reducing the toxicity and glyphosate concentration was also observed.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Parameters that influence the efficiency of the degradation of glyphosate
Protocol	Non-GLP
Test compound	Glyphosate
Test system and conditions	The photodegradation experiments were performed in a lab-scale using a 400 W high pressure mercury vapour lamp as irradiation source. The average irradiance in the UVA furnished by this kind of lamp was estimated as being equal to 1100 W m-2, with a photonic flux of 3.3×10^{-6} einstein s^{-1} between 295 and

	710 nm. These measurements were done using a Solar Light PMA 2100 photometer/radiometer, equipped with an UVA detector (320-400 nm) and a radiometric/photometric setup built. A total volume of 4 L of aqueous solution containing commercial glyphosate was recirculated by pumping at a flow rate of 2.37 L min^{-1} after the addition of iron solution, pH adjustment between 2.8 ± 0.2 and addition of H_2O_2. The lamp was only turned on when the reactor was filled with solution. The solution temperature was controlled using a thermostatic bath, keeping close to 40 ± 2 ºC. Aliquots (25 mL) of the solutions containing the photodegraded material were collected at 15 min intervals up to 60 min, and, at 30 min intervals up to 120 min. Using the lab-scale setup, the following parameters were evaluated: (i) the use of Fe^{2+} (0.27 mmol L^{-1} - 15 mg L^{-1}) and Fe^{2+}/Fe^{3+} (0.27 and 0.135 mmol L^{-1} of each specie); (ii) total iron concentration (Fe^{2+}/Fe^{3+}): from 0.18 to 1.78 mmol L^{-1} (between 10.0 and 100.0 mg L^{-1}); (iii) oxalate concentration: 0.225 to 2.25 mmol L^{-1} (37.5 to 375.0 mg L^{-1}) and (iv) H_2O_2 concentration: 5.2 to 15.5 mmol L^{-1} (176.8 to 527.0 mg L^{-1}). Thus, kinetic experiments were carried out using 0.59 mmol L^{-1} (100 mg L^{-1}) glyphosate, 0.27 mmol L^{-1} Fe^{2+}/Fe^{3+}, 1.13 mmol L^{-1} oxalate (187.6 mg L^{-1}) and 10.3 mmol L^{-1} (350.2 mg L^{-1}) H_2O_2. After sampling and before analysis, a calculated volume of 2.0 mol L-1 Na_2SO_3 aqueous solution was added to the samples according to the stoichiometry between H_2O_2 and Na_2SO_3, and H_2O_2 concentration, ensuring the removal of the remaining H_2O_2, stopping the Fenton reaction. The experiments using solar radiation were carried in the winter under clear sky conditions, using a solar pilot plant. It consists of a compound parabolic collector (CPC) with an irradiated surface of 1.62 m^2 (irradiated volume of 12 L) and a reservoir with maximum capacity of 120 L. A volume of 50 L of glyphosate solution (0.59 mmol L^{-1}, 100 mg L^{-1}) circulates under turbulent flow into the CPC absorber tubes in a closed recirculating system. The solar irradiance was measured using the same radiometer applied in the lab-scale experiments, placed at the same angle of inclination of the reactor, being that an average solar irradiance of 40 ± 5 W m^{-2} was obtained. The photoreactor hydraulic circuit consists of a continuously stirred tank and a 0.50 HP centrifugal recirculation pump. At the beginning of the experiment, with the collectors covered, the same initial conditions defined for the reagents in kinetic experiments under lab-scale were used (0.27 mmol L^{-1} Fe^{2+}/Fe^{3+}, 1.13 mmol L^{-1} oxalate, 10.3 mmol L^{-1} H_2O_2 and pH 2.8 ± 0.2). The cover was then removed and the samples were collected at intervals of 100 kJ m^{-2} of UVA dose up to 800 kJ m^{-2} (30 min up to 240 min of irradiation). The same treatment using the Na_2SO_3 solution (previously described) was done to stop the Fen-

	ton reaction.
Statistical design	
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Duke et al. (2012)

Title: Effects of Glyphosate on the Mineral Content of Glyphosate-Resistant Soybeans (Glycine max)	
Author: Stephen O. Duke, Krishna N. Reddy, Kaixuan Bu and James V. Cizdziel	
Reference: J. Agric. Food Chem. 2012, 60, 6764−6771	
Year: 2012	
Results and conclusion: This article describes experiments designed to determine the effects of a recommended rate (0.86 kg ha^{-1}) of glyphosate applied once or twice on the mineral content of young and mature leaves, as well as in seeds produced by GR soybeans (Glycine max) in both the greenhouse and field using inductively coupled plasma mass spectrometry (ICP-MS). In the greenhouse, there were no effects of either one application (at 3 weeks after planting, WAP) or two applications (at 3 and 6 WAP) of glyphosate on Ca, Mg, Mn, Zn, Fe, Cu, Sr, Ba, Al, Cd, Cr, Co, or Ni content of young or old leaves sampled at 6, 9, and 12 WAP and in harvested seed. Se concentrations were too low for accurate detection in leaves, but there was also no effect of glyphosate applications on Se in the seeds. In the field study, there were no effects of two applications (at 3 and 6 WAP) of glyphosate on Ca, Mg, Mn, Zn, Fe, Cu, Sr, Ba, Al, Cd, Cr, Co, or Ni content of young or old leaves at either 9 or 12 WAP. There was also no effect on Se in the seeds. There was no difference in yield between control and glyphosate-treated GR soybeans in the field. The results indicate that glyphosate does not influence mineral nutrition of GR soybean at recommended rates for weed management in the field.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Roundup WeatherMax, Monsanto Agricultural Co., St. Louis, MO, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Duke et al. (2012)

Title: Glyphosate Effects on Plant Mineral Nutrition, Crop Rhizosphere Microbiota, and Plant Disease in Glyphosate-Resistant Crops	
Author: S. O. Duke, J. Lydon, W. C. Koskinen, T. B. Moorman, R. L. Chaney and R. Hammerschmidt	
Reference: dx.doi.org/10.1021/jf302436u | J. Agric. Food Chem. 2012, 60, 10375−10397	
Year: 2012	
Results and conclusion: Claims have been made recently that glyphosate-resistant (GR) crops sometimes have mineral deficiencies and increased plant disease. This review evaluates the literature that is germane to these claims. Our conclusions are: (1) although there is conflicting literature on the effects of glyphosate on mineral nutrition on GR crops, most of the literature indicates that mineral nutrition in GR crops is not affected by either the GR trait or by application of glyphosate; (2) most of the available data support the view that neither the GR transgenes nor glyphosate use in GR crops increases crop disease; and (3) yield data on GR crops do not support the hypotheses that there are substantive mineral nutrition or disease problems that are specific to GR crops.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Review article
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Duke and Powles (2008)

Title: Mini-review: Glyphosate: a once-in-a-century herbicide
Author: Stephen O Duke and Stephen B Powles
Reference: Pest Manag Sci 64:319–325
Year: 2008
Results and conclusion: Since its commercial introduction in 1974, glyphosate [N-(phosphonomethyl)glycine] has become the dominant herbicide worldwide. There are several reasons for its success. Glyphosate is a highly effective broad-spectrum herbicide, yet it is very toxicologically and environmentally safe. Glyphosate translocates well, and its action is slow enough to take advantage of this. Glyphosate is the only herbicide that targets 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), so there are no competing herbicide analogs or classes. Since glyphosate became a generic compound, its cost has dropped dramatically. Perhaps the

most important aspect of the success of glyphosate has been the introduction of transgenic, glyphosate-resistant crops in 1996. Almost 90% of all transgenic crops grown worldwide are glyphosate resistant, and the adoption of these crops is increasing at a steady pace. Glyphosate/glyphosate-resistant crop weed management offers significant environmental and other benefits over the technologies that it replaces. The use of this virtually ideal herbicide is now being threatened by the evolution of glyphosate-resistant weeds. Adoption of resistance management practices will be required to maintain the benefits of glyphosate technologies for future generations.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Review article: Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Eker et al. (2006)

Title: Foliar-Applied Glyphosate Substantially Reduced Uptake and Transport of Iron and Manganese in Sunflower (Helianthus annuus L.) Plants
Author: SELIM EKER, LEVENT OZTURK, ATILLA YAZICI, BULENT ERENOGLU, VOLKER ROMHELD, AND ISMAIL CAKMAK
Reference: J. Agric. Food Chem. 54, 119-125
Year: 2006
Results and conclusion: Evidence clearly shows that cationic micronutrients in spray solutions reduce the herbicidal effectiveness of glyphosate for weed control due to the formation of metal-glyphosate complexes. The formation of these glyphosate-metal complexes in plant tissue may also impair micronutrient nutrition of non-target plants when exposed to glyphosate drift or glyphosate residues in soil. In the present study, the effects of simulated glyphosate drift on plant growth and uptake, translocation, and accumulation (tissue concentration) of iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) were investigated in sunflower (Helianthus annuus L.) plants grown in nutrient solution under controlled environmental conditions. Glyphosate was sprayed on plant shoots at different rates between 1.25 and 6.0% of the recommended dosage (i.e. 0.39 and 1.89 mM glyphosate isopropylamine salt). Glyphosate applications significantly decreased root and shoot dry matter production and chlorophyll concentrations of young leaves and shoot tips. The basal parts of the youngest leaves and shoot tips were severely chlorotic. These effects became apparent within 48 h after the glyphosate spray. Glyphosate also caused substantial decreases in leaf concentration of Fe and Mn while the concentration of Zn and Cu was less affected. In short-term uptake ex-

periments with radiolabelled Fe (^{59}Fe), Mn (^{54}Mn), and Zn (^{65}Zn), root uptake of ^{59}Fe and ^{54}Mn was significantly reduced in 12 and 24 h after application of 6% of the recommended dosage of glyphosate, respectively. Glyphosate resulted in almost complete inhibition of root-to-shoot translocation of ^{59}Fe within 12 h and ^{54}Mn within 24 h after application. These results suggest that glyphosate residues or drift may result in severe impairments in Fe and Mn nutrition of nontarget plants, possibly due to the formation of poorly soluble glyphosate-metal complexes in plant tissues and/or rhizosphere interactions.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Roundup Ultra [active ingredient N-[phosphonomethyl]glycine isopropylamine salt, Monsanto Co.], CAS-no.: 38641-91-0; radiolabelled Fe (59Fe), Mn (54Mn), and Zn (65Zn)
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Ermakova et al. (2010)

Title: Bioremediation of glyphosate-contaminated soils
Author: Inna T. Ermakova, Nina I. Kiseleva, Tatyana Shushkova, Mikhail Zharikov, Gennady A. Zharikov, Alexey A. Leontievsky
Reference: Appl Microbiol Biotechnol 88:585–594
Year: 2010
Results and conclusion: Based on the results of laboratory and field experiments, we performed a comprehensive assessment of the bioremediation efficiency of glyphosate-contaminated soddypodzol soil. The selected bacterial strains Achromobacter sp. Kg 16 (VKM B-2534D) and Ochrobactrum anthropi GPK 3 (VKM B-2554D) were used for the aerobic degradation of glyphosate. They demonstrated high viability in soil with the tenfold higher content of glyphosate than the recommended dose for the single in situ treatment of weeds. The strains provided a two- to threefold higher rate of glyphosate degradation as compared to indigenous soil microbial community. Within 1-2 weeks after the strain introduction, the glyphosate content of the treated soil decreased and integral toxicity and phytotoxicity diminished to values of non-contaminated soil. The decrease in the glyphosate content restored soil biological activity, as is evident from a more than twofold increase in the dehydrogenase activity of indigenous soil microorganisms and their biomass (1.2-fold and 1.6-fold for saprotrophic bacteria and fungi, respectively). The glyphosate-degrading strains used in this study are not pathogenic for mammals and do not exhibit integral toxicity and phytotoxicity. There-

fore, these strains are suitable for the efficient, ecologically safe, and rapid bioremediation of glyphosate-contaminated soils.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Isopropylamine ammonium salt of glyphosate as a component of commercial Ground Bio herbicide, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Fan et al. (2012)

Title: Isolation, identification and characterization of a glyphosate-degrading bacterium, Bacillus cereus CB4, from soil	
Author: Jieyu Fan, Guoxia Yang, Haoyu Zhao, Guanying Shi, Yucong Geng, Taiping Hou, and Ke Tao	
Reference: J. Gen. Appl. Microbial., 58, 263-271 (2012)	
Year: 2012	
Results and conclusion: A bacterial strain named CB4, with highly effective glyphosate degradation capability, was isolated from soil after enrichment. On the basis of the Biolog omniLog Identification system (Biolog) and 165 ribosomal RNA (rRNA) gene sequencing methods, strain CB4 was identified as Bacillus cereus. Further experiments were carried out to optimize the growth of strain CB4 and the glyphosate degradation activity by high performance liquid chromatography (HPLC). The optimal conditions were found as follows: initial pH 6.0, incubation temperature 35°C, glyphosate concentration 6 g L^{-1}, inoculation amount 5% and incubation time 5 days. Under the optimal conditions, stain CB4 utilized 94.47% of glyphosate. This is the first report on B. cereus with a capacity to utilize herbicide glyphosate, and it can degrade glyphosate concentrations up to 12 g L^{-1}. Metabolization of glyphosate by strain B. cereus CB4 was studied. Results indicated that two concurrent pathways were capable of degrading glyphosate to AMPA, glyoxylate, sarcosine, glycine and formaldehyde as products. Glyphosate breakdown in B. cereus CB4 was achieved by the Cop lyase activity and the glyphosate oxidoreductase activity.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable

Endpoint	Not applicable
Protocol	Not applicable
Test compound	No test design
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Fobbe et al. (2006)

Title: Polar Herbicides and Metabolites	
Author: Rita Fobbe, Birgit Kuhlmann, Jürgen Nolte, Gudrun Preuß, Christian Skark, and Ninette Zullei-Seibert	
Reference: Organic Pollutants in the Water Cycle. T. Reemtsma and M. Jekel (Eds.). Copyright © 2006 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim ISBN: 3-527-31297-8	
Year: 2006	
Results and conclusion: Overview article: Polar herbicides are widely used man-made substances. Because of their chemical diversity, different analytical methods are required for monitoring purpose. As a consequence, only a small fraction of all the herbicides in use is analyzed in groundwater, surface water, and drinking water, or in research work. The few comprehensive investigations show as a rule that the occurrence of polar herbicides in water bodies is strictly related to the amount applied as well as to failures in good application practice. Thus, positive findings mostly concern atrazine, simazine, terbutylazine and its metabolites, bentazone, isoproturon, diuron, chlorotoluron, MCPP, 2,4-D or glyphosate, and rarely sulfonylureas. Even if raw water used for drinking water production is contaminated by polar herbicides, the drinking water itself is usually not affected. Natural as well as technical filtration and oxidation steps or combined purification techniques are able to solve the problems. Therefore, in Europe only minor cases of exceeding the drinking water standard of 0.1 µg L^{-1} are reported. Globally, cases of contamination of drinking water always involve areas where the use of herbicides is intensive and the water treatment is inadequate.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	No test design
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	

Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Franco et al. (2012)

Title: Evaluation of Glyphosate Application on Transgenic Soybean and its Relationship with Shikimic Acid	
Author: FRANCO, D.A.S., ALMEIDA, S.D.B., CERDEIRA, A.L., DUKE, S.O., MORAES, R.M., LACERDA, A.L.S. and MATALLO, M.B.	
Reference: Planta Daninha, Viçosa-MG, v. 30, n. 3, p. 659-666, 2012	
Year: 2012	
Results and conclusion: A field experiment was conducted at Engenheiro Coelho-SP, Brazil, during the agricultural year 2007/2008 to evaluate the effect of glyphosate on the growth, development, and seed quality of GRC soybean variety BRS Valiosa RR. A randomized block design was used with four replications. Glyphosate was applied at 720 and 960 g a.e. ha-1 (acid equivalent) and in sequence at the doses 720/720, 960/720, and 960/720/720 g a.e. ha-1 (acid equivalent). To evaluate transfer from GRC soybean to non GRC soybean cultivated in nutrient solution, a pot experiment was conducted at Instituto Biológico, SP, Brazil. Glyphosate was applied on the GRC soybean (M8045RR) at 2,400 g a.e. ha-1. Both GRC soybean and non GRC soybean were sown in the same box with nutrient solution. At 0, 1, 3, 7, and 10 days after application, shikimic acid was measured by HPLC and the glyphosate and aminomethylphosphonic acid (AMPA) levels in nutrient solution were determined by GC-MS. The results showed that yield, plant height, seed oil, and protein contents were not affected by glyphosate application. GRC soybean accumulated shikimic acid in the field. Glyphosate and AMPA were released through the roots of GRC soybean, and subsequently taken up by non-GRC soybean, exerting inhibitory effects on their shikimic pathway.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	No test design
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Geng et al. (2015)

Title: Modeling the release of organic contaminants during compost decomposition in soil	
Author: C. Geng, C.-S. Haudin, Y. Zhang, G. Lashermes, S. Houot, P. Garnier	
Reference: Chemosphere 119 (2015) 423–431	
Year: 2015	
Results and conclusion: Composts, incorporated in soils as amendments, may release organic contaminants during their decomposition. COP–Soil is presented here as a new model to simulate the interaction between organic contaminants and compost, using one module for organic matter and one for organic pollutants, with these modules being linked by several assumptions. Published results of laboratory soil incubations using labeled carbon pollutants from compost were used to test the model for one polycyclic aromatic hydrocarbon (PAH), two surfactants and one herbicide. Several simulation scenarios were tested using (i) the organic pollutant module either alone or coupled to the organic matter module, (ii) various methods to estimate the adsorption coefficients (Kd) of contaminants on organic matter and (iii) different degrading biomasses. The simulations were improved if the organic pollutant module was coupled with the organic matter module. Multiple linear regression model for Kd as a function of organic matter quality yielded the most accurate simulation results. The inclusion of specific biomass in the model made it possible to successfully predict the PAH mineralization.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate amongst others
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Gerhardt et al. (2012)

Title: Active in Situ Biomonitoring of Pesticide Pulses Using Gammarus spp. in Small Tributaries of Lake Constance	
Author: A. Gerhardt, M. Koster, F. Lang, V. Leib	
Reference: Journal of Environmental Protection, 2012, 3, 573-583, doi:10.4236/jep.2012.37069 Published Online July 2012 (http://www.SciRP.org/journal/jep)	
Year:	
Results and conclusion: Gammarids are important members of a stream’s macrozoobenthos biocoenosis and food web. Moreover, they proved to be very sensitive towards different types of pollution. GamToxTM is a new in situ ecotoxicity test, based on survival and	

feeding behavior of caged gammarids for active monitoring of small streams in agricultural areas. GamToxTM has been applied in two streams with specific pollution problems in the catchment of Lake Constance. Ten organisms were exposed in 5 replicates in flow through test tubes containing one conditioned alder leaf, placed in baskets which were attached in the stream bottom and on the banks. Each week, the number of living animals was counted, the percentage of leaf skeletized estimated in semi-quantitative classes and a new elder leaf provided. Dead organisms were removed. Simultaneously, chemical analyses of pesticides and nutrients (N-compounds, P) were performed on cumulative water samples over one week. Moreover, macrozoobenthos was collected and determined according to the IBCH method, and the SPEAR index calculated. GamToxTM proved to be very sensitive to detect pesticides, copper as well as nutrients, both during acute pollution pulses and chronic exposures of up to 6 weeks. Survival turned out to be a more sensitive and less variable parameter than feeding. GamToxTM is easy to perform and directly provides a measure of ecotoxicological effects of toxicant/nutrient mixtures, which cannot be predicted by biological indices based on macrozoobenthos data such as IBCH and SPEAR-index. This study was co-financed by the InterReg IV project "Ökotoxikologischer Index im Bodenseeraum", no. 227 (2011-2013) supported by the EFRE.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Not applicable
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Greenpeace International (2011)

Title: Herbicide tolerance and GM crops – Why the world should be Ready to Round Up glyphosate
Author: Greenpeace International
Reference: -
Year: 2011
Results and conclusion: Review – Regarding environmental fate the following statements are made: General: It is glyphosate's capacity to bind tightly to soil particles that prevents it from being highly mobile. Binding can immobilise it in the soil provided that there are sufficient suitable sites. This varies depending on the soil type and composition. Studies have found that binding of glyphosate is greater in soils with lower pH (i.e. more acidic) (Gimsing et al. 2004) and that phosphates (Simonsen et al. 2008) can compete for binding sites.

Water:
A report by the World Health Organisation (WHO 2005) confirmed that glyphosate is found in surface waters at levels between 0.5 µg/l and 1 µg/l and its environmental breakdown product, AMPA, was present at levels around 6 µg/l. The levels of glyphosate exceed the maximum allowed for pesticides in drinking water under EU law and would require water companies to undertake expensive filtration before the water could be supplied to the public.
In streams in the Midwest US, glyphosate was detected during every season up to a maximum concentration of 8.7 µg/l. The maximum concentration of AMPA recorded in this study was 3.67 µg/l.
In Alberta, Canada glyphosate was found in 8 out of 13 sites and in 22 % of samples taken in wetland and streams, with a peak concentration of 6.07 µg/l.
In Denmark, glyphosate was detected to the depth of the drainage system. Furthermore, loamy soils were found to be more prone to leaching of glyphosate and AMPA than coarse sandy soils, where matrixes of aluminium and iron provide the right conditions for sorption and degradation. On loamy soils, autumn application resulted in detectable concentrations of glyphosate and AMPA in the drainage water in the upper metre of soil, often at concentrations exceeding the EU's maximum concentration for drinking water. The maximum concentrations of glyphosate recorded in drainage water at the two most vulnerable sites were found in 2009 (31 µg/l and 4.7 µg/l respectively). Average concentrations of glyphosate in drainage water following the first drainage after application were well above 0.1 µg/l for some crops, for instance maize in 2005 (4.04 µg/l) and peas in 2001 (0.54 µg/l), both following the application RoundUp. Detection of glyphosate and AMPA was mainly confined to drainage water although it was detected at three sites below the drainage system. At one site in the wet August of 2008, glyphosate was frequently detected in groundwater, with a maximum concentration of 0.67 µg/l.
Small catchment studies in Sweden, France and Greece have confirmed that glyphosate can leach into drainage systems and surface waters.
A study in France showed that glyphosate can enter watercourses more readily from urban areas via the sewerage system than in rural environments due to applications on roads and railways. High levels were linked to rainfall events. Glyphosate is banned from use on hard surfaces in Denmark and by half of Swedish municipalities.
In general, the statements are supported by citations.

Proposed action:
Consider as additional information due to the fact that the article is a review and data are cited only. Furthermore, some monitoring data reviewed are obtained at sites outside the EU.

Type of information (critical, high/low weight, supporting, additional):
Additional information

Reliability	
Endpoint	Behaviour of glyphosate in soil and water as well as results from surface water and groundwater monitoring
Protocol	No detailed information in the report on analysed studies
Test compound	Glyphosate and AMPA
Test system and conditions	No information in the report about the analysed studies
Statistical design	Not provided
Relevance	
Environmental relevance	Partly, since some monitoring sites are outside the EU.
Weight of evidence	

"Positive"/"Negative" evidence	Detailed information on the analysed studies is not provided in the report. Therefore, the data in the report cannot be considered for endpoint derivation and/or further risk assessment.

Hadi et al. (2013)

Title: New bacterial strain of the genus Ochrobactrum with glyphosate-degrading activity	
Author: F. HADI, A. MOUSAVI, K. A. NOGHABI, H. G. TABAR and A. SALMANIAN	
Reference: Journal of Environmental Science and Health, Part B (2013) 48, 208–213	
Year: 2013	
Results and conclusion: Thirty bacterial strains with various abilities to utilize glyphosate as the sole phosphorus source were isolated from farm soils using the glyphosate enrichment cultivation technique. Among them, a strain showing a remarkable glyphosate-degrading activity was identified by biochemical features and 16S rRNA sequence analysis as Ochrobactrum sp. (GDOS). Herbicide (3 mM) degradation was induced by phosphate starvation, and was completed within 60 h. Aminomethylphosphonic acid was detected in the exhausted medium, suggesting glyphosate oxidoreductase as the enzyme responsible for herbicide breakdown. As it grew even in the presence of glyphosate concentrations as high as 200 mM, Ochrobactrum sp. could be used for bioremediation purposes and treatment of heavily contaminated soils.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Not applicable
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Harris et al. (2012)

Title: Computer Simulation of the Interactions of Glyphosate with Metal Ions in Phloem
Author: Wesley R. Harris,R. Douglas Sammons, Raymond C. Grabiak, Akbar Mehrsheikh and Marian S. Bleeke‡
Reference: J. Agric. Food Chem. 2012, 60, 6077−6087
Year: 2012
Results and conclusion: Essential nutrients such as trace metal ions, amino acids, and sugars are transported in the phloem from leaves to other parts of the plant. The major chelating agents in phloem include nicotianamine, histidine, cysteine, glutamic acid, and citrate. A computer model for the speciation of metal ions in phloem has been used to assess the degree to which the

widely used herbicide glyphosate binds to Fe3+, Fe2+, Cu2+, Zn2+, Mn2+, Ca2+, and Mg2+ in this fluid over the pH range of 8 to 6.5. The calculations show that glyphosate is largely unable to compete effectively with the biological chelating agents in phloem. At a typical phloem pH of 8, 1.5 mM glyphosate binds 8.4% of the total Fe3+, 3.4% of the total Mn2+, and 2.3% of the total Mg2+ but has almost no effect on the speciation of Ca2+, Cu2+, Zn2+, and Fe2+. As the pH decreases to 6.5, there are some major shifts of the metal ions among the biological chelators, but only modest increases in glyphosate binding to 6% for Fe2+ and 2% for Zn2+. The calculations also indicate that over 90% of the glyphosate in phloem is not bound to any metal ion and that none of the metal−glyphosate complexes exceed their solubility limits.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Haudin et al. (2013)

Title: Fate of ^{14}C-organic pollutant residues in composted sludge after application to soil	
Author: C.-S. Haudin,Y. Zhang, V. Dumény, G. Lashermes, V. Bergheaud, E. Barriuso, S. Houot	
Reference: Chemosphere 92 (2013) 1280–1285	
Year: 2013	
Results and conclusion: Organic micropollutants may be present in biosolids, leading to soil contamination when they are recycled in agriculture. A sludge spiked with ^{14}C-labelled glyphosate (GLY), sodium linear dodecylbenzene sulphonate (LAS), fluoranthene (FLT) or 4-n-nonylphenol (NP) was composted with green waste and the fate of the ^{14}C-micropollutant residues remaining after composting was assessed after the compost application to the soil. ^{14}C-residues were mineralised in the soil and represented after 140 d 20–32% of the initial activity for LAS, 16–25% for GLY, 6–9% for FLT and 4–7% for NP. The ^{14}C-residues at the end of composting that could not be extracted with methanol or ammonia were minimally remobilised or even increased for FLT. After 140 d, non-extractable residues represented 38–52% of all of the 14C-residues remaining in the soil for FLT, 50–67% for GLY, 91–92% for NP and 94–97% for LAS and in most cases, less than 1% of the 14C-residues were water soluble, suggesting a low direct availability for leaching and microbial or plant assimilation. FLT was identified as the main compound among the methanol-extractable ^{14}C-residues that may be potentially available. The fate of the ^{14}C-organic pollutant residues in composts after application to soil could be	

assessed through a sequential chemical extraction scheme and depended on the chemical nature of the pollutant. In detail: The fate of the ^{14}C-organic pollutant residues depended on the pollutant and its distribution between the available and non-available fractions in the composts. The residues of each pollutant mineralised to different extents (LAS > GLY > FLT = NP). The composting appeared to have an impact on the stabilisation of the ^{14}C-residues because the non-extractable residues were apparently poorly remobilisable at this time scale (140 d). Nevertheless a part of GLY and FLT residues remained potentially available.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Fate of ^{14}C-organic pollutant residues in composted sludge
Protocol	Non-GLP
Test compound	Four organic pollutants: ^{14}C-labelled glyphosate (GLY), sodium linear dodecylbenzene sulphonate (LAS), fluoranthene (FLT) or 4-n-nonylphenol (NP)
Test system and conditions	A sludge spiked with ^{14}C-labelled glyphosate (GLY), sodium linear dodecylbenzene sulphonate (LAS), fluoranthene (FLT) or 4-n-nonylphenol (NP) was composted with green waste and the fate of the ^{14}C-micropollutant residues remaining after composting was assessed after the compost application to the soil.
Statistical design	Not given in the paper
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Henry et al. (2011)

Title: Glyphosate's Effect Upon Mineral Accumulation in Soybean
Author: Ryan S. Henry, Kiersten A. Wise, and William G. Johnson
Reference: Crop Management doi:10.1094/CM-2011-1024-01-RS
Year: 2011
Results and conclusion: Glyphosate has been demonstrated to reduce the macronutrient and micronutrient content of glyphosate-susceptible (GS) and first generation glyphosate-resistant (GR) or Roundup Ready (RR) soybean, possibly by complexation of the herbicide molecule with the nutrient. The recent release of newer GR soybean cultivars, second generation Roundup Ready 2 Yield (RR2Y), provides growers with newer technology for weed management programs, but it is unclear how the nutrient content of these cultivars is affected by glyphosate in a field setting. The objective of this experiment was to identify the effect of glyphosate on the concentration of macronutrient and micronutrients in RR and RR2Y soybean when grown using standard agronomic practices in Indiana. The macronutrients analyzed were nitrogen, phosphorus, potassium, sulphur, magnesium, and calcium. The micronutrients analyzed were boron, zinc, manganese, iron, copper, and aluminium. Our results indicate that while differences in accumulation of macro and micronutrients exist between the two

cultivars tested, there was no consistent effect due to glyphosate treatment. Glyphosate-induced deficiency symptoms observed in previous reports were not observed in this study. Growers should continue to monitor soil nutrient levels to identify and correct nutrient deficiencies.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Jolley et al. (2004)

Title: Nutritional and management related interactions with iron-deficiency stress response mechanism	
Author: V.D. Jolley, N.C. Hansen, A.K. Shiffler	
Reference: Soil Sci Plant Nutr, 50 (7), 973-981	
Year: 2004	
Results and conclusion: Review article: Iron (Fe) deficiency symptoms develop in many agricultural and horticultural settings and generally occur when susceptible genotypes are grown in calcareous soils where Fe availability is limited. I some situations, Fe deficiency develops as a result of biological interactions with factors other than limited available Fe. We review physiological explanations for some factors known to interact with iron-deficiency stress. The discussion includes interactions with macronutrients and micronutrients, management factors such as grazing and companion cropping, and symbiotic nitrogen fixation. We also refer to several field observed interactions with Fe deficiency in soybean, where physiological explanations are yet to be identified. These include interactions with seeding rate and application of the herbicide glyphosate on glyphosate tolerant varieties.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and con-	Not applicable

ditions	
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Junges et al. (2013)

Title: Effectiveness evaluation of glyphosate oxidation employing the H_2O_2/UVC process: Toxicity assays with Vibrio fischeri and Rhinella arenarum tadpoles	
Author: C. M. JUNGES, E. E. VIDAL, A. S M. ATTADEMO, M. L. MARIANI, L. CARDELL, A. C. NEGRO, A. CASSANO, P. M. PELTZER, R. C. LAJMANOVICH and C. S. ZALAZAR	
Reference: Journal of Environmental Science and Health, Part B (2013) 48, 163-170	
Year: 2013	
Results and conclusion: The H_2O_2/UVC process was applied to the photodegradation of a commercial formulation of glyphosate in water. Two organisms (Vibrio fischeri bacteria and Rhinella arenarum tadpoles) were used to investigate the toxicity of glyphosate in samples M_1, M_2, and M_3 following different photodegradation reaction times (120, 240 and 360 min, respectively) that had differing amounts of residual H_2O_2. Subsamples of M_1, M_2, andM_3 were then used to create samples $M_{1,E}$, $M_{2,E}$ and$M_{3,E}$ in which the H_2O_2 had been removed. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities were measured in tadpoles to determine possible sub-lethal effects. In V. fischeri, $M_{1,E}$, which was collected early in the photodegradation process, caused 52% inhibition, while $M_{3,E}$, which was collected at the end of the photodegradation process, caused only 17% inhibition. Survival of tadpoles was 100% in samples M_2, M_3, and in $M_{1,E}$, $M_{2,E}$ and $M_{3,E}$. The lowest percentages of enzymatic inhibition were observed in samples without removal of H_2O_2: 13.96% (AChE) and 16% (BChE) for M2, and 24.12% (AChE) and 13.83% (BChE) for M_3. These results show the efficiency of the H_2O_2/UVC process in reducing the toxicity of water or wastewater polluted by commercial formulations of glyphosate. According to the ecotoxicity assays, the conditions corresponding to M_2 (11 ± 1 mg a.e. L^{-1} glyphosate and 11 ± 1 mg L^{-1} H_2O_2) could be used as a final point for glyphosate treatment with the H_2O_2/UV process.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Glyphosate oxidation by the H_2O_2/UVC process and toxicity assays
Protocol	Not given
Test compound	(a) glyphosate (AccuStandard) as a standard chromatographic and (b) glyphosate as the commercial herbicide Eskoba®, 35.6% (w/v) as acid or 48% as a monoisopropylamine salt (MIPA)
Test system and conditions	The H_2O_2/UVC process was applied to the degradation of a commercial formulation of glyphosate in water. Two different bioassays were used for determining sample toxicity at different stages

	of mineralization. The bioassays used the luminescence bacterium Vibrio fischeri (a traditional assay for evaluating AOPs) and tadpoles of Rhinella arenarum, a common anuran that is frequently found in forests, wetlands, agricultural land and urban territories and has an extensive Neotropical distribution. Rhinella was used to assess acute toxicity. In addition, total AChE and BChE activities were evaluated as possible indicators of sub-lethal toxicity of both untreated wastewater and wastewater treated by the H_2O_2/UVC process.
Statistical design	In the R. arenarum survival tests, the percentage of mortality expressed as the mean ± standard error of measurement (SEM) was recorded. The differences in the mortality proportions were estimated using a Chi-Squared Goodness of Fit model (with Yates correction). Enzymatic activities were expressed as the mean ± SEM. The influence of treatments on the B-esterase enzyme activities were analyzed statistically using the non-parametric Kruskal-Wallis ANOVA. Pairwise comparisons between samples from the all treatment were tested by the Dunnett's test for post-hoc multiple comparisons. Statistical significance was held at $\alpha = 0.05$. Analyses were performed with GraphPad InStats®.
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Kempenaar et al. (2007)

Title: Trade off between costs and environmental effects of weed control on pavements
Author: C. Kempenaar, L.A.P. Lotz, C.L.M. van der Horst, W.H.J. Beltman, K.J.M. Leemans, A.D. Bannink
Reference: Crop Protection 26, 430–435
Year: 2007
Results and conclusion: An actor-participative project on sustainable weed control on pavements was started in 2000 in the Netherlands. The aim of the project was to develop a new concept of weed management that provides cost-effective and environmentally sound weed control. Early in 2002, practical guidelines were drawn up in support of decision making by managers of pavements, and weed control contractors. The guidelines are focused mainly on reduction of herbicide use and emission thereof. The new concept was tested in 2002 and 2003 in nine Dutch municipalities on defined urban areas of 5-25 ha, which formed units from a construction, hydrology and management point of view. Use of herbicides (mainly glyphosate) was reduced by 11-66% compared to standard practice. Levels of weed control remained good and ecological threshold concentrations in surface waters were not exceeded. Monitoring showed a glyphosate emission factor via the sewage water system of 2% on average. Costs of weed control with the new concept were higher (10-25%) compared to the standard practice control of weeds (using herbicides) on pavements, but much lower compared to alternative (non-herbicide) weed control systems. It is concluded that the new concept provides a useful framework for finding a good trade off between economical and

ecological aspects of weed control on pavements.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; and AMPA, CAS-no.: 1066-51-9
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Kirk et al. (2013)

Title: Glyphosate and fungicide effects on Cercospora leaf spot in four glyphosate-resistant sugar beet (Beta vulgaris) varieties	
Author: W. W. Kirk, L. E. Hanson, C. L. Sprague	
Reference: Crop Protection 44 (2013) 38-43	
Year: 2013	
Results and conclusion: The potential for improved management of Cercospora leaf spot (CLS), caused by Cercospora beticola, using the herbicide glyphosate in glyphosate-resistant sugar beet varieties was investigated. Controlled field experiments were conducted in 2008 and 2009 to determine if glyphosate and glyphosate-fungicide combinations improved the management of CLS in four commercial varieties of glyphosate-resistant sugar beet. Variety and fungicide main effects were significant for CLS development. However, regardless of the herbicide program, glyphosate or a conventional herbicide program, CLS development was not affected. Therefore, results from of this research indicate that glyphosate and glyphosate-fungicide combinations do not significantly contribute to CLS management.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate and glyphosate-fungicide combinations
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	

Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Kleter et al. (2008)

Title: Review: Comparison of herbicide regimes and the associated potential environmental effects of glyphosate-resistant crops versus what they replace in Europe	
Author: Gijs A Kleter, Caroline Harris, Gerry Stephenson, John Unsworth	
Reference: Pest Manag Sci 64:479–488	
Year: 2008	
Results and conclusion: Review: While cultivation of transgenic crops takes place in seven of the EU member states, this constitutes a relatively limited part of the total acreage planted to these crops worldwide. The only glyphosate-resistant (GR) crop grown commercially until recently has been soybean in Romania. In addition, large-scale experimental European data exist for GR sugar and fodder beets, and, to a lesser extent, GR oilseed rape. These GR crops are likely to have an impact both on the use of herbicides and on the environmental impact of the latter. From the data on these GR crops, it appears that quantities of herbicides applied to GR beets are decreased while those on GR soybean are slightly increased compared with their conventional counterparts. Depending on the parameters used for prediction or measurement of environmental impacts of GR crops, generally similar or less negative impacts were observed compared with conventional crops. Favourable environmental effects of the glyphosate-containing herbicide regimes on GR crops appear feasible, provided appropriate measures for maintaining biodiversity and prevention of volunteers and gene flow are applied.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Not applicable, no test design
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Kremer et al. (2005)

Title: Glyphosate affects soybean root exudation and rhizosphere micro-organisms
Author: ROBERT J. KREMER, NATHAN E. MEANS and SUJUNG KIM

Reference: Intern. J. Environ. Anal. Chem. Vol. 85, No. 15, 1165–1174	
Year: 2005	
Results and conclusion: Glyphosate is a non-selective, broad-spectrum herbicide that kills plants by inhibiting the enzyme 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS), which is necessary for synthesis of aromatic amino acids. A secondary mode of action involves infection of roots of glyphosate-susceptible plants by soil-borne micro-organisms due to decreased production of plant protection compounds known as phytoalexins. Varieties of several crops, including glyphosate-resistant (GR) or Roundup Ready soybean, are genetically modified to resist the herbicidal effects of glyphosate and provide farmers with an effective weed-management tool. After glyphosate is applied to GR soybean, glyphosate that is not bound to glyphosate-resistant EPSPS is translocated throughout the plant and accumulates primarily in meristematic tissues. We previously reported that fungal colonization of GR soybean roots increased significantly after application of glyphosate but not after conventional post-emergence herbicides. Because glyphosate may be released into soil from GR roots, we characterized the response of rhizosphere fungi and bacteria to root exudates from GR and non-GR (Williams 82; W82) cultivars treated with and without glyphosate at field application rates. Using an immunoassay technique, glyphosate at concentrations >1000 ng/plant were detected in exudates of hydroponically grown GR soybean at 16 days post-glyphosate application. Glyphosate also increased carbohydrate and amino acid contents in root exudates in both soybean cultivars. However, GR soybean released higher carbohydrate and amino acid contents in root exudates than W82 soybean without glyphosate treatment. In vitro bioassays showed that glyphosate in the exudates stimulated growth of selected rhizosphere fungi, possibly by providing a selective C and N source combined with the high levels of soluble carbohydrates and amino acids associated with glyphosate treatment of the soybean plants. Increased fungal populations that develop under glyphosate treatment of GR soybean may adversely affect plant growth and biological processes in the soil and rhizosphere.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Roundup Ultra®: Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Kryuchkova et al. (2014)

Title: Isolation and characterization of a glyphosate-degrading rhizosphere strain, Enterobacter cloacae K7

Author: Y. V. Kryuchkova, G. L. Burygin, N. E. Gogoleva, Y. V. Gogolev, M. P. Chernyshova, O. E. Makarov, E. E. Fedorov, O. V. Turkovskaya	
Reference: Microbiological Research 169 (2014) 99-105	
Year: 2014	
Results and conclusion: Plant-growth-promoting rhizobacteria exert beneficial effects on plants through their capacity for nitro-gen fixation, phytohormone production, phosphate solubilization, and improvement of the water and mineral status of plants. We suggested that these bacteria may also have the potential to express degradative activity toward glyphosate, a commonly used organophosphorus herbicide. In this study, 10 strains resistant to a 10 mM concentration of glyphosate were isolated from the rhizoplane of various plants. Five of these strains – Alcaligenes sp. K1, Comamonas sp. K4, Azomonas sp. K5, Pseudomonas sp. K3, and Enterobacter cloacae K7 – possessed a number of associative traits, including fixation of atmospheric nitro-gen, solubilization of phosphates, and synthesis of the phytohormone indole-3-acetic acid. One strain, E.cloacae K7, could utilize glyphosate as a source of P. Gas–liquid chromatography showed that E. Cloacae growth correlated with a decline in herbicide content in the culture medium (40% of the initial 5 mM content), with no glyphosate accumulating inside the cells. Thin-layer chromatography analysis of the intermediate metabolites of glyphosate degradation found that E. cloacae K7 had a C–P lyase activity and degraded glyphosate to give sarcosine, which was then oxidized to glycine. In addition, strain K7 colonized the roots of common sunflower (Helianthus annuus L.) and sugar sorghum (Sorghum saccharatum Pers.), promoting the growth and development of sunflower seedlings. Our findings extend current knowledge of glyphosate-degrading rhizosphere bacteria and may be useful for developing a biotechnology for the cleanup and restoration of glyphosate-polluted soils.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	The source of glyphosate in the study was the commercial formulation Roundup produced and packed by the ZAO Avgust (Russia) under a license agreement with Monsanto Europe S.A. (Belgium).
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Laboski et al. (2012)

Title: Effect of soybean variety, glyphosate use, and manganese application on soybean yield
Author: Carrie A.M. Laboski, Todd Andraski, Shawn Conley and John Gaska
Reference: Proc. of the 2012 Wisconsin Crop Management Conference, Vol. 51

Year: 2012	
Results and conclusion: Application of Mn in starter or as foliar at R1, R3, or R1 + R3 did not increase soybean yield at locations where Mn was expected to be a problem based on low soil test levels or at locations with optimum soil test levels. At all of these locations, R1 tissue Mn concentrations were considered low based on current UW plant analysis interpretation guidelines; however there were no visual Mn deficiency symptoms. It should be noted that some Mn treatments at some locations may have increased yield by a couple bushels, yield reductions with Mn application were also observed. At some tissue sampling times in Outagamie and Walworth, the non-glyphosate resistant variety had greater tissue Mn concentrations compared to the glyphosate resistant variety with either conventional herbicides or glyphosate. The opposite of this was true at Dodge and Jefferson. Overall, these data do not suggest that glyphosate resistant soybean varieties are more sensitive to Mn, or benefit from foliar applications after glyphosate application. These data suggest that a tissue Mn sufficiency concentration range of 54 to 300 ppm may be too high because all sites had R1 tissue Mn concentrations below this range but did not respond to Mn applications. These data also suggest that even on soils where Mn deficiency has the potential to be a problem (low Mn soil test or pH over 6.9 on soils with OM greater than 6.0%), if no visual deficiency symptoms are apparent, then application of Mn is likely not economical.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

LALLF (2008)

Title: Ergebnisse und Empfehlungen zum Integrierten Pflanzenschutz im Ackerbau 2009
Author: LALLF (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern)
Reference: Report of the Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern, Rostock, Germany
Year: November 2008
Results and conclusion: Based on experiences and investigation results general recommendations for the use of plant protection products in agricultural practices are given in the report of the federal authorities in Mecklenburg-West Pomerania in Germany. In this context, the relevance of glyphosate containing plant protection products as non-selective herbicides are emphasized

Results from a federal monitoring programme regarding entries of plant protection products in surface water bodies are additionally summarized. Finally, glyphosate was found in 105 of 180 samples with concentrations > 0.1 µg/L in 2008. This finding rate gives evidence for a high frequency of glyphosate deposition into small and medium sized surface water bodies in intensive used agricultural areas.	
Proposed action: To be used as additional information.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information	
Reliability	Reliable
Endpoint	Monitoring, summary information on measured concentrations in surface water bodies in Germany
Protocol	No detailed information available
Test compound	Glyphosate
Test system and conditions	No information available
Statistical design	Not provided
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	The glyphosate findings in surface water bodies with concentrations > 0.1 g/L in intensive used agricultural areas in Mecklenburg-West Pomerania in Germany 2008 are in line with the results from other monitoring programmes. However, no details are provided in the report about the measurements, the finding localities and special causes. The information should be considered as additional.

Laitinen et al. (2008)

Title: Effects of Soil Phosphorus Status on Environmental Risk Assessment of Glyphosate and Glufosinate-Ammonium
Author: Pirkko Laitinen, Katri Siimes, Sari Rämö and Lauri Jauhiainen, Liisa Eronen, Seija Oinonen, Helinä Hartikainen
Reference: J. Environ. Qual. 37:830–838
Year: 2008
Results and conclusion: The increased use of herbicides poses a risk to the aquatic environment. Easy and economical methods are needed to identify the fields where specific environment protection measures are needed. Phosphorus (P) and organophosphorus herbicides compete for the same adsorption sites in soil. In this study the relationship between P obtained in routine Finnish agronomic tests (acid ammonium acetate [PAC]) and adsorption of glyphosate and glufosinate-ammonium was investigated to determine whether PAC values could be used in the risk assessment. The adsorption of glyphosate ((N-(phosphonomethyl)glycine) and glufosinate-ammonium (2-amino-4-(hydroxymethylphosphinyl)butanoic acid) was studied in a clay and a sandy loam soil enriched with increasing amounts of P added as potassium dihydrogen phosphate. Desorption was also determined for some P-enriched soil samples. The adsorption of both herbicides diminished with increasing PAC value. The correlations between Freundlich adsorption coefficients obtained in the adsorption tests and PAC were nonlinear but significant (r > 0.98) in both soils. The exponential models of the relationship between soil PAC values and glyphosate adsorption were found to fit well to an independ-

ent Finnish soil data set (P < 0.1 for glyphosate and P < 0.01 for glufosinate-ammonium). The desorption results showed that glufosinate-ammonium sorption is not inversely related to soil P status, and the high correlation coefficients obtained in the test of the model were thus artefacts caused by an abnormal concentration of exchangeable potassium in soil. The solved equations are a useful tool in assessing the leaching risks of glyphosate, but their use for glufosinate-ammonium is questionable.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; Glufosinate-ammonium, CAS-no.: 77182-82-2
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Lin et al. (2011)

Title: Reducing Herbicides and Veterinary Antibiotics Losses from Agroecosystems Using Vegetative Buffers
Author: Chung-Ho Lin, Robert N. Lerch, Keith W. Goyne, and Harold E. Garrett
Reference: J. Environ. Qual. 40:791–799
Year: 2011
Results and conclusion: Multiple species vegetative buffer strips (VBSs) have been recommended as a cost-effective approach to mitigate agrochemical transport in surface runoff derived from agronomic operations, while at the same time offering a broader range of long-term ecological and environmental benefits. However, the effect of VBS designs and species composition on reducing herbicide and veterinary antibiotic transport has not been well documented. An experiment consisting of three VBS designs and one continuous cultivated fallow control replicated in triplicate was conducted to assess effectiveness in reducing herbicide and antibiotic transport for claypan soils. The three VBS designs include (i) tall fescue, (ii) tall fescue with a switchgrass hedge barrier, and (iii) native vegetation (largely eastern gamagrass). Rainfall simulation was used to create uniform antecedent soil moisture content in the plots and to generate runoff. Our results suggested that all VBS significantly reduced the transport of dissolved and sediment-bound atrazine, metolachlor, and glyphosate in surface runoff by 58 to 72%. Four to 8 m of any tested VBS reduced dissolved sulfamethazine transport in the surface runoff by more than 70%. The tall fescue VBS was overall most effective at reducing dissolved tylosin and enrofloxacin transport in the runoff (>75%). The developed exponential regression models can be used to predict expected field-scale results and provide design criteria for effective field implementation of grass

buffers. Our study has demonstrated that an optimized VBS design may achieve desired agrochemical reductions and minimize acreage removed from crop production.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; atrazine, CAS-no.: 1912-24-9; s-metolachlor, CAS-no.: 87392-12-9
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Machado et al. (2009)

Title: Absorption, Translocation and Radicular Glyphosate Exudation in Eucalyptus sp. Clones	
Author: MACHADO, A.F.L., FERREIRA, L.R., SANTOS, L.D.T., SANTOS, J.B., FERREIRA, F.A. and VIANA, R.G	
Reference: Planta Daninha, Viçosa-MG, v. 27, n. 3, p. 549-554	
Year: 2009	
Results and conclusion: To evaluate absorption, translocation and radicular glyphosate exudation in two Eucalyptus sp. clones (2277 and 531), ^{14}C-glyphosate at 1440 g ha^{-1} were distributed on the third and fourth leaf blade, under 0,030 µCi of radioactivity. Evaluations were performed 0, 2, 8, 32 and 72 hours after herbicide application-HAA. After 8 HAA, ^{14}C-glyphosate on the leaf was similar in both clones. However, considering the plant, it was higher in 2277, at any evaluation time. After washing the leaves, higher amount of ^{14}C-glyphosate was verified in the water of 531, indicating its smaller herbicide absorption. In the ground tissue and in the roots, ^{14}C-glyphosate was similar in both clones, at any application time though, showing higher concentrations in the roots. Between 0.78 and 1.16% any of the applied herbicide was exuded into the nutritive solution, without showing difference on translocation and radicular exudation in both clones. The different absorption between the clones can be a likely explanation for the different tolerance among genotypes.	
Proposed action: Not to be considered as publication does not focuses on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable

Test compound	^{14}C-Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Mallmann et al. (2013)

Title: Effects of swine wastewater on glyphosate leaching by liquid chromatography	
Author: L. S. Mallmann, S. C. Sampaio, S. R. Machado Coelho, M. Sorace and L. H. Andrade	
Reference: Journal of Food, Agriculture & Environment Vol.11 (2):908-914. 2013	
Year: 2013	
Results and conclusion: Chemical application on crops comes as an alternative of development and expansion due to the increase in food demand and yield. An example is the use of glyphosate herbicide, applied mainly in soybeans. The West of Paraná State presents itself as a major producer of grain and pork, but the effluents generated in the activity of swine present themselves as potential environmental pollutants when improperly used. An alternative for this is the use of micro-organisms to degrade herbicide in soil. This study aimed at evaluating glyphosate behavior in soil, using swine wastewater as a source of micro-organisms and organic matter. There were four acrylic columns containing soil (sterile or not), with distilled water or wastewater and glyphosate solution (112.5 g L^{-1}). The columns were incubated for seven days in a control environmental at 23°C, so that micro-organisms could adapt themselves to these conditions. Then, it was made the leaching test in order to analyze the samples at high performance liquid chromatography. The treatments showed similar behavior among themselves, with no peak which represented glyphosate. This suggests that it has been adsorbed or mineralized, but organic matter had no influence on the studied treatments. Based on the results found in the work, it can be concluded: Swine wastewater, in the amount used in this study, did not affect the dynamics of herbicide glyphosate in clayey Oxisol. Glyphosate does not have sufficient mobility to contaminate groundwater, in this type of soil, if soil profile has 30 cm or more.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	

Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Mamy et al. (2010)

Title: Comparative environmental impacts of glyphosate and conventional herbicides when used with glyphosate-tolerant and non-tolerant crops	
Author: Laure Mamy, Benoît Gabrielle, Enrique Barriuso	
Reference: Environmental Pollution 158, 3172-3178	
Year: 2010	
Results and conclusion: The introduction of glyphosate-tolerant (GT) crops is expected to mitigate the environmental contamination by herbicides because glyphosate is less persistent and toxic than the herbicides used on non-GT crops. Here, we compared the environmental balances of herbicide applications for both crop types in three French field trials. The dynamic of herbicides and their metabolites in soil, groundwater and air was simulated with PRZM model and compared to field measurements. The associated impacts were aggregated with toxicity potentials calculated with the fate and exposure model USES for several environmental endpoints. The impacts of GT systems were lower than those of non-GT systems, but the accumulation in soils of one glyphosate metabolite (aminomethylphosphonic acid) questions the sustainability of GT systems. The magnitude of the impacts depends on the rates and frequency of glyphosate application being highest for GT maize monoculture and lowest for combination of GT oilseed rape and non-GT sugar beet crops.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Sulcotrione Cas-no.:99105-77-8, Metamitron Cas-no.:41394-05-2, Trifluralin Cas-no.:1582-09-8, Metazachlor Cas-no.:67129-08-2, Glyphosate CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Massot et al. (2012)

Title: Biodegradation of phytosanitary products in biological wastewater treatment	
Author: A. Massot, K. Estéve, P. Noilet, C. Méoule C. Poupot and M. Mietton-Peuchot	

Reference: J. Environ. Qual. 33:816–824	
Year: 2012	
Results and conclusion: Agricultural activity generates two types of waste: firstly, biodegradable organic effluents generally treated by biological processes and, secondly, phytosanitary effluents which contain residues of plant protection products. The latter are collected and treated. Current technological solutions are essentially based on concentration or physicalechemical processes. However, recent improvements in the biodegradability of pesticides open the way to the consideration of alternative, biological, treatment using mixed liquor from wastewater plant activated sludge. The feasibility of the biological treatment of viticultural effluents has been evaluated by the application of pesticides to activated sludge. The necessity for selection of a pesticide-resistant biomass has been highlighted. The elimination of the phytosanitary products shows the potential of a resistant biomass in the treatment of pesticides. The aerated biological storage ponds at three wineries, followed by a sand or reed-bed filter, were used for the treatment of the total annual volume of the viticulture effluents and validate the laboratory experiments. The results show that the biological purification of pesticides by activated sludge is possible by allowing approximately 8 days for biomass adaptation. Stability of purification occurs between 20 and 30 days.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Not applicable
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Mazzei and Piccolo (2012)

Title: Quantitative Evaluation of Noncovalent Interactions between Glyphosate and Dissolved Humic Substances by NMR Spectroscopy
Author: Pierluigi Mazzei and Alessandro Piccolo
Reference: Environ. Sci. Technol. 2012, 46, 5939−5946
Year: 2012
Results and conclusion: Interactions of glyphosate (N-phosphonomethylglycine) herbicide (GLY) with soluble fulvic acids (FAs) and humic acids (HAs) at pH 5.2 and 7 were studied by ^{1}H and ^{31}P NMR spectroscopy. Increasing concentrations of soluble humic matter determined broadening and chemical shift drifts of proton and phosphorus GLY signals, thereby indicating the occurrence of weak interactions between GLY and humic superstructures. Binding was larger for FAs and pH 5.2 than for HAs and pH 7, thus suggesting formation of hydrogen

bonds between GLY carboxyl and phosphonate groups and protonated oxygen functions in humic matter. Changes in relaxation and correlation times of ^{1}H and ^{31}P signals and saturation transfer difference NMR experiments confirmed the noncovalent nature of GLY−humic interactions. Diffusion-ordered NMR spectra allowed calculation of the glyphosate fraction bound to humic superstructures and association constants (Ka) and Gibbs free energies of transfer for GLY−humic complex formation at both pH values. These values showed that noncovalent interactions occurred most effectively with FAs and at pH 5.2. Our findings indicated that glyphosate may spontaneously and significantly bind to soluble humic matter by noncovalent interactions at slightly acidic pH and, thus, potentially pollute natural water bodies by moving through soil profiles in complexes with dissolved humus.	
Proposed action: Not to be considered for endpoint and PEC-assessment as sorption of glyphosate to fulvic and humic acids has been investigated.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information only	
Reliability	Low
Endpoint	Adsorption of glyphosate to fulvic and humic acids
Protocol	NMR method
Test compound	Glyphosate was added (no purity given); CAS-no.: 1071-83-6
Test system and conditions	^{1}H and ^{31}P NMR techniques were applied to study the occurrence and type of interactions between glyphosate and water-soluble humic substances and calculate the corresponding thermodynamic parameters. On the basis of previous approaches, the adopted NMR techniques consisted of measurements of relaxation times to extrapolate nuclear correlation times and of self-diffusion values to calculate the association constants for humic−glyphosate complexes. Moreover, the homonuclear proton saturation transfer difference (STD) technique was employed here for the first time to prove the formation of noncovalent host−guest complexes between relatively large humic associations and the small glyphosate ligand.
Statistical design	Not reported
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Not directly comparable to publications dealing with Glyphosate sorption; no negative evidence.

Mbanaso et al. (2014)

Title: Potential microbial toxicity and non-target impact of different concentrations of glyphosate-containing herbicide (GCH) in a model pervious paving system
Author: F.U. Mbanaso, S.J. Coupe, S.M. Charlesworth, E.O. Nnadi, A.O. Ifelebuegu
Reference: Chemosphere 100 (2014) 34–41
Year: 2014
Results and conclusion: Pervious Pavement Systems are Sustainable Drainage devices that meet the three-fold SUDS functions of stormwater quantity reduction, quality improvement and amenity benefits. This paper reports on a study to determine the impact of different concentrations of glyphosate-containing herbicides on non-target microorganisms

and on the pollutant retention performance of PPS. The experiment was conducted using 0.0484 m^2 test rigs based on a four-layered design. Previous studies have shown that PPS can trap up to 98.7% of applied hydrocarbons, but results of this study show that application of glyphosate-containing herbicides affected this capability as 15%, 9% and 5% of added hydrocarbons were released by high (7200 mg L^{-1}), medium (720 mg L^{-1}) and low (72 mg L^{-1}) glyphosate-containing herbicides concentrations respectively. The concentrations of nutrients released also indicate a potential for eutrophication if these effluents were to infiltrate into aquifers or be released into surface waters. The effect of glyphosate- containing herbicides application on the bacterial and fungal communities was slightly different; fungi exhibited a ‘‘top-down’’ trend as doses of 7200 mg L^{-1} glyphosate-containing herbicides yielded the highest fungal growth whilst those with a concentration of 720 mg L^{-1} glyphosate-containing herbicides applied yielded the highest bacterial growth. In the case of protists, doses of glyphosate-containing herbicides above 72 mg L^{-1} were fatal, but they survived at the lower concentration, especially the ciliates Colpoda cucullus and Colpoda steinii thus indicating potential for their use as biomarkers of herbicide-polluted environments. Data also showed that at the lowest concentration of glyphosate-containing herbicides (72 mg L^{-1}), biodegradation processes may not be affected as all trophic levels required for optimum biodegradation of contaminants were present.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate-containing herbicide (GCH)
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Motavalli et al. (2004)

Title: Impact of Genetically Modified Crops and Their Management on Soil Microbially Mediated Plant Nutrient Transformations
Author: P. P. Motavalli, R. J. Kremer, M. Fang, and N. E. Means
Reference: J. Environ. Qual. 33:816–824
Year: 2004
Results and conclusion: One of the potential environmental effects of the recent rapid increase in the global agricultural area cultivated with transgenic crops is a change in soil microbial mediated processes and functions. Among the many essential functions of soil biota are soil organic matter decomposition, nutrient mineralization and immobilization, oxidation-reduction reactions, biological N fixation, and solubilisation. However, relatively little research has examined the direct and indirect effects of transgenic crops and their management on microbial medi-

ated nutrient transformations in soils. The objectives of this paper are to review the available literature related to the environmental effects of transgenic crops and their management on soil microbial mediated nutrient transformations, and to consider soil properties and climatic factors that may affect the impact of transgenic crops on these processes. Targeted genetic traits for improved plant nutrition include greater plant tolerance to low Fe availability in alkaline soils, enhanced acquisition of soil inorganic and organic P, and increased assimilation of soil N. Among the potential direct effects of transgenic crops and their management are changes in soil microbial activity due to differences in the amount and composition of root exudates, changes in microbial functions resulting from gene transfer from the transgenic crop, and alteration in microbial populations because of the effects of management practices for transgenic crops, such as pesticide applications, tillage, and application of inorganic and organic fertilizer sources. Possible indirect effects of transgenic crops, including changes in the fate of transgenic crop residues and alterations in land use and rates of soil erosion, deserve further study. Despite widespread public concern, no conclusive evidence has yet been presented that currently released transgenic crops, including both herbicide and pest resistant crops, are causing significant direct effects on stimulating or suppressing soil nutrient transformations in field environments. Further consideration of the effects of a wide range of soil properties, including the amount of clay and its mineralogy, pH, soil structure, and soil organic matter, and variations in climatic conditions, under which transgenic crops may be grown, is needed in evaluating the impact of transgenic crops on soil nutrient transformations. Future environmental evaluation of the impact of the diverse transgenic crops under development could lead to an improved understanding of soil biological functions and processes.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Not applicable
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Neumann et al. (2006)

Title: Relevance of glyphosate transfer to non-target plants via the rhizosphere
Author: G. NEUMANN, S. KOHLS, E. LANDSBERG, K. STOCK-OLIVEIRA SOUZA, T. YAMADA, V. RÖMHELD
Reference: Journal of Plant Diseases and Protection, Special Issue/Sonderheft XX, 963-969 (2006), ISSN 1861-4051
Year: 2006
Results and conclusion:

There is a common understanding that the widely used herbicide glyphosate is easily degraded and adsorbed in soils and thus, harmless for use in agriculture. We can demonstrate, however, that this conclusion is wrong and dangerous for farmers because in former risk assessments the behaviour of glyphosate in the rhizosphere was not properly considered. In nutrient solution, rhizobox and pot experiments we can show that foliar applied glyphosate to target plants is released into the rhizosphere after a fast translocation from shoots to roots. In the rhizosphere glyphosate can obviously be stabilized long enough to achieve negative effects on non-target plants. Such a negative side effect is for example inhibited acquisition of micronutrients such as Mn, but also Zn, Fe and B, which are involved in plant own disease resistance mechanisms. From this glyphosate transfer from target to non-target plants (e.g. from weed to trees in orchards) we predict an increase in disease problems, particularly on soils with low micronutrient availability as already reported in the USA. In view of plant and soil health, we urgently call for a re-assessment of glyphosate as herbicide.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Roundup-Ultra (Monsanto, St. Louis, USA)
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Ndjeri et al. (2013)

Title: Degradation of glyphosate and AMPA (amino methylphosphonic acid) solutions by thin films of birnessite electrodeposited: A new design of material for remediation processes?
Author: M. Ndjeri, A. Pensel, S. Peulon, V. Haldys, B. Desmazières, A. Chaussé
Reference: Colloids and Surfaces A: Physicochem. Eng. Aspects 435 (2013) 154-169
Year: 2013
Results and conclusion: The paper focuses on the possibility to use birnessite thin films for remediating aqueous solutions containing glyphosate and AMPA, the most found pollutants in environment. Indeed, glyphosate is the pesticide the most used in the world, and AMPA is its main metabolite, more toxic and more persistent than its parent. However, AMPA can also mainly come from the degradation of phosphonic acids present in detergents. We show that birnessite, electrodeposited as thin films onto a cheap transparent semiconductor substrate (SnO_2), can significantly degrade and mineralise glyphosate and AMPA. Glyphosate is spontaneously degraded with simultaneous production of AMPA, formaldehyde, phosphate ions, nitrate ions and ammonium ions, without macroscopic modification of birnessite. The last four by-products are also obtained during the degradation of

AMPA by birnessite. Various experimental parameters such as temperature, concentration of pollutant, stirring of solution, presence or not of oxygen were studied and a schematic summary of observed evidences was proposed. The good mineralisation yields obtained during glyphosate/birnessite and AMPA/birnessite interactions allow us to envisage a possible application of these thin films for remediation.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate and AMPA
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Nourouzi et al. (2011)

Title: Glyphosate Utilization as the Source of Carbon: Isolation and Identification of new Bacteria	
Author: M. MOHSEN NOUROUZI, T.G. CHUAH, THOMAS S.Y. CHOONG and C.J. LIM	
Reference: E-Journal of Chemistry 8(4), 1582-1587	
Year: 2011	
Results and conclusion: Mixed bacteria from oil palm plantation soil (OPS) were isolated to investigate their ability to utilize glyphosate as carbon source. Results showed that approximately all of the glyphosate was converted to aminomethylphosphonic acid (AMPA) (99.5%). It is worthy to note that mixed bacteria were able to degrade only 2% of AMPA to further metabolites. Two bacterial strains i.e. Stenotrophomonas maltophilia and Providencia alcalifaciens were obtained from enrichment culture. Bacterial isolates were cultured individually on glyphosate as a sole carbon source. It was observed that both isolates were able to convert glyphosate to AMPA.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate-contaminated soil and Glyphosate, CAS-no.: 1071-83-6

Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Nourouzi et al. (2012)

Title: Modeling biodegradation and kinetics of glyphosate by artificial neural network	
Author: MOHSEN M. NOUROUZI, TEONG G. CHUAH, THOMAS S.Y. CHOONG and F. RABIEI	
Reference: Journal of Environmental Science and Health, Part B (2012) 47, 455–465	
Year: 2012	
Results and conclusion: The result showed that ANN model was able to accurately predict the experimental results. A low ratio of self-inhibition and half saturation constants of Haldane equations (< 8) exhibited the inhibitory effect of glyphosate on bacteria growth. The value of Ki/Ks increased when the mixed inoculum size was increased from 104 to 106 bacteria/mL. It was found that the percentage of glyphosate degradation reached a maximum value of 99% at an optimum pH 6-7 while for pH values higher than 9 or lower than 4, no degradation was observed.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Low weight	
Reliability	Medium
Endpoint	Not applicable, model development study
Protocol	Not applicable, model development study
Test compound	Not applicable, model development study
Test system and conditions	An artificial neural network (ANN) model was developed to simulate the biodegradation of herbicide glyphosate [2-(Phosphonomethylamino) acetic acid] in a solution with varying parameters pH, inoculum size and initial glyphosate concentration. The predictive ability of ANN model was also compared with Monod model.
Statistical design	Not applicable, model development study
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable, model development study

Olesen and Cedergreen (2010)

Title: Glyphosate uncouples gas exchange and chlorophyll fluorescence	
Author: Charlotte F Olesen and Nina Cedergreen	
Reference: Pest Manag Sci 66: 536–542	
Year: 2010	
Results and conclusion: Background: Changes in chlorophyll fluorescence have often been advocated as a sensitive biomarker of plant stress, assuming that any kind of plant stress serious enough to affect plant growth will also affect photosynthesis. Glyphosate affects photosynthetic electron transport indirectly by inhibiting sink processes. The question is how fast this inhibition can be observed on CO_2 assimilation and ultimately on chlorophyll fluorescence? Results: Experiments measuring CO_2 assimilation, conductance and chlorophyll fluorescence using four Kautsky curve parameters on barley (Hordeum vulgare L.) exposed to increasing doses of glyphosate showed a total cessation of CO_2 fixation and conductance without significant changes in chlorophyll fluorescence. The decrease in CO_2 fixation and conductance was significant 1 day after spraying and corresponded well to the decrease in biomass 5–7 days after spraying. Conclusion: A total cessation of CO_2 assimilation can take place without affecting chlorophyll fluorescence. Hypotheses concerning what happens to the energy from the photosynthetic apparatus that is not used for CO_2 assimilation are discussed. The results question the use of chlorophyll fluorescence as a universal indicator of stress on photosynthetic processes. Also, they demonstrate that changes in gas-exchange parameters are more sensitive biomarkers for glyphosate toxicity compared with chlorophyll fluorescence.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; glyphosate (isopropylamine salt), CAS-no.:38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Oliver et al. (2014)

Title: Banded applications are highly effective in minimising herbicide migration from furrow-irrigated sugar cane
Author: D.P. Oliver, J. S. Anderson, A. Davis, S. Lewis, J. Brodie, R. Kookana
Reference: Science of the Total Environment 466–467 (2014) 841–848
Year: 2014

Results and conclusion: Runoff from farm fields is a common source of herbicide residues in surface waters in many agricultural industries around the world. In Queensland, Australia, the runoff of PSII inhibitor herbicides (in particular diuron and atrazine) is a major concern due to their potential impact on the Great Barrier Reef. This study compared the conventional practice of broadcast application of herbicides in sugarcane production across the whole field with the banded application of particular herbicides onto raised beds only using a shielded sprayer. This study found that the application of two moderately soluble herbicides, diuron and atrazine, to only the raised beds decreased the average total load of both herbicides moving off-site by N90% compared with the conventional treatment. This was despite the area being covered with the herbicides by the banded application being only 60% less than with the conventional treatment. The average total amount of atrazine in drainage water was 7.5% of the active ingredient applied in the conventional treatment compared with 1.8% of the active ingredient applied in the banded application treatment. Similarly, the average total amount of diuron in drainage water was 4.6% of that applied in the conventional treatment compared with 0.9% of that applied in the banded application treatment. This study demonstrates that the application of diuron and atrazine to raised beds only is a highly effective way of minimising migration of these herbicides in drainage water from furrow irrigated sugarcane. Furthermore, the study found that glyphosate concentrations in drainage water from the Banded treatment bays were below the detection limit suggesting that it would be a good alternative herbicide to atrazine and diuron for use in furrows. However, other studies have found detectable concentrations of glyphosate in tailwater draining from furrow-irrigated sugarcane (Davis, unpublished data), which would suggest that further work on different soil types is required before glyphosate can be fully endorsed as an alternative to diuron and atrazine in the furrows.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Atrazine, diuron, 2,4-D, paraquat, glyphosate
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Orcaray et al. (2012)

Title: Impairment of carbon metabolism induced by the herbicide glyphosate
Author: Luis Orcaray, Amaia Zulet, Ana Zabalza, Mercedes Royuela
Reference: Journal of Plant Physiology 169, 27– 33
Year: 2012
Results and conclusion:

The herbicide glyphosate reduces plant growth and causes plant death by inhibiting the biosynthesis of aromatic amino acids. The objective of this work was to determine whether glyphosate-treated plants show a carbon metabolism pattern comparable to that of plants treated with herbicides that inhibit branched-chain amino acid biosynthesis. Glyphosate-treated plants showed impaired carbon metabolism with an accumulation of carbohydrates in the leaves and roots. The growth inhibition detected after glyphosate treatment suggested impaired metabolism that impedes the utilization of available carbohydrates or energy at the expected rate. These effects were common to both types of amino acid biosynthesis inhibitors. Under aerobic conditions, ethanolic fermentative metabolism was enhanced in the roots of glyphosate-treated plants. This fermentative response was not related to changes in the respiratory rate or to a limitation of the energy charge. This response, which was similar for both types of herbicides, might be considered a general response to stress conditions.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate (commercial formula, Glyfos, BayerGarden, Valencia, Spain), CAS-no.: 1071-83-6; glufosinate (commercial formula, Finale, BayerCropscience, Valencia, Spain), CAS-no.: 51276-47-2
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Ozturk et al. (2007)

Title: Glyphosate inhibition of ferric reductase activity in iron deficient sunflower roots
Author: Levent Ozturk, Atilla Yazici, Selim Eker, Ozgur Gokmen, Volker Römheld and Ismail Cakmak
Reference: New Phytologist doi: 10.1111/j.1469-8137.2007.02340.x
Year: 2007
Results and conclusion: Iron (Fe) deficiency is increasingly being observed in cropping systems with frequent glyphosate applications. A likely reason for this is that glyphosate interferes with root uptake of Fe by inhibiting ferric reductase in roots required for Fe acquisition by dicot and nongrass species. This study investigated the role of drift rates of glyphosate (0.32, 0.95 or 1.89 mM glyphosate corresponding to 1, 3 and 6% of the recommended herbicidal dose, respectively) on ferric reductase activity of sunflower (Helianthus annuus) roots grown under Fe deficiency conditions. Application of 1.89 mM glyphosate resulted in almost 50% inhibition of ferric reductase

within 6 h and complete inhibition 24 h after the treatment. Even at lower rates of glyphosate (e.g. 0.32 mM and 0.95 mM), ferric reductase was inhibited. Soluble sugar concentration and the NAD(P)H oxidizing capacity of apical roots were not decreased by the glyphosate applications. To our knowledge, this is the first study reporting the effects of glyphosate on ferric reductase activity. The nature of the inhibitory effect of glyphosate on ferric reductase could not be identified. Impaired ferric reductase could be a major reason for the increasingly observed Fe deficiency in cropping systems associated with widespread glyphosate usage.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate formulated as Roundup Ultra (active ingredient N-[phosphonomethyl]glycine isopropylamine salt; Monsanto Ltd, Adana, Turkey), CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Pesce et al. (2008)

Title: Longitudinal changes in microbial planktonic communities of a French river in relation to pesticide and nutrient inputs
Author: Stéphane Pesce, Céline Fajon, Corinne Bardot, Frédérique Bonnemoy, Christophe Portelli, Jacques Bohatier
Reference: Aquatic Toxicology 86, 352–360
Year: 2008
Results and conclusion: To determine the effects of anthropic activities on river planktonic microbial populations, monthly water samples were collected for 11 months from two sampling sites characterized by differing nutrient and pesticide levels. The difference in trophic level between the two stations was particularly pronounced from May to November. Total pesticide concentrations were notably higher at the downstream station from April to October with a clear predominance of herbicide residues, especially the glyphosate metabolite aminomethylphosphonic acid (AMPA). From spring, algal biomass and density were favored by the high orthophosphate concentrations recorded at the downstream location. However, isolated drops in algal biomass were recorded at this sampling station, suggesting an adverse effect of herbicides on algal communities. No major difference was observed in bacterial heterotrophic production, density, or activity (CTC reduction) between the two sampling stations. No major variation was detected using the fluorescent in situ hybridization (FISH) method, but shifts in bacterial community composition were recorded by PCR-

TTGE analysis at the downstream station following high nutrient and pesticide inputs. However, outside the main anthropic pollution period, the water's chemical properties and planktonic microbial communities were very similar at the two sampling sites, suggesting a high recovery potential for this lotic system.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; AMPA, CAS-no.: 1066-51-9
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Pipke and Amrhein (1988)

Title: Isolation and Characterization of a Mutant of *Arthrobacter* sp. Strain GLP-1 Which Utilizes the Herbicide Glyphosate as Its Sole Source of Phosphorus and Nitrogen	
Author: Pipke, R. And Amrhein, N.	
Reference: Applied and Environmental microbiology, Nov. 1988, Vol. 54, No. 11, 2868-2870	
Year: 1988	
Results and conclusion: *Arthrobacter* sp. strain GLP-1, grown on glucose as a carbon source, utilizes the herbicide glyphosate [N-(phosphonomethyl)glycine] as its sole source of phosphorus as well as its sole source of nitrogen. The mutant strain GLP-1/Nit-1 utilizes glyphosate as its sole source of nitrogen as well. In strain GLP-1, Pi was a potent competitive inhibitor of glyphosate uptake (Ki, 24 ,uM), while the affinity of Pi for the uptake system of strain GLP-1/Nit-1 was reduced by 2 orders of magnitude (Ki, 2.3 mM). It is concluded that the inability of strain GLP-1 to utilize glyphosate as a source of nitrogen is due to the stringent control of glyphosate uptake by excess phosphate released during the degradation of the herbicide.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; AMPA, CAS-no.:1066-51-9
Test system and con-	Not applicable

ditions	
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Puértolas et al. (2010)

Title: Evaluation of side-effects of glyphosate mediated control of giant reed (Arundo donax) on the structure and function of a nearby Mediterranean river ecosystem	
Author: Laura Puértolas, Joana Damásio, Carlos Barata, Amadeu M.V.M. Soares, Narcís Prat	
Reference: Environmental Research 110, 556–564	
Year: 2010	
Results and conclusion: The aim of this study was to evaluate the effect of the application of the herbicide Herbolex (Aragonesas Agro, S.A., Madrid, Spain) to control giant reed (Arundo donax), which has glyphosate as active ingredient, on the structure and function of a nearby river ecosystem. Specifically, we assessed glyphosate environmental fate in the surrounding water and its effects on transplanted Daphniamagna, field collected caddisfly (Hydropsyche exocellata) and on benthic macroinvertebrate structure assemblages. Investigations were conducted in the industrialized and urbanized Mediterranean river Llobregat (NE Spain) before and after a terrestrial spray of glyphosate. Four locations were selected to include an upstream site and three affected ones. Measured glyphosate levels in river water following herbicide application were quite high (20-60 mg/l) with peak values of 137 mg/l after three days. After 12 days of its application, leaching of glyphosate from sprayed river banks was quite high in pore water (20-85 mg/l) but not in the river. Closely linked with the measured poor habitat and water physico-chemical conditions, macroinvertebrate communities were dominated by taxa tolerant to pollution and herbicide application did not affect the abundance or number of taxa in any location. Nevertheless, significant specific toxic effects on transplanted D.magna and field collected H. exocellata were observed. Effects included D. magna feeding inhibition and oxidative stress related responses such as increased antioxidant enzyme activities related with the metabolism of glutathione and increased levels of lipid peroxidation. These results emphasize the importance of combined chemical, ecological and specific biological responses to identify ecological effects of pesticides in the field.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; AMPA, CAS-no.:1066-51-9
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	

Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Qin et al. (2013)

Title: Can rainwater induce Fenton-driven degradation of herbicides in natural waters?	
Author: J. Qin, H. Li, C. Lin, G. Chen	
Reference: Chemosphere 92 (2013) 1048–1052	
Year: 2013	
Results and conclusion: Microcosm experiments were conducted to examine Fenton reaction-driven degradation of three common herbicides exposed to a variety of $Fe2^{+}$–H_2O_2 combinations that are likely to be encountered in natural water environments. The results show that these combinations had significant ($P < 0.05$) effects on removing the water-borne herbicides. This discovery sheds some light on the possible role of rainwater-borne H_2O_2 in inducing Fenton reaction in many natural waters such as lakes, streams, estuaries and tidal zones, fishponds and paddy fields that may contain ferrous ion at micromolar levels. The research findings obtained from this preliminary work provide a rationale for undertaking further study to confirm the presence of an overlooked naturally-occurring process that may lead to rapid dissipation of many herbicides and other organic pollutants in open water environments.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Effect of $Fe2^{+}$–H_2O_2 combinations on removing herbicides
Protocol	Not given
Test compound	Diuron, butachlor and glyphosate (purity 98 %, 98 % and 97 %)
Test system and conditions	A total of nine Fe^{2+}-H_2O_2 combinations were set for the herbicide degradation experiment with the concentration of both Fe^{2+} and H_2O_2 ranging from 5 to 50 µM. In addition, three concentration levels were also set for ''Fe^{2+} only'' and ''H_2O_2 only'' systems. The aqueous system without the added Fe^{2+} and H_2O_2 served as the control. Each experiment was performed in triplicate in a room with the temperature being controlled at 25 ± 1 °C. Centrifuge tubes (capacity: 10 mL) were used as batch reactors. For each reactor, 4 mL of a relevant herbicide stock solution with appropriate concentration were added into the tube, followed by simultaneous addition of 3 mL of an appropriate Fe^{2+} stock solution and 3 mL of an appropriate H_2O_2 stock solution. After addition of all the ingredients, the tube was capped, hand shaken for 30 s, and stood for 1 h before taking samples for determinations of residual herbicides. Glyphosate in the solution was determined using a DIONEX ICS-900 ion chromatography system.
Statistical design	The statistical significance of difference between the treatment means was determined by the Duncan's multiple range test.

Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Rampoldi et al. (2011)

Title: The Fate of Glyphosate in Crop Residues	
Author: E. Ariel Rampoldi, Susana Hang, Enrique Barriuso	
Reference: Soil Sci. Soc. Am. J. 75, Number 2:553–559	
Year: 2011	
Results and conclusion: The environmental fate of glyphosate [N-(phosphonomethyl)glycine] was studied in six crop residue (CR) types, three from maize (Zea mays L.) (M1, M2, and M3) and three from soybean [Glycine max (L.) Merr.] (S1, S2, and S3). Glyphosate adsorption was characterized through isotherms. The glyphosate distribution in CRs was characterized through the balance of ^{14}C-glyphosate radioactivity among the mineralized fraction, the extractable fractions (water and NH_4OH), and the non-extractable fraction. Crop residues were characterized by elemental composition, organic C, total N, and biochemical parameters (soluble fraction, cellulose, hemicellulose, and lignin). Total microbial activity (TMA) was also assessed. Limited and reversible glyphosate adsorption on soybean and maize CRs was determined. The sorption coefficient K_f index range for maize CR was 1.5 to 8.3 L/kg and 2.6 to 7.4 L/kg for soybean CR. Organic C and hemicelluloses partially explained adsorption variability. The addition of mineralized and non-extractable fractions of the initial ^{14}C-glyphosate applied on the CRs averaged 56%; however, differences were detected between soybean and maize CRs. Mineralization and non-extractable residues were 30.7 ± 11 and 32.5 ± 6% (soybean CR) and 44.3 ± 12 and 17 ± 7% (maize CR), respectively. We hypothesized that glyphosate molecules could be used initially by microorganisms as a labile C source. High variability in ^{14}C-glyphosate mineralization was observed in all crop residues, suggesting that the magnitude of the glyphosate mineralization process would be regulated by accessibility and the lability of other carbonate sources.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	^{14}C-Glyphosate and unlabeled glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Reddy et al. (2010)

Title: Glyphosate Effect on Shikimate, Nitrate Reductase Activity, Yield, and Seed Composition in Corn	
Author: KRISHNA N. REDDY, NACER BELLALOUI, AND ROBERT M. ZABLOTOWICZ	
Reference: J. Agric. Food Chem. 58, 3646–3650	
Year: 2010	
Results and conclusion: When glyphosate is applied to glyphosate-resistant (GR) crops, drift to non-glyphosate-resistant (non-GR) crops may cause significant injury and reduce yields. Tools are needed to quantify injury and predict crop losses. In this study, glyphosate drift was simulated by direct application at 12.5% of the recommended label rate to non-GR corn (Zea mays L.) at 3 or 6 weeks after planting (WAP) during two field seasons in the Mississippi delta region of the south-eastern USA. Visual plant injury, shikimate accumulation, nitrate reductase activity, leaf nitrogen, yield, and seed composition were evaluated. Effects were also evaluated in GR corn and GR corn with stacked glufosinate-resistant gene at the recommended label rate at 3 and 6 WAP. Glyphosate at 105 g ae/ha was applied once at 3 or 6 weeks after planting to non-GR corn. Glyphosate at 840 (lower label limit) or 1260 (upper label limit) g ae/ha was applied twice at 3 and 6 WAP to transgenic corn. Glyphosate caused injury (45-55%) and increased shikimate levels (24-86%) in non-GR compared to non-treated corn. In non-GR corn, glyphosate drift did not affect starch content but increased seed protein 8-21% while reducing leaf nitrogen reductase activity 46-64%, leaf nitrogen 7-16%, grain yield 49-54%, and seed oil 18-23%. In GR and GR stacked with glufosinate-resistant corn, glyphosate applied at label rates did not affect corn yield, leaf and seed nitrogen, or seed composition (protein, oil, and starch content). Yet, nitrate reductase activity was reduced 5-19% with glyphosate at 840 + 840 g/ha rate and 8-42% with glyphosate at 1260 + 1260 g/ha rate in both GR and GR stacked corn. These results demonstrate the potential for severe yield loss in non-GR corn exposed to glyphosate drift.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Potassium salt of glyphosate (Roundup Weathermax, Monsanto Agricultural Co., St. Louis,MO), CAS-no.: 40465-60-5
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Rodríguez-Rodríguez et al. (2013)

Title: On-farm biopurification systems: role of white rot fungi in depuration of pesticide-containing wastewaters	
Author: C. E. Rodríguez-Rodríguez, V. Castro-Gutiérrez, J. S. Chin-Pampillo & K. Ruiz-Hidalgo	
Reference: FEMS Microbiol Lett 345 (2013) 1–12	
Year: 2013	
Results and conclusion: Environmental contamination with pesticides is an undesired consequence of agricultural activities. Biopurification systems (BPS) comprise a novel strategy to degrade pesticides from contaminated wastewaters, consisting of a highly active biological mixture confined in a container or excavation. The design of BPS promotes microbial activity, in particular by white rot fungi (WRF). Due to their physiological features, specifically the production of highly unspecific ligninolytic enzymes and some intracellular enzymatic complexes, WRF show the ability to transform a wide range of organic pollutants. This minireview summarizes the potential participation of WRF in BPS. The first part presents the potential use of WRF in biodegradation of pollutants, particularly pesticides, and includes a brief description of the enzymatic systems involved in their oxidation. The second part presents an outline of BPS, focusing on the elements that influence the participation of WRF in their operation, and includes a summary of the studies regarding the fungal-mediated degradation of pesticides in BPS biomixtures and other solid-phase systems that mimic BPS.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Review article
Test compound	Not applicable
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Rojano-Delgado et al. (2012)

Title: Limited uptake, translocation and enhanced metabolic degradation contribute to glyphosate tolerance in Mucuna pruriens var. utilis plants
Author: Antonia María Rojano-Delgado, Hugo Cruz-Hipolito, Rafael De Prado, María Dolores Luque de Castro and Antonio Rodríguez Franco
Reference: Phytochemistry 73 (2012) 34–41
Year: 2012
Results and conclusion: Velvet bean (Mucuna pruriens, Fabaceae) plants exhibits an innate, very high resistance

(i.e., tolerance) to glyphosate similar to that of plants which have acquired resistance to this herbicide as a trait. We analyzed the uptake of [14C]-glyphosate by leaves and its translocation to meristematic tissues, and used scanning electron micrographs to further analyze the cuticle and 3D capillary electrophoresis to investigate a putative metabolism capable of degrading the herbicide. Velvet bean exhibited limited uptake of glyphosate and impaired translocation of the compound to meristematic tissues. Also, for the first time in a higher plant, two concurrent pathways capable of degrading glyphosate to AMPA, Pi, glyoxylate, sarcosine and formaldehyde as end products were identified. Based on the results, the innate tolerance of velvet bean to glyphosate is possibly a result of the combined action of the previous three traits, namely: limited uptake, impaired translocation and enhanced degradation.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Rosolem et al. (2009)

Title: Manganese uptake and redistribution in soybeans as affected by glyphosate
Author: Rosolem, C A, Andrade, Gabriel JM, Lisboa, Izaias P, Zoca, Samuel M
Reference: The Proceedings of the International Plant Nutrition Colloquium XVI, Department of Plant Sciences, UC Davis, UC Davis http://escholarship.ucop.edu/uc/item/3f53794z
Year: 2009
Results and conclusion: A detrimental effect of glyphosate on soybean Mn nutrition has been reported, which could happen even when applying the herbicide to weeds infesting soybean crops. Three experiments were conducted to study the effect of glyphosate on Mn absorption kinetics, accumulation, distribution within the soybean plant and soybean response to Mn as affected by this herbicide. In a nutrient solution experiment, using the solution depletion technique, Mn uptake kinetics (V_{max}, K_m and C_{min}) were determined for a conventional and its near-isogenic glyphosate-resistant counterpart cultivar as affected by glyphosate applied to the nutrient solution. In another nutrient solution experiment, differential Mn accumulation and distribution were studied for the same cultivars. In the third experiment, with Mn-deficient soil in pots, the response of glyphosate-resistant soybean cultivars to Mn application was studied in the presence of the herbicide. A few days after herbicide treatment, soybean plants developed yellowish leaves, a symptom that, in the field, could be misinterpreted as

Mn deficiency. But there was no evidence of deleterious effects of glyphosate on Mn absorption, accumulation, distribution in the plant and response by soybean cultivars.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Rosolem et al. (2010)

Title: MANGANESE UPTAKE AND REDISTRIBUTION IN SOYBEAN AS AFFECTED BY GLYPHOSATE
Author: Ciro Antonio Rosolem, Gabriel José Massoni de Andrade, Izaias Pinheiro Lisboa & Samuel Menegatti Zoca
Reference: R. Bras. Ci. Solo, 34:1915-1922
Year: 2010
Results and conclusion: Detrimental effects of glyphosate on plant mineral nutrition have been reported in the literature, particularly on Mn uptake and redistribution. However, in most of the experiments conducted so far glyphosate-susceptible plants were used. Effects of glyphosate on Mn absorption kinetics, accumulation, and distribution within the plant, as well as soybean response to Mn as affected by glyphosate were studied in three experiments. In the first experiment, in nutrient solution, the effect of glyphosate on soybean Mn uptake kinetic parameters (I_{max}, K_m and C_{min}) was determined. In a second experiment, also in nutrient solution, differential Mn accumulation and distribution were studied for a conventional soybean cultivar and its near-isogenic glyphosate-resistant counterpart as affected by glyphosate. In a third experiment, response of glyphosate-resistant soybean cultivars to Mn application was studied in the presence of glyphosate, in pots with Mn-deficient soil. Maximum Mn influx (Imax) was higher in the herbicide-resistant (GR) cultivar than in its conventional counterpart. Glyphosate applied to nutrient solution at low rates decreased K_m and C_{min}. A few days after herbicide treatment, RR soybean plants developed yellowish leaves, a symptom which, in the field, could be misinterpreted as Mn deficiency, but herbicide application had no effect on Mn uptake or distribution within the plant. In the soil experiment, soybean Mn uptake was increased by Mn application, with no effect of glyphosate. Under greenhouse conditions, there was no evidence of deleterious effects of glyphosate on Mn absorption, accumulation and distribution in the plant and on soybean cultivars response to Mn application.
Proposed action:

Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Santos et al. (2007)

Title: Effects of Glyphosate Formulations on Transgenic Soybean	
Author: SANTOS, J.B., FERREIRA, E.A., REIS, M.R., SILVA, A.A., FIALHO, C.M.T. e FREITAS, M.A.M.	
Reference: Planta Daninha, Viçosa-MG, v. 25, n. 1, p. 165-17	
Year: 2007	
Results and conclusion: This study aimed to evaluate the effects of three glyphosate formulations (Roundup Ready® and R. Transorb® - both with isopropylamine salt and Zapp Qi®, formulated as potassium salt), on transgenic soybean. CD 219RR variety soybean plants displaying the CP4Epsps gene, tolerant to glyphosate, were cultivated. At 25 days after emergence (DAE), when plants showed the second trifolium completely expanded (stadiums V2-V3), formulations were applied at 2,000 g/ha. Plants intoxication was evaluated 15 days after application as well as the number and dry matter of leaflets, number of radicular nodules and foliar content of N, P, K, S, Ca, Mg, Fe, Cu, Zn, and Mn at flowering and grain yield at the end of the cycle. Soil basal respiration rate, microbial biomass carbon and metabolic quotient were evaluated through soil samples collected during soybean flowering. Isopropylamine salt, present in the Roundup Transorb formulation, was more harmful to the soybean plants, also providing a negative effect on the soil microbiota. Roundup Ready formulation, registered as transgenic soybean, should not be applied on this crop at a higher rate, since it could alter the content of some nutrients, such as N, Ca, Mg, Fe and Cu, besides causing intoxication in the plants.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Roundup Ready® and R. Transorb® - both with isopropylamine salt CAS-no.: 38641-94-0 and Zapp Qi®, formulated as potassium

	salt Glyphosate, CAS-no.: 70901-12-1
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Sarigiannis et al. (2013)

Title: Inventory of pesticide emissions into the air in Europe

Author: D.A. Sarigiannis, P. Kontoroupis, E.S. Solomou, S. Nikolaki, A.J. Karabelas

Reference: Atmospheric Environment 75 (2013) 6-14

Year: 2013

Results and conclusion: Creation of a reliable and comprehensive emission inventory of the pesticides used in Europe is a key step towards quantitatively assessing the link between actual pesticide exposure and adverse health effects. An inventory of pesticide emissions was generated at a 1 × 1 km grid, for the year 2000. The emission model comprises three components: estimates of active substance (AS) wind drift taking into account crop type, volatilization during pesticide application and volatilization from the crop canopy. Results show that atmospheric emission of pesticides varies significantly across Europe. Different pesticide families are emitted from different parts of Europe as a function of the main crop(s) cultivated, agro-climatic conditions and production intensity.
The pesticide emission inventory methodology developed herein is a valuable tool for assessing air quality in rural and peri-urban Europe, furnishing the necessary input for atmospheric modelling at different scales. Its estimates have been tested using global sensitivity and Monte Carlo analysis for uncertainty assessment and they have been validated against national and local surveys in four European countries; the results demonstrate the robustness and reliability of the inventory. The latter may therefore be readily used for exposure and health risk assessment studies targeting farmers, applicators, but also bystanders and the general population in Europe.

Results in deatil: Glyphosate emissions do not exceed 70 kg yr^{-1} km^2. The highest values are computed in parts of Portugal, Germany, the UK and Denmark.

Quantities used and emissions for 1,3-dichloropropene, mancozeb, chlorpyrifos and glyphosate in Europe (total):

1,3-dichloropropene		Mancozeb		Chlorpyrifos		Glyphosate	
Quantities in tons yr^{-1}	Emissions in tons yr^{-1}	Quantities in tons yr^{-1}	Emissions in tons yr^{-1}	Quantities in tons yr^{-1}	Emissions in tons yr^{-1}	Quantities in tons yr^{-1}	Emissions in tons yr^{-1}
939	520	6960	2505	253	84	13335	3393

Proposed action:
To be considered as additional information.

Type of information (critical, high/low weight, supporting, additional):
Additional information

Reliability	
Endpoint	Pesticide emissions into the air in Europe
Protocol	Not given
Test compound	Pesticides, e.g. glyphosate (herbicide), chlorpyrifos (insecticide), mancozeb (fungicide)
Test system and conditions	Overview: The multi-step methodology developed to create the EU-wide pesticide emission inventory at a 1×1 km grid is as follows: (a) Starting from data on crop cultivated areas per grid cell, pesticide AS lists per crop and country are created, taking into account the current legislation. The final AS list encompasses thus the effect of use restrictions of hazardous AS. (b) Annual pesticide usage data at the country level are disaggregated to a fine 1×1 km resolution via a spatial allocation algorithm. (c) On that basis, an emission model is developed capturing the main physico-chemical processes that govern pesticide emission into the air. (d) The effects of model input to the estimated emission are investigated using global sensitivity methods. On the basis of the relative weight of the input parameters the spatially distributed consumption data are used as input to the emission model to deduce yearly air emission per crop in each grid cell. (e) Pesticide input data are validated against pesticide usage and sales data in locations in North and South Europe at different spatial resolutions. Corrective steps are taken when necessary to increase the robustness and accuracy of the final estimates. (f) Emission rate output is validated via Monte Carlo simulation using its inputs to a dispersion model to deduce concentration in a rural site for a number of pesticides found in the inventory.
Statistical design	Global sensitivity analysis according to the Sobol method (Sobol, 1993) was used to quantify the variation in emission due to model inputs such as ambient temperature, fraction of pesticide lost due to drift, fraction intercepted by the crop and lateral distance from the source. After sensitivity analysis, uncertainty was assessed via Monte Carlo Simulation (MCS). It involved a large number of samples (typically hundreds of thousands) from the distribution of the input parameters (i.e. crop fraction per cell, the fraction intercepted by crop) that were combined to obtain probability distributions for the emission rate output and thus statistically quantify the residual uncertainty.
Relevance	

Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Schönherr and Schreiber (2004)

Title: Interactions of Calcium Ions with Weakly Acidic Active Ingredients Slow Cuticular Penetration: A Case Study with Glyphosate	
Author: JÖRG SCHÖNHERR AND LUKAS SCHREIBER	
Reference: J. Agric. Food Chem. 52, 6546-6551	
Year: 2004	
Results and conclusion: Potassium and calcium salts of glyphosate were obtained by titrating glyphosate acid with the respective bases to pH 4.0, and rates of penetration of these salts across isolated astomatous cuticular membranes (CMs) were measured at 20 °C and 70, 80, 90, and 100% humidity. K-glyphosate exhibited first-order penetration kinetics, and rate constants (k) increased with increasing humidity. Ca-glyphosate penetrated only when the humidity above the salt residue was 100%. At 90% humidity and below, Ca-glyphosate formed a solid residue on the CMs and penetration was not measurable. With Ca-glyphosate, the k value at 100% humidity decreased with time and the initial rates were lower than for K-glyphosate by a factor of 3.68. After equimolar concentrations of ammonium oxalate were added to Ca-glyphosate, high penetration rates close to those measured with K-glyphosate were measured at all humidities. Adding ammonium sulfate or potassium carbonate also increased rates between 70 and 100% humidity, but they were not as high as with ammonium oxalate. The data indicate that at pH 4.0 one Ca^{2+} ion is bound to two glyphosate anions. This salt has its deliquescence point near 100% humidity. Therefore, it is a solid at lower humidity and does not penetrate. Its molecular weight is 1.82 times larger than that of K-glyphosate, and this greatly slows down rates of penetration, even at 100% humidity. The additives tested have low solubility products and form insoluble precipitates with Ca^{2+} ions, but only ammonium oxalate binds Ca^{2+} quantitatively. The resulting ammonium salt of glyphosate penetrates at 70-100% humidity and at rates comparable to K-glyphosate. The results contribute to a better understanding of the hard water antagonism observed with glyphosate. It is argued that other pesticides and hormones with carboxyl functions are likely to respond to Ca^{2+} ions in a similar fashion. In all of these cases, ammonium oxalate is expected to overcome hard water antagonism.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	^{14}C-Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable

Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Schuette (1998)

Title: Environmental fate of glyphosate	
Author: Jeff Schuette	
Reference: Revised Report of the Environmental Monitoring & Pest Management Department of Pesticide Regulation Sacramento, CA 95824-5624, California, US	
Year: November 1998	
Results and conclusion: A summary of the physical-chemical properties, the environmental fate of glyphosate in air, in water and sediment, in soil and in biota and the toxicity of the active substance are provided based on different studies from regulatory context and/or open literature. An overview of results from eight studies conducted in the forest environment is additionally provided. The summarized studies indicate that glyphosate is adsorbed to mineral clays and organic matter and is excluded from these sites by inorganic phosphate. Glyphosate has limited pre-emergence herbicidal activity in most soils because of its tendency to adsorb strongly to soil. The K_{oc} values indicate that glyphosate will not move readily through soil, and under conditions of the summarized studies, glyphosate would not leach into non-target areas. Glyphosate is inactivated in soil and water by microbial degradation. When applied to foliage, glyphosate is readily absorbed and translocated to various parts of plant via the phloem.	
Proposed action: To be used as supporting information.	
Type of information (critical, high/low weight, supporting, additional): Low weight, supporting information, review report on environmental fate studies	
Reliability	High, review report from a regulatory authority
Endpoint	Range of degradation and adsorption endpoints in soil, water, air
Protocol	No detailed information in the report on analysed studies
Test compound	Glyphosate
Test system and conditions	No information in the report about the analysed studies
Statistical design	Not provided
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	Detailed information on the analysed studies is not provided in the report. Therefore, the data in the report cannot be considered for endpoint derivation and/or further risk assessment.

Sebiomo et al. (2012)

Title: The Impact of Four Herbicides on Soil Minerals
Author: A. Sebiomo, V.W. Ogundero and S.A. Bankole
Reference: Research Journal of Environmental and Earth Sciences 4(6): 617-624, 2012
Year: 2012
Results and conclusion:

The aim of this study was to investigate the interaction of atrazine, primextra, paraquat and glyphosate with soil minerals. The treatments were carried out for a period of 6 weeks; at company recommended rates of 4l/h (at 350 mL in 15 L sprayer) for paraquat, glyphosate and primeextra while recommended rate of 3 kg/h (atrazine powder) was used for atrazine treatment (soil treatments were carried out in triplicates). Soil moisture content was determined using Satorious Moisture content Analyser. Soil mineral concentration were then determined by injecting sample solutions (extract) and standard solution for each mineral into the atomic absorption spectrophotometer into sample fray and the mean signal response was recorded for each of the element at their respective wavelength. The concentrations of the minerals were then calculated. Herbicide treated soils showed reduction in the moisture content from the second to the sixth weeks of treatment. There was significant ($p<0.001$) reduction in Sodium ion (Na) and Calcium ion (Ca) concentration compared to the control. The potassium, Magnesium, Iron and Zinc (K, Mg, Fe and Zn) increased significantly ($p<0.001$) compared to the control. Ths study has elucidated the ability of herbicides to chelate with soil minerals thereby reducing their availability for uptake by plants. It has also been shown that soil minerals are utilised by plants and microbes during microbial degradation.	
Proposed action: Not to be considered as the study was performed under outdoor conditions in Nigeria; not representative for EU.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information on Glyphosate sorption to soil minerals	
Reliability	Low
Endpoint	Mineral contents of the soils
Protocol	Non-GLP
Test compound	Glysate, Nantong Ji Angshan Agrochemicals (glyphosate, CAS-no.: 1071-83-6)
Test system and conditions	Soil treatments: The treatments were carried out for a period of 6 weeks, at company recommended rates of 4l/h (at 350 mL in 15 L sprayer) for paraquat, glyphosate and primeextra while recommended rate of 3 kg/h (atrazine powder) was used for atrazine treatment (soil treatments were carried out in triplicates). Soil sampling: Top soil up to 5 cm depth samples was collected from cassava farm in Ijebu-Ode (Ogun State, Nigeria) with no prior pesticide treatment. The soil samples were sieved through a 2.0 mm width mesh to remove stones and plant debris.
Statistical design	Not specified
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Other studies support the results; no negative evidence.

Serra et al. (2011)

Title: Glyphosate influence on nitrogen, manganese, iron, copper and zinc nutritional efficiency in glyphosate resistant soybean
Author: Ademar Pereira Serra, Marlene Estevão Marchetti, Ana Carina da Silva Candido, Ana Caroline Ribeiro Dias, Pedro Jacob Christoffoleti

Reference: Ciência Rural, v.41, n.1, p. 77-84	
Year: 2011	
Results and conclusion: After development of glyphosate-resistant (GR) soybean, there is a considerable raise in the use of this herbicide, with three to four applications during the culture cycle. Thus, these applications may be influencing the mineral nutrition of the crop. So, the aim of this research was evaluate the glyphosate influence on uptake, translocation and use efficiency of N, Mn, Cu, Zn and Fe by (GR) soybean 'P98R31' cultivar. The experiment was conducted in the greenhouse at ESALQ/USP, Piracicaba, State of São Paulo, Brazil, in 2009. The experimental unit was formed by 11kg/vase of soil (Rhodic Paleudult) with two plants in each vase. The treatments have been arranged in a factorial pathway 5X5, with five levels of the factor Mn (0, 20, 40, 60 and 80mg/dm³) and five of glyphosate drifts (0; 0,648; 1,296; 1,944 e 2,592 kg e.a./ha) and the Mn was supplied by the manganese sulphate. The experimental design was randomized blocks, with four repetitions. There was no influence on response from plants concerning the levels of Mn used into the experiment. The application of glyphosate interfered on mineral nutrition of soybean and the total contents of N, Mn, Cu, Zn and Fe. The use of glyphosate has caused reduction of the nodules number and reduced the output of dry mass.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Shushkova et al. (2010)

Title: Glyphosate bioavailability in soil
Author: Tatyana Shushkova, Inna Ermakova, Alexey Leontievsky
Reference: Biodegradation 21:403–410
Year: 2010
Results and conclusion: Biodegradation of glyphosate in sod-podzol soil by both the indigenous micro flora and the introduced strain Ochrobactrum anthropi GPK 3 was studied with respect to its sorption and mobility. The experiments were carried out in columns simulating the vertical soil profile. Soil samples studied were taken from soil horizons 0-10, 10-20, and 20-30 cm deep. It was found out that the most of the herbicide (up to 84%) was adsorbed by soil during the first 24 h; the rest (16%) remained in the soluble fraction. The adsorbed glyphosate was completely extractable by alkali. No irreversible binding of glyphosate was observed. By

the end of the experiment (21st day), glyphosate was only found in extractable fractions. The comparison of the effect of the introduced O. anthropi GPK 3 and indigenous microbial community on the total toxicant content (both soluble and absorbed) in the upper 10 cm soil layer showed its reduction by 42% (21 mg/kg soil) and 10-12% (5 mg/kg soil), respectively. Simultaneously, 14-18% glyphosate moved to a lower 10-20 cm layer. Watering (that simulated rainfall) resulted in a 20% increase of its content at this depth; 6–8% of herbicide was further washed down to the 20-30 cm layer. The glyphosate mobility down the soil profile reduced its density in the upper layer, where it was available for biodegradation, and resulted in its concentration in lower horizons characterized by the absence (or low level) of biodegradative processes. It was shown for the first time how the herbicide biodegradation in soil can be increased manifold by introduction of the selected strain O. anthropi GPK 3.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Herbicide Ground Bio (Tekhnoexport, Russia), containing GP as its isopropylamine salt, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Soltani et al. (2011)

Title: Influence of manganese on efficacy of glyphosate in glyphosate-resistant soybean
Author: Nader Soltani, Christy Shropshire, and Peter H. Sikkema
Reference: Can. J. Plant Sci. 91: 1061-1064
Year: 2011
Results and conclusion: Four field trials were conducted from 2007 to 2010 in Ontario to evaluate the effect of various manganese (Mn) formulations (Mn1, Ecoman 5% Mn; Mn2, MangaMax 5.5% Mn; Mn3, ManMax 5.5% Mn; Mn4, Superman 5% Mn; Mn5, Stoller This 5% Mn; Mn6, Nortrace 6% Mn-EDTA (ethylenediaminetetraacetate); Mn7, Nortrace 22% Mn and Mn8, WolfTrax 33% Mn) applied at 2.0 kg actual Mn/ha on glyphosate efficacy at 900 g a.e./ha in glyphosate-resistant soybean. The tank mix of glyphosate plus Mn4, Mn6 or Mn8 caused as much as 6, 17 and 4% injury in soybean, respectively. There was minimal crop injury (0-1.4%) with other Mn tank mixes. The addition of Mn4 or Mn6 to glyphosate did not antagonize glyphosate efficacy on the weeds evaluated (AMARE, AMBEL, CHEAL and SETVI). The other Mn formulations antagonized glyphosate efficacy for the control of AMARE, AMBEL, CHEAL or SETVI under some environments. The addition of Mn3 or Mn6 to glyphosate reduced soybean yield as much as 15 and 10% compared with glypho-

sate alone, respectively. Based on these results, it is recommended that glyphosate and manganese applications be applied sequentially to avoid weed control antagonism and maximize soybean yield.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate (WeatherMax)
Test system and con-ditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental rele-vance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Sorvari and Jaakkonen (2011)

Title: Environmental Risks Caused by Pesticides at Forest Nurseries in Finland
Author: Jaana Sorvari and Satu Jaakkonen
Reference: Human and Ecological Risk Assessment, 17: 431–466
Year: 2011
Results and conclusion: Previously, various persistent pesticides were used extensively in the production of seed-lings at Finnish forest nurseries. The extent and magnitude of the risks arising from the consequent environmental contamination are largely unknown. Therefore, we selected two representative nurseries for which we conducted tiered health risk assessments (HRA) us-ing risk-based benchmarks and two calculation tools (SSL and Risc-Human software). Eco-logical risk assessments (ERA) involved comparisons of environmental concentrations with ecotoxicological benchmarks. Site investigations revealed that the concentration of several pesticides exceeded the Finnish soil quality guidelines in some places. The compost pile for organic residues and the pond receiving runoffs contained traces of pesticides and the max-imum concentration of atrazine and terbuthylazine in groundwater exceeded the corre-sponding guideline for household water. Hexachloro-benzene proved to pose the highest health risks, the maximum hazard quotient being around 10 (carcino-genity-based) in the residential land use scenario. Owing to the conservative assumptions, health risks are ex-pected to remain insignificant, however. Risks to the local terrestrial ecosystem would also remain low, while only further studies will reveal the actual risks to the adjoining aquatic ecosystem. Both calculation tools showed shortcomings that generate uncertainty in the HRA, whereas the ERA was hampered particularly by the lack of benchmarks.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; AMPA, CAS-no.: 1066-51-9 and other 68 pesticides
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Sprague et al. (2012)

Title: Relating Management Practices and Nutrient Export in Agricultural Watersheds of the United States	
Author: L. A. Sprague and J. A. M. Gronberg	
Reference: J. Environ. Qual. 41, doi:10.2134/jeq2012.0073	
Year: 2012	
Results and conclusion: Relations between riverine export (load) of total nitrogen (N) and total phosphorus (P) from 133 large agricultural watersheds in the United States and factors affecting nutrient transport were evaluated using empirical regression models. After controlling for anthropogenic inputs and other landscape factors affecting nutrient transport—such as runoff, precipitation, slope, number of reservoirs, irrigated area, and area with subsurface tile drains—the relations between export and the area in the Conservation Reserve Program (CRP) (N) and conservation tillage (P) were positive. Additional interaction terms indicated that the relations between export and the area in conservation tillage (N) and the CRP (P) progressed from being clearly positive when soil erodibility was low or moderate, to being close to zero when soil erodibility was higher, to possibly being slightly negative only at the 90th to 95th percentile of soil erodibility values. Possible explanations for the increase in nutrient export with increased area in management practices include greater transport of soluble nutrients from areas in conservation tillage; lagged response of stream quality to implementation of management practices because of nitrogen transport in groundwater, time for vegetative cover to mature, and/or prior accumulation of P in soils; or limitations in the management practice and stream monitoring data sets. If lags are occurring, current nutrient export from agricultural watersheds may still be reflecting the influence of agricultural landuse practices that were in place before the implementation of these management practices.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Not applicable
Test system and con-	Not applicable

ditions	
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Stefanello et al. (2011)

Title: Effect of glyphosate and manganese on nutrition and yield of transgenic glyphosate-resistant soybean	
Author: Fabio Fernando Stefanello; Marlene Estevão Marchetti; Eulene Francisco da Silva; Josemar Stefanello; Rafael Bonifácio Sabino Doreto; Jose Oscar Novelino	
Reference: Ciências Agrárias, Londrina, v. 32, n. 3, p. 1007-1014, jul/set. 2011	
Year: 2011	
Results and conclusion: Research suggests that the application of glyphosate on transgenic glyphosate-resistant soybean can cause induced deficiency of Mn. Thus, the aim of this work was to evaluate the application of glyphosate and manganese in post-emergence on different phenological growth stages of RR soybean and its effects on leaf nutrient contents and productivity of grains. The experiment was carried out at two farms in Rio Brilhante-MS, both with randomized block experimental design with six replications at Lages de Pedra farm and four replications at São Manoel farm. Treatments were established in 3 x 8 factorial schemes, where the factor A consisted of three treatments with glyphosate (without the application of glyphosate, application of 720 g i.a. in the growth stage V2 + 480 g a. in V4, and application of 1.200 g i.a. in V4 growth stage). The factor B consisted of eight treatments with foliar application of Mn being without application, and seven Mn application was sprayed the leaves with 332 g/ha, divided into different growth stages. The application of glyphosate on transgenic soybean did not have effect on leaf nutrient contents, including the absorbing of Mn. Yield and mass of 100 grains were not influenced by applying of glyphosate neither by leaf fertilization with Mn, and leaf applying of Mn influenced only the leaf contents of Mn and Fe.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Roundup Ready®, isopropylammmonium salt, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	

"Positive"/"Negative" evidence	Not applicable

Steinmann et al. (2013)

Title: Glyphosat – ein Herbizid in der Diskussion und die Suche nach dem „Notwendigen Maß"	
Author: H.-H. Steinmann	
Reference: Gesunde Pflanzen (2013) 65:47–56, DOI 10.1007/s10343-013-0297-2	
Year: 2013	
Results and conclusion: Glyphosate is the most widely used herbicidal active ingredient in the world. In Germany, the distributed amounts have been doubled during the last ten years. There is public concern and criticism on this extensive use and use limitations are claimed. However, also loss of efficacy as reported from countries with high use intensity of glyphosate reduces long term use of these herbicides. Recently, scientific studies aimed to quantify the economic benefits of glyphosate to estimate the costs of losing or banning this herbicide. In this text, possibilities of reductions of glyphosate use in arable farming are discussed to obtain a necessary extent. It is pointed out, that those uses are preferential, that enable minimum soil cultivation that minimize soil erosion. Post-harvest applications have the potential for use reductions, due to replacement techniques such as soil cultivation that are available. Pre-harvest applications, which are targeted for crop maturation only, should not be used as a routine. It is suggested to seek for best management practices of glyphosate use.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	-
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Swift and Bell (2011)

Title: What are the environmental consequences of using silviculturally effective forest vegetation management treatments?
Author: Kathie I. Swift and F. Wayne Bell
Reference: THE FORESTRY CHRONICLE, VOL. 87, NO.2
Year: 2011
Results and conclusion: In this paper, we present examples of stand-level consequences of using forest vegetation

management treatments in boreal and temperate forest ecosystems in Canada. Specifically, we address several selected indicators: air and water quality, soils and nutrients, plant diversity, and wildlife habitat. For each of these, we discuss direct and indirect effects of five broad categories of treatments: (1) silviculture and harvesting systems and (2) physical, (3) thermal, (4) cultural, and (5) chemical/biological treatments. Our emphasis is on forest vegetation management treatments that are currently used in Canada to manage conifers. By applying regulations and best management practices, conducting landscape-level analyses and developing longer-term monitoring programs resource managers can minimize the effects of FVM treatments on the environmental indicators presented in this paper. Continued monitoring of abiotic and biotic indicators will be needed to reduce uncertainty related to the use of FVM treatments in Canadian forests is recommended. Although the literature available does point to limited short-term effects, an understanding of the cumulative effects of FVM treatments over multiple rotations is lacking. In addition, FVM treatments continue to evolve and uncertainties about the effects of new or modified treatments will arise. For example, demand for fibre as an energy source is on the rise in the boreal forests of Canada and will affect the volume of woody and structural material removed from boreal ecosystems.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Not applicable
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Syversen (2005)

Title: Cold-climate vegetative buffer zones as pesticide-filters for surface runoff	
Author: N. Syversen	
Reference: Water Science & Technology Vol 51 No 3-4 pp 63–71	
Year: 2005	
Results and conclusion: Vegetative buffer zones adjacent to watercourses can be effective filters for diffuse pollution from agriculture. Several investigations, even during snowmelt season, have shown that retention of sediments and sediment-bound nutrients in runoff water has been high through buffer zones (BZ). It is likely that BZ also can be effective filters for sediment-bound pesticides. The retention of glyphosate, propiconazole, fenpropimorph and soil particles was studied in surface runoff experiments with 5m wide buffer zones. Volume proportional samples were collected after each runoff episode (1999–2002). The distribution coefficient (K_d) shows moderate to high adsorption of the pesticides to the experimental	

soil. Results show average retention efficiency of about 51%, 48%, 85% and 34% for particles, glyphosate, propiconazole and fenpropimorph, respectively. The amount of AMPA (which is a degradation product of glyphosate), entering the BZ was high; approximately the same amount as for glyphosate. The retention efficiency through the BZ for AMPA was about 67%. There were no significant differences in removal efficiency (in %) between winter with snowmelt and summer. This is possibly due to detachment of coarser aggregates during winter, which trap more easily in the BZ. The conclusion based on this study suggests BZ to be contributors to reduced pesticide input to surface waters.	
Proposed action: Not to be considered as publication deals with risk mitigation. Thus, no fate related endpoints or the monitoring chapter are affected.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information about buffer zones could be contributors to reduce pesticide input to surface waters. Glyphosate and AMPA are trapped in the BZ, because they are adsorbed to soil particles.	
Reliability	Medium
Endpoint	Average removal efficiency in %
Protocol	Norwegian Standard 4733
Test compound	Glyphosate (CAS-no.: 1071-83-6), propiconazole and fenpropimorph
Test system and conditions	Four study plots with an upper supply area of 10m x 45m each with cereal production (barley), and a lower part with a buffer zone area of 10m x 5m (two plots) and no buffer zone (two reference plots), respectively, were used. The application rate represented a normal pesticide application in the area. Volume proportional mixed samples were taken after every runoff event or as frequently as 1–2 times a day during the snowmelt period. From the sampling tank, water samples were collected for laboratory analysis of glyphosate, fenpropimorph, propiconazole, suspended solids (SS), and soil texture.
Statistical design	Glyphosate results were adjusted according to R = -9,64 Ln(SS) + 113 and also corrected for recovery of the analysis itself. A simple linear regression model (Eq. 2) was used to correct for differences in runoff between the plots
Relevance	
Environmental relevance	Environmental parameters are measured and reported.
Weight of evidence	
“Positive”/”Negative” evidence	No negative evidence; results supported by other publications.

Syversen and Bechmann (2004)

Title: Vegetative buffer zones as pesticide filters for simulated surface runoff
Author: Nina Syversen, Marianne Bechmann
Reference: Ecological Engineering 22, 175–184
Year: 2004
Results and conclusion: Losses of pesticides from agricultural land to surface waters can cause environmental harm to fish and other aquatic organisms. Vegetated buffer zones (BZ) between agricultural land and surface waters have proved to be effective filters for sediments and sediment-bound

nutrients. It is therefore; likely that BZ also can be effective filters for pesticides, especially sediment-bound pesticides. The retention of glyphosate, fenpropimorph, propiconazole and soil particles was studied in short-term BZ experiments with simulated surface runoff. Runoff water containing pesticides and soil particles was added directly to the BZ. The BZ was 5m wide and consisted of natural grass/herbaceous vegetation. To calculate retention efficiency of pesticides and particles through the BZ, surface runoff was collected before entering and after passing the BZ. The average removal efficiency was 39, 71, 63 and 62% for glyphosate, fenpropimorph, propiconazole and soil particles, respectively. Aminomethylphosphonic acid (AMPA), which is a degradation product of glyphosate, constituted only a small part of glyphosate (about 10%) in this short-term experiment. Based on this study BZ can serve as contributors to reduce pesticide input to surface waters. .	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, Round UpEco; fenpropimorph and propiconazole, Tilt-Top
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Tang et al. (2010)

Title: Study on the Degradation Characteristics of Yeast Strain S-2 on Glyphosate Herbicide	
Author: Tang *et al.*	
Reference: Journal of Anhui Agri. Sci. 2010, 38 (4): 1992-1994	
Year: 2010	
Results and conclusion: Study is written in Chinese language.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Not applicable, study is written in Chinese language.	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Not applicable
Test system and conditions	Not applicable

Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Tesfamariam et al. (2009)

Title: Fate of glyphosate stored in weed residues and the potential of phytotoxicity for following crops	
Author: Tesfamariam, T., Bott, S., Roemheld, V., Neumann, G.	
Reference: The Proceedings of the International Plant Nutrition Colloquium XVI, Department of Plant Sciences, UC Davis, UC Davis	
Year: 2009	
Results and conclusion: Glyphosate, a broad spectrum, non-selective herbicide, is the world´s most important and widely used herbicide. The globally increasing adoption of no-till or reduced tillage systems is becoming a driving force for enhanced glyphosate use. In such systems, glyphosate is applied pre-sowing for weed control and glyphosate may remain in root and shoot residues. To evaluate potential risks associated with glyphosate residues, a pot experiment was conducted under controlled greenhouse conditions with two contrasting soils: weakly buffered acidic Arenosol and highly buffered Luvisol. Glyphosate was supplied as glyphosate enriched rye grass straw (1.2 g DM kg^{-1} soil) prior to sowing sunflower as a non-target plant. Several physiological parameters, such as intracellular shikimate accumulation as a metabolic indicator for glyphosate toxicity, biomass production and micronutrient status were analyzed. Detrimental effects on sunflower plants linked to glyphosate toxicity were observed only in the Arenosol but not in the Luvisol. This is most probably related to the difference in soil properties. The detoxification capacity of the fine-textured Luvisol, with a high clay content, was high enough for an adequate immobilization and inactivation of glyphosate. On the sandy Arenosol, the level of glyphosate supply exceeded the detoxification capacity. In addition to the difference in detoxification capacity, differences in nutrient bio-availability might also have aggravated the observed inhibition of nutrient acquisition. Thus, the findings suggest the importance of weed residues in transferring glyphosate from target to nontarget plants, particularly in no-till or reduced tillage systems, with the consequence of detrimental effects on following crop plants.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable

Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Todorovic et al. (2010)

Title: Dispersion of glyphosate in soils through erosion	
Author: Gorana Rampazzo Todorovic, Axel Mentler, Nicola Rampazzo, Winfried E.H. Blum, Alexander Eder, Peter Strauss	
Reference: Environmental Quality 4, 125-138	
Year: 2010	
Results and conclusion: For a better understanding of the influence of erosion processes on glyphosate behaviour and dispersion under rainy conditions after application in the practice, two rain simulation experiments were conducted on two different locations in Austria with complete different soil types in September 2008. The results of the experiments showed that under normal practical conditions, the potential adsorption capacity of the Kirchberg soil is confirmed compared to the low adsorption Chernosem soil (about 8,000 ppm pedogenic Fe-oxides). Considering the enormous differences in the run-off amounts between the two sites Pixendorf and Kirchberg it can be concluded how important the soil surface conditions and vegetation cover of the agricultural fields for erosion risk and pollution risk of surface water are. In the rainfall simulation experiments under comparable simulation conditions, the amount of run-off at Kirchberg was app. 10 times higher than at the Pixendorf site, due to its better infiltration rate. Moreover, the total loss of glyphosate (NT+CT) through run-off was more than double on the Kirchberg site, which confirms the higher risk of pesticide pollution for surface waters on the agricultural fields with higher erosion intensity.	
Proposed action: Not to be considered, no data for recalculation of endpoint, furthermore, no raw data are reported with sufficient precision.	
Type of information (critical, high/low weight, supporting, additional): Low weight, only information of the importance of the soil surface conditions and vegetation cover of the agricultural fields for erosion risk and pollution risk of surface water are presented.	
Reliability	Low
Endpoint	Figures of time dependent glyphosate concentration
Protocol	Non-GLP
Test compound	Round up (450 g glyphosate /L)
Test system and conditions	The rain simulation experiments took place in 3 field replications of the Conventional Tillage (CT)- and No Tillage (NT)-plots. Before starting the rain simulation, erosion plots were installed in each field repetition in a dimension of 2m x 2m. The culture type at time of the experiments was different in both sites (Pixendorf had a green cover after the wheat yield of July whereas Kirchberg stood immediately after the maize yield). For the rain simulation experiments a 2% herbicide solution was sprayed homogeneously by hand pump in the same concentration and amounts as in practice (180 mg glyphosate/m²). Immediately after application the rain simulation started (60 minutes, 30 mm). Run-off-fractions and soil samples were collected.
Statistical design	No data
Relevance	
Environmental relevance	No environmental are data reported.
Weight of evidence	
“Positive”/”Negative”	No negative evidence.

evidence	

Trinelli et al. (2013)

Title: Co-biosorption of copper and glyphosate by Ulva lactuca	
Author: M. A. Trinelli, M. M. Areco, M. dos Santos Afonso	
Reference: Colloids and Surfaces B: Biointerfaces 105 (2013) 251– 258	
Year: 2013	
Results and conclusion: This study investigated the adsorption of glyphosate (PMG) onto the green algae Ulva lactuca. PMG was not adsorbed by U. lactuca but PMG was adsorbed when the process was mediated by Cu(II) with molar ratios Cu(II):PMG ≥ 1.5:1. U. lactuca was characterized by water adsorption surface area, FTIR, SEM and EDS. The Langmuir and Freundlich models were applied. Results showed that the biosorption processes for copper and PMG in the presence of copper were described described by the Langmuir model (qmax = 0.85±0.09 mmol g^{-1}, KL = 0.55±0.14 l $mmol^{-1}$ and qmax = 3.65±0.46 mmol g^{-1}, KL = 0.103±0.03 l $mmol^{-1}$, respectively). Copper adsorption was greater in the presence of PMG than in the absence of the pesticide and the adsorption can only be represented by the Freundlich model (KF = 0.08±0.01, 1/n = 1.86±0.07). In all cases studied, the maximum metal uptake (qmax) increased with increasing pH. Surface complexes with a stoichiometry ranging from Cu PMG Cu to Cu PMG Cu3 are suggested as reaction products of the process. Due to the increasing amounts of PMG applied in Argentina, natural reservoirs present considerable amounts of this herbicide. The value of this work resides in using U. lactuca, a marine seaweed commonly found along coastlines all over the world, as a biosorbent for PMG.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate (PMG)
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Tsui et al. (2005)

Title: Influence of glyphosate and its formulation (Roundup®) on the toxicity and bioavailability of metals to Ceriodaphnia dubia
Author: Martin T.K. Tsui, Wen-Xiong Wang, L.M. Chu
Reference: Environmental Pollution 138, 59-68
Year: 2005
Results and conclusion:

This study examined the toxicological interaction between glyphosate (or its formulation, Roundup®) and several heavy metals to a freshwater cladoceran, Ceriodaphnia dubia. We demonstrated that all binary combinations of Roundup® and metals (Cd, Cu, Cr, Ni, Pb, Se and Zn) exhibited ‘‘less than additive’’ mixture toxicity, with 48-h LC50 toxic unit>1. Addition of glyphosate alone could significantly reduce the acute toxicity of Ag, Cd, Cr, Cu, Ni, Pb and Zn (but not Hg and Se). The ratio between glyphosate and metal ions was important in determining the mitigation of metal toxicity by glyphosate. A bioaccumulation study showed that in the presence of glyphosate the uptake of some metals (e.g. Ag) was halted but that of others (e.g. Hg) was increased significantly. Therefore, our study strongly suggests that glyphosate and its commercial formulations can control the toxicity as well as the bioavailability of heavy metals in aquatic ecosystems where both groups of chemicals can co-occur.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Roundup (41% active ingredient and 10-20% POEA) Glyphosate, CAS-no.: 1071-83-6 and isopropylamine (IPA) salt of glyphosate, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Tuffi Santos et al. (2005)

Title: Root Exudation of Glyphosate by Brachiaria decumbens and its Effects on Eucalypt Plants and Microbial Soil Respiration
Author: TUFFI SANTOS, L.D., FERREIRA, F.A., BARROS, N.F., SIQUEIRA, C.H., SANTOS, I.C., MACHADO, A.F.L.
Reference: Planta Daninha, Viçosa-MG, v. 23, n. 1, p. 143-152
Year: 2005
Results and conclusion: This study aimed to evaluate root exudation of the herbicide glyphosate by Brachiaria decumbens and its effects on eucalypt cultivated in soil and in nutritive solution; and to quantify microbial respiration in soil under different managements. One Eucalyptus grandis and four Brachiaria decumbens seedlings were planted in pots with lids with five perforations holding 8.0 L of the nutritive solution. A randomized block design in six replications was used, each pot being considered an experimental plot. The eucalypt and brachiaria plants were interplanted in a hydroponic solution for 30 days. Fifteen days after the transplant, the brachiaria plants were pruned to stimulate tillering. After this period, glyphosate treatments of 0, 720, 1440, 2160, and 2880 g a.e./ha were applied to the brachiaria plants. In the soil

experiment, E. grandis seedlings were planted in 72 10-liter pots, half containing sandy soil and half clayey soil. The experiment was set up in a randomized block design with six replications, in a 2 x 6 factorial scheme (two soil types and six management combinations). Following the eucalypt seedlings, five Brachiaria decumbens seedlings per pot were planted in 48 pots (24 of each soil), and interplanted with a eucalypt seedling. The remaining eucalypt pots were cultivated in monoculture. The tested treatments were: 1- interplanted eucalypt and brachiaria (control); 2- Eucalypt without brachiaria + 1440 g a.e./ha of glyphosate applied in the soil; 3- interplanted eucalypt and brachiaria cut after spray with 1440 g/ha glyphosate; 4, 5 and 6- interplanted eucalypt and sprayed brachiaria with 720, 1440, and 2880 g a.e/ha glyphosate, respectively. In treatments 4, 5, and 6 the eucalypt plants were protected from contact with the herbicide applied to the brachiaria plants. In treatment 2, glyphosate was applied directly to soil. The eucalypt plants in treatment 3 were treated with the brachiaria plant cut shoots, seven days after the latter had been sprayed with 1440 g/ha glyphosate. All the tested rates controlled over 95% of the grass species in both assays, and no toxicity symptoms were verified in the eucalypt plants. The microbial activity was greater in the sandy soil, mainly with the increase of the glyphosate rates applied to the brachiaria plants.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate isopropylammonium salt, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Tuffi Santos et al. (2009)

Title: Leaf anatomy and morphometry in three eucalypt clones treated with glyphosate	
Author: Tuffi Santos, LD., Sant’Anna-Santos, BF., Meira, RMSA., Ferreira, FA., Tiburcio, RAS. and Machado, AFL.	
Reference: Braz. J. Biol., 69(1): 129-136	
Year: 2009	
Results and conclusion: This work aimed to evaluate the effects of simulated drift of glyphosate on the morphoanatomy of three eucalypt clones and to correlate the intoxication symptoms on a microscopic scale with those observed in this visual analysis. The effects of glyphosate drift were proportional to the five doses tested, with Eucalyptus urophylla being more tolerant to the herbicide than E. grandis and urograndis hybrid. The symptoms of intoxication which were similar for the different clones at 7 and 15 days after application were characterized by leaf wilting, chlorosis and curling and, at the highest rates, by necrosis, leaf senescence and	

death. Anatomically glyphosate doses higher than 86.4 g/ha caused cellular plasmolysis, hypertrophy and hyperplasia, formation of the cicatrization tissue and dead cells on the adaxial epidermis. The spongy parenchyma had a decrease, and the palisade parenchyma and leaf blade thickness had an increase. The increased thickness in leaf blade and palisade parenchyma may be related to the plant response to glyphosate action, as a form of recovering the photosynthetically active area reduced by necroses and leaf senescence caused by the herbicide.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate: Commercial brand name Roundup SC, Monsanto of Brazil Ltd,
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Tuffi Santos et al. (2008)

Title: Radicular Exudation of Glyphosate by Brachiaria decumbens and Its Effects on Eucalypt Plant
Author: TUFFI SANTOS, L.D., SANTOS, J.B., FERREIRA, F.A., OLIVEIRA, J.A., BENTIVENHA, S., e MACHADO, A.F.L.
Reference: Planta Daninha, Viçosa-MG, v. 26, n. 2, p. 369-374
Year: 2008
Results and conclusion: Eucalypt plants commonly present symptoms of intoxication in areas where glyphosate is used. One possible way of contamination is through radicular exudation of glyphosate by the treated weed and later, plant absorption. This study aimed to evaluate glyphosate exudation by Brachiaria decumbens and its effects on eucalypt plants when ^{14}C -glyphosate, mixed to the solution of the commercial product Scout® was applied. Seedlings of two eucalypt clones (UFV05 and UFV06) were cultivated in pots, intercropped with Brachiaria decumbens, on two types of soil (clayey and sandy). At 35 days after transplantation, 50 µL of the mixture was applied on brachiaria by using a precision micro-syringe. After application, 2, 8, 16 and 24 days, samples of eucalypt plants were collected and fractioned in the primary apices, secondary apices, leaves and roots, following the usual methodology to determine radioactivity. Symptoms of intoxication were not observed in any eucalypt plant evaluation. However, ^{14}C-glyphosate was found in all plants, regardless of the soil type, clone or evaluation time, with the highest concentration being found in the sandy soil. Results show radicular exudation of glyphosate by B. decumbens and its absorption by eucalypt plants through roots. However, concentrations lower than necessary may cause crop

intoxication.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	^{14}C-Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Veiga et al. (2001)

Title: Dynamics of glyphosate and aminomethylphosphonic acid in a forest soil in Galicia, north-west Spain
Author: F. Veiga, J.M. Zapata, M.L. Fernandez Marcos_, E. Alvarez
Reference: The Science of the Total Environment 271(2001)135-144
Year: 2001
Results and conclusion: Only glyphosate was quantified in the solid phase. Only a semi-quantitative estimation of AMPA could be carried out. Determined glyphosate concentrations in the soil solid phase ranged from 0 to 6.9 µg g^{-1}, averaging 0.85 µg g^{-1}. The glyphosate concentrations show a trend to decrease along the monitoring period, being very low from 1 month after the treatment, both in solid and liquid phases of the forest soil studied. The AMPA concentrations, after increasing during the first fortnight, while the glyphosate decomposed, decreased until the end of the experiment. Despite its adsorption onto soil components, glyphosate and AMPA quickly reached a 30-cm depth in soil solution. At this depth they are degraded more slowly than in the surface layer Ž0-20 cm, as a consequence of lower biological activity. Glyphosate concentrations in the soil are lower in the lower slope position as a consequence of its distribution in a thicker soil layer. The higher dose of herbicide Ž8 l ha^{-1} did not result in significantly higher concentrations in soil.
Proposed action: Consider as additional information. Refers to soil monitoring and stability in soil. However, the authors did not calculate any DT_{50} values according to the recommended FOCUS procedure.
Type of information (critical, high/low weight, supporting, additional): Additional information
Reliability

Endpoint	Analysis of processes involved in microbial glyphosate degradation
Protocol	Non-GLP study
Test compound	Non-labelled glyphosate (CAS 1071-83-6), non-labelled AMPA

	(CAS 1066-51-9)
Test system and conditions	The soil used in the study is an umbric Regosol, according to FAO classificationŽFAO 1990., developed on green shales. It is located in the range of MeiraŽLugo, north-west Spain, at 625 m altitude, in a humid, temperate climate; the slope is approximately 20%. Previously to herbicide application, two soil depths 0-20 and 20-35 cm. were sampled and analysed for general properties. In spring 1996 *Eucalyptus nitens* seedlings were planted in rows along the maximum slope. Each row, consisting of 18 trees, was considered an experimental unit. After planting, two different Roundup doses (5 and 8 l/ha) were randomly applied to tree rows, with three replicates and leaving three control rows.
Statistical design	Three replicates
Relevance	
Environmental relevance	There is relevance for this specific type of application in railway tracks. However, the authors did not calculate any DT_{50} values according to the recommended FOCUS procedure.
Weight of evidence	
"Positive"/"Negative" evidence	The monitoring principally supports the results of mobility and degradation studies performed with glyphosate.

Wang et al. (2005)

Title: Influence of sediment on the fate and toxicity of a polyethoxylated tallowamine surfactant system (MON 0818) in aquatic microcosms
Author: Ning Wang, John M. Besser, Denny R. Buckler, Joy L. Honegger, Chris G. Ingersoll, B.T. Johnson, Mitchell L. Kurtzweil, Jon MacGregor, Michael J. McKee
Reference: Chemosphere 59, 545–551
Year: 2005
Results and conclusion: The fate and toxicity of a polyethoxylated tallowamine (POEA) surfactant system, MON 0818, was evaluated in water–sediment microcosms during a 4-d laboratory study. A surfactant solution of 8 mg/l nominal concentration was added to each of nine 72-l aquaria with or without a 3-cm layer of one of two natural sediments (total organic carbon (TOC) 1.5% or 3.0%). Control well water was added to each of nine additional 72-l aquaria with or without sediment. Water samples were collected from the microcosms after 2, 6, 24, 48, 72, and 96 h of aging to conduct 48-h toxicity tests with Daphnia magna and to determine surfactant concentrations. Elevated mortality of D. magna (43–83%) was observed in overlying water sampled from water-only microcosms throughout the 96-h aging period, whereas elevated mortality (23–97%) was only observed in overlying water sampled from water–sediment microcosms during the first 24 h of aging. Measured concentrations of MON 0818 in water-only microcosms remained relatively constant (4–6 mg/l) during the 96-h period, whereas the concentrations in overlying water from microcosms containing either of the two types of sediment dissipated rapidly, with half-lives of 13 h in the 3.0% TOC sediment and 18 h in the 1.5% TOC sediment. Both toxicity and the concentration of MON 0818 in overlying water decreased more rapidly in microcosms containing sediment with the higher percent TOC and clay and with a higher microbial biomass. Mortality of D. magna was significantly correlated with surfactant concentrations in the overlying water. These results indicate that the toxicity of the POEA surfactant in water rapidly declines in the presence of sediment due to a reduction in the surfactant concentration in the overlying water above the sediment.

Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	MON 0818 contains a complex polyethoxylated tallowamine (POEA) surfactant mixture used in Roundup
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Wang et al. (2009)

Title: The Inhibition of the Combined Pollution of Copper and Glyphosate to the Seed Germination and Root Elongation of Wheat	
Author: Wang Mi-dao, Cheng Feng-xia, Si You-bin	
Reference: Asian Journal of Ecotoxicology Vol. 4, No. 4, 591-596	
Year: 2009	
Results and conclusion: Interaction of pollutions is one of the research focuses in current environmental sciences. The combined toxicity of copper and glyphosate to the inhibition rates of wheat germination, sprout length and root elongation was studied. Results indicated that Cu^{2+} had no obvious effect on the wheat germination but could inhibit the root elongation and sprout length significantly. Glyphosate had obvious inhibition effect on the wheat germination, sprout length and root elongation. When Cu^{2+} and glyphosate were combined, the presence of Cu^{2+} decreased the inhibition of glyphosate to wheat germination and sprout length. But for the root elongation, the Cu^{2+} increased the toxicity of glyphosate when glyphosate was at low concentrations and decreased the toxicity of glyphosate to root elongation when glyphosate was at high concentrations. The possible reason of Cu^{2+} decreased the ecotoxicity of glyphosate is the complexation reaction of Cu^{2+} and glyphosate.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable

Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Warnemuende et al. (2007)

Title: Effects of tilling no-till soil on losses of atrazine and glyphosate to runoff water under variable intensity simulated rainfall	
Author: Elizabeth A. Warnemuende, Judodine P. Patterson, Douglas R. Smith, Chi-hua Huang	
Reference: Soil & Tillage Research 95 (2007) 19–26	
Year: 2007	
Results and conclusion: This study focuses on the viability of glyphosate tolerant cropping systems as an alternative to atrazine-based systems, and the impact of tilling historically no-till ground on the runoff pollution potential of these systems. Variable intensity field rainfall simulations were performed on 2 m long x 1 m wide plots within a field in first-year disk and harrow following no-till (CT), and within a long-term no-tilled (NT) field, both treated with atrazine and glyphosate according to label. Rainfall sequence was: 50 mm/h for 50 min followed by 75 mm/h for 15 min, 25 mm/h for 15 min, and 100 mm/h for 15 min. Runoff was collected at regular time intervals during two simulated rainfall events and analyzed for herbicide concentration, sediment content, and volume. Maximum glyphosate concentration in runoff was 233 mg/L for NT and 180 mg/L for CT (approximately 33% and 26% of the maximum contaminant limit (MCL) for glyphosate (700 mg/L), respectively, while maximum atrazine concentrations in runoff was 303 mg/L for NT and 79 mg/L for CT (approximately 100 times and 26 times the atrazine MCL (3 mg/L)). Atrazine concentration and loading were significantly higher in runoff from NT plots than from CT plots, whereas glyphosate concentration and loading were impacted by tillage treatment to a much lesser degree. Results suggest that glyphosate-based weed management may represent a lower drinking water risk than atrazine-based weed management, especially in NT systems.	
Proposed action: Not to be considered as publication deals with risk mitigation. Thus, no fate related endpoints or off-site monitoring are affected.	
Type of information (critical, high/low weight, supporting, additional): Low weight, only additional information about tillage or no-tillage management	
Reliability	Medium
Endpoint	Average runoff rates; Runoff herbicide concentrations are given as a function of time
Protocol	EPA Drinking Water Method for Chemical Contaminants #547; modified EPA method 525.2; soil samples: glyphosate (method: Monsanto RES-014-91) and atrazine (method:FAO PAM 302/SPE/NPD)
Test compound	Bicep II Magnum (33% atrazine); Roundup Ultra Max (41% glyphosate, CAS-no.: 1071-83-6)
Test system and conditions	The experimental design was a randomized complete block. Each block contained three plots, 2 m long and 1 m wide, representing three replications of CT and NT treatments. All plots were planted in glyphosate-tolerant corn, in annual rotation with soybeans.

	Herbicides were applied by a certified pesticide applicator to all plots 24 h prior to the first rainfall event according to label: Bicep II Magnum (33% atrazine) at a rate 1621 g atrazine/ha and Round-up Ultra Max (41% glyphosate) at a rate of 709 g glyphosate/ha. Immediately before herbicide application and immediately prior to the rainfall, soil was sampled and analyzed for herbicide levels to establish initial soil concentrations and confirm uniform and precise application. Two rainfall events were performed (rainfall simulator) on each plot, at 1 day and 8 days after herbicide application. Runoff samples were collected at 5 min intervals from the onset of runoff to 50 min and 3 min intervals from 53 min to 95 min.
Statistical design	Statistical analyses were performed using SAS 9.1, and SigmaPlot V. 6.0; runoff volumes, and concentrations and mass losses of sediment, glyphosate, and atrazine were determined using PROCGLM with $P \leq 0.05$. Regression analyses were performed using linear and logarithmic functions in SigmaPlot V. 6.0
Relevance	
Environmental relevance	Given; influencing endpoints are measured and partly reported.
Weight of evidence	
"Positive"/"Negative" evidence	The results are supported by other publications.

Watts (2009)

Title: Glyphosate
Author: Meriel Watts (Pesticide Action Network Asia and the Pacific (PAN AP))
Reference: -
Year: 2009
Results and conclusion: Review - Regarding environmental fate the following statements are made: Soils: Glyphosate is relatively persistent in soil, with residues still found up to 3 years later in cold climates. It is less persistent in warmer climates, with a half-life between 4 and 180 days. It is bound onto soil particles, and this was once thought to mean that glyphosate is not biologically active within soil, nor will it leach to groundwater. However it is now known that it can easily become unbound again, be taken up by plants or leach out, indicating a greater risk of groundwater contamination. It can reduce nitrogen and phosphate fertility of soils. Water: Glyphosate is soluble in water, and slowly dissipates from water into sediment or suspended particles. Although it does break down by photolysis and microbial degradation, it can be persistent for some time in the aquatic environment, with a half-life of up to nearly 5 months, and still be present in the sediment of a pond after 1 year. Residues of glyphosate have been found in a wide range of drains, streams, rivers and lakes, in many countries including Canada, China, France, Netherlands, Norway, USA, and the UK. Urban use on road and rail sides is contributing significantly to this contamination, with residues being found in sewage sludge and wastewater treatment plants. Contamination of 'vernal pools' – pools that are shallow and disappear in dry weather – are a concern for amphibia, for which these water sources are critical. Residues have also been found in

groundwater in Canada, Denmark, the Netherlands, and USA. They have been detected in the marine environment off the Atlantic Coast of France; and in the rain in Belgium and Canada. In general, the statements are supported by citations.	
Proposed action: Consider as additional information due to the fact that the article is a review and data are cited only.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Degradation in soil and water, groundwater contamination, findings in of drains, streams, rivers and lakes, residues being found in sewage sludge and wastewater treatment plants
Protocol	No detailed information in the report on analysed studies
Test compound	Glphosate
Test system and conditions	No information in the report about the analysed studies
Statistical design	Not provided
Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	Detailed information on the analysed studies is not provided in the report. Therefore, the data in the report cannot be considered for endpoint derivation and/or further risk assessment.

Zabaloy et al. (2011)

Title: Herbicides in the Soil Environment: Linkage Between Bioavailability and Microbial Ecology
Author: M. Celina Zabaloy, Graciela P. Zanini, Virginia Bianchinotti, Marisa A. Gomez and Jay L. Garland
Reference: Herbicides, Theory and Applications ISBN 978-953-307-975-2
Year: 2011
Results and conclusion: Although desorption has been considered a pre-requisite for biodegradation of soil-bound herbicides, there is increasing evidence that sorbed compounds may still be degraded by attached cells. However, there is still considerable work ahead for researchers to understand the mechanisms and populations intervening in these processes. Integrative approaches are essential to study physicochemical and biological factors that affect sorption, bioavailability and biodegradation of herbicides in soil. Development of new molecular methods coupling function and structure may improve our understanding of the role of microbial populations in herbicides degradation and how these compounds affect non-degrading members of the microbial community. Overall, a number of studies have shown that the herbicides 2,4–D, metsulfuron methyl and glyphosate at recommended rates have only transient impacts on soil microbial communities, being glyphosate the one with larger effects, while metsulfuron methyl may be toxic under certain soil conditions (e.g. high pH).
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional):

Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	No test design
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zablotowicz and Reddy (2004)

Title: Impact of Glyphosate on the Bradyrhizobium japonicum Symbiosis with Glyphosate-Resistant Transgenic Soybean: A Minireview	
Author: Robert M. Zablotowicz and Krishna N. Reddy	
Reference: J. Environ. Qual. 33:825–831	
Year: 2004	
Results and conclusion: Glyphosate-resistant (GR) soybean [Glycine max (L.) Merr.] expressing an insensitive 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS) gene has revolutionized weed control in soybean production. The soybean nitrogen fixing symbiont, Bradyrhizobium japonicum, possesses a glyphosate-sensitive enzyme and upon exposure to glyphosate accumulates shikimic acid and hydroxybenzoic acids such as protocatechuic acid (PCA), accompanied with B. japonicum growth inhibition and death at high concentrations. In a series of greenhouse and field experiments, glyphosate inhibited nodulation and nodule leghemoglobin content of GR soybean. Glyphosate accumulated in nodules of field-grown GR soybean, but its effect on nitrogenase activity of GR soybean was inconsistent in field studies. In greenhouse studies, nitrogenase activity of GR soybean following glyphosate application was transiently inhibited especially in early growth stages, with the greatest inhibition occurring under moisture stress. Studies using bacteroid preparations showed that the level of glyphosate inhibition of bacteroid nitrogenase activity was related to invitro glyphosate sensitivity of the B. japonicum strains. These studies indicate the potential for reduced nitrogen fixation in the GR soybean system; however, yield reductions due to this reduced N_2 fixation in early stages of growth have not been demonstrated.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Review article, no study design; Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable

Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2010)

Title: Glyphosate affects micro-organisms in rhizospheres of glyphosate-resistant soybeans	
Author: L.H.S. Zobiole, R.J. Kremer, R.S. Oliveira Jr and J. Constantin	
Reference: Journal of Applied Microbiology 110, 118–127	
Year: 2010	
Results and conclusion: Aims: Glyphosate-resistant (GR) soybean production increases each year because of the efficacy of glyphosate for weed management. A new or 'second' generation of GR soybean (GR2) is now commercially available for farmers that is being promoted as higher yielding relative to the previous, 'first generation' (GR1) cultivars. Recent reports show that glyphosate affects the biology and ecology of rhizosphere micro-organisms in GR soybean that affect yield. The objective of this research was to evaluate the microbiological interactions in the rhizospheres of GR2 and GR1 soybean and the performance of the cultivars with different rates of glyphosate applied at different growth stages. Methods and Results: A greenhouse study was conducted using GR1 and GR2 soybean cultivars grown in a silt loam soil. Glyphosate was applied at V2, V4 and V6 growth stages at three rates. Plants harvested at R1 growth stage had high root colonization by Fusarium spp.; reduced rhizosphere fluorescent pseudomonads, Mn-reducing bacteria, and indoleacetic acid–producing rhizobacteria; and reduced shoot and root biomass. Conclusions: Glyphosate applied to GR soybean, regardless of cultivar, negatively impacts the complex interactions of microbial groups, biochemical activity and root growth that can have subsequent detrimental effects on plant growth and productivity. Significance and Impact of the Study: The information presented here will be crucial in developing strategies to overcome the potential detrimental effects of glyphosate in GR cropping systems.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Potassium salt of glyphosate (Roundup WeatherMax®; Monsanto, St Louis, MO), CAS-no.: 70901-12-1
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative"	Not applicable

evidence	

Zobiole et al. (2010)

Title: Glyphosate Affects Seed Composition in Glyphosate-Resistant Soybean	
Author: LUIZ H. S. ZOBIOLE, RUBEM S. OLIVEIRA JR., JESUI V. VISENTAINER, ROBERT J. KREMER, NACER BELLALOUI, AND TSUIOSHI YAMADA	
Reference: J. Agric. Food Chem. 58, 4517–4522	
Year: 2010	
Results and conclusion: The cultivation of glyphosate-resistant (GR) soybeans has continuously increased worldwide in recent years mainly due to the importance of glyphosate in current weed management systems. However, not much has been done to understand eventual effects of glyphosate application on GR soybean physiology, especially those related to seed composition with potential effects on human health. Two experiments were conducted to evaluate the effects of glyphosate application on GR soybeans compared with its near-isogenic non-GR parental lines. Results of the first experiment showed that glyphosate application resulted in significant decreases in shoot nutrient concentrations, photosynthetic parameters, and biomass production. Similar trends were observed for the second experiment, although glyphosate application significantly altered seed nutrient concentrations and polyunsaturated fatty acid percentages. Glyphosate resulted in significant decreases in polyunsaturated linoleic acid (18:2n-6) (2.3% decrease) and linolenic acid (18:3n-3) (9.6% decrease) and a significant increase in monounsaturated fatty acids 17:1n-7 (30.3% increase) and 18:1n-7 (25% increase). The combined observations of decreased photosynthetic parameters and low nutrient availability in glyphosate-treated plants may explain potential adverse effects of glyphosate in GR soybeans.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Isopropylamine salts of glyphosate, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Zobiole et al. (2010)

Title: Glyphosate affects lignin content and amino acid production in glyphosate-resistant soybean
Author: Luiz Henrique Saes Zobiole, Edicléia Aparecida Bonini, Rubem Silvério de Oliveira Jr., Robert John Kremer, Osvaldo Ferrarese-Filho

Reference: Acta Physiol Plant 32:831–837	
Year: 2010	
Results and conclusion: Farmers report that some glyphosate-resistant soybean varieties are visually injured by glyphosate. Glyphosate is the main herbicide that directly affects the synthesis of secondary compounds. In this work, we evaluated the effect of increasing rates of glyphosate on lignin and amino acid content, photosynthetic parameters and dry biomass in the early maturity group cultivar BRS 242 GR soybean. Plants were grown in half-strength complete nutrient solution and subjected to various rates of glyphosate either as a single or in sequential applications. All parameters evaluated were affected by increasing glyphosate rates. The effects were more pronounced as glyphosate rates increased, and were more intense with a single total application than sequential applications at lower rates.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Isopropylamine salts of glyphosate, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Zobiole et al. (2010)

Title: Glyphosate reduces shoot concentrations of mineral nutrients in glyphosate-resistant soybeans
Author: Luiz Henrique Saes Zobiole, Rubem Silvério de Oliveira Jr, Don Morgan Huber, Jamil Constantin, César de Castro, Fábio Alvares de Oliveira, Adilson de Oliveira Jr.
Reference: Plant Soil 328:57–69
Year: 2010
Results and conclusion: Although glyphosate-resistant (GR) technology is used in most countries producing soybeans (Glycine max L.), there are no particular fertilize recommendations for use of this technology, and not much has been reported on the influence of glyphosate on GR soybean nutrient status. An evaluation of different cultivar maturity groups on different soil types, revealed a significant decrease in macro and micronutrients in leaf tissues, and in photosynthetic parameters (chlorophyll, photosynthetic rate, transpiration and stomatal conductance) with glyphosate use (single or sequential application). Irrespective of glyphosate applications, concentrations of shoot macro- and micronutrients were found lower in the near-isogenic GR-cultivars compared to their respective non-GR parental lines Shoot and root dry biomass were reduced by glyphosate with all GR cultivars evaluated in both soils. The lower biomass in GR soybeans compared to their isogenic normal lines probably represents

additive effects from the decreased photosynthetic parameters as well as lower availability of nutrients in tissues of the glyphosate treated plants.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Isopropylamine salt of glyphosate, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2010)

Title: Water use efficiency and photosynthesis of glyphosate-resistant soybean as affected by glyphosate	
Author: Luiz Henrique Saes Zobiole, Rubem Silvério de Oliveira Jr., Robert John Kremer, Jamil Constantin, Carlos Moacir Bonato, Antonio Saraiva Muniz	
Reference: Pesticide Biochemistry and Physiology , 97 182–193	
Year: 2010	
Results and conclusion: Previous studies comparing cultivars of different maturity groups in different soils demonstrated that early maturity group cultivars were more sensitive to glyphosate injury than those of other maturity groups. In this work, we evaluated the effect of increasing rates of glyphosate on water absorption and photosynthetic parameters in early maturity group cultivar BRS 242 GR soybean. Plants were grown in a complete nutrient solution and subjected to a range of glyphosate rates either as a single or sequential leaf application. Net photosynthesis, transpiration rate, stomatal conductance, sub-stomatal CO_2, carboxylation efficiency, fluorescence, maximal fluorescence and chlorophyll content were monitored right before and at different stages after herbicide application; water absorption was measured daily. All photosynthetic parameters were affected by glyphosate. Total water absorbed and biomass production by plants were also decreased as glyphosate rates increased, with the affect being more intense with a single full rate than half the rate applied in two sequential applications. Water use efficiency (WUE) was significantly reduced with increasing rates of glyphosate.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related end-point.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable

Protocol	Not applicable
Test compound	Isopropylamine salt of glyphosate (Roundup Ready®, Monsanto Company), CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2010)

Title: NUTRIENT ACCUMULATION AND PHOTOSYNTHESIS IN GLYPHOSATE-RESISTANT SOYBEANS IS REDUCED UNDER GLYPHOSATE USE	
Author: Luiz Henrique Saes Zobiole, Rubem Silvério de Oliveira Junior, Robert John Kremer, Antonio Saraiva Muniz, and Adilson de Oliveira Junior	
Reference: Journal of Plant Nutrition, 33:1860–1873	
Year: 2010	
Results and conclusion: Global production of glyphosate-resistant (GR) soybean [Glycine max (L.) Merr.] continues to increase annually; however, there are no particular specific fertilizer recommendations for the transgenic varieties used in this system largely because reports of glyphosate effects on mineral nutrition of GR soybeans are lacking. Several metabolites or degradation products of glyphosate have been identified or postulated to cause undesirable effects on GR soybeans. In this work we used increasing glyphosate rates in different application on cv. 'BRS 242 GR' in order to evaluate photosynthetic parameters, macro- and micronutrient uptake and accumulation and shoot and root dry biomass production. Increasing glyphosate rates revealed a significant decrease in photosynthesis, macro and micronutrients accumulation in leaf tissues and also decreases in nutrient uptake. The reduced biomass in GR soybeans represents additive effects from the decreased photosynthetic parameters as well as lower availability of nutrients in tissues of the glyphosate treated plants.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Isopropylamine salt of glyphosate, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative"	Not applicable

evidence	

Zobiole et al. (2010)

Title: Glyphosate affects photosynthesis in first and second generation of glyphosate-resistant soybeans	
Author: Luiz Henrique Saes Zobiole, Robert John Kremer, Rubem Silvério de Oliveira Jr, Jamil Constantin	
Reference: Plant Soil 336:251–265	
Year: 2010	
Results and conclusion: The crop area planted to conventional soybeans has decreased annually while that planted to glyphosate-resistant (RR) soybean has drastically increased mainly due to the wide adoption of glyphosate in current weed management systems. With the extensive use of glyphosate, many farmers have noted visual plant injury in RR soybean varieties after glyphosate application. A new generation designated as "second generation-RR2" has been recently developed and these RR2 cultivars already are commercially available for farmers and promoted as higher yielding relative to the previous RR cultivars. However, little information is currently available about the performance of RR2 soybean beyond commercial and farmer testimonial data. Thus, an evaluation of different glyphosate rates applied in different growth stages of the first and second generation of RR soybeans, revealed a significant decrease in photosynthesis. In general, increased glyphosate rate and late applications (V6) pronounced decrease photosynthetic parameters and consequently decreased in leaf area and shoot biomass production. In contrast, low rate and early applications were less damage for the RR soybean plants, suggesting that with early applications (V2), plants probably have more time to recover from glyphosate or its metabolites effects regarding late applications.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Potassium salt of glyphosate (Roundup Weather Max®, Monsanto Company), CAS-no.: 70901-12-1
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2011)

Title: Glyphosate affects chlorophyll, nodulation and nutrient accumulation of ''second generation'' glyphosate-resistant soybean (Glycine max L.)

Author: Luiz H.S. Zobiole, Robert J. Kremer, Rubem S. Oliveira Jr., Jamil Constantin	
Reference: Pesticide Biochemistry and Physiology 99, 53–60	
Year: 2011	
Results and conclusion: The recently developed ‘‘second generation’’ of Roundup Readysoybean (RR2) cultivars commercially available for farmers in 2008 were promoted as higher yielding relative to the ‘‘first generation’’ RR cultivars (RR1). Previous studies showed that glyphosate reduced such yield components as photosynthesis, water absorption, nutrient uptake and symbiotic N2 fixation in RR soybean cultivars; however, no data are available regarding the glyphosate effects on these physiological factors in RR2 soybean. Thus, the objective of this research was to evaluate the nutrient accumulation and nodulation of both generations of RR soybeans at different rates of glyphosate applied at various growth stages. In general, increased glyphosate rates and late applications decreased the nutrient accumulation, nodulation, and shoot and root biomass in both RR1 and RR2. All macro- and micronutrients, with exception of N and K, accumulated more in RR1 than RR2. Although this result may be an individual cultivar characteristic, it suggests that the RR2 cultivar was also inefficient in nutrient uptake and translocation or was unable to rapidly recover from potential chelating effects of glyphosate. These studies suggest that applying glyphosate at early growth stages using the lowest glyphosate rate might have less damage on growth and productivity of RR soybeans.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Potassium salt of glyphosate (Roundup Weather Max®, Monsanto Company) , CAS-no.: 70901-12-1
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Zobiole et al. (2011)

Title: Prevention of RR Soybean Injuries Caused by Exogenous Supply of Aminoacids
Author: ZOBIOLE, L.H.S., OLIVEIRA JR., R.S., CONSTANTIN, J., BIFFE, D.F.
Reference: Planta Daninha, Viçosa-MG, v. 29, n. 1, p. 195-205
Year: 2011
Results and conclusion: Glyphosate-resistant (RR) soybean crop areas have expanded every year. However, as a result of this expansion, the use of glyphosate has significantly increased, with the appearance of visual injuries in RR soybeans immediately after post-emergence application of the herbicide. Thus, two experiments were conducted in different years with different objec-

tives. The first experiment aimed to evaluate the influence of glyphosate on photosynthetic variables and biomass production. The second experiment aimed to re-evaluate the same parameters affected in RR soybeans by glyphosate, as well as the use of various methods of amino acid application, as a form of a likely recovery of the soybean plants following these exogenous applications. The photosynthetic rate and SPAD index decreased as the glyphosate rate increased, with a pronounced decrease after a single herbicide application. Overall, due to a decrease in the photosynthetic rate and chlorophyll production, as well as to a likely immobilization of shoot nutrient concentration by glyphosate, a significant biomass decrease was verified in the treatments with glyphosate application. However, the use of exogenous amino acids may be a strategy to safeguard the undesirable effects of this herbicide on RR soybean.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Isopropylammmonium salt of glyphosate, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Zobiole et al. (2010)

Title: Use of Exogenous Amino Acid to Prevent Glyphosate Injury in Glyphosate-Resistant Soybean
Author: ZOBIOLE, L.H.S., OLIVEIRA JR., R.S., CONSTANTIN, J., BIFFE, D.F., KREMER, R.J.
Reference: Planta Daninha, Viçosa-MG, v. 28, n. 3, p. 643-653
Year: 2010
Results and conclusion: Cultivation of glyphosate-resistant (GR) soybeans has increased in Brazil as a result of the application of this technology in weed management systems developed for this crop. However, the expansion of GR soybean production has significantly increased the use of glyphosate and, in some cases, resulted in injury symptoms observed in GR soybean, known as “yellow flashing” or yellowing of the upper leaves. Thus, two experiments were conducted in different years. The first experiment aimed to evaluate the influence of glyphosate on GR soybeans regarding the photosynthetic variables, nodule parameters, and shoot and root dry biomass by comparing cultivar BRS 242 GR without glyphosate and BRS 242 RR + glyphosate at 1.200 g/ha at V4 growth stage, to the near isogenic non-GR parental line cv. Embrapa 58. The second experiment aimed to reassess the same parameters in GR soybeans at the V4 stage treated with glyphosate, plus the application of various amino acids, to evaluate the expected recovery of soybean growth under the exogenous use

of supplemental amino acids. In general, the photosynthetic variables, nodulation parameters and shoot and root dry biomass were affected by glyphosate; however, the use of amino acids may be a strategy to prevent the undesirable effects of this herbicide on GR soybean.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Zobiole et al. (2010)

Title: Effect of glyphosate on symbiotic N_2 fixation and nickel concentration in glyphosate-resistant soybeans	
Author: L.H.S. Zobiole, R.S. Oliveira Jr., R.J. Kremer b, J. Constantin, T. Yamada, C. Castro, F.A. Oliveira, A. Oliveira Jr.	
Reference: Applied Soil Ecology 44, 176–180	
Year: 2010	
Results and conclusion: Decreased biological nitrogen fixation in glyphosate-resistant (GR) soybeans has been attributed directly to toxicity of glyphosate or its metabolites, to N_2-fixing microorganisms. As a strong metal chelator, glyphosate could influence symbiotic N_2 fixation by lowering the concentration of nickel (Ni) that is essential for the symbiotic microorganisms. Evaluation of different cultivars grown on different soil types at the State University of Maringá, PR, Brazil during the summer 2008 revealed, significant decreases in photosynthetic parameters (chlorophyll, photosynthetic rate, transpiration and stomatal conductance) and nickel content with glyphosate use (single or sequential application). This work demonstrated that glyphosate can influence the symbiotic N_2 fixation by lowering nickel content available to the symbiotic microorganisms.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Isopropylamine salts of glyphosate, CAS-no.: 38641-94-0

Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2012a)

Title: AMINO ACID APPLICATION CAN BE AN ALTERNATIVE TO PREVENT GLYPHOSATE INJURY IN GLYPHOSATE-RESISTANT SOYBEANS	
Author: L. H. S. Zobiole, R. S. de Oliveira Jr., J. Constantin, R. J. Kremer and D. F. Biffe	
Reference: Journal of Plant Nutrition, 35:268–287, 2012	
Year: 2012	
Results and conclusion: Glyphosate-resistant (GR) soybeans have continuously increased; however, this expansion significantly increased the use of glyphosate and therefore, in some cases, has resulted in injury symptoms observed in GR soybean, known as "yellow flashing". Previous reports of interference of glyphosate with nutrient availability and utilization by GR soybean may be linked to this injury symptom. Also, because glyphosate interferes with amino acid synthesis, supplementation with exogenous amino acids may help GR soybean recover from adverse effects of glyphosate. Therefore, an experiment was designed to evaluate different amino acid concentrations. Near-isogenic and GR soybean varieties were grown in the greenhouse in two soils with and without glyphosate at different rates and amino acids were foliarly applied with and without glyphosate. In general, the photosynthetic variables, nutrient contents, and shoot and root dry biomass parameters were affected by glyphosate, however, use of amino acid formulations suppressed harmful effects of glyphosate on these parameters.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Isopropylamine salts of glyphosate (480 g a.e. L^{-1})
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2012b)

Title: Glyphosate effects on photosynthesis, nutrient accumulation, and nodulation in glyphosate-resistant soybean	
Author: L. H. S. Zobiole, R. J. Kremer, R. S. de Oliveira Jr., and J. Constantin	
Reference: J. Plant Nutr. Soil Sci. 2012, 175, 319–330	
Year: 2012	
Results and conclusion: Previous greenhouse studies have demonstrated that photosynthesis in some cultivars of first-(GR1) and second-generation (GR2) glyphosate-resistant soybean was reduced by glyphosate. The reduction in photosynthesis that resulted from glyphosate might affect nutrient uptake and lead to lower plant biomass production and ultimately reduced grain yield. Therefore, a field study was conducted to determine if glyphosate-induced damage to soybean (Glycine max L. Merr. cv. Asgrow AG3539) plants observed under controlled greenhouse conditions might occur in the field environment. The present study evaluated photosynthetic rate, nutrient accumulation, nodulation, and biomass production of GR2 soybean receiving different rates of glyphosate (0, 800, 1200, 2400 g a.e. ha^{-1}) applied at V2, V4, and V6 growth stages. In general, plant damage observed in the field study was similar to that in previous greenhouse studies. Increasing glyphosate rates and applications at later growth stages decreased nutrient accumulation, nodulation, leaf area, and shoot biomass production. Thus, to reduce potential undesirable effects of glyphosate on plant growth, application of the lowest glyphosate rate for weed-control efficacy at early growth stages (V2 to V4) is suggested as an advantageous practice within current weed control in GR soybean for optimal crop productivity.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Potassium salts of glyphosate 540 g a.e. L^{-1} (Roundup Weather Max®, Monsanto Company)
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2012c)

Title: Nutrient Accumulation in Conventional and Glyphosate-Resistant Soybean under Different Types of Weed Control
Author: ZOBIOLE, L.H.S., OLIVEIRA JR., R.S., CONSTANTIN, J., OLIVEIRA JR., A., CASTRO, C., OLIVEIRA, F.A., KREMER, R.J., MOREIRA, A. and ROMAGNOLI, L.M
Reference: Planta Daninha, Viçosa-MG, v. 30, n. 1, p. 75-85, 2012
Year: 2012

Results and conclusion: The cultivation of soybean-Glycine max (Roundup Ready® – RR) has increased and little has been reported on the influence of glyphosate on the nutritional status of the plants. The aim of this work was to compare nutrient accumulation at different phenological stages between the cultivars BRS 184 (conventional) and BRS 243 RR (transgenic), with the same crop cycle, under different weed management systems (hand weed and herbicide). Nutrient accumulation and dry matter in conventional soybean was superior to that in the glyphosate-treated RR soybean, indicating that a higher level of nutrients might be required for the RR cultivars to achieve physiological efficiency and a new fertilizer recommendation for RR crops may be considered, due to the reduced nutritional efficiency imposed by glyphosate.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	-
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

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Renewal Assessment Report

18 December 2013

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29 January 2015
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Glyphosate

Volume 3

Annex B.9

Ecotoxicology

RMS: Germany **Co-RMS: Slovakia**

Version history (Volume 3, chapter B.9)

Date	**Reason for revision**
19 December 2013	Initial Renewal Assessment Report
29 January 2015	Updated following RAR peer review, taking into account the reporting table, the evaluation table and additional data
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B.9 Ecotoxicology

B.9.1 Effects on birds (Annex IIA 8.1; Annex IIIA 10.1)

B.9.1.1 Acute oral toxicity

Birds are exposed to residues of glyphosate on their food items following spraying of the formulated product.

According to current data requirements in Commission Regulation (EC) No 1107/2009 of 21 October 2009 amending Council Directive 91/414/EEC the acute oral toxicity of an active substance to a quail species (Japanese quail, *Coturnix coturnix japonica* or bobwhite quail, *Colinus virginianus*) or to mallard duck (*Anas platyrhynchos*) must be determined. New studies not evaluated during the first evaluation of glyphosate in the EU peer review of 2001, have been included ([redacted] 1997, BVL no 2310906; [redacted] 1996, BVL no 2310909; [redacted] 1996, BVL no 2310912; [redacted] 1999, BVL no 2310910; [redacted] 2003, BVL no 2310915).

KIIA 8.1.1/01

Author:	[redacted]
Title:	Glyphosate acid. Acute oral toxicity (LD_{50}) to bobwhite quail
Date:	03.02.1997
Doc ID:	2310906 /ISN 400/963858
Guidelines:	US EPA Guideline, Section E, Series 71.1, Avian single dose oral LD_{50} test (1982)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	P24
Purity:	95.6 %
2. Vehicle and/or positive control:	Methylcellulose (1% w/v) as vehicle and negative control
Species:	Bobwhite quail (*Colinus virginianus*)
Age:	Young adults, approximately 4 - 6 month old on arrival
Weight:	175 - 213 g (15 days prior to test initiation)
Source:	[redacted]
Diet/Food:	Standard HRC layer diet in pellet form obtained from Parker Brothers Ltd. (Lark Mills, Mildenhall, Suffolk, UK). Food was offered ad libitum, with the exception of an overnight starvation period of approximately 21 hours prior to dosing. Water was available at all times.
Acclimatisation:	15 days
Temperature:	17 – 19 °C
Relative humidity:	68%
Photoperiod:	10 hours light / 14 hours darkness

Methods:	Young adult Bobwhite quails (5 males and 5 females per dosage) received a single dose of the test substance or vehicle by oral intubation using a disposable syringe and a Ch 10 Nelaton plastic catheter. The test consisted of three dosage groups and a control group. Nominal dosages used in the study were 500, 1000 and 2000 mg a.s./kg bw (dosage concentrations: 5%, 10% and 20% w/v). A constant dose volume of 10 mL/kg bodyweight was used for all treatment groups. The control birds received an equivalent volume of methylcellulose only. During the test all mortalities, bird health and clinical signs of the birds were observed daily. Body weights were measured individually 15 and 7 days prior to test start, at the initiation of the test (immediately prior to dosing) and on days 7, and 14 of the test. Feed consumption was determined by cage of each dosage group and the control group 15, 8, 7 and 1 day(s) prior to test start and on days 1 to 7 and 8 to 14 of the test. Post mortem examination was carried out on all ten control birds and all ten birds from the highest dose group. Since no mortality was reported, no statistical calculation of LD50 values was possible. The NOEC was determined by visual interpretation of the mortality and observation data.

Results

Evaluation according to current OECD 223 test validity criteria was performed. Validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups.

There were no mortalities observed in any treatment and no clinical signs of toxicity were observed. Body weight changes were similar in all groups and there was no evidence of any treatment-related effects. Group mean food consumption was similar in all groups and there was no evidence of any treatment-related effects. No abnormalities were detected in any birds during *post mortem* examination at termination of the study.

Table B.9.1-1: Effects of glyphosate acid on body weight and food consumption of bobwhite quail

Glyphosate acid (mg a.s./kg bw)			**Control**	**500**	**1000**	**2000**
Average body weight per animal (g) (± SD)						
Body weight	Day -15	male	192 ± 5.9	195 ± 5.9	192 ± 3.7	195 ± 4.9
		female	191 ± 11.4	191 ± 15.6	191 ± 13.3	190 ± 8.9
	Day -7	male	196 ± 5.7	196 ± 6.5	194 ± 4.1	198 ± 5.6
		female	190 ± 10.2	190 ± 18.2	192 ± 7.8	189 ± 11.6
	Day 0	male	194 ± 4.7	197 ± 6.9	193 ± 4.8	198 ± 5.9
		female	190 ± 9.1	189 ± 17.1	192 ± 10.6	186 ± 10.5
	Day 7	male	198 ± 2.5	199 ± 6.1	196 ± 4.3	198 ± 8.8
		female	192 ± 13.0	192 ± 18.9	197 ± 13.3	191 ± 9.7
	Day 14	male	200 ± 2.3	199 ± 4.9	196 ± 3.8	196 ± 7.0
		female	192 ± 8.6	194 ± 17.0	198 ± 10.6	189 ± 9.5
Body weight change	Days 0-14	male	6.0 ± 2.4	2.0 ± 2.0	3.0 ± 1.0	-2.0± 1.1
		female	2.0 ± 0.5	5.0 ± 0.1	6.0 ± 0.0	3.0 ± 1.0

Glyphosate acid (mg a.s./kg bw)			**Control**	**500**	**1000**	**2000**
Mean food consumption per animal (g/bird/day)						
Food consumption	Day -15 to -8	male	13	13	12	13
		female	13	13	12	13
	Day -7 to -1	male	13	13	12	13
		female	13	13	13	13
	Day 1 to 7	male	14	15	14	13
		female	16	15	15	15
	Day 8 to 14	male	14	14	14	13
		female	15	13	14	14
Group mean	Day 1-14	male	14	14.5	14	13
		female	15.5	14	14.5	14.5

RMS Conclusions

The acute oral LD_{50} for bobwhite quail exposed to glyphosate acid was determined to be > 2000 mg a.s./kg bw. The NOEL in the study was determined to be 2000 mg a.s./kg bw.
The study is considered to be acceptable and valid.

KIIA 8.1.1/02

Author:	[redacted]
Title:	Glyphosate: Acute oral toxicity to Japanese quail
Date:	23.07.1996
Doc ID:	2310909 / 1413/4-1011
Guidelines:	US EPA Guideline, Section E, Series 71.1, Avian single dose oral LD_{50} test (1982)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Description:	White/off-white crystalline powder
Lot/Batch #:	H95 D161A
Purity:	95.3 %
Vehicle and/or positive control:	0.5% carboxymethyl cellulose (CMC) as vehicle and negative control
Species:	Japanese quail (Coturnix coturnix japonica)
Age:	Young adults, approx. 23 weeks old
Weight:	202 - 300 g (at test initiation)
Source:	[redacted]
Diet/Food:	Proprietary avian food, *ad libitum*
Acclimation period:	5 weeks prior to dosing
Fasting	16 to 17 hours prior to dosing
Temperature:	15 – 20°C

Humidity:	40 – 78%
Photoperiod:	8 hours light / 16 hours dark
Methods:	Based on the results of a range finder study, an acute oral toxicity test was performed by administering a single limit dose of 2000 mg glyphosate a.s./kg bw (dissolved in 0.5% carboxymethyl cellulose) by oral intubation to ten adult Japanese quails (5 males and 5 females)in one treatment group. In addition, one control group was administered an equivalent volume of the vehicle (CMC) only as the test groups, at a dose rate of 2 mL/kg bw. After dosing, birds where fed ad libitum throughout the study. Birds were caged and observed continuously for signs of toxicity, abnormal behaviour and mortality for one hour after dosing, then at intervals throughout day 0 and twice daily thereafter. Food consumption was measured covering day 0-7, and day 7-14. Each animal was weighed at least on day 0, 3,7 and 14. On day 14, all surviving animals were sacrificed and a gross macroscopic examination was carried out. The necropsy comprised a general inspection of major visceral organs. Since the mortality was < 50%, no statistical calculation of LC50 values was possible. The NOEC was determined by visual interpretation of the mortality and observation data.

Results

Evaluation according to current OECD 223 test validity criteria was performed. Validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups.

There was no treatment-related mortality, except for one bird in treatment group found dead due to trauma of the reproductive tract. Furthermore, no adverse effects were observed on bodyweight or food intake. No findings at necropsy were considered to be treatment-related.

Table B.9.1-2: Effects of glyphosate acid on body weight and food consumption of Japanese quail

Glyphosate acid (mg a.s./kg bw)			**Control**	**2000**
Average body weight per animal (g) (± SD)				
Body weight	Day 0	male	249 ± 27.1	228 ± 22.3
		female	257 ± 15.3	260 ± 28.0
	Day 3	male	270 ± 31.4	231 ± 22.2
		female	268 ± 18.5	272 ± 36.1
	Day 7	male	275 ± 31.8	239 ± 17.3
		female	271 ± 18.5	271 ± 32.8
	Day 14	male	276 ± 33.5	243 ± 18.5
		female	276 ± 18.2	288 ± 28.7
Body weight change	Days 0-14	male	26 ± 12	15 ± 5.7
		female	19 ± 13.6	23 ± 3.0
Mean food consumption per animal (g/bird/day)				
Food consumption	Day 0-7	male	64.4	39.9
		female	56.1	60.9
	Day 7-14	male	50.0	41.8
		female	58.0	67.9
Group mean	Day 0-14	mean	57.2	52.0

RMS Conclusions

The acute oral LD_{50} for Japanese quail exposed to glyphosate acid was determined to be > 2000 mg a.s./kg bw. The NOEL in the study was determined to be 2000 mg a.s./kg bw.
The study is considered to be acceptable and valid.

KIIA 8.1.1/03

Author:	[redacted]
Title:	Avian single-dose acute oral toxicity test in Japanese quail with the chemical product Glifosate Técnico Nufarm
Date:	20.12.1999
Doc ID:	2310910 / D.8.1 - 382/99
Guidelines:	Not stated (The OECD guideline number is not explicitly mentioned but it is highly probable that the study was carried out according to OECD 223.)
GLP:	NO
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	037-919-113
Purity:	95 % (nominal) 954.9 g/kg acid equivalent (measured)
2. Vehicle and/or positive control:	Gelatin capsules as vehicle and negative control
Species:	Japanese quail (*Coturnix coturnix japonica*)
Age:	Young adults, at least 16 weeks old
Weight:	Males: 100 – 130 g at test start Females: 114 – 140 g at test start
Source:	[redacted]
Diet/Food:	Commercial diet (GUABI ration) and water ad libitum.
Acclimatisation:	At least 15 days
Temperature:	25 – 28 °C
Relative humidity:	30 – 70 %
Photoperiod:	10 hours light / 14 hours dark
Methods:	Young adult Japanese quails (5 males and 5 females per treatment) received a single limit dose of 2000 mg/kg bw of the test substance, enclosed in gelatin capsules. A control group received empty capsules by oral gavage. During the 15 days of the test, mortality, behaviour, clinical symptoms and anatomopathological alterations were observed daily. Birds were weighed at the beginning and at the end of test. Since no mortality was reported, no statistical calculation of LD50 values was possible. The NOEC was determined by visual interpretation of the mortality and observation data.

Results

Evaluation according to current OECD 223 test validity criteria was performed. All validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups.

There were no mortalities observed in any treatment. All control and test birds remained in good health following dosing, and no clinical signs of toxicity were observed. Body weight changes were similar in all groups and there was no evidence of any treatment-related effects. Group mean food consumption was similar in all groups and there was no evidence of any treatment-related effects. No abnormalities were detected in any birds during *post mortem* examination at termination of the study.

Table B.9.1-3: Effects of glyphosate acid on body weight and food consumption of Japanese quail

Glyphosate acid (mg a.s./kg bw)			**Control**	**2000**
Average body weight per animal (g) (± SD)				
Body weight	Day 0	male	109 ± 9.3	123 ± 5.3
		female	121 ± 5.8	116 ± 3.9
	Day 7	male	113 ± 11.1	121 ± 6.9
		female	122 ± 10.2	114 ± 9.9
	Day 14	male	119 ± 9.5	126 ± 6.9
		female	130 ± 9.6	123.8 ± 7.6
Body weight change	Days 0-14	male	10.2 ± 5.0	3.4 ± 5.5
		female	8.8 ± 7.4	7.8 ± 6.3
Mean food consumption per animal (g/bird/day)				
Food consumption	Day 0-7		88.7	99.4
	Day 7-14		77.2	99.6
Group mean	Day 0-14	mean	83.0	99.5

RMS Conclusions

The acute oral LD_{50} for Japanese quail exposed to glyphosate acid was determined to be > 2000 mg a.s. /kg bw. The NOEL in the study was determined to be 2000 mg a.s./kg bw.
The study is considered to be acceptable and valid.

KIIA 8.1.1/04

Author:	[redacted]
Title:	Glyphosate: Acute oral toxicity to Mallard Duck
Date:	18.07.1996
Doc ID:	2310912 /1413/5-1011
Guidelines:	US EPA Guideline, Section E, Series 71.1, Avian single dose oral LD_{50} test (1982)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	H95 D161A
Purity:	95.3 % w/w

2.Vehicle and/or positive control:	0.5% carboxymethyl cellulose (CMC) (vehicle)
Species:	Mallard duck (*Anas platyrhynchos*)
Age:	Young adults, approx. 23 weeks old
Sex	Males and females
Weight	903 - 1114 g (at test initiation)
Source:	[redacted]
Loading	Approx. 4.5 m^2 for 5 birds
Diet/Food:	Proprietary avian food, *ad libitum*
Acclimation period:	5 weeks prior to dosing
Fasting	16 to 17 hours prior to dosing
Temperature:	15 – 22°C
Humidity:	42 – 74 %
Photoperiod:	14 hours light / 10 hours dark
Methods:	Based on the results of a range finding study, an acute oral toxicity test was performed as a limit test by administering a single limit dose of 2000 mg a.s./kg bw. (dissolved in 0.5% carboxymethyl cellulose) by direct intubation to ten juvenile Mallard ducks (5 males and 5 females)in one treatment group. In addition, one control group comprising 5 males and 5 females was administered an equivalent volume of the vehicle (CMC) only, at a dose rate of 2 mL/kg bw. After dosing, birds where fed ad libitum throughout the study. Birds were caged and observed for signs of toxicity, abnormal behaviour and mortality continuously for one hour after dosing, then at intervals throughout day 0 and twice daily thereafter. Food consumption was measured per time interval, covering day 0-7, and day 7-14.Each animal was weighed at least on day 0, 5, 11 and 14. On day 14, all surviving animals were sacrificed and a gross macroscopic examination was carried out. The necropsy comprised a general inspection of major visceral organs. Since no mortality was reported, no statistical calculation of LD50 values was possible. The NOEC was determined by visual interpretation of the mortality and observation data.

Results

Evaluation according to current OECD 223 test validity criteria was performed. All validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups.

No mortalities and no post-dosing signs of toxicity were observed in any treatment and all animals remained in good health throughout the study. Furthermore, the body weight was not adversely affected by the treatment. Similarly, there were no treatment-related effects on food consumption and no abnormalities were detected at necropsy of the animals 14 days after treatment.

Table B.9.1-4: Effects of glyphosate acid on body weight and food consumption of mallard duck

Glyphosate acid (mg a.s./kg bw)			**Control**	**2000**
Average body weight per animal (g) (± SD)				
Body weight	Day 0	male	1011 ± 41.5	1012 ± 76.4
		female	1072 ± 128.4	1018 ± 81.1
	Day 5	male	1101 ± 33.5	1048 ± 49.6
		female	1170 ± 160.0	1082 ± 60.8
	Day 11	male	1096 ± 54.8	1052 ± 69.6
		female	1191 ± 155.0	1175 ± 41.4
	Day 14	male	1104 ± 51.8	1053 ± 65.9
		female	1171 ± 122.6	1156 ± 66.5
Body weight change	Days 0-14	male	93 ± 30.9	42 ± 12.2
		female	99 ± 86.0	138 ± 110.8
Mean food consumption per animal (g/bird/day)				
Food consumption	Day 0-7	male	79	80
		female	131	121
	Day 7-14	male	72	76
		female	130	138

RMS Conclusions

The acute oral LD_{50} for Mallard duck exposed to glyphosate acid was determined to be > 2000 mg a.s./kg bw. The NOEL was determined to be 2000 mg a.s./kg bw.
The study is considered to be acceptable and valid.

KIIA 8.1.1/05

Author:	[redacted]
Title:	MON78623: An acute oral toxicity study with the Northern Bobwhite
Date:	10.10.2003
Doc ID:	2310915 /WL-2002-151
Guidelines:	OPPTS 850.2100, FIFRA subdivision E, Section 71-1
GLP:	NO
Validity:	YES

Materials and Methods

Test item:	MON 78623
Lot/Batch #:	GLP-0108-11688-F
Purity:	47.7 % glyphosate acid
Vehicle and/or positive control:	Deionised water
Species:	Northern bobwhite, Bobwhite quail (*Colinus virginianus*)
Age:	Young adults, 30 weeks
Sex	5 male, 5 female per treatment/ control group

Weight	176 - 248 g (at test initiation)
Source:	[redacted]
Diet/Food:	Game bird ration, *ad libitum* during acclimation and during the test, 18 h fasting prior to test start. Birds were given water soluble antibiotic in their drinking water for seven days after arrival in the laboratory.
Loading:	5 male and 5 female / pen, Pen size: 58 x 71 cm
Acclimation period:	Approx. 4 months
Fasting	18 hours prior to dosing
Temperature:	22.0 ± 0.2 °C
Relative humidity:	43 % ± 8 %
Photoperiod:	8 h light, 16 h dark
Methods:	In an acute oral toxicity test, bobwhite quail were given nominal doses of 291, 484, 807, 1344 and 2241 mg glyphosate acid equivalent/kg bw by oral gavage and observed the following 14 d for mortality, clinical signs of toxicity, abnormal behaviour, body weight change and feed consumption. Ten quails (5 male, 5 female) were assessed per dose and control group. The control group was given diluent only. After test initiation, birds were observed twice daily for mortality, clinical signs of toxicity and abnormal behaviour. Body weights were measured at study initiation and after 3, 7 and 14 d. Average feed consumption was determined by pen for each group for day 0 – 3, 4 – 7 and 8 – 14, by measuring the weight change of the presented feed. Since the mortality was < 50 %, no statistical calculation of LC_{50} values was possible. The NOEC was determined by visual interpretation of the mortality and observation data.

Results

According to OECD guideline this study is designed to have birds housed individually and housing conditions should be within optimal limits for the test species and minimum recommended space per bird is 1000 cm^2 for quail. In the present study the loading was 10 birds 58 x 71 cm, probably leading to unfavourable conditions to test animals.

One control male suffered a leg injury during body weight procedures and lost weight afterwards. Nevertheless, there were no mortalities in the control group. There were no mortalities in any treatment group either. During the course of the test foot lesions with associated lameness and/or ruffled appearance were noted for two birds and 484 mg a.e./kg dosage, for two birds and 807 mg a.e. /kg dosage, 5 birds and 1344 mg a.e./kg dosage and two birds at 2241 mg a.e./kg dosage. A treatment related effect could not be precluded for these dosages. Treatment related loss in mean body weight was noted at 2241 mg a.e./kg dosage from day 0 to day 3.

Table B.9.1-5: Effects of glyphosate K-salt on body weight, food consumption of Northern bobwhite quail

Glyphosate acid equivalent (mg/kg bw)	**Control**	**291**	**484**	**807**	**1344**	**2241**
Mortality						
Day 14	0	0	0	0	0	0
Clinical signs						
Ruffled appearance	0	0	2[1]	4*	5*	2*
Lethargy	0	0	0	0	1	1

Glyphosate acid equivalent (mg/kg bw)	**Control**	**291**	**484**	**807**	**1344**	**2241**
Mean body weight (g) (male/female)						
Day 0	224/197	221/208	222/207	219/206	219/221	225/212
Day 14	221/201	224/209	223/210	221/209	221/226	223/216
Feed consumption (g) (male/female)						
Day 0 - 3	31/15	28/21	27/26	18/23	21/15	17/23
Day 4 - 7	28/21	29/22	23/26	20/24	23/23	25/28
Day 8 - 14	25/16	24/17	19/20	21/20	20/18	17/18

[1] Not considered to be treatment related due to the timing and isolated nature of the signs noted.

* A treatment related effect could not be precluded for these clinical signs.

RMS Conclusions

The validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups. However it should be considerd in the present study that the housing conditions were not within optimal limits for the test species.

The acute LD_{50} for northern bobwhite exposed to glyphosate K-salt was determined to be > 2241 mg glyphosate acid equivalent/kg bw (nominal). The NOEC was determined to be 484 mg glyphosate acid equivalent/kg bw (nominal).

B.9.1.2 Dietary toxicity

The assessment of short term toxicity data for birds is not considered to be generally necessary following the guidance document on Risk Assessment for Birds and Mammals (EFSA, 2009), in particular if there are no indications that the dietary LD_{50} will be lower than the LD_{50} based on an acute oral study. The 5-day dietary toxicity of technical glyphosate was tested in bobwhite quail, Japanese quail and mallard duck during the first EU peer reviewed evaluation in 2001 and considered to be > 4640 ppm for glyphosate technical. In none of these studies any effects were reported. AMPA, the main metabolite of glyphosate, also caused no effects up to the top concentration 5620 ppm (DAR Glyphosat B-8:Ecotoxicolocy 1998 and Review Report 6511/IV/99-final, 21 January 2002).

~~**Author:**~~	[redacted]
~~**Title:**~~	~~Eight-day dietary LC50 – bobwhite quail technical CP67573~~
~~**Date:**~~	~~29.05.1973~~
~~**Doc ID:**~~	~~2243258 / HL-73-76 ! 241-106~~
~~**Guidelines:**~~	~~-~~
~~**GLP:**~~	~~-~~
~~**Validity:**~~	~~-~~

~~**Author:**~~	[redacted]
~~**Title:**~~	~~Eight-day dietary LC50 – mallard ducks technical CP67573~~
~~**Date:**~~	~~9.05.1973~~
~~**Doc ID:**~~	~~2243257 / HL-73-15 ! 241-107~~
~~**Guidelines:**~~	~~-~~
~~**GLP:**~~	~~-~~
~~**Validity:**~~	~~-~~

B.9.1.3 Subchronic toxicity and reproduction

KIIA 8.1.4/01

Author:	[redacted]
Title:	Glyphosate Acid: A reproduction study with the Northern Bobwhite (*Colinus virginianus*)
Date:	13.05.1999
Doc ID:	2310916 /123-186
Guidelines:	FIFRA Guideline 71-4 OECD Guideline 206
GLP:	YES
Validity:	NO

Materials and Methods

Test item:	Glyphosate acid
Purity:	95.6 %
Positive control:	None
Species:	Bobwhite quail (*Colinus virginianus*)
Age:	Young adults, 30 weeks (at test initiation)
Sex	Males and females
Weight	196 to 250 g (at test initiation)
Source:	[redacted]
Loading	Approx. 0.138 m^2 for 2 birds (1 males and 1 female per pen)
Feed/Diet:	Game bird ration, *ad libitum*
Acclimation period:	10 weeks
Temperature:	23.1 ± 1.8 °C (adults); 27.3± 1.2 °C (hatchling) 38 °C (brooding compartment)
Humidity:	66 ± 12 % (adults); 40 ± 17 % (hatchling)
Photoperiod:	17 hours light / 7 hours dark, (approx. 265 lux), 8 hours light / 16 hours dark (first seven weeks)
Methods:	A reproductive toxicity study was performed by feeding adult bobwhite quail ad libitum on a series of 3 nominal dietary doses, encompassing 0, 500, 1000 and 2250 mg /kg diet. Sixteen replicates (1 male and 1 female per pen) were used for each treatment group and control. The birds were exposed to the treated diets for approximately 20 weeks and were evaluated for treatment-related effects upon bird health and reproduction. Eggs were collected daily and stored at 13.6 ± 0.6 °C and 82 ± 8 % relative humidity. All eggs laid within a week were considered as one lot and incubated in a Petersime Incubator. On day 21 of incubation, eggs were placed in a Petersime Hatcher and allowed to hatch. The hatchlings were maintained on untreated diet until 14 days of age. Homogeneity of the test substance in treated diets was evaluated by collecting 6 samples of each treatment group on day 0 of week 1. During weeks 2, 3, 4, 8, 12, 16 and 20 of the test, a single sample was collected from the control diet and an additional duplicate sample was collected from treatment group diet, to measure and/ or verify test concentrations. Adult birds were observed daily for signs of toxicity and abnormal behaviour throughout the study. Adult body weight was measured at study initiation and termination, in addition to on weeks 2, 4, 6, and 8. For each pen, food consumption was measured weekly throughout the study except for the last interval, where food consumption was measured over a 6 day period. At the end of each week, all collected eggs were counted and a single egg was randomly selected for eggshell thickness

	measurements. The remaining eggs were candled to detect egg shell cracks or abnormal eggs before incubation. During the incubation period, eggs were candled again on day 11 or 12 to evaluate embryo viability and on day 21 to determine embryo survival. During the study, total egg production, number of eggs cracked, eggshell thickness, embryo viability, embryo survival, number of hatchlings, body weight of new hatchlings, body weight of 14 day old hatchlings and survivorship of 14 day old hatchlings were determined. An analysis of variance (ANOVA) was used to determine significant differences among the groups followed by Dunnett's multiple comparison procedure as the post-hoc test.

Results

Evaluation according to current OECD 206 test validity criteria was performed. According to OECD 206 the age of the test organsims should range between 20-24 weeks.
Adult bobwhite quail birds in the present study were 30 weeks. Moreover, the test guideline recommends a minimun floor area of pen per pair of 0.25 m^2, whereas in the present study the loading was 2 birds on 0.138 m^2, probably leading to unfavourable conditions to test animals. For the test to be valid, the mortality in the controls should not exceed 10 % at the end of the test. At start of week 19, 12 mortalities occurred, all of which were hens. Of the 12 mortalities, six occurred in the control group and two occurred in each of the three treatment groups, probably due to limited space leading to stress.

RMS Conclusions

Validity criteria according to OECD 206 were not fulfilled, as the mortality of the control exceeded 10 % at the end of the test. Therefore, the test is considered as not valid. In addition, the minimum floor area of pen per pair was too small (0.138 instead of 0.25 m^2). This minor space could be a reason why six birds died in the control group and two in each treatment group. *The study is not considered to be acceptable and valid.*

KIIA 8.1.4/02

Author:	[redacted]
Title:	Glyphosate Acid: A reproduction Study with the Mallard (*Anas platyrhynchos*)
Date:	11.01.1999
Doc ID:	2310918 /123-187
Guidelines:	FIFRA Guideline 71-4 OECD Guideline 206
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	P24
Purity:	95.6%
Positive control:	None
Species:	Mallard duck (*Anas platyrhynchos*)
Age:	21 weeks (at test initiation)
Sex:	Males and females

Weight:	868 to 1259 g (at test initiation)
Source:	[redacted]
Loading:	Approx. 0.675 m^2 for 2 birds (1 male and 1 female per pen)
Feed/Diet:	Game bird ration, *ad libitum*
Acclimation period:	6 weeks
Temperature:	22.4 ± 0.9 °C (adults); 29 °C (hatchling); 38 °C (brooding compartment)
Humidity:	69 ± 13 % (adults); 61 ± 15 % (hatchling)
Photoperiod:	17 hours light / 7 hours dark, (approx. 292 Lux)
Methods:	A reproductive toxicity study was performed by feeding young adult mallard ducks ad libitum on a series of 3 nominal dietary doses, encompassing 500, 1000, and 2250 mg glyphosate acid/kg feed. Sixteen replicates (1 male and 1 female per pen, 16 pen per treatment group) were used for each treatment group and control. The birds were exposed to the treated diets for approximately 21 weeks, and were evaluated for treatment-related effects on bird health and reproduction. Eggs were collected daily, washed and stored in a cold room at 13.6 ± 0.6 °C and 82 ± 8 % relative humidity. All eggs laid within a week were considered as one lot and were incubated in a Petersime incubator. On day 24 of incubation, eggs were placed in a Petersime hatcher and were allowed to hatch. The hatchlings were maintained on untreated diet until 14 days of age. Homogeneity of the test substance in treated diet was evaluated by collecting 6 samples from each treatment group on day 0 of week 1. During weeks 2, 3, 4, 8, 12, 16 and 20 of the test, a single sample was collected from the control diet and an additional duplicate sample was collected from treatment group diet, to measure and/ or verify test concentrations. :Parental birds were observed daily throughout the study for signs of toxicity and abnormal behaviour. Adult body weights were measured at study initiation and termination in addition to on weeks 2, 4, 6, and 8 of the adult in-life period. For each pen, feed consumption was measured weekly. At the end of each week, all eggs collected were counted and selected by indiscriminate draw for eggshell thickness measurement. The remaining eggs were candled to detect egg shell cracks or abnormal eggs before incubation. During the incubation period, eggs were candled again on day 14 to investigate embryo viability and on day 21 to determine embryo survival. During the study, total egg production, number of eggs cracked, eggshell thickness, embryo viability, embryo survival, number of hatchlings, body weight of new hatchlings, body weight of 14 day old hatchlings and survivorship of hatchlings after 14 days were determined. An analysis of variance (ANOVA) was used to determine significant differences among the groups and Dunnett's multiple comparison procedure was used as post-hoc test.

Results

All validity criteria according to OECD 206 were fulfilled, as the mortality of the control group did not exceed 10 % at the end of the test and the average number of 14-day-old survivors per hen in the control was greater than 14. Also, the average egg shell thickness for the control group was greater than 0.34 and the lowest treatment level did not result in compound-related mortality or observable toxic effects.

There were no treatment related mortalities at any of the concentrations. However, three incidental adult mortalities occurred during the course of the study. One incidental mortality occurred in the control group and in both the 1000 ppm treatment groups. Except for incidental clinical findings, all birds appearded normal throuout the study. Clinical signs as

lameness and wing droop were observed and frequently were associated with the incidental injuries.

There were no treatment related effects upon reproductive performance at any of the concentrations tested. However, offspring in the 2250 ppm treatment group did show a slight, but statistically significant ($p<0.05$) reduction in the mean body weight of 14-day old survivors when compared to the control. The mean body weight value for 14 day old survivors in the control group was 262 ± 32 g while mean values for the 500, 1000 and 2250 ppm treatment groups were 236 ± 35 g, 260 g ± 16 g, 235 g ± 23 g, respectively. As especially the parameter concerning hatchling weight were affected at 2250 ppm, it can not be excuded that the observed changes in hatchling weight do not represent a population relevant adverse effect. Therefore, this endpoint will be considered as a NOAEL of 1000 ppm, corresponding to 116 mg/kg/bw/d.

RMS Conclusion

Due to signficant reduction in mean body weight in the 2250 ppm treatment group in 14-day old survivors, the NOEL for mallard ducks exposed to glyphosate acid in a reproduction study was determined to be 1000 mg glyphosate acid/kg feed, corresponding to 116 mg/kg/bw/d.
The study is considered to be valid.

KIIA 8.1.4/03

Author:	[redacted]
Title:	One-Generation Reproduction Study – Bobwhite Quail; Glyphosate Technical.
Date:	01.11.1978
Doc ID:	2310921 /139-141
Guidelines:	Not stated
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	XHI 162
Purity:	83% (measured)
Vehicle and/or positive control:	Corn oil (vehicle)
Species:	Bobwhite quail (*Colinus virginianus*)
Age:	5 months old (young adults)
Sex	Males and females
Weight	192-203 g (at test initiation)
Source:	[redacted]
Loading	1 males and 2 females per pen
Diet/Diet:	Game bird breeder ration, *ad libitum*
Acclimation period:	Not stated
Temperature:	21.1 – 26.7 °C (research facility) 15.6 °C (eggs storage), 37.4 - 37.6 °C (eggs incubation)

Humidity:	55 % (eggs storage)
Photoperiod:	9 hours light / 15 hours dark (first 6 weeks) 17 hours light / 7 hours dark (following 16 weeks)
Methods:	A reproductive toxicity study was performed by feeding three adult Bobwhite quails(1 male and 2 females per pen) per replicate ad libitum on a series of 3 nominal dietary doses, encompassing 50, 200 and 1000 mg glyphosate technical/kg diet. The diet was prepared by incorporating appropriate concentrations of the test item and corn oil into the aliquots of basal diet. Twelve replicates were exposed per treatment group and control. The birds were exposed for nine weeks to the treated diet prior to egg deposition and for additional eight weeks during egg collection. Eggs were collected daily, stored at 15.6 °C and 55% relative humidity and were cleaned weekly. The eggs were then incubated at 37.5 ± 0.06 °C. On day 19 of incubation, the eggs were placed in a Humidaire hatcher and allowed to hatch. All hatchlings were housed according to the appropriate parental grouping and maintained on control diet until 14 days of age. Body weights were recorded at study initiation, 5 weeks after study initiation prior to onset of egg deposition and at termination of the study. Food consumption was recorded every second week throughout the study. All eggs were candled on day 0 of incubation for eggshell cracks, on day 14 to measure embryo viability, and on day 19 to measure embryo survival. Weekly throughout the egg deposition period, one egg of each pen in each group was randomly selected for egg weight and eggshell thickness measurement. During the study total egg production, number of eggs cracked, egg set, embryo viability, embryo survival, number of hatchlings, body weight of new hatchlings, body weight of 14 days-old hatchlings, 14 day survival, egg weight and eggshell thickness were determined. To evaluate differences between reproductive parameters, Student's t-test was used.

Results

Evaluation according to current OECD 206 test validity criteria was performed. All current validity criteria were fulfilled, as the mortality of the control did not exceed 10 % at the end of the test and the average number of 14-day-old survivors per hen in the control was ≥ 14. Also, the average egg shell thickness for the control group was ≥ 0.34 mm and the lowest treatment level did not result in compound-related mortality or observable toxic effects.

Table B.9.1-6: Effects of glyphosate on reproductive parameters of bobwhite quail

Glyphosate technical (mg test/kg diet)	**Control**	**50**	**200**	**1000**
Reproductive success				
Number of eggs laid per hen in 8 weeks (mean)	31.9	28.0	28.0	32.5
Number of eggs cracked (%)	9.7	7.6	9.2	6.3
Viable embryos of egg set (%)	91.9	80.6	91.7	87.0
Live 3-week embryos of viable embryos (%)	97.3	97.2	97.5	96.5
Hatchlings of live 3-week embryos (%)	81.5	70.3	73.4	74.4
14-day-old survivors of normal hatchlings (%)	95.5	93.1	95.7	93.5
14- day-old survivors per hen [a]	18.7	12.3	14.8	16.7
Egg weight				
Mean egg weight (g)	10.3	9.9	10.2	9.5 *
Eggshell thickness				
Mean eggshell thickness (mm)	0.214	0.204*	0.211	0.224
Body weight of representative hatchling				
Mean body weight (g)	6.8	6.9	6.9	6.7
Body weight of representative 14-day old survivors				
Mean body weight (g)	22.0	22.2	22.6	22.0

[a] based on 24 hens
* Statistically significant compared to control (Student's t-test)

There were no statistically significant impacts on reproductive parameters with two exceptions. A statistically significant reduction in egg weight occurred at the highest test dose of 1000 mg glyphosate technical/kg diet. However, changes in egg weight are not considered a standard endpoint in avian reproduction studies according to the actual guideline OECD 206. Other relevant endpoints determined did not show any statistically significant differences differences compared to the control treatment – including No. of eggs, No. of 14 d old survivals and hatchlings weight. Egg weight is not a standard endpoint in guideline avian reproduction studies, it is not included in OECD 206, and was a carryover from poultry performance studies.
Since especially all parameters concerning hatchling weight and survival were not affected at any concentration, it can be assumed that the observed changes in egg weight do not represent a population relevant adverse effect. Therefore, this endpoint will be considered as a NOAEL of 1000 ppm and will be used for the assessment of the chronic risk for birds exposed to glyphosate.
A statistically significant impairment was observed in eggshell thickness at the test dose of 50 mg glyphosate technical/kg diet. No effect on eggshell thickness was noted at any other dose level. Therefore, this impact was not considered to be biologically relevant. A high incidence of eggshell cracks was noted during the course of the study. This can be attributed to the fact that the bobwhite quail utilized for this study were inadvertently not debeaked prior to study initiation. In fact, caged quail have a natural propensity to peck at their eggs causing cracks.

RMS Conclusions
Based on the overall results of this study, the NOEAL for bobwhite quail exposed to glyphosate acid in a one-generation reproduction study was determined to be 1000 mg

glyphosate technical/kg feed, corresponding to 96.3 mg/kg/bw/d and will be used for the assessment of the chronic risk for birds exposed to glyphosate.

The study is considered to be acceptable and valid.

KIIA 8.1.4/04

Author:	[redacted]
Title:	One-Generation Reproduction Study - Mallard Duck; Glyphosate technical
Date:	25.09.1978
Doc ID:	2310923 / 139-143
Guidelines:	Not stated
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	XHI 162
Purity:	83% a.s.
Vehicle and/or positive control:	Corn oil (vehicle)
Species:	Mallard duck (*Anas platyrhynchos*)
Age:	6 months old (adults, at test initiation)
Sex	Males and females
Weight	1047 - 1257 g (at test initiation)
Source:	[redacted]
Loading	Approx. 8.2 m^2 for 7 specimens (2 males and 5 females per pen)
Diet/Diet:	Game bird breeder ration, *ad libitum*
Temperature:	37.4 – 37.6 °C (eggs incubation)
Humidity:	55 % (eggs storage)
Photoperiod:	outdoor (natural daylight/photoperiod)
Methods:	A reproductive toxicity study was performed by feeding 7 adult mallard ducks (2 males and 5 females per replicate) *ad libitum*, on a series of 3 nominal dietary doses encompassing 50, 200 and 1000 mg glyphosate acid/kg diet. The diet was prepared by incorporating appropriate concentrations of the test item and corn oil into the aliquots of basal diet. Five replicates were used for each treatment group and the control. The birds were exposed to the treated diet for 9 weeks prior to egg deposition and for additional 8 weeks during egg collection. Eggs were collected daily and stored at 15.6 °C and 55 % relative humidity and were cleaned weekly. The clean eggs were then incubated at 37.5 ± 0.06 °C. On day22 or 23 of incubation, the eggs were allowed to hatch. The hatchlings were housed according to the appropriate parental grouping and maintained on control diet until 14 days of age. Observations: Body weights were recorded at study initiation, 5 weeks after study initiation, prior to the onset of egg deposition, and at termination of the study. Food consumption was recorded bi-weekly throughout the study. All eggs were candled on day 0 of incubation for eggshell cracks, on day 14 to measure embryo viability and to remove any E coli-contaminated eggs, and on day 21 to measure embryo survival.

	Weekly throughout egg deposition period, one egg from each pen in each experimental group and the controls was randomly selected for egg weight and eggshell thickness measurement. During the study, the total egg production, the number of eggs cracked, embryos viability, embryos survival, number of hatchlings, body weight of representative new hatchling, body weight of representative 14 days-old hatchlings, 14 day-old survivorship, egg weight and the eggshell thickness were determined. Statistical calculations: To evaluate the differences between each of the above-mentioned reproductive parameters, Student's t-test was used.

Results

Evaluation according to current OECD 206 test validity criteria was performed. All validity criteria according to current guidelines were fulfilled, as the mortality of the control did not exceed 10 % at the end of the test and the average number of 14-day-old survivors per hen in the control was ≥ 14. Also, the average egg shell thickness for the control group was ≥ 0.34 mm and the lowest treatment level did not result in compound-related mortality or observable toxic effects.

Table B.9.1-7: Effects of glyphosate on reproductive parameters of Mallard duck

Glyphosate technical (mg test/kg diet)	**Control**	**50**	**200**	**1000**
Reproductive success				
Number of eggs laid per hen in 8 weeks	28	23	28	29
Number of eggs cracked (%)	3	5	5	6
Viable embryos of egg set	90	93	85	86
Live 3-week embryos of viable embryos (%)	96	93	95	95
Hatchlings of live 3-week embryos (%)	74	77	77	81
14-day-old survivors of normal hatchlings (%)	97	99	98	96
14- day-old survivors per hen [a]	16	14	15	16
Egg weight				
Number of eggs analysed	38	38	38	39
Mean egg weight(g)	57.5	58.3	56.3	58.9
Eggshell thickness				
Number of eggs analysed	38	38	38	39
Mean shell thickness (mm)	0.394	0.375	0.372	0.375
Body weight of representative hatchling				
Number of ducklings analysed	72	73	72	73
Mean body weight(g)	33	33	32	34
Body weight of representative 14-day old survivors				
Number of ducklings analysed	72	72	72	73
Mean body weight(g)	217	206	208	205

For the parental birds exposed to glyphosate, no symptoms of toxicity or behavioural abnormalities were recorded at any of the dietary doses tested or the control treatments for the entire test duration. In addition, no mortality was observed in control and treatments groups, except for the highest test dose, at which a single mortality was observed on week 12 after study initiation. This death was however considered incidental, and not compound related. The evaluation of the reproductive data and statistical analysis of above-mentioned

reproductive parameters indicates that glyphosate caused no reproductive impairment at the dose levels tested.

RMS Conclusions
Based on the results of this study, the NOEC for Mallard duck exposed to glyphosate acid in a one-generation reproduction study was determined to be 1000 mg glyphosate acid/kg diet.
The study is considered to be acceptable and valid.

B.9.1.4 Toxicity of the formulation

For animal welfare reasons no acute oral toxicity studies or chronic studies were conducted with the formulation MON 52276. Evaluation of toxicity is based on the assessment of effects of the active substance.

B.9.1.5 Literature Review

There was no critical peer-reviewed published data that changed the outcome of the environmental risk assessment for the active substance glyphosate. Often it was not possible to distinguish between the effect of the technical glyphosate and the surface-active substances added to the commercial formulations by the experimental designs used. Two publications pointed towards indirect effects of glyphosate use on the biodiversity of birds on a regional scale on the long-term by subtle alterations of the vegetation structure (please refer to chapter B.9.1.7.7).

B.9.1.6 Summary of effects on birds

A large number of acute studies in birds without any mortality at limit doses were submitted. EFSA guidance document 1438/2009 indicates that "it is permissible to extrapolate an LD_{50} value in cases where there is no mortality or a single mortality at a limit dose in an acute avian toxicity study". Using the study with the Bobwhite quail with a limit dose of 2000 mg/kg bw, the extrapolation factor for no mortalities at the limit dose and 20 birds per dose group (the actual number of birds tested at this limit dose exceeded 20), the acute LD_{50} to be used in a bird risk assessment according to EFSA guidance document 1438/2009 is proposed to be 2000 x 2.167 = 4334 mg/kg bw.

Table B.9.1-8: Summary of avian acute toxicity endpoints for glyphosate, glyphosate salts and the metabolite AMPA.

Species	**Substance**	**Test design**	**LD_{50} (mg a.s./ kg bw)**	**NOEL (mg a.s./kg bw)**	**Reference**
Colinus virginianus	Glyphosate Acid	Acute, 14 days	> 2000	-	[redacted] 1997, BVL no 2310906
Coturnix coturnix japonica	Glyphosate Acid	Acute, 14 days	> 2000	-	[redacted] 1996, BVL no 2310909

Species	**Substance**	**Test design**	**LD_{50} (mg a.s./ kg bw)**	**NOEL (mg a.s./kg bw)**	**Reference**
Coturnix coturnix japonica	Glyphosate Acid	Acute, 14 days	> 2000	-	[redacted] 1999, BVL no 2310910
Anas platyrhynchos	Glyphosate Acid	Acute, 14 days	> 2000	-	[redacted], 1996, BVL no 2310912
Colinus virginianus	Glyphosate Acid	Acute, 14 days	> 2000	-	[redacted] 1991; CHV 48/91266
Colinus virginianus	Glyphosate K-salt	Acute, 14 days	> 2241	484	[redacted] 2003, BVL no 2310915
Colinus virginianus	AMPA	Acute, 14 days	> 2250	-	[redacted] 1991; AVS95-00222

Studies shaded in grey have been reviewed as part of the first EU peer reviewed evaluation in 2001.

Avian long-term (one-generation) reproduction studies have been conducted with bobwhite quail and mallard duck for the active substance glyphosate.

Conversion of endpoints from ppm to mg a.s./kg bw/d was performed according to EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009). The daily dose for birds in each treatment group of each test, expressed as test substance (TS) intake, was calculated by treatment group using the following formula:

$$\text{Test substance intake (mg TS/g bw/day)} = (\text{Consumption}_{mean} \times \text{Conc}_{Feed}) / \text{BW}_{mean}$$

$\text{Consumption}_{mean}$= Group Mean Feed Consumption (g/bird/day)
Conc_{Feed} = Concentration (mg TS/kg feed);
BW_{mean}= Group Mean Body Weight for Start of Treatment and Exposure Termination (g)

The values used in the calculations and the daily dose values are presented in the tables below.

Table B.9.1-9: Daily dose from glyphosate acid avian reproduction studies

Nominal Dose (mg a.s./kg feed)	**Daily mean food consumption (g feed/bird/day)**	**Mean body weight (g)**	**Daily dose (mg a.s./kg bw/day)**
B.9.1.6.1.1.1 KIIA 8.1.4/03, Bobwhite quail; [redacted] 1978, BVL no 2310921			
Control	21.1	218.5	0
50	20.3	208.0	4.9
200	19.8	218.3	18.1
1000	20.4	211.8	96.3
B.9.1.6.1.1.2 KIIA 8.1.4/04 Mallard duck; [redacted], 1978, BVL no 2310923			
Control	131.1	1154.3	0
50	118.8	1078.3	5.5
200	128.9	1135.5	22.7

1000	145.5	1161.3	125.3
B.9.1.6.1.1.3 KIIA 8.1.4/02 Mallard duck, [redacted] 1999, BVL no 2310918			
Control	140.6	1098	0
500	140.0	1083	64.6
1000	128.0	1093	117
2250	142.4	1069	300

Concerning the effects of glyphosate on bird reproduction, studies have been conducted with bobwhite quail ([redacted] 1978, BVL no 2310921 and [redacted] 1999, BVL no 2310916) and mallard duck ([redacted] 1978, BVL no 2310923, [redacted] 1999, BVL no 2310918) for the active substance glyphosate. For mallard duck, no effect were observed up to the highest concentration tested (NOEC = 1000 ppm).
The study by [redacted] 1978, BVL no 2310921 is proposed for risk assessment. A significant reduction in egg weight was observed at the highest concentration tested (1000 ppm). Therefore, a NOEC of 18.1 mg a.s./kg bw/d was determined and agreed during the EU review process. However, changes in egg weight are not considered a standard endpoint in avian reproduction studies anymore according to guideline OECD 206 and has therefore not been adressed in other studies testing higher concentrations. Other relevant endpoints determined in the study of [redacted] (1978, BVL no 2310921) did not show any unacceptable differences compared to the control treatment – including No. of eggs, No. of 14 d old survivals and hatchlings weight. The differences in egg weight between control and the treatment with 1000 ppm amounted to a decrease of approx. 7.5 % (10.26 g ± 0.38 g vs. 9.48 g ± 0.47 g in control and 1000 ppm treatment, respectively). Since all parameters concerning hatchling weight and survival were not affected, it can be assumed that the observed changes in egg weight are statistically significant but do not represent a population relevant adverse effect. Therefore, this endpoint will be considered as a NOAEL of 1000 ppm and is proposed for the assessment of the chronic risk for birds exposed to glyphosate.

Taking into account the aforementioned considerations, the endpoints as documented below are proposed by the RMS to be used for performing the risk assessment.

Table B.9.1-10: Summary of avian long-term toxicity endpoints for glyphosate acid.

Species	**Substance**	**Test design**	**NOEL/NOAEL (mg a.s./ kg feed)**	**NOEL/NOAEL (mg a.s./kg bw/day)**	**Reference**
Colinus virginianus	Glyphosate Acid	Long-term	**1000ppm**	**96.3**	[redacted] 1978, BVL no 2310921
Anas platyrhynchos	Glyphosate Acid	Long-term	1000ppm	125.3	[redacted] 1978, BVL no 2310923
Anas platyrhynchos	Glyphosate Acid	Long-term	1000ppm	117	[redacted] 1999, BVL no 2310918

B.9.1.7 Risk assessment

The first-tier risk assessment for birds is performed by calculating TER values based on dietary exposure according to the scenarios provided by the current EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009).

Table B.9.1-11: End point values used for the assessement of the risk for birds arising from the exposure to glyphosate

Time scale and type of risk assessment	Test species	Proposed endpoint	Explanation/justification
Acute toxicity	Bobwhite quail *(Colinus virginianus)*	LD_{50} = 4334 mg/kg bw	A large number of acute studies in birds without any mortality at limit doses were submitted. According to EFSA guidance document 1438/2009 the LD_{50} value was extrapolated and is proposed to be LD_{50} = 4334 mg/kg bw
Reproductive toxicity (long-term)	Bobwhite quail (*Colinus virginianus*)	NOAEL = 1000ppm corr: 96.3 mg/kg bw/d	A NOAEL of 1000 ppm, corresponding to 96.3 mg/kg/bw/d is proposed.

For the assessment of the risk arising from the intended uses of glyphosate, the relevant crop scenarios, as well as the corresponding shortcut values for estimating the daily dietary dose from uptake of contaminated food, and the resulting TER values are summarised in the tables below for acute and long-term/reproductive toxicity, respectively.

B.9.1.7.1 Screening step

In the screening step, the risk to indicator bird species from an exposure to glyphosate is assessed. These indicators are considered to have highest exposure in a specific crop at a particular time due to their size and feeding habits and represent a worst case scenario.

To estimate the daily dietary doses, following equations were used:

Daily dietary dose (DDD):

$$DDD_{single\ application} = \text{application rate (kg a.s./ha)} \times \text{shortcut value}^{1}$$

[1] see section 4.1 of EFSA/2009/1438

In case of multiple applications, the daily dietary dose for a single application is multiplied with an appropriate multiple application factor for 90th percentile residue data (MAF_{90}; see Table 7 of EFSA/2009/1438).

$$DDD_{multiple\ application} = DDD_{single\ application} \times MAF_{90}{}^{1}$$

Toxicity exposure ratio (acute):

$$TER_A = \frac{LD_{50}\ (\text{mg/kg bw/day})}{\text{Acute DDD}\ (\text{mg/kg bw/day})}$$

As listed in the GAP-table, intended uses of glyphosate acid in the product MON 52276 cover several crops in pre-planting, pre-emergence, pre-harvest and row-application in orchards and vineyards.
Regarding the proposed indication preplanting (post-emergence of weeds), no bare soil is considered as general scenario since the mode of action of glyphosate is via uptake by green tissues of leaves and stems of treated plants. Therefore, a leaf development is assumed in the assessment scenario.
The maximum cumulative application rate per year is set to 4.32 kg glyphosate/ha. The maximum application rate per treatment is 2.16 kg glyphosate/ha, except for spot applications in orchards and vines where the maximum application rate is 2.88 kg glyphosate/ha.

Table B.9.1-12: Avian screening risk assessment for dietary exposure to glyphosate (acute toxicity, worst-case indicator species)

Crop and application timing	**Applic. Rate per treated area (ARTreated) (g a.s. /ha)**	**Applic. rate /cropped area (ARCropped) (g a.s. /ha)** (50 % of **(ARTreated)**	**Scenario**	**Indicator species**	**Shortcut value × MAF**	**Daily dietary dose (mg a.s./kg bw/d)**	**TER**
Acute toxicity: LD_{50} > 4334 mg a.s./kg bw							
All crops (all seeded or trans-planted crops) Pre planting of crop	Max. 2 × 2160 g a.s./ha, Min. 21 d interval	-	Grassland	Large herbiv. bird	30.5 x 1.2	79.06	55
			Bare soil	Small graniv. bird	24.7 x 1.2	64.02	68
			Leafy vegetables, cereals, bulbs and onion like crops	Small omniv. bird	158.8 x 1.2	411.6	11
All crops (all seeded crops) Post planting/ pre emergence of crop	1× max. 1080 g a.s./ha	-	Grassland	Large herbiv. bird	30.5	32.9	132
			Leafy vegetables, cereals, bulbs and onion like crops	Small omniv. bird	158.8	171.5	25
Cereals (pre harvest) wheat, rye, triticale, barley and oats Crop maturity	1 × max. 2160 g a.s./ha	-	cereals	Small omniv. bird	158.8	343.0	13
Oilseed (pre harvest) rapeseed, mustard seed, linseed Crop maturity	1 × max. 2160 g a.s./ha	-	Oilseed rape	Small omniv. bird	158.8	343.0	13
Orchard crops	1 × max.	1 × max. 1440	orchards	Small	46.8	67.4	64

Crop and application timing	**Applic. Rate per treated area (ARTreated) (g a.s. /ha)**	**Applic. rate /cropped area (ARCropped) (g a.s. /ha) (50 % of (ARTreated)**	**Scenario**	**Indicator species**	**Shortcut value × MAF**	**Daily dietary dose (mg a.s./kg bw/d)**	**TER**
(vines including citrus & tree nuts) Post emergence of weeds 28 days interval between applications **Intrarow & Spot treatment (50% applic. rate) ***	2880 g a.s./ha	g a.s./ha		insectiv. bird			
	1- 3 applic., e.g. 2 × max. 720 g a.s./ha +1× max. 2880 g a.s./ha	1- 3 applic., e.g. 2 × max. 360 g a.s./ha +1× max. 1440 g a.s./ha			46.8 x 1.2 + 46.8	19.9 + 67.4 = 87.3 *	50
	2 × max. 2160 g a.s./ha	2 × max. 1080 g a.s./ha			46.8 x 1.2	60.7 *	71
	3 × max. 1440 g a.s./ha	3 × max. 720 g a.s./ha			46.8 x 1.3	43.8 *	99
Orchard crops (vines including citrus & tree nuts) Post emergence of weeds **Intrarow & Spot treatment (50% applic. rate) ***	1 × max. 2880 g a.s./ha	1 × max. 1440 g a.s./ha	vineyard	Small omniv. bird	95.3	137.2	32
	1- 3 applic., e.g. 2 × max. 720 g a.s./ha +1 × max. 2880 g a.s./ha	1- 3 applic., e.g. 2 × max. 360 a.s./ha +1 × max. 1440 g a.s./ha			95.3 x 1.2 + 95.3	41.7 + 137.2= 178.9 *	24
	2 × max. 2160 g a.s./ha	2 × max. 1080 g a.s./ha			95.3 x 1.2	123.5 *	35
	3 × max. 1440 g a.s./ha	3 × max. 720 g a.s./ha			95.3 x 1.3	89.2 *	49
TER in bold do not meet the acceptability criteria.							

* Because applications are made round base of trunk and to the intra-rows, (inner strips between two trees within a row), application rates per ha are expressed per 'unit of treated surface area' the actual application rate per ha orchard or vineyard will only be 50% of the reported rate.

Table B.9.1-13: Avian screening risk assessment for dietary exposure to glyphosate (long-term/reproductive toxicity, worst-case indicator species)

Crop and application timing	**Applic. Rate per treated area (ARTreated) (g a.s. /ha)**	**Applic. rate /cropped area (ARCropped) (g a.s. /ha) (50 % of (ARTreated)**	**Scenario**	**Indicator species**	**Shortcut value × (MAF × TWA)**	**Daily dietary dose (mg a.s./kg bw/d)**	**TER**
Long-term/reproductive toxicity: NOAEL = 96.3 mg a.s./kg bw/d							
All crops (all seeded or trans-planted	Max. 2 × 2160 g a.s./ha Min 21 d	-	Grassland	Large herbiv. bird	16.2 × 1.4 × 0.53	25.96	**4**

Crop and application timing	**Applic. Rate per treated area (ARTreated) (g a.s. /ha)**	**Applic. rate /cropped area (ARCropped) (g a.s. /ha) (50 % of (ARTreated)**	**Scenario**	**Indicator species**	**Shortcut value × (MAF × TWA)**	**Daily dietary dose (mg a.s./kg bw/d)**	**TER**
crops) Pre planting of crop	interval		Bare soil	Small graniv. birds	11.4 × 1.4 × 0.53	18.27	5
			Leafy vegetables, cereals, bulbs and onion like crops	Small omniv. bird	64.8 × 1.4 × 0.53	103.86	**1**
All crops (all seeded crops) Post planting/pre emergence of crop	1 × max. 1080 g a.s./ha	-	Grassland	Large herbiv. bird	16.2 × 0.53	9.27	11
			Leafy vegetables, cereals, bulbs and onion like crops	Small omniv. bird	64.8 × 0.53	37.09	**3**
Cereals (pre harvest) wheat, rye, triticale, barley and oats Crop maturity	1 × max. 2160 g a.s./ha	-	Cereals	Small omniv. bird	64.8 × 0.53	74.18	**1**
Oilseed (pre harvest) rapeseed, mustard seed, linseed Crop maturity	1 × max. 2160 g a.s./ha	-	Oilseed rape	Small omniv. bird	64.8 × 0.53	74.18	**1**
Orchard crops (vines including citrus & tree nuts) Post emergence of weeds 28 days interval between applications **Intrarow & Spot treatment (50% applic. rate) ***	1 × max. 2880 g a.s./ha	1 × max. 1440 g a.s./ha	Orchards	Small insectiv. bird	18.2 × 0.53	19.9	5
	1- 3 applic., e.g. 2 × max. 720 g a.s/ha +1 × max. 2880 g a.s./ha	1- 3 applic., e.g. 2 × max. 360 g a.s./ha +1× max. 1440 g a.s./ha			18.2 × 1.4 × 0.53 + 18.2 × 0.53	4.8 + 13.9 = 18.7	5
	2 × max. 2160 g a.s./ha	2 × max. 1080 g a.s./ha			18.2 × 1.4 × 0.53	14.6	7
	3 × max. 1440 g a.s./ha	3 × max. 720 g a.s./ha			18.2 × 1.5 × 0.53	10.4	9
Orchard crops (vines including citrus & tree nuts) Post emergence of	1 × max. 2880 g a.s./ha	1 × max. 1440 g a.s./ha	Vineyard	Small omniv. bird	38.9	29.7	**3**
	1- 3 applic. e.g. 2 × max. 720 g a.i/ha +1 × max.	1- 3 applic., e.g. 2 × max. 360 g a.s./ha +1× max. 1440			38.9 × 1.4 × 0.53 + 38.9 × 0.53	10.4 + 29.7 = 40.1	**2**

Crop and application timing	**Applic. Rate per treated area (ARTreated) (g a.s. /ha)**	**Applic. rate /cropped area (ARCropped) (g a.s. /ha) (50 % of (ARTreated)**	**Scenario**	**Indicator species**	**Shortcut value × (MAF × TWA)**	**Daily dietary dose (mg a.s./kg bw/d)**	**TER**
weeds 28 days interval between applications **Intrarow & Spot treatment (50% applic. rate) ***	2880 g a.s./ha	g a.s./ha					
	2 × max. 2160 g a.s./ha	2 × max. 1080 g a.s./ha			38.9 × 1.4 × 0.53	31.2	**3**
	3 × max. 1440 g a.s./ha	3 × max. 720 g a.s./ha			38.9 × 1.5 × 0.53	22.3	**4**
TER in bold do not meet the acceptability criteria.							

***** Because applications are made round base of trunk and to the intra-rows , (inner strips between two trees within a row), application rates per ha are expressed per 'unit of treated surface area' the actual application rate per ha orchard or vineyard will only be 50% of the reported rate.

The screening steps showed an acceptable acute risk for all indicator species for the maximal application rates indicated above. Concerning the long-term risk, the indicator species large herbivorous bird and small omnivorous bird did not met the Annex VI trigger values.

B.9.1.7.2 Tier 1 risk assessment

In the Tier1 risk assessment step, the defined daily dietary doses and TER values were calculated for so-called generic focal species (see EFSA 1438/2009, Annex I). As for the indicator species, the generic focal species are considered to be representative for all species potentially at risk. In the Tier 1 assessment, a mixed diet approach is followed when appropriate and interception of the spray by the crop is taken into account for the calculation of residue levels for different food types. MAF values for the application interval of 21 and 28 days was calculated using the formula in Appendix H, which results in a MAF of 1.23 and 1.14 for two applications and a MAF of 1.29 and 1.16 for three applications respectively.

For those intended uses, for which the calculated TER values were <5 in the long-term scenarios of the screening steps, respective generic focal species according to the EFSA guidance document were selected. For different scenarios (bulbs and onions, cereals early, fruiting vegetables, hop, leafy vegetables, legume, legume forage, maize, oilseed rape, orchard, potatoes, pulses, root & stem vegetables, strawberries, sugar beet, sunflower and vineyard) we considered birds species in early BBCH stages (BBCH 10-19, BBCH 10-29 and BBCH 10-49) with high, worse case residues on food items after application (RUD).

For the indication „Oilseed preharvest" Scenario 122 (BBCH 80-99, late with seeds) is relevant and results in the highest default shortcut values (mean SV= 11.4, 90th centile SV = 24.7). With an application rate 2.16 kg/ha, SCV of 11.4 and ftwa value of 0.53 results in a DDD value of 13.1 and a TER of 7 thus passing the risk assessment. The scenarios 120, 134, and 138 result in a less risk, but were included although they are not worse –case.

For the indication "Orchard crops (vines including citrus & tree nuts)/ Post emergence of weeds" sceanrio 142 and 146 are relevant according to the GAP, but 146 presents the worst case and passes the risk assessment. For the indication" Vinyard" it is proposed to include scenario 231 (Small omnivorous bird "lark") and furthermore to no interception to take into account an application to the ground for vineyard herbicide uses and a diet composition of 25% grass, 25% weed seeds without interception and 50% ground arthropods without interception was assumed.

Table B.9.1-14: Avian higher tier (Tier 1) risk assessment for dietary exposure to glyphosate (long-term/reproductive toxicity, worst-case generic focal species)

Crop and application timing	**Applic. Rate / treated area (ARTreated) (g a.s. /ha)**	**Scenario**	**Generic focal species**	**Shortcut value × (MAF × TWA)**	**Daily dietary dose (mg a.s./ kg bw/d)**	**TER**
Long-term/reproductive toxicity: NOAEL = 96.3 mg a.s./kg bw/d						
All crops (all seeded or trans-planted crops) Pre planting of crop	Max. 2 × 2160 g a.s./ha Min 21 d interval	Grassland, growing shoots	Large herbiv. bird "goose" Pink-foot goose *(Anser brachyrhynchus)*	16.2 × 1.23× 0.53	22.81	**4.2**
		Bare soil, BBCH<10	Small graniv. bird "finch" Linnet *(Carduelis cannabina)*	11.4 × 1.23× 0.53	16.05	6
		Bulbs and onions BBCH 10-39	Small graniv. bird "finch" Linnet *(Carduelis cannabina)*	11.4 × 1.23× 0.53	16.05	6
		Cereals early (shoots) autumn-winter BBCH 10-29	Large herbiv. bird "goose" Pink-foot goose *(Anser brachyrhynchus)*	16.2 × 1.23× 0.53	22.81	**4.2**
		Fruiting vegetables BBCH 10-49	Small graniv. bird "finch" Linnet *(Carduelis cannabina)*	11.4 × 1.23× 0.53	16.05	6
		Hop BBCH 10-19	Small graniv.bird "finch" Goldfinch *(Carduelis carduelis)*	11.4 × 1.23× 0.53	16.05	6
		Leafy vegetables Leaf development BBCH 10-49	Med. Herbiv./graniv. bird "pigeon" Wood pidgeon *(Columba palumbus)*	22.7 × 1.23× 0.53	31.96	**3**
		Legume forage BBCH 10-49	Small graniv. bird "finch" Linnet *(Carduelis cannabina)*	11.4 × 1.23× 0.53	16.05	6
		Maize BBCH 10-29	Med. herbiv./ graniv. bird "pigeon" Wood pidgeon *(Columba palumbus)*	22.7 × 1.23× 0.53	31.96	**3**
		Oilseed rape early (shoots) BBCH 10-19	Large herbiv. bird "goose" Greylag goose *(Anser anser)*	15.9 × 1.23× 0.53	22.38	**4.3**
		Oilseed rape BBCH	Med. herbiv./ graniv. bird	22.7 × 1.23×	31.96	**3**

Crop and application timing	**Applic. Rate / treated area (ARTreated) (g a.s. /ha)**	**Scenario**	**Generic focal species**	**Shortcut value × (MAF × TWA)**	**Daily dietary dose (mg a.s./ kg bw/d)**	**TER**
		10-19	"pigeon" Wood pidgeon *(Columba palumbus)*	0.53		
		Potatoes BBCH 10-39	Small omniv. bird "lark" Woodlark *(Lullua arborea)*	10.9 × 1.23× 0.53	15.35	6.3
		Pulses, leaf development, BBCH 10-19	Med. herbiv./ graniv. bird "pigeon" Wood pidgeon *(Columba palumbus)*	22.7 x 1.23x 0.53	31.96	**3**
		Root & stem vegetables BBCH 10-39	Small graniv. bird "finch" Linnet *(Carduelis cannabina)*	11.4 × 1.23× 0.53	16.05	6
		Strawberries BBCH 10-19	Small insectiv. bird "wagtail" Yellow wagtail *(Motacilla flava)*	11.3 × 1.23× 0.53	15.99	6
		Sugar beet early (spring) BBCH 10-19	Small omniv. bird "lark" Woodlark *(Lullua arborea)*	10.9 × 1.23× 0.53	15.35	6
		Sunflower, early Germinantion/ leaf development BBCH 00-19	Small insectiv. bird "wagtail" Yellow wagtail *(Motacilla flava)*	11.3 × 1.23× 0.53	15.99	6
All crops (all seeded crops) Post planting/pre emergence of crop	1 × max. 1080 g a.s./ha	Bulbs and onions BBCH 10-39	Small graniv. bird "finch" Linnet *(Carduelis cannabina)*	11.4 × 0.53	6.52	16
		Cereals early (shoots) autumn-winter BBCH 10-29	Large herbiv. bird "goose" Pink-foot goose *(Anser brachyrhynchus)*	16.2 × 0.53	9.27	11
		Fruiting vegetables BBCH 10-49	Small graniv. bird "finch" Linnet *(Carduelis cannabina)*	11.4 × 0.53	6.52	16
		Hop BBCH 10-19	Small graniv. bird "finch" Goldfinch *(Carduelis carduelis)*	11.4 × 0.53	6.52	16
		Leafy vegetables Leaf development BBCH 10-49	Med. herbiv./ graniv. bird "pigeon" Wood pidgeon *(Columba palumbus)*	22.7 × 0.53	12.99	8
		Legume forage BBCH 10-49	Small graniv. bird "finch" Linnet *(Carduelis cannabina)*	11.4 × 0.53	6.52	16
		Maize BBCH 10-29	Med. herbiv./graniv. bird "pigeon" Wood pidgeon *(Columba palumbus)*	22.7 × 0.53	12.99	8
		Oilseed rape BBCH 10-19	Med. herbiv./graniv. bird "pigeon" Wood pidgeon *(Columba palumbus)*	22.7 × 0.53	12.99	7
		Potatoes BBCH 10-19	Small insectiv. bird "wagtail" Yellow wagtail	11.3 × 0.53	6.47	16

Crop and application timing	**Applic. Rate / treated area (ARTreated) (g a.s. /ha)**	**Scenario**	**Generic focal species**	**Shortcut value × (MAF × TWA)**	**Daily dietary dose (mg a.s./ kg bw/d)**	**TER**
			(Motacilla flava)			
		Pulses, leaf development, BBCH 10-19	Med. herbiv./graniv. bird "pigeon" Wood pidgeon *(Columba palumbus)*	22.7 × 0.53	12.99	8
		Root & stem vegetables BBCH 10-39	Small graniv. bird "finch" Linnet *(Carduelis cannabina)*	11.4 × 0.53	6.52	16
		Strawberries BBCH 10-19	Small insectiv. bird "wagtail" Yellow wagtail *(Motacilla flava)*	11.3 × 0.53	6.47	16
		Sugar beet early (spring) BBCH 10-19	Small omniv. bird "lark" Woodlark *(Lullua arborea)*	10.9 × 0.53	6.24	16
		Sunflower, early Germinantion/ leaf development BBCH 00-19	Small insectiv. bird "wagtail" Yellow wagtail *(Motacilla flava)*	11.3 × 0.53	6.47	16
Cereals pre harvest / crop maturity	1 × max. 2160 g a.s./ha	Cereals (wheat, rye, triticale, barley and oats shoots	Small graniv./insectiv. bird "bunting"	2.3** × 0.53	2.63	37
			Small insectiv. bird "pas-serine"	22.4 × 0.53	25.486	**3.8**
Oilseed (pre harvest) Crop maturity	1 × max. 2160 g a.s./ha	Oilseed rape , late with seeds (rapeseed, mustard seed, linseed) BBCH 88-99	Small graniv. bird "finch"*(Carduelis cannabina)*	11.4 × 0.53	13.05	7
		Late - late (with seeds) (BBCH 30-99)	Small insectiv. bird "dunnock"	2.7 × 0.53	3.091	31
		BBCH ≥ 40	Small omniv. bird "lark"	2.7 × 0.53	3.091	31
		BBCH ≥ 40	Medium herbiv./graniv. bird "pigeon"	0.9 × 0.53	1.024	94

Crop and application timing	**Applic. Rate / treated area (ARTreated) (g a.s. /ha)**	**Applic. rate /cropped area (ARCropped) (g a.s. /ha) (50 % of (ARTreated)**	**Scenario**	**Generic focal species**	**Shortcut value × (MAF × TWA)**	**Daily dietary dose (mg a.s./ kg bw/d)**	**TER**
Orchard crops (vines including citrus & tree	1 × max. 2880 g a.s./ha	1 × max. 1440 g a.s./ha	Orchard, not crop directed, application all season	Small graniv. bird "finch" Serin *(Serinus serinus)*	12.6 × 0.53	9.6*	10

Crop and application timing	**Applic. Rate / treated area (ARTreated) (g a.s. /ha)**	**Applic. rate /cropped area (ARCropped) (g a.s. /ha) (50 % of (ARTreated)**	**Scenario**	**Generic focal species**	**Shortcut value × (MAF × TWA)**	**Daily dietary dose (mg a.s./ kg bw/d)**	**TER**
nuts) Post emergence of weeds **Intrarow & Spot treatment (50% applic. rate) ***	2 × max. 2160 g a.s./ha 28 days interval	2 × max. 1080 g a.s./ha 28 days interval			12.6 × 1.14× 0.53	8.22*	12
	3 × max. 1440 g a.s./ha 28 days interval	3 × max. 720 g a.s./ha 28 days interval			12.6 × 1.16 × 0.53	5.58*	17
	1 × max. 2880 g a.s./ha	1 × max. 1440 g a.s./ha		Small insectiv./ve-miv. bird "thrush"	2.7 × 0.53	2.06*	46
	2 × max. 2160 g a.s./ha 28 days interval	2 × max. 1080 g a.s./ha 28 days interval			2.7× 1.14× 0.53	1.761*	55
	3 × max. 1440 g a.s./ha 28 days interval	3 × max. 720 g a.s./ha 28 days interval			2.7 × 1.16 × 0.53	1.195*	81
Orchard crops (vines including citrus & tree nuts) Post emergence of weeds; **Intrarow & Spot treatment (50% applic. rate) ***	1 × max. 2880 g a.s./ha	1 × max. 1440 g a.s./ha	vineyard	Small insectiv. species "redstart" (*Phoenicurus ochrurus)*	11.5 × 0.53	8.8*	11
	2 × 2160 g a.s./ha 28 days interval	2 × max. 1080 g a.s./ha 28 days interval			11.5 × 1.14× 0.53	8.10*	12
	3 × 1440 g a.s./ha 28 days interval	3 × max. 720 g a.s./ha 28 days interval			11.5 × 1.16 × 0.53	5.6*	17
	1 × max. 2880 g a.s./ha	1 × max. 1440 g a.s./ha	vineyard	Small omniv. bird "lark" *(Lullula arborea)*	RUD: (54.2+7.5+40.2) × 0.53	10.21*	9

Crop and application timing	**Applic. Rate / treated area (ARTreated) (g a.s. /ha)**	**Applic. rate /cropped area (ARCropped) (g a.s. /ha) (50 % of (ARTreated)**	**Scenario**	**Generic focal species**	**Shortcut value × (MAF × TWA)**	**Daily dietary dose (mg a.s./ kg bw/d)**	**TER**
	2 × 2160 g a.s./ha 28 days interval	2 × max. 1080 g a.s./ha 28 days interval			(54.2+7.5+40.2) × 0.6	7.66*	13
	3 × 1440 g a.s./ha 28 days interval	3 × max. 720 g a.s./ha 28 days interval			(54.2+7.5+40.2) × 0.61	5.10*	19
TER in bold do not meet the acceptability criteria.							

***** Because applications are made round base of trunk and to the intra-rows , (inner strips between two trees within a row), application rates per ha are expressed per 'unit of treated surface area' the actual application rate per ha orchard or vineyard will only be 50% of the reported values. For application it is recommend that only the strip along the tree line will be treated (approx. 33% to max. 50% of the cropped area) to create a weed free area. Consequently the alleys (inter-rows) are left as untreated permanent grassy strips with no exposure to glyphosate. In case the whole area is treated the full dose should be taken into account.

** The scenario "crop maturity/late season" is meant to address birds feeding on cereal seeds that are on the plant, not lying on the ground (food category "Grains/ear"). In this assessment, the scenario was amended by excluding two non-representative measurements from the dataset for deriving the RUD for "Grains/ear". The original dataset consists of the following 21 values: 2.93 - 3.27 - 4.32 - 4.80 - 5.08 - 5.20 - 5.20 - 5.55 - 5.60 - 6.51 - 7.54 - 7.90 - 8.60 - 9.50 - 11.06 - 11.22 - 12.71 - 12.96 - 12.97 - 57.80 - 114.00 (mean = 15.0; s.d. = 25.4; 90th %-ile 13.0), but the two highest values were obtained with an unrealistically low application volume of 6 L/ha, whereas all other values were obtained with realistic application volumes of 460-1100 L/ha. Considering the 19 reliable values ranging from 2.93 to 12.97, a mean of 7.5 with a standard deviation of 3.3 and a 90th percentile of 12.8 are obtained, which are obviously more meaningful than the previous values. Based on those RUDs, the appropriate shortcuts for the scenario of birds feeding on grains that are still on the plant are 2.3 (mean RUD) and 4.0 (90th percentile RUD; same value as in the GD).

Based on Tier 1 assessment step, the calculated TER values for the long-term risk resulting from an exposure of birds to glyphosate according to the intended use in oilseed rape (late with seeds, rapeseed, mustard seed, linseed, BBCH 88-99) and cereals (cereals, late season) as well as in orchards (vines including citrus & tree nuts, post emergence of weeds; 28 days interval between applications with spot treatment round of trunks or application to the intra-rows) achieve the acceptability criteria TER ≥ 5 according to Regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2. for long-term effects. The results of the assessment indicate an acceptable risk for birds due to the intended uses according to the label.

The calculated TER values for the long-term risk resulting from an exposure of birds to glyphosate according to the intended use in the scenarios "all crops post planting/pre emergence of crop" towards the "large herbivorous bird goose (*Anser brachyrhynchus*)" and the "medium herbivorous/granivorous bird pidgeon (*Columba palumbus*)" with an application rate of 2 x 2160 g a.s./ha and "cereals pre harvest /crop maturity" towards the "small insectivorous bird passerine" with an application rate of 1 x 2160 g a.s./ha do not achieve the acceptability criteria TER ≥ 5. The results of the assessment indicate an unacceptable risk for birds due to the intended use according to the label. A refined risk assessment is necessary.

B.9.1.7.3 Higher Tier: Refined risk assessment

A refinement for the long-term risk has been presented by the notifier. The methodology used to calculate the TWA for glyphosate in grass foliage for the long-term mammalian risk assessment follows the procedure described in the Guidance Document on Terrestrial Ecotoxicology (91/414/EEC, September 2002, SANCO/4145/2000). The decline of glyphosate residue in grass was characterised using data from 22 residue trials each of which had a day 0 value. **The average DT_{50} for the 22 trials was 2.8 days.** The 21-day time weighted average (twa) for glyphosate in grass foliage has been used to calculate a refined f_{twa}. The 21-day twa is calculated to be **0.19** for the active substance glyphosate acid. A f_{twa} of 0.19 will be used in the risk assessment for birds feeding on grass foliage.
Please refer to chapter B.9.13 for details on the calculation of the 21day time-weighted – average (twa) for glyphosate in grass foliage used in the mammalian risk assessment.

For the scenario "Cereals pre harvest /crop maturity" the GAP indicates that cereal applications are made for pre-harvest uses and desiccation (starting with BBCH 89), which is why the Late season- seed heads scenario is considered the most relevant. Including the scenarion which takes into account BBCH 71 -89, the "Small insectivorous bird "passerine" does not achieve the acceptability criteria TER ≥ 5. for long-term effects.
To evaluate the long term risk of Glyphosate for insectivourous birds, an assessment can consider the following:

- Birds are highly mobile and often travel considerable distances between breeding habitat and feeding habitat. Hence, a refinement of PT to 0.5 can be proposed.
- Particularly in the case of preharvest application it must be assument that habitat loss due to harvest takes place inbetween a short period and therfore longterm feeding on contaminated foliar insects displays a worse case.
- There is only one application per crop.
- The generic focal species "Small insectivorous bird "passerine" corresponds to a Fan-tailed Warbler *Cisticola juncidis* displaying a species found in South-central and south-east Europe. In Europe, most habitats are coastal primarily in Mediterranean and neighbouring warm temperate lowlands, both continental and oceanic.
- According to „Birds of the Western Palearctic 1.00" ©2004 BirdGuides Ltd. *Cisticola juncidis* feeds Chiefly insects, also taken on or near ground. Therfore, a mixed diet of 75% foliar-dwelling invertebrates and 25% ground-dwelling invertebrates with interception of 90% can be proposed and is shown in the follong table:

Table B.9.1-15: Higher tier (Tier 2) risk assessment for birds exposed to glyphosate (long-term/reproductive toxicity, worst-case generic focal species)

Applic. rate (g a.s./ha)	Scenario	Generic focal species	Shortcut value × (MAF × TWA)	Daily dietary dose (mg a.s./kg bw/d)	TER
Max. 2 × 2160 g a.s./ha Min 21 d interval	Grassland, growing shoots	Large herbiv.bird "goose" Pink-foot goose *(Anser brachyrhynchus)*	16.2 × 0.19	6.73	14
	Cereals early (shoots) autumn-winter BBCH 10-29	Large herbiv. bird "goose" Pink-foot goose *(Anser brachyrhynchus)*	16.2 × 0.19	6.73	14
	Leafy vegetables Leaf development	Medium herbiv./ graniv.bird "pigeon" Wood pidgeon	22.7 × 0.19	9.43	10

	BBCH 10-49	*(Columba palumbus)*			
	Maize BBCH 10-29	Medium herbiv./ graniv.bird "pigeon" Wood pidgeon *(Columba palumbus)*	22.7 × 0.19	9.43	10
	Oilseed rape early (shoots) BBCH 10-19	Large herbiv. bird "goose" Greylag goose *(Anser anser)*	15.9 × 0.19	6.61	15
	Oilseed rape BBCH 10-19	Medium herbiv./ graniv.bird "pigeon" Wood pidgeon *(Columba palumbus)*	22.7 × 0.19	9.43	10
	Pulses, leaf development, BBCH 10-19	Medium herbiv./ graniv.bird "pigeon" Wood pidgeon *(Columba palumbus)*	22.7 × 0.19	9.43	10
1 × max. 2160 g a.s./ha	Cereals pre harvest / crop maturity	Small insectivorous bird "pas-serine" (*Cisticola juncidis*)	RUD foliar invert.: 21 RUD ground invert:; 3.5/7.5	DDD foliar invert.: 19.078 DDD ground invert.: 1.060/ 0.227	4.8-5
TER in bold do not meet the acceptability criteria					

Based on refined assessment step, the calculated TER values for the long-term risk resulting from an exposure of birds to glyphosate according to the intended use in the scenario "all crops (all seeded or transplanted crops) " with an application rate of max. 2 x 2160 g a.s./ha and in the use in the scenario "Cereals pre harvest /crop maturity" with an application rate of 1 x 2160 g a.s./ha achieve the acceptability criteria TER ≥ 5 according to Regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2. for long-term effects.

B.9.1.7.4 Risk from exposure to contaminated drinking water

In addition to their diet, birds may also be exposed to glyphosate via drinking water.

As pointed out in the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) specific calculations of exposure and TER values are only necessary when the ratio of effective application rate (in g a.s./ha) to relevant endpoint (in mg a.s./kg bw/d) exceeds 50 in the case of less sorptive (K_{OC} < 500 L/kg) or 3000 in the case of more sorptive (K_{OC} ≥ 500 L/kg) substances. For glyphosate, the ratio of highest application rate (4320 g a.s./ha) to lowest relevant endpoint (NOAEL = 96.3 mg a.s./kg bw/d) is 55; therefore, the risk can be considered acceptable without the need for further calculations.

B.9.1.7.5 Risk assessment for metabolites

The primary metabolite of glyphosate is aminomethylphosphonic acid (AMPA). Most of the parent glyphosate is eliminated unchanged and only a small amount (less than 1 % of the applied dose) is transformed to aminomethylphosphonic acid (AMPA). The metabolite AMPA has been tested in several toxicity studies which demonstrated that it is of lower toxicity than glyphosate acid (see Volume 1, chapter 2.6.8). Avian toxicity tests with metabolites of glyphosate showed equally low acute toxicity as glyphosate.

Following direct treatment via foliar, trunk, stem or hydroponic treatment, unchanged glyphosate was the only significant residue. In presence of soil as a substrate the active substance is quickly degraded, leaving AMPA at rates comparable or even higher than parent glyphosate. However, the uptake via the roots and the translocation in the plants was very low, not resulting in significant residue levels as confirmed by plant metabolism and confined rotational crop studies. A major part of the glyphosate was degraded into CO_2 (for details please refer to Volume 1, chapter 2.7.2). Therfore it can be concluded that the risk to birds will be acceptably low and no further quantitative risk assessment is conducted.

B.9.1.7.6 Bioaccumulation and food chain behaviour

According to the EFSA Guidance Document, substances with a log $P_{ow} \geq 3$ are likely to possess a potential for bioaccumulation that might result in unacceptable risks for organisms at higher trophic levels. Hence, a specific risk assessment ('secondary poisoning') must be performed for these substances. Since the log K_{ow} values of glyphosate is log P < –3.2 (pH 2–5, 20 °C), the active substance is deemed to have a negligible potential to bioaccumulate in animal tissues. No formal risk assessment from secondary poisoning is therefore required. The primary metabolite of glyphosate is aminomethylphosphonic acid (AMPA). Most of the parent glyphosate is eliminated unchanged and only a small amount (less than 1 % of the applied dose) is transformed to aminomethylphosphonic acid (AMPA). The metabolite AMPA has been tested in several toxicity studies which demonstrated that it is of lower toxicity than glyphosate acid (see Volume 1, chapter 2.6.8).
Furthermore, the log K_{ow} for AMPA – estimated via EpisuiteProgramm and SMILES code (C(N)P(=O)(O)O) – is -2.47 and does not indicate a potential for bioaccumulation.

B.9.1.7.7 Indirect effects via trophic interactions

In the following, we discuss the evidence of an impact of herbicide use on the biodiversity of terrestrial non-target species with an emphasis on farmland bird species. Even though these effects are not substance-specific but rather caused by the use of herbicides *per se*, the RMS considers it adequate to raise this issue here, as glyphosate is by far the most extensively used herbicide in the EU.

The existing impact of herbicide use on farmland vertebrate and invertebrate species is an indirect effect via trophic interactions, starting with the plants at the bottom of the food web. In previous evaluations of active substances, this kind of effect has not been assessed, and standardised assessment methods are not yet available. Nevertheless, we consider adverse indirect effects on the environment as relevant for the regulatory assessment and management of plant protection products (PPP) and their active substances under Commission Regulation (EC) No 1107/2009. Effective and reasonable risk mitigation measures are available (e. g. compensation measures, especially conservation headlands, fallow land and flowering margins) but yet have to be established in the regulation of PPP. As the severity of indirect effects of herbicide use on the conservational status of populations of farmland bird and mammal species strongly depends on agricultural and landscape properties, an assessment considering all different conditions all over the EU is hardly possible. Thus, the RMS limits the assessment to the description of the potential of glyphosate and other broad spectrum herbicides to cause indirect effects and highlights the need for risk mitigation measures by the Member States, proposing compensation measures as a suitable tool.

Scientific evidence of indirect effects of PPP on farmland bird and mammal species

The state of knowledge regarding indirect effects of pesticide use on the populations of farmland birds and mammals has been reviewed recently in a comprehensive report by Jahn et al. (2013). According to this report, several reviews and comprehensive studies on indirect effects are available, demonstrating enduring impact on populations of farmland bird species. These studies mainly focus on arable landscapes in the UK (Campbell et al. 1997; Boatman et al. 2004; DEFRA 2005; Bright et al. 2008). Campbell et al. (1997) demonstrate the clear relationship of adverse pesticide effects with the decline of Grey Partridge populations and assume possible negative effects on eleven further species, such as Turtle Doves, Reed Bunting or Blackbirds. Boatman et al. (2004) expand these results by proving the negative indirect effects of pesticide applications for another three species, the Yellowhammer, Skylark and Corn Bunting.

The impact of insecticides is most studied and study results show adverse effects on bird species like the Yellowhammer or Corn Bunting feeding on insects during the breeding season. However, regarding the most intensively investigated study species, the Grey Partridge, a relationship between pesticides, food availability, breeding performance and population size has been clearly demonstrated with herbicides being the main determining pesticide group (Marshall et al. 2001). The species' population has declined in Europe by at least 83 % since the 1930s (Potts 1991) and underlying negative effects of pesticide applications on Grey Partridges have been demonstrated in an extensive long-term study in Sussex, UK (The Game Conservancy Trust's study). Chick survival is the key factor determining population development and Grey Partridge chicks are highly dependent on invertebrate prey abundance in arable crops, mainly cereals, where they feed on insects and other arthropods along the edges (Potts et al. 1991, Rands 1985). In an experimental study, Rands (1985) found that the abundance of insects and the mean brood size of Partridges were significantly higher in cereal fields where areas were left unsprayed with pesticides (broad-spectrum grass and broad-leaved weed herbicides, fungicides and insecticides). Declines in insect abundances due to herbicide applications correlated to the negative trend of Grey Partridge populations (Potts et al. 1991). By reducing chick food supply, pesticide applications decreased chick survival and therewith brood sizes of the Grey Partridge (Rands 1985). Declined chick survival rates are seen as the major factor responsible for the decrease of Grey Partridge numbers (Rands 1985; Potts et al. 1991). Brood sizes of an average of 3.14 chicks are needed to sustain Partridge populations (Potts 1980). Rands (1985) recorded mean brood sizes of less than 3.0 chicks in sprayed cereal fields, while brood sizes in unsprayed headlands were larger than 5.0. Hence, brood sizes in sprayed habitats were insufficient to maintain Grey Partridge populations. These and further findings are discussed extensively in the review by Campbell et al. (1997) as well as in Marshall et al. (2001) and Bright et al. (2008).

For mammals, thorough studies on the population level are lacking. Individuals can be negatively affected by direct effects such as poisoning but certainly also by indirect effects on habitat quality and food availability. Feeding on wild plants and simultaneously needing a high proportion of ground cover to avoid predation makes small mammals vulnerable towards indirect herbicide effects. Although few studies addressed this issue, several authors mention such a possible negative impact e.g. for the Brown Hare or the Common Shrew. Furthermore, a number of studies from North America have proven adverse indirect herbicide effects on rodents even on the population level. These effects concerned both habitat quality and food availability. However, in total it seems that food availability is the more important ecological

prerequisite that, when altered by pesticide applications, induces negative consequences for both farmland bird and mammal species. Due to the general lack of knowledge on many small mammals species, about their population numbers and trends but also ecological features like habitat occurrence and diet choice, the actual role taken by pesticides in the presumably decline of small mammal populations is hard to estimate.

The importance of glyphosate regarding indirect effects of PPPs

The impact of herbicide use on farmland birds and mammals and invertebrate species is an indirect effect via trophic interactions. The effects trace back to the intended effect of the herbicides – eliminating competing plants – which is virtually the same in all broad spectrum herbicides. Even though indirect effects of herbicides on terrestrial vertebrates are not substance-specific, they are caused by the use of specific plant protection products. Glyphosate is the herbicidal active substance most intensively used worldwide. The industry Task Force on Glyphosate assessed that glyphosate accounts for around 25 % of the global herbicide market (http://www.glyphosate.eu). No further aggregated quantitative use data for glyphosate on global or EU level was available to the RMS, but use data from individual countries clearly indicate the massive extent of its use covering almost all crops. The pesticide use statistics of individual EU member states indicates that glyphosate belongs to one of the top ranked substances of the herbicide marked at EU level (e. g. 30-40 % in Germany). In Germany, large areas of oilseed rape (87 %), pulses (72 %) and winter barley (66 %) are treated with glyphosate. Predominating uses are stubble management, pre-sowing application and applications for pre-harvest crop desiccation in several crops (Dickeduisberg, 2012). Glyphosate must therefore be considered the most significant herbicide regarding indirect effects on terrestrial vertebrates.

Need for regulating indirect effects under Commission Regulation (EC) No 1107/2009

The RMS is aware of the fact that up to now the EU risk assessment of indirect effects of PPP on terrestrial vertebrates has mostly been restricted to indirect poisoning, whilst effects due to trophic interactions have rarely been assessed and regulated. However, it should be noted that the prerequisite for the approval of a PPP according to Commission Regulation (EC) No 1107/2009 – the absence of unacceptable effects – does not explicitly differentiate between direct and indirect effects. In fact, there is no legal basis for a nonconsideration of potentially unacceptable indirect effects. According to the new data requirements (Annex to Commission Regulation (EC) No 283/2013) „the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects via alteration of the food web, shall be considered.“ The EFSA PPR Panel makes allowance for this in its “Scientific Opinion on the development of specific protection goal options for environmental risk assessment of pesticides, in particular in relation to the revision of the Guidance Documents on Aquatic and Terrestrial Ecotoxicology (SANCO/3268/2001 and SANCO/10329/2002)” (EFSA Panel on Plant Protection Products and their Residues (PPR), 2010) by considering “indirect effects via trophic interactions” in the derivation of specific protection goals.

One of the few examples for a consideration of indirect effects due to trophic interactions in the present risk assessment of PPP, is the use of mesocosm tests in the higher tier aquatic risk assessment. According to the OECD “Draft Guidance Document on Simulated Freshwater Lentic Field Tests” (OECD, 2004), the interpretation of these studies focuses on effects at the community and ecosystem level, potential indirect effects and the recovery potential of

sensitive endpoints.”. To some extent, indirect effects are also considered in arthropod field studies on the community level.

One reason for the fact that indirect effects of herbicide use on terrestrial non-target invertebrates and vertebrates have not yet been assessed in the EU risk assessment of PPP might be that these effects trace back to direct effects on in-field non-target plants which have not been explicitly considered a subject of protection so far. Here, it is relevant that in the course of the recent revision of the PPP data requirements (Annex to Commission Regulation (EC) No 284/2013), the former phrase “non-target plants are plants outside the cropped area” in the non-target higher plants chapter has been deleted.

Even though the endangerment of farmland invertebrate and vertebrate species is known to be a multicausal problem, it is obvious that the effects of pesticide use on the availability of in-field plants and insects as a food source can be a limiting factor to the viability of farmland bird populations. Consequently a consideration of indirect effects in the environmental risk assessment of PPPs as well as in the risk management is necessary from both the scientific as well as the legal perspective.

Scope of regulation of indirect effects

Many typical farmland insect and vertebrate species are adopted to other man-made habitats than arable land in a strict sense. The condition of the populations of such species is affected by an alteration of the landscape structure (e.g. loss of off-field habitats and grassland) rather than by the application of pesticides on arable land. Such effects are not in the scope of a regulatory risk management of PPPs. Thus, the risk assessment and management under Commission Regulation (EC) No 1107/2009 should concentrate on those species adapted to arable land like Grey Partridge and other species mentioned above. Those species depend on arable land as a foraging habitat, thus being affected by the use of PPP and other agricultural measures reducing food availability (i.e. insect abundance).

Potential risk mitigation measures for the regulation of indirect effects of herbicide application on birds and mammals – ecological suitability and practicablity

Depending on the ecological and agricultural frame conditions, indirect effects of broad spectrum herbicide use on farmland birds and mammals can exceed the level of acceptability. In such cases, risk mitigation measures (RMM) have to be applied in order to reduce the effects to an acceptable level. The most severe RMM in the regulatory framework of PPP is non-approval. Regarding the regulation of negative impacts due to indirect effects, though, non-approval is certainly not a reasonable option as this type of effect is not substances-specific, so that substitution by another substance in fact would not reduce the risk. The same applies to other established RMMs as limitations to the field rate or to the time of application as such measures would mostly be incompatible with the intended plant protection effect – effectively eliminating weeds. Thus, it appears necessary to expand the range of risk mitigation options when it comes to the addressing of indirect effects. In principle, two options are available:

- *compensating indirect effects* by measures integrated into the agricultural scheme

- *mitigating indirect effects* by e. g. increasing the proportion of low pesticide-input agriculture as organic farming in order to reduce impact on the potentially affected species.

Although not yet established in the risk regulation of PPP, comprehensive practical experiences with potential compensation measures exists in the context of landscape conservation and species protection in the EU countries. Potential options for the compensation of indirect effects of PPPs on birds and mammals have recently been evaluated comprehensively regarding their ecological suitability and the prospects of their practical implementation in Germany (Jahn et al., 2013). According to this study, the most effective compensation measures for the risk management of farmland bird species include:

- Creation of extensive field crops without application of pesticide and with reduced sowing density and fertilisation (very similar: creation of sparsely sown cereal crops (defined areas or strips) – to date little known and tested)
- Creation of flowering areas or strips
- Keeping stubble fields with self-greening and as appropriate with maintenance measures
- Creation of road, water and bank verges with extensive grassland
- Creation of biotope networks (e.g. sowing of wild herbs from autochthonous seeds)

These five measures can be implemented generally in almost all arable countryside in the EU. In their report, Jahn et al. (2013) also discuss the minimum extent of ecological compensation areas needed to sustainably secure the populations of many farmland species. The kind and extent of necessary compensation measures are essentially dependent on the affected species and the given ecological and agricultural conditions. For instance, the population relevance of indirect effects will be more severe in intensive agricultural landscapes with a scarcity of suitable refugium habitats. Thus, no concrete recommendations for the risk management in the Member States can be given here. Rather, the concrete risk assessment regarding indirect effects as well as the derivation of a suitable risk mitigation strategy for their management should be done on the level of the single Member States. Nevertheless, the methodology should be harmonised in order to ensure consistent risk assessment and management in the single Member States.

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B.9.2 Effects on aquatic organisms (Annex IIA 8.2; Annex IIIA 10.2)

B.9.2.1 Toxicity data

A summary of all available relevant and compliant toxicity data for aquatic species exposed to glyphosate acid, its salts as well as the metabolite AMPA are presented below including data already reviewed during the first EU peer reviewed evaluation of glyphosate in 2001.

The toxicity of glyphosate acid, the isopropylamine (IPA) salt, the potassium (K) salt and its metabolites AMPA and HMPA to aquatic organisms as well as the representative formulation in the present approval renewal of glyphosate (MON 52276) was investigated in a series of laboratory studies with representative species from different trophic levels of the aquatic food chain (i.e. fish, aquatic invertebrates, algae and aquatic plants). As algae and aquatic plants are most sensitive to the metabolites of glyphosate; AMPA and HMPA, for animal welfare reasons no tests on aquatic vertebrates (fish) were conducted on HMPA.

Table B.9.2-1: Acute toxicity of glyphosate acid, glyphosate IPA salt, K-salt and metabolites AMPA for different fish species. Results of studies submitted for the present application for approval renewal of glyphosate

Species	Substance	Test design	EC_{50} (mg a.s./L)	NOEC (mg a.s./L)	Reference
Oncorhynchus mykiss	Glyphosate acid	96 h static	130	32	IIA 8.2.1/01 BL5552/B [redacted] 1995, BVL no 2310924
Lepomis macrochirus	Glyphosate acid	96 h static	47	32	IIA 8.2.1/02 BL5553/B [redacted] 1995, BVL no 2310926
Danio rerio	Glyphosate acid	96 h semi-static	122.91	56	IIA 8.2.1/03 RF-D61.47/99 D[redacted] 2000 BVL no 2310928
Cyprinus carpio	Glyphosate acid	96 h semi-static	>100	100	IIA 8.2.1/04 2060/015 [redacted] 2006 BVL no 2310930
Oncorhynchus mykiss	AMPA	96 h static	>100	100	IIA 8.2.1/05 232469 [redacted], 1998 BVL no 2310933
Oncorhynchus mykiss	AMPA	96 h static	>180	18	IIA 8.2.1/06 BL5070/B [redacted] 1991 BVL no 2310934
Oncorhynchus mykiss	Glyphosate K-salt	96 h static	1227	157	IIA 8.2.1/07 WL-2002-149 [redacted] 2003 BVL no 2310937

Table B.9.2-2: Acute toxicity of glyphosate acid, glyphosate IPA salt, K-salt and metabolites AMPA for different fish species. Results of tests already reviewed as part of the first EU peer review of glyphosate in 2001

Species	**Substance**	**Test design**	**EC_{50} (mg a.s./L)**	**NOEC (mg a.s./L)**	**Reference**
Oncorhynchus mykiss	Glyphosate acid	96 h static	71.4	34.9	AB-78-165 [redacted], 1978/95-00014
Oncorhynchus mykiss	Glyphosate acid	96 h static	95 - 171	95	271631 [redacted] 1990/yes 95-00011
Oncorhynchus mykiss	Glyphosate acid	96 h static	>100	>100	[redacted] 1995 95-00536
Oncorhynchus mykiss	Glyphosate acid	96 h static	**38**	10	[redacted] 1972 95-00016
Lepomis macrochirus	Glyphosate acid	96 h static	120	-	AB-78-1123 [redacted] 1978 95-00013
Lepomis macrochirus	Glyphosate acid	96 h static	133.3 - 200	133.3	271642 [redacted], 1991 95-00012
Cyprinus carpio	Glyphosate acid	96 h static	115		[redacted] 1990 95-00015
Oncorhynchus mykiss	Glyphosate IPA-salt	96 h static	2192 1624 a.e	517 383 mg a.s./L	80-91-2328-03-93 [redacted] 1993 94-00157
Oncorhynchus mykiss	Glyphosate IPA-salt	96 h static	**> 1000** > 741 a.e	1000 741 mg a.s./L	94-01161 [redacted], 1981
Leuciscus idus	Glyphosate IPA-salt	96 h static	> 5000 > 3704 a.e	5000 3704 mg a.s./L	80-91-2328-02-93 [redacted] 1993 94-00156
Lepomis macrochirus	Glyphosate IPA-salt	96 h static	**> 1000** > 741 mg a.e	560 415 mg a.s./L	95-00712 [redacted], 1981
Oncorhynchus mykiss	AMPA	96 h static	**520**	32	AB-90-402 [redacted] 1991 94-01162
Oncorhynchus mykiss	MON 52276	96 h static	> 989 mg/L > 306 a.e	989 mg/L 306 mg a.s./L	TO-91-296 [redacted] 1992, BVL no 2317591

Species	**Substance**	**Test design**	**EC_{50} (mg a.s./L)**	**NOEC (mg a.s./L)**	**Reference**
Cyprinus carpio	MON 52276	96 h static	> 895 mg/L 277 a.e	895 mg/L 277 mg a.s./L	TO-91-298 [redacted] 1992, BVL no 2317595

Values in grey: confirmed EU endpoints in first EU Review Monograph and SANCO/6511/VI/99-final
a.e.: acid equivalents

B.9.2.1.1 Fish – acute toxicity

KIIA 8.2.1/01

Author:	[redacted]
Title:	Glyphosate acid: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*)
Date:	5.09.1995
Doc ID:	2310924 /BL5552/B
Guidelines:	OECD 203/ EPA FIFRA; Subdivision E, 72-1
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	P24
Purity:	95.6%
Control:	Filtered and dechlorinated tap water
Species:	Rainbow trout (*Oncorhynchus mykiss*)
Age:	Juvenile
Size:	40 – 71 mm (mean: 57 mm)
Body weight of the animals:	1.16 – 4.56 g/ fish (mean: 2.68 g)
Loading:	0.89 g fish/L (10 fish per 30 litres of test medium)
Source:	[redacted]
Diet/Food:	no feeding for 48 hours prior to test and during the total test period
Acclimation period:	32 days
Temperature:	11.5 – 12.6°C
Photoperiod:	16 hours
pH:	Control (start – 96 h): 7.7 - 7.0 32 mg/L (start – 96 h): 6.4 – 6.2 56 mg/L (start – 96 h): 5.9 – 6.0 100 mg/L (start – 96 h): 4.7 – 5.1 180 mg/L(start – 24 h): 3.5 320 mg/L (start – 24 h): 3.0 560 mg/L (start – 24 h): 2.8 – 2.7
Dissolved oxygen:	6.2 – 10.4 mg O_2/L
Conductivity:	281 µS/cm³ in the dilution water

Hardness:	56.3 mg $CaCO_3$/L
Methods:	The toxicity test was performed at nominal concentrations of 32, 56, 100, 180, 320 and 560 mg glyphosate acid/L prepared using filtered and dechlorinated tap water treated with ultra violet steriliser. The test was conducted under static test conditions. A negative control (dilution water only) was also prepared. A single replicate vessel was prepared for the control and at each treatment level, each containing ten fish (added to 40 L glass aquariums containing 30 L test medium). Fish in all vessels were observed for sublethal effects and mortality after 24, 48, 72 and 96 hours. Temperature, pH-value and oxygen saturation of test solutions were measured on a daily basis. Hardness and conductivity of the test water was measured at test initiation. At test termination, the ten fish from the dilution water control were weighed and measured. Analytical measurements were performed by HPLC analysis at test initiation and after 48 and 96 hours. The LC_{50} values and their 95% confidence intervals were calculated using non-linear interpolation. The NOEC was determined by visual interpretation of the mortality and observation data.

Results

The mean measured concentrations of glyphosate acid ranged from 91 to 100 %. As the measured concentrations of glyphosate were between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.
All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60 % of air saturation and constant exposure conditions have been maintained.
Until 100 mg test item/L, no mortality occurred, but all fish died at the test concentrations of 180 mg test item/L and higher. Sublethal effects like dark discolouration and loss of balance were observed at 56 and 100 mg test item/L respectively.

Table B.9.2-3: Effects of glyphosate acid on rainbow trout

Glyphosate acid (mg/L)	% of dead fish and observed symptoms			
	24 h	**48 h**	**72 h**	**96 h**
Control	< 10%	< 10%	< 10%	< 10%
32	< 10%	< 10%	< 10%	< 10%
56	> 30%	< 30%/DC	< 30%/DC	< 10%
100	> 30%/DC	< 30%/ DC, LB	< 10%	< 10%
180	100%	100%	100%	100%
320	100%	100%	100%	100%
560	100%	100%	100%	100%

DC: Dark colouration; LB: Loss of balance

RMS Conclusion

The 96 hour LC_{50} value for rainbow trout (*Oncorhynchus mykiss*) exposed to glyphosate acid was calculated to be 130 mg glyphosate acid/L (nominal) with 95 % confidence interval of 100 to 180 mg/L. The NOEC after 96 h was 32 mg glyphosate acid/L.

The study is considered to be acceptable and valid.

KIIA 8.2.1/02

Author:	[redacted]
Title:	Glyphosate acid: Acute toxicity to Bluegill Sunfish (*Lepomis macrochirus*)
Date:	21.12.1995
Doc ID:	2310926 /BL5553/B
Guidelines:	OECD 203/FIFRA Guideline 72-1
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	P24
Purity:	95.6 % a.s.
Control:	Filtered and dechlorinated tap water
Species:	Bluegill sunfish (*Lepomis macrochirus*)
Age:	Juvenile
Size:	30 mm (mean)
Body weight:	0.54 g (mean)
Loading:	10 test individuals for 20 L test solution
Source:	[redacted]
Diet/Food:	no feeding for 48 hours prior to test and during the total test period
Acclimation period:	19 days at 22 °C prior to the test initiation
Temperature:	22 ± 1 °C
Photoperiod:	16 hours with 20 min transition period
pH:	Control (start – 96 h): 7.3–6.8 10 mg/L (start – 96 h): 5.9 – 6.4 18 mg/L (start – 96 h): 5.2 – 5.8 32 mg/L (start – 96 h): 4.6 – 4.8 56 mg/L(start – 96 h): 3.8 – 3.9 100 mg/L (start – 24 h): 3.4 180 mg/L (start – 24 h): 3.1
Dissolved oxygen:	6.2 – 9.0 mg/L
Conductivity:	100 µS/cm
Hardness:	16.0 mg $CaCO_3$/L.
Methods:	The acute toxicity test was performed at nominal concentrations of 10, 18, 32, 56, 100 and 180 mg test item/L prepared using filtered and dechlorinated tap water treated with ultra violet steriliser. The test was conducted under static test conditions (no media renewal). A negative control group (dilution water only) was also prepared. A single vessel was prepared for the control and each test media group, each containing ten fish (27.5 L borosilicate glass vessels containing 20 L test medium). Observations: All fish were observed for sublethal effects and mortality after 24, 48, 72 and 96 hours. Temperature, pH-value and oxygen saturation of test solutions were measured on a daily basis. Hardness and conductivity of the test water was measured at test initiation. Samples of test media were analysed for glyphosate acid content using HPLC analysis at test initiation and after 48 and 96 hours. Statistical calculations: The 96 hour LC50 values and 95 % confidence intervals were calculated using non-linear interpolation. The NOEC was determined by visual interpretation of the mortality and observation data.

Results

The measured concentrations of glyphosate acid in fresh media at test initiation ranged between 96.9 and 110 % of nominal. In aged test media at 96 hours, mean measured glyphosate acid concentrations ranged between 94.4 and 97.0 % of nominal. At 100 and 180 mg/L, no chemical analysis was performed at 48 and 96 hours, as all fish died within the first 24 hours following addition. As measured concentrations of glyphosate acid were between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60 % of air saturation and constant exposure conditions have been maintained.

There were no mortalities in the control or the 10, 18 and 32 mg /L treatments. At 56 mg test item/L, there was 90 % mortality. There was 100% mortality at 100 mg/L and higher test concentrations that occurred after 24 hours. There was a strong negative correlation between pH value and test item concentrations observed. At 56 mg test item/L, the pH was reduced to 3.8 and lower.

Table B.9.2-4: Effects of glyphosate acid on Bluegill sunfish (Lepomis macrochirus)

Glyphosate acid (mg/L)	**% of dead fish and observed symptoms**			
	24 h	**48 h**	**72 h**	**96 h**
Control	< 10%	< 10%	< 10%	< 10%
10	< 10%	< 10%	< 10%	< 10%
18	< 10%	< 10%	< 10%	< 10%
32	< 10%	< 10%	< 10%	< 10%
56	40%	80%	90%	90%
100	100%	100%	100%	100%
180	100%	100%	100%	100%

RMS Conclusions

The 96 hour LC_{50} value for bluegill sunfish (*Lepomis macrochirus)* exposed to glyphosate acid was 47 mg glyphosate acid/L (nominal) with a 95 % confidence interval of 35 to 66 mg/L, with a 96 hour NOEC values of 32 mg glyphosate acid/L.

The study is considered to be acceptable and valid.

KIIA 8.2.1/03

Author:	[redacted]
Title:	Acute Toxicity of Glifosate Técnico Nurfarm to Zebrafish (*Brachydanio rerio*)
Date:	26.01.2000
Doc ID:	2310928 /RF-D61.47/99
Guidelines:	OECD Guideline 203 (1993)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	037-919-113
Purity:	95.0 % a.s. (nominal), 95.49% a.s.(analysed)
Control:	Reconstituted water; Reference: Potassium dichromate ($K_2Cr_2O_7$)
Species:	Zebra fish (*Danio rerio*)
Age:	Not stated
Size:	Not stated
Body weight of the animals:	0.191 -0.239 g
Loading:	(0.38 to 1.44 g fish/L) based on 10 specimens exposed in 3 L test solution
Source:	[redacted]
Diet/Food:	no feeding during the total test period
Acclimation period:	72 h (to dilution water) prior to the test initiation (no feeding 24 h prior to test start and during the test)
Temperature:	24.1 – 24.5 °C
Photoperiod:	16 hours
pH:	Control (start – 96 h): 7.4 – 7.5 10 mg/L (start – 96 h): 7.3 – 7.1 32 mg/L (start – 96 h): 7.0 – 6.6 56 mg/L(start – 96 h): 6.5 – 5.3 100 mg/L (start – 96 h): 5.1– 4.8 180 mg/L (start – 24 h): 4.1 – 4.0 320 mg/L (start – 24 h): 3.5 – 3.6
Dissolved oxygen:	4.9 – 5.8 mg O_2/L (61.72 % - 73.06 % of saturation value at 24.5 °C)
Conductivity:	691 - 711 µS/cm
Hardness:	229.7 – 249.9 mg $CaCO_3$/L.
Methods:	Based on the results of a range finding test, a definite toxicity test was performed using nominal concentrations of 10, 32, 56, 100, 180, 320 mg test item/L in a semi-static test setup, with test media renewal after 48 hours. A negative control (reconstituted water only) was also prepared. There were two vessels per treatment, containing ten fish each (4000 mL glass vessels containing 3000 mL test medium). All fish were observed for sublethal effects and mortality after 3, 24, 48, 72 and 96 hours. Temperature, pH-value and oxygen saturation of test solutions were measured on a daily basis. Weight measurements were conducted of each individual fish at test initiation. Samples of test media were analysed using HPLC analysis at test initiation and after 48 and 96 hours. LC_{50} values, along with respective 95% confidence limits were calculated using the Trimmed Spearman-Karber Method. The NOEC was determined by visual interpretation of the mortality and observation data.

Results

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 10 % of air saturation and constant exposure conditions have been maintained. The 96 h LC_{50} (95 % CL) for the reference product was calculated to be 79.54 (68.87 – 91.88) mg/L.

At the 180 mg glyphosate acid/L concentrations and higher, 100 % mortality was observed after 24 hours exposure. At 100 mg glyphosate acid/L, there was 20 % mortality after 72

hours and 30 % mortality after 96 hours, with hyperactivity observed in test fishes at 48 hours onwards. At 56 mg test item/L and lower, no fish mortalities or sub-lethal effects were observed throughout the test period.

Table B.9.2-5: Effects of glyphosate acid on Zebra fish (*Danio rerio*)

Glyphosate acid (mg/L)	Number of dead fish /observed symptoms				
	3 h	24 h	48 h	72 h	96 h
Control	0	0	0	0	0
10	0	0	0	0	0
32	0	0	0	0	0
56	0	0	0	0	0
100	0	0	0/HA	2/HA	3/HA
180	0/LE	10	10	10	10
320	9/LE	10	10	10	10

HA: Hyperactivity ; LE: Loss of equilibrium

RMS Conclusions

The 96 hour LC_{50} for zebra fish (*Danio rerio*) exposed to glyphosate acid was 122.91 mg glyphosate acid/L (nominal) with a 95% confidence interval of 111.97 to 134.92mg/L. The 96 hour NOEC was 56 mg glyphosate acid/L.

The study is considered to be acceptable and valid.

KIIA 8.2.1/04

Author:	[redacted]
Title:	Glyphosate Technical: Acute Toxicity to Common Carp (*Cyprinus carpio*)
Date:	03.01.2006
Doc ID:	2310930 /2060/015
Guidelines:	OECD Guideline 203 (1992)/ JMAFF Testing Guideline for Toxicology Studies, 12 NohSan No. 8147, Guideline 2-7-1(2000)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	H05H016A
Purity:	95.7 %
Control:	Dechlorinated tap water; Pentachlorophenol sodium salt (reference, tested in a different study)
Species:	Common carp (*Cyprinus carpio*)
Age:	Juvenile
Size:	4.2 ± 0.1 cm
Body weight:	2.05 ± 0.13 g

Loading:	0.72 g body weight/L test solution
Source:	[redacted]
Diet/Food:	no feeding during the total test period
Acclimation:	12 days at test conditions
Temperature:	20.6 – 21.2°C
Photoperiod:	16 hours light / 8 hours dark, with 20 minutes dawn and dusk transition
pH:	7.4 – 8.3 (control), 6.3 – 8.0 (treatment)
Dissolved oxygen:	8.1 - 8.8 mg/L (91 - 99% saturation at 20.6 – 21.2°C)
Conductivity:	359 – 610 µS/cm
Hardness:	Approx. 100 mg $CaCO_3$/L.
Methods:	Based on the results of a range finding test, a final toxicity test was performed under semi-static test design as limit test using a single nominal concentration of 100 mg test item/L. The control and test media at 100 mg/L were renewed at 48 hours. A negative control group (dilution water only) was also prepared in parallel. There were duplicate glass vessels for the test concentration and control, each containing seven test fish in 20 L test medium. All fish were observed for sub-lethal effects and mortality after 3, 6, 24, 48, 72 and 96 hours after test initiation (fish addition). Test solutions were renewed after 48 hours. Water temperature, pH-value and oxygen saturation of the test solutions were measured on a daily basis. Water hardness was measured in fresh media only. Samples of fresh media were taken at o hours and samples of old test media were taken at 24 and 96 hours to be analysed for glyphosate using a HPLC method of analysis. Since the mortality was < 50%, no statistical calculation of LC_{50} values was possible. Therefore, NOEC and LC_{50} were determined by visual interpretation of the mortality and observation data.

Results

Mean measured test item concentrations ranged from 90 % to 98 % of nominal test concentrations. Therefore, endpoints were evaluated using nominal test item concentrations.
The 96 h LC_{50} for the reference item pentachlorophenol was 0.26 mg/L, which is within the normal range of the reference material. The reference item was tested in a separate study.
All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60 % of air saturation and constant exposure conditions have been maintained.
At the 100 mg glyphosate acid/L concentration, there was no mortality during the 96 hours of exposure. In addition, no sub-lethal effects were observed.

RMS Conclusions

The 96 h LC_{50} for common carp (*Cyprinus carpio*) exposed to glyphosate acid in a limit test was determined to be > 100 mg a.s./L, with a 96 hour NOEC of 100 mg glyphosate a.s./L.

The study is considered to be acceptable and valid.

KIIA 8.2.1/05

Author:	[redacted]
Title:	96-Hour Acute Toxicity Study in Rainbow trout with (Aminomethyl) Phosphonic Acid (Static).
Date:	29.06.1998

Doc ID:	2310933 / 232469
Guidelines:	OECD203/ EEC directive 92/69, Part C.1
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	(Aminomethyl)phosphonic acid (AMPA)
Lot/Batch #:	A010047101
Purity:	99 %
Control:	Tap water; Reference item: Pentachlorophenol
Species:	Rainbow trout (*Oncorhynchus mykiss*)
Age:	Juveniles
Size:	4.14 ± 0.34 cm
Body weight of the animals:	0.54 ± 0.20 g (mean weight of 10 representative individuals)
Loading:	0.38 g fish/L (7 fish per 10 L of test medium)
Source:	[redacted]
Diet/Food:	Last feeding at about 30 h prior to test initiation and no feeding during the test period
Acclimation period:	At least 12 days after delivery
Temperature:	14.2 – 14.8 °C
Photoperiod:	16 hours light
pH:	7.3 – 8.4
Dissolved oxygen:	9.3 – 9.7 mg O_2/L
Conductivity:	Not stated
Hardness:	2.4 mmol/L
Methods:	The test was conducted as a static (without renewal) 96 h limit test at a nominal test concentration of 100 mg AMPA/L, based on the results of a range finding test. The test media was prepared by direct addition of AMPA to tap water. A negative control (dilution water only) was prepared in parallel. Single vessels (18-L glass aquariums) containing 10 litres of control, or test media were prepared. Seven fish were added to each vessel at the start of the test. All fish were observed for sub-lethal effects and mortalities after 2, 24, 48, 72 and 96 hours. Temperature, pH-value and oxygen saturation of the test solutions were measured on a daily basis. Hardness of the test water was measured at test initiation only. Prior to the start of the test, ten representative fish from the fish stock used in the test were weighed (wet weight (g)) and measured (total length (cm)). Samples of control or test media were taken at test start (0 hours) before fish addition and at 96 hours (test end). Concentrations of AMPA in each sample were determined using an HPLC method of analysis. Since the mortality was < 50%, no statistical calculation of LC_{50} values was possible. Therefore, NOEC and LC_{50} were determined by visual interpretation of the mortality and observation data.

Results

Measured concentrations of AMPA in media samples taken at the start of the test before fish introduction were 105 % of nominal. At the end of the test, concentrations in the aged test media were 97 % of nominal. The mean measured concentration of AMPA ranged between

80 and 120 % of nominal, therefore the ecotoxicological endpoints were evaluated based on the nominal AMPA concentrations.
The determined 96h-LC_{50} for the reference item pentachlorophenol was 0.30 mg/L.
There were no sub-lethal effects or mortality observed in fish exposed to AMPA during the 96 hours limit test at 100 mg AMPA/L.
All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60 % of air saturation and constant exposure conditions have been maintained.

RMS Conclusions
The 96 h LC_{50} for rainbow trout exposed to AMPA was determined to be >100 mg/L. The 96 hour NOEC was considered to be 100 mg AMPA/L, the maximum concentration tested.

The study is considered to be acceptable and valid.

KIIA 8.2.1/06

Author:	[redacted]
Title:	AMPA: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*)
Date:	10.12.1993
Doc ID:	2310934 /BL5070/B
Guidelines:	OECD 203
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	(Aminomethyl)phosphonic acid (AMPA)
Lot/Batch #:	X582
Purity:	85 %
Control:	Dechlorinted tap water
Species:	Rainbow trout (*Oncorhynchus mykiss*)
Age:	Juveniles
Size:	45 -66mm
Body weight of the animals:	1.7g (mean weight of 10 representative individuals)
Loading:	0.85 g fish/L
Source:	[redacted]
Diet/Food:	Last feeding at about 24 h prior to test initiation and no feeding during the test period
Acclimation period:	At least 18 days after delivery
Temperature:	15 °C
Photoperiod:	16 hours light
pH:	7.22 – 7.66
Dissolved oxygen:	9.4 – 10 mg O_2/L
Conductivity:	277µS/cm, 25 °C

Hardness:	41.3 mg/L as CaCo3
Methods:	The test was conducted as a static (without renewal) 96 h limit test at a nominal test concentration of 32, 56, 100 and 180 mg AMPA/L together with a dilution water control. The test media was prepared by direct addition of AMPA exposure vessels. Single vessels (27-L glass aquariums) containing 20 litres of control, or test media were prepared.Ten fish were used in each test concentration and in the dilution control. Fish were randomly assigned to the tes vessels All fish were observed for sub-lethal effects and mortalities after 24, 48, 72 and 96 hours. Temperature, pH-value and oxygen saturation of the test solutions were measured on a daily basis. Hardness of the test water was measured at test initiation only. Samples of control or test media were taken daily and Concentrations of AMPA were determined using an HPLC method of analysis. Since the mortality was < 50%, no statistical calculation of LC50 values was possible. Therefore, NOEC and LC50 were determined by visual interpretation of the mortality and observation data.

Results

The overall mean concentration ranged from 100 to 111 % of nominal values. Therefore, the results of this study were reported on the basis of nominal concentrations.

There were no mortalities in the dilution control or in the nominal concentrations of 180 mg/L or less during the 96 hour exposure.

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60 % of air saturation and constant exposure conditions have been maintained.

Sub-lethal effects or mortality observed in fish exposed to AMPA during the 96 hours were sounding, loss of balance and dark discolouration. Symptoms of toxicity observed are described in the following table.

Table B.9.2-6: Effects of AMPA on Rainbow trout (*Oncorhynchus mykiss*)

AMPA technical (mg/L)	Number of dead fish /observed symptoms			
	24 h	**48 h**	**72 h**	**96 h**
Control	< 10%	< 10%	< 10%	< 10%
18	< 10%	< 10%	<10%	< 10%
32	< 10%	< 10%	< 30%/ SO/LE	< 30%/ SO/LE
56	< 10%	< 10%	< 10%	< 10%
100	< 10%	< 10%	< 10%/ SO/LE	< 10%/ SO/LE
180	10	10	< 30%/ SO/LE	> 30%/ SO/LE

SO: Sounding ; LE: Loss of equilibrium

RMS Conclusions

The 96 hour no observed effect concentration (NOEC) of AMPA technical, based on mortalities and symptoms of toxicity, was found to be 18 mg/L on the basis of nominal concentrations. The LC_{50} value determined based on nominal concentrations was LC_{50} > 180 mg AMPA/L.

The study is considered to be acceptable and valid.

KIIA 8.2.1/07

Author:	[redacted]
Title:	MON 78623: A 96-hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)
Date:	28.08.2003
Doc ID:	2310937 /WL-2002-149
Guidelines:	OECD Guideline 203 OPPTS 850.1075
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	MON 78623 (Glyphosate K-salt)
Lot/Batch #:	GLP-0108-11688-F
Purity:	47.7 %
Control:	Dechlorinated and filtered tap water
Species:	Rainbow trout (*Oncorhynchus mykiss*)
Age:	Juvenile
Size (mean standard length):	43 mm (38 – 56 mm)
Weight (mean wet weight):	0.94 g (0.59 – 1.3 g)
Loading:	0.47 g fish/L
Source:	[redacted]
Acclimation:	5 weeks prior to the test initiation
Temperature:	12.2 – 12.7 °C
Photoperiod:	16 h light, with a 30 min transition period
pH:	Control (start – 96 h): 8.2 – 8.0 156 mg/L (start – 96 h): 7.5 – 8.1 313 mg/L (start – 96 h): 7.1 – 8.0 625 mg/L (start – 96 h): 6.7 – 7.9 1250 mg/L(start – 96 h): 6.2 – 7.1 2500 mg/L (start – 96 h): 5.7 – 5.8
Dissolved oxygen:	≥ 7.3 mg/L (≥ 67% saturation)
Conductivity:	280 µS/cm
Hardness:	144 mg $CaCO_3$/L.
Alkalinity:	184 mg $CaCO_3$/L
Methods:	A definite toxicity test was performed using nominal concentrations of 156, 313, 625, 1250 and 2500 mg test item/L (mean measured: 159, 329, 646, 1302 and 2573 mg test item/L) in a static test setup, based on the results of a range finding test,. A negative control group (dilution water only) was prepared in parallel. Duplicate vessels (38 L glass vessels containing 20-L control water or test medium) were prepared for the control and treatment groups, each containing ten fish (20 fish per treatment). Observations for sub-lethal effects and mortality were performed at 4, 24, 48, 72 and 96 hours after test initiation (fish addition). The pH-value and oxygen saturation of the test solutions were measured at test initiation and on each observation date. Temperature was measured at test initiation and termination. Hardness, alkalinity and specific conductivity of the test water were measured at the start of the test only. Fish

	wet weights and total lengths were measured in the control. Samples of control or test media from all vessels was taken at 0 (before fish addition) 48 and 96 hours and analysed to determine the to measure glyphosate K salt concentration. Since the mortality was < 50%, no statistical calculation of LC_{50} values was possible. The NOEC was determined by visual interpretation of the mortality and observation data.

Results

Measured concentrations were between 99.8 and 109% of nominal. The ecotoxicological endpoints are based on the mean measured concentrations of 159, 329, 646, 1302 and 2573 mg glyphosate K-salt/L, (equivalent to 75.8, 156.9, 308.1, 621.1 and 1227.3 mg glyphosate acid/L).

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60 % of air saturation and constant exposure conditions have been maintained.

There was no mortality or sub-lethal effects in the negative control and at the mean measured concentrations of 159 and 329 mg glyphosate K-salt/L. At 646, 1302 and 2573 mg glyphosate K-salt/L, 0, 5 and 15 % mortality were observed respectively. At the three highest test concentrations, sub-lethal effects were noted within 15 minutes after test initiation (including surfacing, laying on the bottom of test chamber, erratic swimming, loss of equilibrium).

The severity of effect generally increased with increasing concentration, which correlated to the concentration-responsive decrease in pH. The pH at 0 h decreased from 8.2 for the controls to 5.7 at the highest test concentration. All surviving fish in 646 and 1302 mg test item/L appeared normal by 24 h and appeared normal for the remainder of the test. Effects were still evident in three of the 17 surviving fish in 2573 mg test item/L at test termination. The pH remained below 6 in the highest test concentration throughout the test.

Table B.9.2-7: Lethal effects of glyphosate K-salt on rainbow trout (*Oncorhynchus mykiss*)

Glyphosate K-salt (mg/L)	**Glyphosate acid (mg a.s./L)**	**Number of dead fish / Number of fish with intoxication symptoms and observed symptoms**					
		0 h	**4 h**	**24 h**	**48 h**	**72 h**	**96 h**
Control		0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
159	**75.8**	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
329	**156.9**	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
646	**308.1**	0 / 0	0 / 20 A	0 / 11 A	0 / 0	0 / 0	0 / 0
1302	**621.1**	0 / 3 R / 17 E,N	1 / 17 A / 2R	1 / 0	1 / 0	1 / 0	1 / 0
2573	**1227.3**	0 / 8 R / 12 E,N	0 / 7 R / 13 A,E,N	0 / 6 R / 4 A / 2 E,N	3 / 0	3 / 3 R / 1 C	3 / 3 R

A = surfacing; R= laying at bottom of test chamber; E = erratic swimming, N = loss of equilibrium

RMS Conclusions

96 hour LC_{50} for rainbow trout (*Oncorhynchus mykiss*) exposed to the glyphosate K-salt was determined to be > 2573 mg /L, equivalent to >1227.3 mg a.s/L. The 96 hour NOEC was determined to be 329 mg glyphosate K-salt/L, equivalent to 156.9 mg a.s./L.

The study is considered to be acceptable and valid.

KIIIA 10.2.2.1/01

Author:	[redacted]
Title:	MON 52276: Acute toxicity to rainbow trout, *Oncorhynchus mykiss*, under flow-through test conditions
Date:	1992
Doc ID:	2317591 /TO-91-296
Guidelines:	US EPA FIFRA 72-1 (1982), OECD 203, and EEC Method C.1.
GLP:	YES
Validity:	YES

Materials and Methods

Test item::	MON 52276
Active substance	Glyphosate (isopropylamine salt)
Lot/Batch #:	LLN-9105-3135F
Purity:	30.95 %
Species:	Rainbow trout (*Oncorhynchus mykiss*)
Age:	Juveniles
Size:	3.1 – 4.1 cm
Loading:	10 test individual for 15 L test solution
Source:	[redacted]
Acclimation period:	48 hours prior to the test initiation
Body weight of the animals:	0.35 – 0.95 g
Temperature:	11.5 – 13.8 °C
Photoperiod:	16 hours, 392 – 500 lux
pH:	8.1 – 8.3 (control); 7.3 – 7.4 (119 mg test item/L); 5.9 (989 mg/L)
Dissolved oxygen:	≥7.1 mg/L
Conductivity:	382 – 705 µmhos/cm
Hardness:	38 - 116 mg $CaCO_3$/L
Alkalinity:	57 - 77 mg $CaCO_3$/L
Methods:	Two groups of ten fish each were exposed under flow-through conditions in a proportional diluter system (4.8 cycles/h) for 96 hours to nominal concentrations of MON 52276 at 0 (controls), 130, 216, 360, 600 and 1000 mg/L. The test water was a blend of treated municipal water and treated well water. During the 14-day holding period prior to test initiation, fish were fed daily and were in good health. There were two vessels per treatment, each containing ten fish (appr.24 L glass vessels containing 15 L test medium). Mortality and signs of toxicity were recorded at 24, 48, 72 and 96 hours after test initiation. Water temperature in a control vessel was measured hourly throughout the test, and water pH and dissolved oxygen were measured daily in all test vessels. Hardness, total alkalinity and specific conductivity were measured at test initiation and test termination. At 0, 48 and 96 hours, samples of test medium were taken for quantification of glyphosate by HPLC. LC50 values were calculated along with the 95% confidence limits using non-linear interpolation.

Results

One fish died at the lowest test concentration (119 mg a.e./L), but it was judged to be not treatment-related. No mortality was observed at the higher test concentrations. No sublethal effects were observed at any test concentration.

RMS Conclusions

The pH of the test system was correlated with MON 52276 concentration, and varied by more than 1 unit across the 5 dose levels. Within each test concentration, the pH variation was less than one unit. The range of temperature during the test was 2.3 ºC, rather than the maximum range of 2 ºC specified in the guideline. The dissolved oxygen concentration during the holding period was not reported. Fish length ranged from 3.1 – 4.1 cm, outside the recommended length of 4.0 – 8.0 cm. Fish were not inspected after the first 2 to 4 hours of the test. These deviations were not considered to have affected the outcome of the study.
The present study is considered valid according to OECD guideline 203.
Based on mean measured concentrations, the 96-hour LC_{50} for rainbow trout (*Oncorhynchus mykiss*) exposed to MON 52276 in a flow-through test system was > 989 mg/L (> 306 mg a.s./L). The corresponding no observed effect concentration (NOEC) was 989 mg/L (306 mg a.s./L), based on the absence of mortality and abnormal sublethal effects at this concentration.

KIIIA 10.2.2.1/02

Author:	[redacted]
Title:	MON 52276: Acute toxicity to the common carp, *Cyprinus carpio*, under flow-through test conditions.
Date:	1992
Doc ID:	2317595 /TO-91-298
Guidelines:	US EPA FIFRA 72-1 (1982), OECD 203, and EEC Method C.1.
GLP:	YES
Validity:	YES

Materials and Methods

Test item::	MON 52276
Active substance	Glyphosate as (isopropylamine salt)
Lot/Batch #:	LLN-9105-3135F
Purity:	30.95 %
2. Vehicle and/or positive control:	None
Species:	Common carp (*Cyprinus carpio*)
Age:	Juveniles
Size:	2.7 – 5.0 cm
Loading:	10 test individuals for 15 L test solution (0.93 g fish/L)
Source:	[redacted]
Acclimation period:	48 hours prior to the test initiation
Body weight of the animals:	0.57 – 2.97 g
Temperature:	21.7 – 23.8 °C

Photoperiod:	16 hours light, 350 - 425 lux
pH:	7.2 – 8.1 (control); 7.1 (98 mg test item/L); 5.7 (895 mg/L)
Dissolved oxygen:	6.7 – 8.7 mg/L
Conductivity:	1614 - 1688 µmhos/cm
Hardness:	184 - 192 mg $CaCO_3$/L
Alkalinity:	34 - 45 mg $CaCO_3$/L
Methods:	Two groups of ten fish each were exposed under flow-through conditions using a proportional diluter system (3.8 daily volume turnover) for 96 hours to nominal concentrations of MON 52276 at 0 (controls), 130, 216, 360, 600 and 1000 mg/L. The test water was a blend of treated municipal water and treated well water. During the 14-day holding period prior to test initiation, fish were fed daily and were in good health. There were two vessels per treatment, each containing ten fish (appr.24 L glass vessels containing 15 L test medium). Mortality and signs of toxicity were recorded at 24, 48, 72 and 96 hours after test initiation. Water temperature in a control chamber was measured hourly throughout the test, and water pH and dissolved oxygen were measured daily in all test chambers. Hardness, total alkalinity and specific conductivity were measured at test initiation and test termination. At 0, 48 and 96 hours, samples of test medium were taken for quantification of glyphosate by HPLC. LC_{50} values were calculated along with the 95% confidence limits using non-linear interpolation.

Results

The mean measured concentrations during the 96-hour exposure ranged from 98 to 895 mg test item/L and from 75 to 90 % of nominal.

Mortality to one fish was observed at the lowest test concentration (119 mg/L), but it was judged to be not treatment-related. No mortality was observed at the higher test concentrations. No sublethal effects were observed at any test concentration.

RMS Conclusions

For a period of 4-6 hours, beginning at 8 hours prior to test termination, only dilution water was delivered to test chambers due to a malfunction in the diluter system. Test fish were exposed to nominal test concentrations for approximately 88 hours, followed by a slow dilution of test concentration for 4 hours, and then the exposure was adjusted to nominal concentrations for the remaining 4 hours of the test. Since there were no indications of stress or any other effects, it is unlikely that the reduction in exposure concentration for this short period had any effect on the outcome of the test. The pH of the test system was correlated with MON 52276 concentration, and varied by more than 1 unit across the 5 dose levels. Within each test concentration, the pH variation was less than one unit. The temperature range during the test was 2.1 ºC, rather than the maximum range of 2 ºC specified in the guideline. The dissolved oxygen concentration during the holding period was not reported. During the test period, the dissolved oxygen during the test fell below 60 % of the air saturation value in at least one replicate at every dose level and in both replicates at the two highest dose levels; Fish length ranged from 2.7 – 5 cm, outside the recommended length of 4.0 – 8.0 cm.
These deviations were not considered to have affected the outcome of the study.

The study is considered valid and acceptable.

Based on mean measured concentrations, the 96-hour LC_{50} for common carp (*Cyprinus carpio*) exposed to MON 52276 in a flow-through test system was > 895 mg/L (> 277 mg a.s./L). The corresponding no observed effect concentration (NOEC) was 895 mg/L (277 mg a.s./L).

B.9.2.1.2 Fish – Early life-stage toxicity test

After the EU peer reviewed evaluation of glyphosate in 2001, the lowest chronic NOEC for a fish study was determined to be 25.7 mg a.s./L (Anonym, 95-00020). New early life-stage toxicity studies have been submitted for the present evaluation: a study with *Oncorhynchus mykiss* investigating effects of glyphosate ([redacted] 2010, BVL no 2310941) and a study with *Pimephales promelas* investigating effects of the metabolite AMPA ([redacted], 2011, BVL no 2310943). Additionally, an early life stage study with *Brachydanio rerio* ([redacted]., 2000, BVL no 2310938) was conducted. The new studies are described in detail below. A summary of data for chronic toxicity of fish, listing the studies reviewed in the 2001 EU glyphosate evaluation as well as the new studies performed with glyphosate and AMPA is shown in the following table.

Table B.9.2-8: Early life-stage toxicity studies with glyphosate acid and metabolites AMPA and fish newly submitted for the present application for renewal of approval

Species	Substance	Test design	EC_{50} (mg a.s./L)	NOEC (mg a.s./L)	Reference
Pimephales promelas	Glyphosate acid	255d	.	25.7	Review Report for the active substance Glyphosate (SANCO/6511/VI/99-final), Anonym, 95-00020
Brachydanio rerio	Glyphosate acid	168 h	25	1	[redacted]2000; BVL no 2310938 /RF-D62.16/99
Oncorhynchus mykiss	Glyphosate acid	85-day (60 days post-hatch	-	**9.63**	[redacted] 2010; BVL no 2310941 /1005.029.321
Pimephales promelas	AMPA	33-day (7days post-hatch	-	12	[redacted] 2011; BVL no 2310943 /WL-2010-328

Values in grey: confirmed EU endpoints (SANCO/6511/VI/99-final, or EU Review Monograph)

KIIA 8.2.3/01

Author:	[redacted]
Title:	Chronic Toxicity of Glifosate Técnico Nufarm to zebra fish larvae (*Brachydanio rerio*)
Date:	13.01.2000
Doc ID:	2310938 /RF-D62.16/99

Guidelines:	OECD 212/ IBAMA 1990: Manual de testes para avaliacao da ecotoxicidade de agentes quimicos
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	037-919-113
Purity:	954.9 g/kg acid equivalent
2. Vehicle and/or positive control:	Tap water; Potassium dichromate ($K_2Cr_2O_7$)
Species:	Zebra fish (*Danio rerio*) larvae
Age:	Larvae, approx. 48 hours old
Size:	Not stated
Loading:	1 L for 10 larvae
Source:	[redacted]
Acclimation period:	48 hours prior to testing during embryo incubation and hatching
Temperature:	23.8-24.3 °C
Photoperiod:	16 hours light / 8 hours dark
Dissolved oxygen:	60-100%
Conductivity:	168 µS/cm
Hardness of test medium:	44.1 mg/L $CaCO_3$
Methods:	The fish early life-stage toxicity test was performed under semi-static exposure conditions renewing the test solution every 48 hours. Following a range finding test, the freshly hatched fry of *Danio rerio* was exposed to test concentrations of 0.32, 0.56, 1.0, 3.2, 5.6, 10 and 32 mg glyphosate acid/L for 168 hours. A control consisting of reconstituted water and five toxic reference concentrations (32, 56, 100, 140 and 180 mg $K_2Cr_2O_7$/L were maintained concurrently. Observations for mortality and sublethal responses were made every 24 hours. Dead individuals were removed at each observation. Temperature, dissolved oxygen, pH and conductivity were measured daily. The active ingredient analysis of stock solutions was performed by liquid chromatography. LC_{50} and its confidence limits were determined using trimmed Spearman-Karber method. Fisher's Exact test was used for determination of significant differences in survival between control and exposure. ~~The NOEC was recalculated by RMS via ToXRatPro Version 2.10~~

Results

The active ingredient concentration in each stock solution was at least 80 % of the nominal concentration. For the reference compound potassium dichromate ($K_2Cr_2O_7$) a 168 hour LC_{50} value of 124.66 mg a.s./L (95 % C.I. 112.08 – 138.67 mg a.s./L) was determined.
With regard to the validity criteria of the pertaining OECD guideline 212 survival of fertilised eggs on successive days was 100 %. Analysis of test item treatments was performed for the stock solutions, the test was caried out in a semistatic system, with renewal of thes test solution each 48 h. The water temperature did not differ more than +/- 1.5 °C between test chambers on successive days at any time during the test at the recommended temperature, as well as pH remained constant. Mortality in control group did not exceed 10 %, dissolved

oxygen concentration was between 60 and 100 % of air saturation. The present study is considered valid according to OECD guideline 212.

A significant increase of mortality was observed at a concentration of 5.6, 10 and 32 mg a.s./L, behavioural responses such as lethargy was observed at 3.2, 5.6, 10 and 32 mg a.s./L. The following observations for mortality were made every 24 h during the 168h test period:

~~The NOEC was recalculated by RMS: Fisher`s Exact Binomial Test with Bonferroni Correction was used with Pair-wise comparisons between treatment and control on the multiple significance level (alpha is 0,05; one-sided greater). Pair-wise comparisons re performed sequentially using the adjusted Alpha* (= alpha/(k-1); k: number of comparisons (after Holm 1979)); Ho (no effect)is accepted, if the probability p > Alpha*. A NOEC of 5.6 mg a.s./L and EC_{50} of 23.6 mg a.s./L is suggested (ToXRatPro Version 2.10).~~

Table B.9.2-9: Lethal effects of glyphosate acid for zebra fish

	Glyphosate acid (mg a.s./L)							
	0 (Control)	**0.32**	**0.56**	**1.0**	**3.2**	**5.6**	**10**	**32**
Introduced	30	30	30	30	30	30	30	30
Survided (168 h)	30	30	30	30	27	25	22	13
Mortality (168 h) (%)	0	0	0	0	10	16.7*	26.7*	56.7*

*statistically significant different from control

RMS Conclusions

~~In an early life-stage toxicity test on fish larvae, the No-Observed-Effect-Concentration (NOEC) for zebra fish (*Danio rerio*) exposed to glyphosate acid was determined to be 5.6 mg a.s./L based on nominal concentrations. The LC_{50} after 168 hours was determined to be 23.6 mg a.s./L.~~

In the guideline OECD 212 it is recommended that the duration of the test should be 30 days post hatch. By contrast, the present study was performed for 168 h. It is also stated that the test is to be continued at least until all the fish in control treatment are free feeding. Moreover, the time of first feeding should start 6-7 days after spawning. In the current test it is not clear, if fish in the control treatment are free feeding totally. Nevertheless, significant increase of mortality was observed at a concentration of 5.6, 10 and 32 mg a.s./L. Despite these defiencies, the study is considered to be valid and acceptable.

In the short term toxicity test on fish larvae, the LC_{50} after 168 hours was determined to be 24.71 mg a.s./L. The No-Observed-Effect Concentration (NOEC) and the Lowest-Observed-Effect Concentration (LOEC) for zebra fish (Danio rerio) exposed to glyphosate acid were determined by the author to be 3.2 mg a.s./L and 5.6 mg a.s./L, respectively, based on nominal concentrations. Nevertheless, the mortality effect in the study with *Danio rerio* followed a dose response relationship and in the treatment level at 3.2 mg/L a mortality of 10% was observed. Considering these biological effects as relevant, although not statistically significant, results in a NOEC of 1.0 mg/L.

KIIA 8.2.4/02

Author:	[redacted]
Title:	Glyphosate acid: Early life-stage toxicity test with rainbow trout (*Oncorhynchus mykiss*) under flowthrough conditions
Date:	23.12.2010
Doc ID:	2310941 /1005.029.321
Guidelines:	OECD Guideline 210 (1992)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	GLP-0807-19475-T
Purity:	96.03 %
Control:	Reconstituted well water
Species:	Rainbow trout (*Oncorhynchus mykiss*) eggs and milt
Age of eggs:	Eggs and milt were less than 36 hours old at fertilization. The time between fertilization and egg addition to test system was less than 3.5 hours
Number of animals/dose level:	40 organisms per replicate i.e. 40 organisms per treatment level and control
Supplier:	[redacted]
Mean loading rate (biomass per volume of test solution)	0.31 g/L per 24 hours
Temperature:	Continuously measured temperature: 9.4 to 13.1 °C Single-point measured temperature: 11.3 to 13.9 ºC)
pH:	7.14 to 8.44
Dissolved oxygen:	> 60% ASV for study duration
Conductivity of test medium:	340 to 450 µS/cm
Hardness of test medium:	153 to 184 mg/L $CaCO_3$
Photoperiod:	16 h with a 30 minute transition from day 32 until test completion (intensity 137 to 377 lux) Eggs and larvae were shielded from light during the incubation and hatching phases until one week after hatching.
Methods:	The fish early life-stage toxicity test was performed under flow-through exposure conditions, using a constant-flow test item delivery system, supplying the appropriate test medium to duplicate exposure vessels at each of the five concentrations and the duplicate negative dilution water control vessels. The fertilized eggs were exposed to test concentrations of 0.095, 0.305, 0.977, 3.125 and 10.0 mg glyphosate acid/L for 85 days. 12 impartially located exposure vessels were maintained in a temperature-controlled water bath designed to maintain the test solution temperatures at 12 ± 2 °C. During the egg exposure phase and until one week after hatching the test area was maintained in continuous darkness. From test day 32 until test completion, the vessels were illuminated to a light intensity of 137 to 377 lux using fluorescent tubes. A photoperiod of 16 hours was employed with a 30 minute (dawn/dusk) transition period. <u>Preparation of test solution:</u> A 1 g glyphosate acid/L stock solution was prepared directly prior to test initiation and as required during the exposure period, by dissolving approximately 11.737 g of glyphosate acid in 10 L of dilution water. The stock solution was further diluted (dilution water) by the test item delivery system to achieve the required concentrations in each of the exposure vessels. For the control group, dilution water only without test item was used.

	Test units: The test vessels measured 39.0 cm x 19.2 cm, with an approximate water depth of 14.6 cm maintained at a constant volume of 10 L. Two replicates (A and B) were maintained for all treatments and the control. Test initiation: Prior to fertilization, freshly collected rainbow trout milt and eggs were acclimatized in their respective delivery containers to the approximate test temperature of 12 ± 2 °C, using a water bath and then mixed carefully together. The 'apparently' fertilized eggs were impartially distributed to egg incubation cups in groups of five, until each cup contained 50 eggs. The incubation cups were suspended in the respective exposure vessel with two cups per replicate vessel, resulting in 100 eggs per replicate. The test was initiated once all vessels contained eggs within 3.5 hours of receipt of the gametes and within two hours of fertilization. Hatching success was determined on days 22 to 26 based on the number of viable eggs. Any eggs exhibiting embryonic development, whether dead or alive, at the time of assessment, were considered fertile for purposes of determining percent viability. All non-viable eggs were counted and discarded at day 26. The percent viability was calculated based on the actual number of fertilized embryos on day 26. Hatching success was calculated based on the actual number of viable embryos. Egg exposure: Dead and alive eggs were counted daily. All eggs observed to be clear were considered to be alive, all eggs observed to be opaque and milky were considered to be dead. All eggs observed to be dead were removed and preserved in Stockard's solution for clearing and determination of embryonic development. Fry which hatched prior to the determination of viability were collected in an auxiliary egg cup. Post hatch exposure: At completion of hatch on day 26, twenty organisms per replicate i.e. 40 organisms per treatment level and control were transferred directly from the first egg cup (i.e., A1 and B1) to the surrounding test media in the test vessels and the egg cups were removed. For replicate A of the control and the 0.095 mg glyphosate acid/L treatment, 20 fry in the auxiliary egg cup containing the early hatched fry were randomly selected. For replicate A of the 10 mg a.s./L treatment, only eight viable eggs hatched of the 20 randomly selected eggs and therefore only eight hatched fry were released into the test vessel. All remaining alive and dead eggs were preserved in Stockard's solution. The remaining fry were recorded and then discarded. After evaluation of the developmental status of the cleared eggs, the viability of all eggs was calculated. During the post-hatch exposure period, developing fry in all vessels were observed daily; recording behaviour and appearance. Dead fry were removed during these observations. Survival was estimated daily throughout the post-hatch period. At 60-days post-hatch exposure (experimental completion), the percentage fish survival was calculated. Fry feeding: At the beginning of fry swim-up, the fry were fed live brine shrimp nauplii (*Artemia salina*), harvested from hydrated cysts (24 to 36 hours post-hydration) three times per day. Fish were not fed during the 24 hours prior to study termination. Length and weight: At day 60 post-hatch all of the surviving fish in each replicate vessel were euthanized with MS-222 (tricain methane-sulfonate), measured and weighed individually to determine fish total lengths and wet weights, respectively for each treatment. The dissolved oxygen (DO) concentrations, pH and temperature were measured and recorded in each test vessel at experimental start and weekly thereafter until test termination (day 85). On test day 75, the DO levels decreased to between 6.31 to 7.50 mg O_2/L, so aeration was provided to each test vessel until test completion. Temperature was continuously monitored in one replicate (replicate A of the control) throughout the study. Total hardness, alkalinity and specific conductivity were monitored at experimental start and on test days 5, 11, 19, 25, 32, 39, 46, 53, 61, 67, 74 and 81 in one replicate of the highest treatment level and the control during the exposure. Analytical measurements: Prior to the start of the exposure phase, i.e., day -2, samples from one replicate of the treatment level solutions and control solutions were collected and analysed for the active ingredient. Results of the pre-test analyses were used to assess correct dosage of the system before test initiation. During the in-life phase, water samples of approximately 10 mL were removed from

	both replicates of each treatment level and control on test days 0, 6, 13, 20, 27, 33, 41, 48, 55, 62, 70, 76 and 85 and the content of glyphosate acid was determined. Samples of the stock solutions were also analysed at each sampling interval. Determination of NOEC and LOEC: Based on the results of statistical analysis performed for normal fry at hatch, hatching success, survival at test termination and growth (total length, wet and dry weight), the No-Observed-Effect Concentration (NOEC) and the Lowest-Observed-Effect Concentration (LOEC) were determined. The data for percent normal fry at hatch, hatching success, survival at test termination and growth (total length, wet and dry weight) were first checked for normality using Shapiro-Wilks' Test (Weber *et al.*, 1989) and for homogeneity of variance using Bartlett's Test (Bartlett, 1937). The data set for hatching success and survival at test termination were arc-sine (square root) transformed prior to determination of the NOEC and the LOEC by using one-way ANOVA and the parametric post-hoc Dunnett's Test (Dunnett, 1955, 1964). The data sets for growth passed the tests for homogeneity and normality, and Dunnett's Test was used to determine the NOEC and the LOEC.

Results

All validity criteria according to OECD 210 were fulfilled, as dissolved oxygen concentration was between 60 % and 100 % of air saturation, water temperature was within the range specified for the test species and constant exposure conditions have been maintained (i.e. within ±20 % of nominal concentration were recovered, except for the lowest concentration which does not affect the results of the study), and overall survival of fertilised eggs in the controls was greater than or equal to the limits defined in Annexes 3 and 6 of OECD 210.

The mean measured concentrations (calculated as geometric means) of 0.305, 0.977, 3.125 and 10.0 mg a.s./L ranged between 85.7 and 96.3 % of the nominal test concentrations, with the exception of the lowest test concentration (0.095 mg a.s./L), where a mean recovery of 66.9 % of the nominal concentration was calculated. Based on these results, the mean measured concentrations (calculated as geometric means) of 0.064, 0.261, 0.846, 2.804 and 9.63 mg a.s./L were used for the evaluation of the biological data.
The water quality parameters measured were not affected by test item concentrations. The results of the water quality measurements carried out during this study established that conditions maintained throughout the 85-day exposure were satisfactory for the promotion of normal rainbow trout embryo hatchability, fry survival and growth.
The effects of glyphosate acid on embryo viability, hatching success, number of normal fry at hatch, survival at test termination and growth (total length, wet and dry weight) are provided below.

Table B.9.2-10: Egg viability, hatching success and normal fry at completion of hatch (test day 26) and survival, total length, wet weight and dry weight of rainbow trout (*Oncorhynchus mykiss*) at test termination of the 85-day exposure to glyphosate acid. Given are means and standard deviations

Mean measured concentration (mg a.s./L)	Egg viability (%)[A]	Hatching success (%)[A]	Normal fry at hatch (%)	60 days post-hatch			
				Survival (%)	Total length (mm)	Wet weight (mg)	Dry weight (mg)
Control	35±3.3	92±6.9	97±0.56	85±7.1	46.38±0.41	942.6±34.9	195.1±14.3
0.064	43±4.9	84±20.2	96±5.2	95±7.1 [B]	45.33±0.83	899.6±10.7	188.7±5.9
0.261	40±4.0	99±1.7	100±0.0	95±0.0	46.75±0.65	932.2±60.5	190.7±7.5
0.846	38±9.9	95±1.5	100±0.0	93±10.6	46.37±1.7	908.6±84.3	189.1±23.0
2.804	41±2.1	91±5.5	99±2.0	95±7.1	46.19±0.33	889.7±23.7	188.4±10.7
9.63	27±9.2	80±28.3	98±2.1	100±0.0	46.38±1.7	947.3±135	203.0±36.5

[A] Based on total number of viable eggs

[B] On test day 59, one fish of replicate A was inadvertently injured during the cleaning process of the test vessel. One day later this fish had died. Since this mortality was not test item related, the fish was therefore excluded from further statistical evaluation.

RMS Conclusions

In a 85-day (60 days post-hatch) chronic study with rainbow trout (*Oncorhynchus mykiss*) exposed to glyphosate acid, the NOEC and LOEC values for percent normal fry at hatch, hatching success, fry survival, length and weight were 9.63 and > 9.63 mg a.s./L, respectively, based on mean measured concentrations.

The study is considered to be acceptable and valid.

KIIA 8.2.4/03

Author:	[redacted]
Title:	AMPA (Aminomethylphosphonic acid): An early lifestage toxicity test with the fathead minnow (*Pimephales promelas*)
Date:	16.06.2011
Doc ID:	2310943 /WL-2010-328
Guidelines:	OECD Guideline 210 (1992), OPPTS 850.1400, ASTM E 1241-05
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	AMPA (Aminomethylphosphonic acid)
Lot/Batch #:	GLP-0908-19984-A
Purity:	98.7 %
2. Vehicle and/or positive control:	Moderately hard well water
Species:	Fathead minnow (*Pimephales promelas*) embryos < 24 hours old
Age of eggs:	< 24 hours old

Number of animals/dose level:	20 organisms per replicate i.e. 80 organisms per treatment level and control
Supplier:	[redacted]
Mean loading rate (biomass per volume of test solution)	0.05 g fish/L per 24 hours; instantaneous loading at the end of test: 0.32 g fish/L
Diet/Food:	live brine shrimp nauplii (*Artemia* sp.), Brine Shrimp Direct, Ogden, Utah, USA
Temperature:	25±1 °C
pH:	7.8 to 8.2
Dissolved oxygen:	≥89 % of saturation (7.3 mg/L)
Conductivity of test medium:	361 - 395 µS/cm
Hardness of test medium:	132 - 140 mg/L $CaCO_3$
Photoperiod:	16 hours with a 30 minute transition period, Light intensity = 296 lux,.
Methods:	The fish early life-stage toxicity test was performed under flow-through exposure conditions, using a constant-flow test item delivery system, supplying the appropriate test medium to the exposure vessels at each of the five concentrations and a negative control (dilution water only) group. The embryos of fathead minnow (*Pimephales promelas*) were exposed to test concentrations of 0.73, 1.5, 2.9, 6.0 and 12.0 mg AMPA/L for 33 days. The test was conducted in a temperature controlled environmental chamber. The test vessels were 9 L glass aquaria with a constant volume of 7 L of test solution. Embryos were held in incubation cups constructed from glass cylinders 50 mm in diameter with 425 µm nylon screen mesh. Four replicates vessels were maintained for all treatments and the control. At test initiation, embryos < 24 hours old were impartially distributed to incubation cups. After a hatching period of 5 days, larvae were released into test chambers. Newly hatched larvae were fed live brine shrimp nauplii (*Artemia sp*.) harvested from hydrated cysts 2 - 3 times per day. During the first day of exposure, embryos were observed twice for mortality and fungal infection. Thereafter, until hatching was complete, observations of embryo mortality and the removal of dead embryos was performed once per day. Once hatching had reached >90% in the control groups on day 5 of the test, the larvae were released into their respective test vessels and the post-hatch period began. During the 28-day post-hatch exposure period, the number of fry mortalities and numbers of individuals exhibiting clinical signs of toxicity or abnormal behaviour was recorded. From these observations, the time to hatch, hatching success, and post-hatch growth and survival were evaluated. On day 28 of the post-hatch exposure period – test termination, the total length for all surviving fish was measured to the nearest 1 mm using a metric ruler and wet and dry weights of all fish was measured to the nearest 0.1 mg using an analytical balance. Fish were euthanized (MS-222) and dried to constant weight in an oven at approximately 60 °C for approximately 47 hours to establish fish dry weight data. Dissolved oxygen, temperature and pH were measured in alternating replicates of each treatment and control group at the beginning of the test, weekly during the test, and at the end of the test. Hardness, alkalinity and specific conductance were measured in alternating replicates of the negative control (dilution water) and the highest concentration treatment group at the beginning of the test, weekly during the test and at the end of the test. Analytical measurements were performed by HPLC analysis using UV detection. Water samples were collected from one test chamber of each treatment and control group four days prior to test initiation to confirm the operation of the diluter. Water samples were collected from alternating replicate test chambers of each treatment and control group on day 0, 7, 14, 21, 28 and 33 (test termination) to determine concentrations of the test substance in the test chambers. All samples were collected at mid-depth in the test chambers, placed in glass vials and analysed immediately. Data were statistically tested using Chi-square and Fisher's Exact test (discrete-

	variable data; α = 0.05) and Dunnett's t-test (one-tailed, normal distributed data; α = 0.05).The NOEC and LOEC were determined by visual interpretation of the observation data.

Results

All validity criteria according to OECD 210 were fulfilled, as dissolved oxygen concentration was between 60 % and 100 % of air saturation, water temperature was within the range specified for the test species and constant exposure conditions have been maintained (i.e. within ± 20 % of nominal concentration were recovered), and overall survival of fertilised eggs/embryos in the controls was greater than or equal to the limits defined in Annexes 3 and 6 of OECD 210.

Analytical measurements were performed on samples of representative test concentrations. Recoveries ranged from 82.5 % to 117 % relative to nominal concentrations for all test concentrations.

The water quality parameters measured were not affected by test item concentrations. The results of the water quality measurements carried out during this study established that conditions maintained throughout the 33-day exposure were satisfactory for the promotion of normal fathead minnow embryo hatchability, fry survival and growth.

The majority of the fish in the control group and in the AMPA treatment groups appeared normal throughout the test. Through day 7 post-hatch, both in the control group and in the AMPA treatment groups, a number of larvae were noted as either weak, lying on the bottom of the test chambers, curled, or having a curled or curved spine/crooked spine. The frequency of curved/curled or curled spine/crooked spine observed in the treatment groups were comparable to historical frequencies observed in control treatments in early life-stage studies with fathead minnows performed at the test facility and consequently concluded to be not treatment related. Additionally, the frequencies of the occurrence of smaller fish visually observed in the control and treatment groups were comparable and consistent with the individual dry weight measurements.

Table B.9.2-11: Hatch success, larval survival and total length, wet weight and dry weight of fathead minnow (*Pimephales promelas*) at test termination of the 33-day exposure to AMPA.

Mean measured concentration	**Hatching success**	**Survival to day 28 post hatch**	**Growth 28 days post-hatch**		
			Mean total length	**Mean wet weight**	**Mean dry weight**
(mg AMPA/L)	**(%)**	**(%)**	**(mm)**	**(mg)**	**(mg)**
Control	99	91	25.2 ±0.57	112.0 ±11.5	24.1 ±1.4
0.73	100	91	25.2 ±0.27	120.7 ±7.4	24.6 ±1.0
1.5	100	93	25.5 ±0.39	119.3 ±14.2	24.9 ±2.1
2.9	100	90	25.7 ±0.62	117.4 ±3.8	23.5 ±0.42
6.0	100	91	25.4 ±0.22	117.4 ±4.2	23.6 ±0.70
12	99	92	26.2 ±0.62	135.2 ±11.0	26.5 ±2.9

RMS Conclusions

In a fish early life stage test (OECD 210) performed using fathead minnow (*Pimephales promelas*) exposed to AMPA, the NOEC and LOEC values for hatching success, fry survival, length and weight were 12 and > 12 mg AMPA/L, respectively, based on mean measured concentrations.

The study is considered to be acceptable and valid.

B.9.2.1.3 Bioconcentration in fish

Glyphosate acid has a log P_{OW} value of < -3.2. Therefore, based on the low log P_{ow}-values the potential for bioconcentration is considered to be negligible. The octanol/water partition coefficient of glyphosate acid, expressed as log P_{ow}, is -3.2. Values less than 3 indicate a low potential for bioaccumulation, therefore no further assessment is necessary.

However, although the potential for bio-concentration is low for glyphosate acid, residues in fish were measured for glyphosate acid and were part of the first EU peer-reviewed glyphosate evaluation in 2001. In several studies ([redacted], Rep. MSL9309, 1989, [redacted] Rep. MSL 2952, 1983; [redacted]., Rep. MSL 5019, 1985, [redacted] Rep. MSL 2937, 1983) on different aquatic organisms, a bioconcentration factors of max. 10 was determined, which is far below the Annex VI BCF trigger value of 1000. Therefore, a study is not necessary to determine bioaccumulation in aquatic non-target organisms.

The primary metabolite of glyphosate is aminomethylphosphonic acid (AMPA). The extent of urinary excretion may be considered the most appropriate measure for oral absorption (please refer to Volume 1 chapter 2.6.2). It is proposed as an appropriate estimate of oral absorption that urinary excretion in the various studies can be estimated to be 20 %. Bioaccumulation correlates with lipophilicity and in general a log K_{ow} > 3 indicates a potential for bioaccumulation. The log K_{ow} for AMPA was estimated to be -2.47 (Episuite Programm and SMILES code (C(N)P(=O)(O)O)). Most of the parent glyphosate is eliminated unchanged and only a small amount (less than 1 % of the applied dose) is transformed to aminomethyl-phosphonic acid (AMPA). The metabolite AMPA has been tested in several toxicity studies which demonstrated that it is of lower toxicity than glyphosate acid (see Volume 1, chapter 2.6.8).

Table B.9.2-12: Bioaccumulation of glyphosate in *Lepomis macrochirus*

Species	Test design	Results	Reference
Lepomis macrochirus	56 d bio-concentration flow-through	BCF = 1.1 ± 0.61 steady state after 120 ± 59 d	Forbis, 1989, ABC#36440 (Part 1) Ridley, Chott, 1989; MSL-9303 (Part 2) 95-00725

Bioconcentration factor (BCF)		BCF = 1.1 ± 0.61; steady state after 120 ± 59 d log P_{ow} of glyphosate acid and its metabolites was < 3, accumulation potential in aquatic non-target organisms is hence considered to be low
Annex VI Trigger for the bioconcentration factor		100
Clearance time	CT_{50}	Not relevant
	CT_{90}	Not relevant
Level of residues (%) in organisms after the 14-day depuration phase		

B.9.2.1.4 Invertebrates – acute toxicity

A summary of all available relevant and compliant data (including data already reviewed during the 2001 EU evaluation of glyphosate) for glyphosate, glyphosate salts and AMPA are

included in Table B.9.2-13. For studies for which the evaluation outcome did not change please refer to the initial monograph and its addenda.

Additionally, a new study was performed with HMPA, an aquatic metabolite of glyphosate.

Table B.9.2-13: Acute toxicity of glyphosate acid, glyphosate IPA salt, K-salt and metabolites AMPA for *Daphnia magna*. Listed are studies already submitted and reviewed as part of the first EU peer review of glyphosate in 2001

Species	Substance	Test design	EC_{50} (mg a.s./L)	NOEC (mg a.s./L)	Reference
Daphnia magna	Glyphosate acid	48 h static	>100	100	96-00067 141863 Bogers, 1995
Daphnia magna	Glyphosate acid	48 h	40	18	95-00537 Handley *et al.*, 1995
Daphnia magna	Glyphosate IPA salt	48 h	930	320	94-01160 Forbis, Boudreau, 1981
Daphnia magna	Glyphosate IPA salt	48 h	>1000		94-00549 Thun, 1993
Daphnia magna	AMPA	48 h static	690	320	94-01163 AB-90-401 Burgess, Hicks, 1991
Daphnia magna	AMPA	48 h	>180	180	94-00500 BL5061/B Anon, 1993

Table B.9.2-14: Acute toxicity of glyphosate acid, glyphosate IPA salt, K-salt and metabolites AMPA and HMPA for *Daphnia magna*. Newly submitted studies fo the present application for glyphosate renewal of approval

Species	Substance	Test design	EC_{50} (mg a.s./L)	NOEC (mg a.s./L)	Reference /GLP
Daphnia magna	Glyphosate acid	48 h static	647.4	464.8	AB-78-201 McAllister, Forbis, 1978, BVL no 2310945 **not acceptable and not valid**
Daphnia magna	Glyphosate acid	48 h static	74 (recalculated by RMS)	53 (recalculated by RMS)	272968 Wüthrich, 1991 BVL no 2310947
Daphnia magna	Glyphosate acid	48 h static	420.6	179.6	RF-D51.39/99 Franco-Perina, 2000 BVL no 2310948
Daphnia magna	Glyphosate acid	48 h static	100 (surrogate proposed by RMS)	100	BL5551/B Morris *et al.*, 1996 BVL no 2310950
Daphnia magna	Glyphosate-IPA salt	48 h static	>61.6 46 a.e.	61.6	83-91-0737-00-93 Jahnke, 1994 BVL no 2310952

Species	Substance	Test design	EC_{50} (mg a.s./L)	NOEC (mg a.s./L)	Reference /GLP
Daphnia magna	Glyphosate-IPA salt	48 h static	>1397 >471 a.e.	471	RF-D51.017/00 Franco-Perina, 2000 BVL no 2310954
Daphnia magna	Glyphosate K-salt	48 h static	592 278 a.e. (recalculated by RMS)	312 (149 a.s.)	WL-2002-150 Palmer *et al.*, 2003 BVL no 2310957
Daphnia magna	AMPA	48 h static	>100		232471 Bogers, 1998 BVL no 2310959
Daphnia magna	HMPA	48 h static	**>100**	100	WL-2010-329 Palmer *et al.*, 2011 BVL no 2310961
Daphnia magna	MON 52276	48 h flow-through	**676** 209 a.e.	356 (110 a.s.)	TO-91-295, Lintott, 1992 BVL no 2317596

a.e.: acid equivalents

KIIA 8.3.1.1/01

Author:	McAllister, W.A., Forbis, A.D.
Title:	Acute Toxicity of Technical Glyphosate (AB-78-201) to *Daphnia magna*
Date:	31.08.1978
Doc ID:	2310945 /AB 78-201
Guidelines:	OECD
GLP:	YES
Validity:	NO

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	XHI-162
Purity:	83.0 %
Positive control:	Well water
Species:	*Daphnia magna*
Age:	Neonates (< 18 h old)
Loading:	10 specimens for 250 mL test solution
Source:	In-house culture
Diet/Food:	None
Acclimation period:	None
Temperature:	19 ± 1 °C
Photoperiod:	16 hours light / 8 hours dark
pH:	8.0 (at test termination, not specified for which group)
Dissolved oxygen:	7.5 mg/L
Conductivity:	Not stated

Hardness:	> 250 mg $CaCO_3$/L.
Methods:	Based on the results of a range finding test, a definitive toxicity test was performed using nominal concentrations of 560, 650, 750, 870, 1000 mg test item/L, (purity corrected concentrations of 464.8, 539.5, 622.5, 722.1 and 830.0 mg glyphosate acid/L) in a static test setup. The test solutions were prepared using test facility well water (Dissolved oxygen = 8.6 mg/L, pH = 7.8, hardness > 250 mg $CaCO_3$/L.). In addition, a control group was exposed to dilution water (negative control). There were three replicates per treatment, each containing ten daphnids. Test chambers were 500 mL glass beakers containing 250 mL of test medium. Total number of immobile *Daphnia magna* was recorded at 24 h and 48 h after the test initiation. Temperature, pH, and oxygen saturation of the test solutions were measured at the test termination. Hardness of the test water was measured at test initiation. EC_{50} values were calculated along with the 95% confidence limits using Probit analysis.

Results

The validity criteria according to OECD 202 concerning the 100 % mobility of daphnids observed in control groups was fulfilled. However, no analytical verification was conducted and only three control replicates were used. Temperature, pH, and oxygen saturation of the test solutions were measured at the test termination, but not specified for which treatment group.

Table B.9.2-15: Effects of glyphosate acid on *Daphnia magna*

Glyphosate concentrations Purity corrected (Glyphosate acid mg/L)	**-**	**464.8**	**539.5**	**622.5**	**722.1**	**830.0**
Immobility (24 h) (%)	0	0	0	6.7	73.3	100
Immobility (48 h) (%)	0	0	3.3	33.3	100	100

RMS Conclusions

The study is not considered to be acceptable and valid. The study does not meet the actual validity criteria of the Guidelines. There is no technical information given concerning the pH-value and no analytic was performed and therfore dose verification is impossible.

KIIA 8.3.1.1/02

Author:	Wüthrich, V.
Title:	48-Hour Acute toxicity of Glyphosate techn. to *Daphnia magna* (OECD-Immobilisation Test)
Date:	09.11.1990
Doc ID:	2310947 /272968
Guidelines:	OECD 202 (1984)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	229-Jak-5-1
Purity:	98.9 %

Positive control:	Reconstituted water (EEC), Potassium dichromate ($K_2Cr_2O_7$)
Species:	*Daphnia magna*
Age:	Neonates (< 24 h old)
Loading:	10 daphnids per 20 mL test medium
Source:	In-house culture
Diet/Food:	Not fed during test or during the 24 hours preceding test initiation.
Acclimation period:	Approximately 24 hours
Temperature:	21.0 ± 0.5 °C
Photoperiod:	16 hours light
pH:	Control:8.4 – 7.9 62.5 mg test item/L:6.3 – 7.6 125 mg test item/L:4.8 – 5.2 250 mg test item/L:3.2 – 3.4 500 mg test item/L: 2.7 – 2.9 1000 mg test item/L:2.3 – 2.6
Dissolved oxygen:	8.3 – 8.1 mg O_2/L (mean)
Conductivity:	Not stated
Hardness:	250 mg $CaC0_3$/L(reconstituted water)
Methods:	The toxicity test was performed with five test nominal glyphosate acid concentrations of 62.5, 125, 250, 500 and 1000 mg glyphosate acid/L, prepared using reconstituted water (EEC). The test was conducted using a static test design (without media renewal) over 48 hours, in duplicate 50 mL beakers each containing 20 mL of the appropriate test or control (reconstituted water only) solution. Juvenile Daphnid (<24 hours old) were added impartially to the test vessels until all contained 10 daphnia. In addition, a test item stability control without daphnids was also prepared at 1000 mg glyphosate acid/L. The number of immobile *Daphnia magna* in each vessel was recorded at 24 h and 48 h after test initiation. The pH-values and oxygen saturation were measured in each test vessel at test initiation and termination. Samples of control and test media were taken at the start – 0 hours (freshly prepared – before animal addition) and end – 48 hours (pooled replicates according to treatment) and analysed for glyphosate content using an HPLC method of analysis. The EC_{50} (immobilisation) was estimated by the authores using the Logit-model, NOEC, EC_{50} and EC_{100} values were determined by linear regression. EC_{50} values were recalculate by RMS via ToXRatPro Version 2.10 using Probit analysis using linear max. likelihood regression and Multiple testing to find the NOEC (Bonferroni-Fisher Test).

Results

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and and dissolved oxygen concentration was ≥ 3 mg/L. Measured concentrations of glyphosate acid in the test media at 62.5, 125, 250 and 500 mg glyphosate acid/L were in the range of 69.7 – 95.2 % of nominal. Authors reported results based on nominal glyphosate acid concentrations. According to the actual criteria in this case results should be based on measured concentrations. Therefore endpoints were recalculated by RMS. Results of the probit analysis using linear max. likelihood regression proposed an EC_{50} value of 74.0 (95 % CL: 16.96 - 130.34). A NOEC of 53.2 mg glyphosate/L is calculated.
The pH in test medium was decreasing due to increasing test concentrations, as the test item is an acid.

Immobilisation of daphnids was observed beginning with 62.5 mg/L test item and all daphnids were immobilised after 48 h at the next higher concentration of 125 mg/L test item.

Table B.9.2-16: Effects of glyphosate on *Daphnia magna*

		Glyphosate acid (mg/L)									
	Control	**62.5**		**125**		**250**		**500**		**1000**	
Mean measured concentrations (mg/L) (% nominal)	**-**	**53.2 (85)**		**97.6 (78)**		**232.3 (93)**		**475.1 (95)**		**775.2 (78)**	
% immobile daphnids after 24 h	0	10	0	30	60	100	100	100	100	100	100
% immobile daphnids after 48 h	0	10	0	100	100	100	100	100	100	100	100
pH after 24 h	8.4	6.3		4.8		3.2		2.7		2.3	
pH after 48 h	7.9	7.6		5.2		3.4		2.9		2.6	

RMS Conclusions

The authors concluded that the 48 hour EC_{50} (immobilisation) value for *Daphnia magna* exposed to glyphosate acid was 84.0 mg glyphosate/L with a 95 % CL of 73.3 to 110.1 mg/L. The 48 hour NOEC value was 60.3 mg glyphosate /L based on nominal concentrations.
These values were recalculated by the RMS. Results of the probit analysis using linear max. likelihood regression proposed and EC_{50} value of 74 mg/L (95% CL: 16.966 - 130.338). A NOEC of 53 mg glyphosate/L is suggested by the program.

The study is considered to be acceptable and valid. Nevertheless to address actual criteria recalculation of the endpoints was necessary.

KIIA 8.3.1.1/03

Author:	Franco Perina, V. C.
Title:	Acute toxicity of glifosate tecnico Nufarm to *Daphnia magna*
Date:	17.01.2000
Doc ID:	2310948 /RF-D51.39/99
Guidelines:	OECD 202 (1984)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	037-919-113
Purity:	95 %
Vehicle and/or positive control:	Reconstituted water, Potassium dichromate ($K_2Cr_2O_7$)
Species:	*Daphnia magna*
Age of animals:	Neonates (< 24 h old)
Loading:	5 organisms per vessel (30 mL glass beakers containing 20 mL test solution)
Supplier:	Carolina Biological Supply Company, Burlington, North Carolina (USA) and maintained as a stock culture at BIOAGRI
Temperature:	20.0 to 21.5°C

pH:	Control(start – 48 h):7.4 – 7.9 100 mg/L(start – 48 h):6.8 – 8.0 180 mg/L(start – 48 h):6.5 – 7.9 320 mg/L(start – 48 h):6.0 – 7.0 560 mg/L (start – 48 h): 5.0 – 5.4 1000 mg/L(start – 48 h):3.1 – 3.4
Dissolved oxygen:	Start of the test: 5.7-6.2 mg O_2/L, End of the test: 4.4-4.6 mg O_2/L
Conductivity:	410 mg/L µS/cm
Hardness:	245 mg $CaCO_3$
Photoperiod:	0 hours (exposure phase of test performed in the dark)
Methods:	The toxicity of glyphosate acid to *Daphnia magna* was evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (comprising 4 replicates of 5 animals per test beaker) were exposed in the negative control group and at each test group. Nominal concentrations were 100, 180, 320, 560, and 1000 mg a.s./L (corresponding to measured concentrations of 103.40, 179.56, 334.11, 597.06, and 1051.12 mg glyphosate acid/L). A reference test using potassium dichromate was performed in parallel to verify the sensitivity of the test system. Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test or during the 24 hour preceding the test. The pH and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, at 24 and 48 hours. Samples for the determination of the concentrations of glyphosate in the test medium were taken from the control and from all test concentrations at the beginning of the test only. The EC_{50} values for glyphosate and reference substance potassium dichromate were calculated by applying Trimmed Spearman-Karber method.

Results

Measured concentrations of glyphosate acid in samples of test media taken at the start of the test ranged between 99.75 and 106.61 % of the nominal values. The ecotoxicological endpoints are evaluated based on the initial measured concentrations of the test item.

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels. The concentration of the test substance in the test media was measured only at the beginning of the study. However, glyphosate acid in aquatic studies has been shown to be stable and not measuring the glyphosate concentration at test termination does not affect the validity of this study.

Table B.9.2-17: Immobilisation of *Daphnia magna* during the 48 hour toxicity test

Nominal concentration (mg a.s./L)	Measured concentration (mg a.s./L)	Exposed *Daphnia* per replicate (No.)	Immobile *Daphnia* after 24 h (No.)	Immobility after 24 h (%)	Immobile *Daphnia* after 48 h (No.)	Immobility after 48 h (%)
Control	-	20	0	0	0	0
100	103.40	20	0	0	0	0
180	179.56	20	0	0	0	0
320	334.11	20	0	0	2	10
560	597.06	20	14	70	20	100
1000	1051.12	20	20	100	20	100

The highest concentration that showed no lethal effects was 179.6 mg a.s./L and the lowest concentration that showed 100 % daphnids immobililty was 597.1 mg a.s./L.

A refererce substance potassium dichromate was used to verify the sensitivity of the daphnids. The EC_{50} found for the reference substance was 0.68 mg/L (95% CL: 0.63 - 0.75 mg/L).

RMS Conclusions

The 48-h EC_{50} (immobilisation) for *Daphnia magna* exposed to glyphosate was calculated to be 420.6 mg glyphosate acid/L based on initial measured concentrations. The 48 hour NOEC was calculated to be 179.6 mg glyphosate acid/L.

The study is considered to be acceptable and valid.

KIIA 8.3.1.1/04

Author:	Morris, D.S., Kent S.J., Banner, A.J., Wallace, S.J.
Title:	Glyphosate acid: Acute toxicity to *Daphnia magna*
Date:	22.02.1996
Doc ID:	2310950 /BL5551/B
Guidelines:	EPA FIFRA, Subdivision E, Guideline 72-2
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	P24
Purity:	95.6 %
Vehicle	Elendt M4
Species:	*Daphnia magna* Straus
Age of animals:	Neonates (< 24 h old)
Loading:	5 organisms per vessel (250 mL glass beakers containing 200 mL test solution) which corresponds to 25 *Daphnia*/L.
Source:	Continuous laboratory cultures
Temperature:	20.5-20.8 °C
pH:	Control(start – 48 h):8.2 – 8.1 10 mg/L(start – 48 h):7.7 – 8.0 18 mg/L(start – 48 h):7.4 – 8.0 32 mg/L(start – 48 h):7.0 – 7.8 56 mg/L (start – 48 h): 6.6 – 7.5 100 mg/L(start – 48 h):5.7 – 6.1 180 mg/L(start – 48 h):4.3 – 4.2 1000 mg/L(pH, adjusted, start – 48 h):9.0 – 8.8
Dissolved oxygen:	8.7-9.0 mg O_2/L
Conductivity:	693 mg/L µS/cm
Hardness:	263 mg $CaCO_3$
Photoperiod:	16 hours light with a 20 minute transition period
Methods:	The toxicity of glyphosate acid on *Daphnia magna* was evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to nominal 10, 18, 32, 56, 100 and 180 mg/L of glyphosate acid and a pH adjusted 1000 mg/L test concentration of glyphosate acid. In addition, 4 x 5 *Daphnia* were exposed in a negative control group (dilution medium only).

	A stock solution was prepared at a nominal concentration of 1000 mg glyphosate acid/L by dissolving 1000 mg of glyphosate acid in 1000 mL dilution water. The 10 to 180 mg glyphosate acid test media were prepared by diluting aliquots of the stock solution with dilution water. A further 1000 mg a.s./L stock solution was prepared by dissolving 1000 mg of glyphosate acid in 1000 mL of dilution water. The pH of this stock solution was adjusted from 2.59 to 8.98 using 12 mL of 1 molar sodium hydroxide. All stock and test mediums were observed to be clear and colourless. The *Daphnia* were randomly placed into the test beakers and exposed to the test item for 48 hours. Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test nor during the 24 hours period prior to test initiation. The pH-values and dissolved oxygen concentrations were determined in the test media at the start and end of the test. The temperature of the test media was measured at the start of the test, at 24, and 48 hours. The concentrations of glyphosate acid in samples of test media were measured at 0 and 48 hours. The LC_{50} values and their 95% confidence intervals were calculated by the authoresusing non-linear interpolation. The NOEC was determined by visual interpretation of the data..

Results

The measured concentrations of glyphosate acid in samples of test media taken at the start and end of the test ranged between 85 and 100 % of the nominal values. The ecotoxicological endpoint results are based on nominal glyphosate acid concentrations.

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels. The pH in test medium was decreasing with increasing test concentrations due to the intrinsic characteristics of the acid test item.

Table B.9.2-18: Effects of glyphosate acid on *Daphnia magna*

Nominal concentration (mg a.s./L)	Exposed *Daphnia* per replicate (No.)	Immobile *Daphnia* after 24 hours (No.)	Immobility after 24 hours (%)	Immobile *Daphnia* after 48 hours (No.)	Immobility after 48 hours (%)
Control	20	0	0	0	0
10	20	0	0	0	0
18	20	0	0	0	0
32	20	0	0	0	0
56	20	0	0	0	0
100	20	0	0	0	0
180	20	20	100	20	100
1000 (pH adjusted)	20	0	0	0	0

Over the 48 hour test duration, in the control and at measured concentrations up to and including 100 mg a.s./L and also in the pH adjusted 1000 mg glyphosate acid/L treatment group, no immobility was observed. At 180 mg glyphosate acid/L, there was 100% immobility of *Daphnia magna* after 24 hours.

RMS Conclusions

The pH adjusted 48-h EC_{50} for *Daphnia magna* exposed to glyphosate acid can not be considered a relevant endpoint as the adjustment of pH is masking the intrinsic biochemical characteristics of glyphosate acid.

Since no dose reponse relationship can be estimaeted from the data, as there is only a 0% and 100 % effect value reported, deriving an EC_{50} is also not plausible. The presented 48-h EC_{50} (immobilisation, 130 mg a.s./L) for *Daphnia magna* exposed to glyphosate acid is not

supported by RMS, but a surrogte EC_{50} > 100 mg a.s./L is proposed. The study is considered to be acceptable and valid.

KIIA 8.3.1.1/05

Author:	Jahnke, M.
Title:	Acute Toxicity in *Daphnia magna*; Test Article: 'Glyphosate isopropyl-amine salt'
Date:	28.01.1994
Doc ID:	2310952 /83-91-0737-00-93
Guidelines:	OECD
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate isopropylamine salt
Lot/Batch #:	01/06/93
Purity:	61.6% Glyphosate isopropylamine salt; 456.43 mg a.s./L
Density:	1.23 g/cm^3 at 20 °C
Vehicle and/or positive control:	Elendt M4, 0.4 and 1.4 mg/L $K_2Cr_2O_7$
Species:	*Daphnia magna* Strauss
Age:	neonates (6 - 24 h old)
Loading:	10 mL for 5 specimens
Source:	Laboratory bred
Diet/Food:	None
Acclimation period:	Daphnids were held in groups of 25-30 organisms in 1000 mL glass vessels at test conditions. Specimens were fed on green algae and water was renewed 3 times a week.
Temperature:	20.5 °C
Photoperiod:	16 hours Light intensity = 600 – 700 lux
pH:	7.41 – 7.66
Dissolved oxygen:	> 60 % of air saturation 8.0 – 8.3 mg O_2/L
Conductivity:	0.049 µS/cm
Hardness:	14.5° dH
Methods:	The acute toxicity test was performed under static conditions as a limit test at a nominal test concentration of 100 mg test item/L, equivalent to 61.6 mg glyphosate isopropylamine salt/L or 45.64 mg glyphosate/L in glass volumetric cylinders containing reconstituted water (Elendt-M4). In addition a negative control group (Elendt-M4) was prepared in parallel. Two reference toxicant groups were exposed to 0.4 and 1.4 mg/L of $K_2Cr_2O_7$, with four vessels per treatment, each containing five *Daphnia magna* (25 mL volumetric cylinder containing 10 mL test medium). All *Daphnia magna* were observed after 24 and 48 hours after initiation of the test. Temperature, pH-value and oxygen saturation of the test solutions were measured at initiation and test termination. Total number of immobile *Daphnia magna* was recorded at 24 h and 48 h after the test initiation.

	Analytical measurement of the test item concentration was performed using an HPLC method of analysis at the start (0 h) and end (48h) of the limit test. Glyphosate isopropylamine salt concentrations were determined based on measured concentrations of glyphosate acid. Since the immobility was < 50 %, no statistical calculation of EC_{50} values was possible.

Results

The measured concentrations of glyphosate acid in test media at the start (0h) and end (48h) of the limit test were 47.322 mg glyphosate acid/L (103.7 %) and 47.091 mg glyphosate acid/L (103.2 %) respectively. As measured concentrations ranged between 80 and 120 % of nominal concentration, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.
The immobility rate in the control group did not exceed 10 % (0% in the test) at any stage of the test. At the concentration level of 100 mg test item/L, none of the daphnids tested were found to be immobilised 24 h and 48 h after the start of the test.

RMS Conclusions

In a 48-hours static acute toxicity study with *Daphnia magna*, the EC_{50} was determined to be > 100 mg test item/L, equivalent to 61.6 mg glyphosate isopropylamine salt/L or 45.64 mg glyphosate acid/L (nominal).

The study is considered to be acceptable and valid.

KIIA 8.3.1.1/06

Author:	Franco Perina, V. C.
Title:	Acute toxicity of glifosato IPA tecnico Nufarm to *Daphnia magna*
Date:	31.07.2000
Doc ID:	2310954 /RF-D51.017/00
Guidelines:	OECD 202 (1984)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate isopropylamine salt
Lot/Batch #:	MJRT 025-201-104
Purity:	612.7 g/kg salt equivalent, 470.9 g a.s./L
Vehicle and/or positive control:	Reconstituted water, Toxic standard (potassium dichromate)
Species:	*Daphnia magna*
Age of animals:	Neonates (< 24 h old)
Loading:	5 organisms per vessel (4x 30 mL glass beakers containing 20 mL test solution)
Source:	Carolina Biological Supply Company, Burlington, North Carolina (USA) and maintained as a stock culture at BIOAGRI
Temperature:	21.1 to 21.2 C°
pH:	Start of the test: 5.56-7.39, End of the test: 5.54-7.81
Dissolved oxygen:	Start of the test: 6.10-6.27 mg O_2/L, End of the test: 5.57-5.67 mg O_2/L

Conductivity:	603.0 mg/L µS/cm
Hardness:	248 mg $CaCO_3$
Photoperiod:	0 hours (exposure phase performed in the dark).
Methods:	The toxicity of glyphosate acid to Daphnia magna was evaluated in a 48-hour static toxicity test. Twenty Daphnia (comprising 4 replicates of 5 animals per test beaker) were exposed in the negative control group and at each test group. Nominal concentrations were 100, 180, 320, 560, and 1000 mg a.s./L (corresponding to measured concentrations of 103.40, 179.56, 334.11, 597.06, and 1051.12 mg glyphosate acid/L). A reference test using potassium dichromate was performed in parallel to verify the sensitivity of the test system. Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test or during the 24 hour preceding the test. The pH and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, at 24 and 48 hours. Samples for the determination of the concentrations of glyphosate in the test medium were taken from the control and from all test concentrations at the beginning of the test only. The EC50 values for glyphosate and reference substance potassium dichromate were calculated by applying Trimmed Spearman-Karber method.

Results

The concentration of the test substance in the test media was measured only at the beginning of the study. However, glyphosate in aquatic studies has been shown to be stable. Omitting the measurents of glyphosate concentration at test termination is in this specific case not considered to affect the validity of this study.

The measured test concentrations ranged between 75.90 and 139.70 % of the nominal values. Therefore, the ecotoxicological endpoints are evaluated using measured concentrations of the test item. The reference substance potassium dichromate resulted in a 48-h EC_{50} (immobilisation) of 1.22 mg/L (95 % CL = 1.12 - 1.35 mg/L). At 48 hours, there were no immobilised *Daphnia magna* observed at any of the test concentrations. All validity criteria according to the guideline OECD 202 were fulfilled.

RMS Conclusions

The 48-h EC_{50} for *Daphnia magna* exposed to glyphosate isopropylamine salt was greater than 1397 mg/L based on measured concentration (corresponding to 470.9 mg glyphosate acid). As the highest concentration showed no immobility on the daphnids, the NOEC after 48 h based on immobilisation was 1397 mg a.s./L based on measured concentration.

The study is considered to be acceptable and valid.

KIIA 8.3.1.1/07

Author:	Palmer, S.J., Kendall, T.Z., Krueger, H.O.
Title:	MON 78623: A 48-Hour Static Acute Toxicity Test with the cladoceran (*Daphnia magna*)
Date:	01.05.2003
Doc ID:	2310957 /WL-2002-150
Guidelines:	OECD 202
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	MON 78623 (Glyphosate K-salt)
Active substance	Glyphosate acid
Lot/Batch #:	GLP-0108-11688-F
Purity:	47.7 %
Vehicle:	Well water
Species:	*Daphnia magna*
Age:	Neonates (< 24 h old)
Loading:	2 × 10 specimens for 250 mL test solution
Source:	In-house culture
Diet/Food:	None
Acclimation period:	None
Temperature:	19.5 – 20.0 °C
Photoperiod:	16 hours light / 8 hours dark with 30 min transition period
pH:	5.7 – 8.1 (test item); 8.1 – 8.2 (control)
Dissolved oxygen:	≥ 8.6 mg/L (≥ 96 % saturation)
Conductivity:	310 µmhos/cm
Hardness:	140 mg $CaCO_3$/L.
Alkalinity:	184 mg $CaCO_3$/L
Methods:	Based on the results of a range finding test, a definitive toxicity test was performed using nominal concentrations of 156, 313, 625, 1250 and 2500 mg test item/L (measured 165, 312, 624, 1285 and 2582 mg test item/L) in a static test. The test solutions were prepared using test facility well water (Dissolved oxygen ≥ 96%, pH = 5.7 – 8.1, hardness 140 mg $CaCO_3$/L.). In addition, a control group was exposed to well water (negative control). There were two replicates per treatment, each containing ten daphnids. Test chambers were 250 mL glass beakers containing approx. 250 mL of test medium. Total number of immobile *Daphnia magna* was recorded at 19h, 24 h and 48 h after the test initiation. Temperature of the test solutions was measured at the test initiation and termination. Hardness, alkalinity and specific conductance of the dilution water were measured at test initiation. The pH value and oxygen saturation were measured at test initiation and at 24h and 48h. For analysis of test substance concentration with HPLC, test medium was collected from the replicate test chambers at 0 and 48 h. Since the mortality/immobility was < 50%, were estimated by the authors to be greater than the highest measured concentration tested. EC_{50} values were recalculate by RMS via ToXRatPro Version 2.10 using Probit analysis using linear max. likelihood regression and Multiple testing to find the NOEC (Bonferroni-Fisher Test).

Results

To be a valid test, a maximum control mortality of 10.0 % is allowed. In the present test 0 % of the introduced animals died.

The analytics confirm the stability of the test substance, since the recovery was 99 – 105 % at test start and 97 – 107 % at test end. Results are based on mean measured concentrations.

Table B.9.2-19: Effects of glyphosate K-salt to *Daphnia magna*

Glyphosate K-salt (MON 78623) mean measured concentrations (mg/L)	Glyphosate acid mean measured concentrations (mg a.s./L)	Daphnia with normal appearance (19 h) (%)	Daphnia with normal appearance (24 h) (%)	Daphnia with normal appearance (48 h) (%)
Control	-	20	20	20
165	78.7-	20	20	20
312	148.8	20	20	20
624	297.6	20	20	7
1285	612.9	20	12	0
2582	1231.6	20	3	0

Results of the probit analysis as recalculated by RMS gave effective concentrations (EC_{50}) of the test item of 592 mg test item /L and a NOEC of 312 mg/L.

RMS Conclusions

The observed effects "lethargic" was not defined in the test protocol. "Lethargic" could mean in case of *Daphnia*" with motionless antenna". In the view of RMS this symptomlogy can be put on a level with immobilisation. Based on these assumptions, EC_{50} values were recalculated.

The 48 h EC_{50} for *Daphnia magna* exposed to glyphosate K-salt was re-calculated to be 592 mg/L, equivalent to 278.24 mg glyphosate acid/L based on mean measured concentrations. The 48- hour no-effect level (NOEC) for glyphosate K-salt was determined to be 312 mg/L, equivalent to 148.8 mg glyphosate acid/L based on mean measured concentrations.

The study is considered valid and acceptable.

KIIA 8.3.1.1/08

Author:	Bogers, M.
Title:	Acute Toxicity Study in *Daphnia magna* with (Aminomethyl) Phosphonic Acid (Static).
Date:	29.06.1998
Doc ID:	2310959 / 232471
Guidelines:	OECD
GLP:	Yes
Validity:	Yes

Materials and Methods

Test item::	Aminomethylphosphonic acid (AMPA)
Lot/Batch #:	A010047101
Purity:	99 %

Vehicle and/or positive control:	Elendt M4; Reference item: $K_2Cr_2O_7$
Species:	*Daphnia magna* Straus
Age:	Neonates (< 24 h old)
Loading:	10 daphnids per 80 mL of test medium
Source:	In-house culture
Temperature:	20.4 – 20.6 °C
Photoperiod:	16 hours light
pH:	8.0 – 8.2 (control), 6.2 - 6.4 (test solution)
Dissolved oxygen:	8.8 – 9.0 mg O_2/L
Hardness:	250 mg $CaC0_3$/L
Methods:	Based on the results of a range finding test, a static 48 hour limit toxicity test performed at a nominal limit concentration of 100 mg AMPA/L, prepared using ISO-medium. A negative control group (ISO-media only) was run in parallel. Two replicates were prepared for the control and per treatment group comprising 100 mL vessels each containing 80 mL of the appropriate test solution with each replicate containing 10 daphnids. The total number of mobile *Daphnia magna* was recorded at 24 h and 48 h after test initiation. The pH-values and oxygen saturation of the test solutions were measured at test initiation and termination. The temperature was measured daily in one control vessel. The concentrations of AMPA in samples of control and test media were taken (from preparation flasks) at the start of the test and from pooled replicate according to treatment at the end of the test (48 hours). Since the immobility was < 50 %, no statistical calculation of EC_{50} values was possible.

Results

At the tested nominal concentration there was no immobilisation of daphnids observed during the 48 h exposure time.

All validity criteria according to OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

RMS Conclusions

Under the conditions of the present test, aminomethylphosphonic acid (AMPA) induced no effects in *Daphnia magna* exposed to a concentration of 100 mg/L. Hence the 48 h EC_{50} for *Daphnia magna* exposed to aminomethylphosphonic acid (AMPA) was > 100 mg/L, the maximum concentration tested.

The study is considered to be acceptable and valid.

KIIA 8.3.1.1/09

Author:	Palmer, S.J., Kendall, T.Z., Krueger, H.O.
Title:	HMPA (Hydroxymethylphosphonic acid): A 48-hour static acute toxicity test with the cladoceran (*Daphnia magna*)
Date:	29.03.2011
Doc ID:	2310961 /WL-2010-329
Guidelines:	OECD 202
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	HMPA(Hydroxymethylphosphonic acid)
Lot/Batch #:	GLP-1003-20448-A
Purity:	97.0 %
Positive control:	Well water
Species:	*Daphnia magna* Straus
Age:	Neonates (< 24 h old)
Loading:	10 daphnids per 220 mL of test medium
Source:	In-house culture
Diet/Food:	None
Acclimation period:	None
Temperature:	19.7 – 20.7 °C
Photoperiod:	16 hours light (light intensity = 323 Lux), with 30 minute transition periods.
pH:	6.9 – 8.5
Dissolved oxygen:	8.3 – 9.4 mg O_2/L (≥92% of O_2 saturation)
Conductivity:	386 µS/cm
Hardness:	140 mg $CaC0_3$/L
Methods:	The toxicity of Hydroxymethylphosphonic acid (HMPA) on neonates of *Daphnia magna* was evaluated in a 48-hour static toxicity test at a single nominal limit

	concentration of 100 mg HMPA/L dissolved in well water. A negative control group (well water only) was prepared in parallel. Thirty daphnids (3 replicates of 10 animals per test beaker) were exposed at the control and at the limit concentration. The total number of immobile *Daphnia magna* was recorded at 2.5, 24 h and 48 h after test initiation. In addition, specimens were observed for clinical signs of toxicity. Temperature, pH-values and oxygen saturation of the test solutions were measured at test initiation, after 24 hours and at test termination (48 h). The temperature of test media was monitored continuously in all test vessels. Hardness, alkalinity, specific conductance and total organic carbon (TOC) were measured at the beginning of the test. Samples of test media were taken from each replicate test chamber at the start and end of the test for the determination of HMPA concentrations. Samples were analysed using an HPLC method of analysis with mass selective detection (LC/MS). Descriptive only since no immobility of daphnids was observed in the test and control treatments.

Results

The measured test concentrations ranged between 86 and 103 % of the nominal values. Therefore, the EC_{50} and NOEC values given below are based on nominal concentrations.

No immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels. Daphnids in the single treatment group appeared normal throughout the test, with no mortalities or overt signs of toxicity.

RMS Conclusions

The 48-hour EC_{50} for *Daphnia magna* exposed to HMPA was > 100 mg a.s./L nominal (>93 mg a.s./L measured) the single limit concentration tested. The 48-h NOEC was determined to be 100 mg HMPA/L (>93 mg a.s./L measured).

The study is considered to be acceptable and valid.

KIIIA 10.2.2.2/01

Author:	Lintott, D.R.,
Title:	MON 52276: Acute toxicity to the water flea, *Daphnia magna*, under flow-through test conditions
Date:	1992
Doc ID:	2317596 /TO-91-295
Guidelines:	US EPA FIFRA 72-1 (1982), OECD 203, and EEC Method C.1.
GLP:	YES
Validity:	YES

Materials and Methods

Test item::	MON 52276
Active substance:	Glyphosate, as 41.5 % IPA-salt
Lot/Batch #:	LLN-9105-3135F
Purity:	30.95 % w/w glyphosate acid
Species:	*Daphnia magna* Straus
Age:	Neonates (< 24 h old)

Loading:	1 daphnid per 30 mL test medium
Source:	In-house culture
Diet/Food:	none
Acclimation period:	Not stated
Temperature:	20.0 – 23.8 °C
Photoperiod:	16 hours light, 384 - 517 lux
pH:	7.1 – 8.6
Dissolved oxygen:	7.4 – 8.7 mg O_2/L
Conductivity:	436 - 644 µS/cm
Hardness:	60 – 96 mg $CaCO_3$/L
Methods:	The effects of MON 52276 (30.95% w/w glyphosate acid) were evaluated in a 48-hour flow-through toxicity test using a proportional diluter system (1.6 cycles/h). Twenty Daphnids (2 replicates of 10 animals per test beaker) were exposed to nominal concentrations of MON 52276 at 0 (controls), 130, 216, 360, 600, and 1000 mg/L dissolved in a blend of treated municipal water and treated well water. In addition, a control group was exposed to test water without test substance (blank control). Total number of immobile *Daphnia magna* was recorded 24 h and 48 h after test initiation. In addition, specimens were observed for clinical signs of toxicity. Water temperature was measured at 0 and 48 hours in each test chamber, as well as hourly in one negative control replicate. Water pH and dissolved oxygen were recorded at test start then every 24 hours. Hardness, alkalinity and specific conductance were measured once in the dilution water at test initiation. At 0 and 48 hours, samples of test medium were taken for quantification of glyphosate by HPLC. EC_{50} values including 95% confidence limit were determined by non-linear interpolation.

Results

The analysed test concentrations ranged between 95 and 105 % of the nominal values.

No mortality of *Daphnia magna* from exposure to MON 52276 was observed at test concentrations < 356 mg/L. At 580 mg/L, 20 % mortality was observed at 48 hours, with 100 % mortality observed at 948 mg/L. Sublethal effects were observed only at the 580 mg/L test concentration. After 48 h, the pH dropped from initial 8.3 to 5.8. These deviations were not considered to have affected the outcome of the study.

Table B.9.2-20: Acute toxicity of MON 52276 for *Daphnia magna* under flow-through conditions

MON 52276 (mg/L) [1]	Time point (h)	Abnormalities/ Sublethal Effects	Daphnia immobilised or dead[2] (No.)	Cumulative mortality (%)
0	48	observed	0	0
133	48	observed	0	0
227	48	observed	0	0
356	48	observed	0	0
580	48	3 lethargic	4	20
948	48	--	20	100

[1] Mean measured values.

[2] Of 20 total *Daphnia* in group.

RMS Conclusions

The study formally does not fulfill the validity criteria concerning the pH. The pH decrease correlated to increasing test concentrations due to the intrinsic characteristics of the acid test item. These deviations were not considered to have affected the acceptability of the study. Based on mean measured concentrations, the 48-hour EC_{50} for *Daphnia magna* exposed to MON 52276 in a flow-through test system was calculated to be 676 mg/L (equivalent to 209 mg a.s./L). The corresponding no observed effect concentration (NOEC) was 356 mg/L (110 mg a.s./L), based on the lack of mortality and sublethal effects at this concentration.

The test is considered to be acceptable and valid.

B.9.2.1.5 Invertebrates – long-term toxicity

As summarised in the first EU peer review evaluation of the active substance glyphosate, the lowest chronic NOEC for a *Daphnia* life-cycle study was 30 mg a.s./L. In addition to the test evaluated in the first monograph, new studies have been submitted. The toxicity of glyphosate acid and its metabolites AMPA was investigated. A summary of data for chronic toxicity of aquatic invertebrates reviewed in the 2001 EU glyphosate evaluation and the new study performed for glyphosate and AMPA is shown in the following table.

Table B.9.2-21: Chronic toxicity of glyphosate acid, glyphosate IPA salt and its metabolite AMPA to aquatic invertebrates. Tests submitted for the first EU peer review evaluation of glyphosate in 2001

Species	Substance	Test design	EC_{50} (mg a.s./L)	NOEC (mg a.s./L)	Reference
Daphnia magna	Glyphosate acid	21 d semi-static	-	**30 mg/L**	250795 Wüthrich, 1990
Daphnia magna	Glyphosate acid	21 d semi-static	>100	52	141874 Bogers, 1995
Daphnia magna	Glyphosate acid	21 d flow-through	-	50	AB 82-036 McAllister, McKee, 1982
Daphnia magna	Glyphosate acid	21 d semi-static	>100	100	AB 89-58 Forbis, 1989
Daphnia magna	Glyphosate acid	21 d	-	95	94-00154 Wüthrich, 1990
Daphnia magna	Glyphosate acid	21 d	-	56	96-00066 Bogers, 1995
Daphnia magna	Glyphosate-IPA salt	21 d semi-static	-	57.9	89-91-2328-05-93 Thun, 1993; 95-00549

Values in grey: confirmed EU endpoints (SANCO/6511/VI/99-final, or EU Review Monograph)

Table B.9.2-22: Chronic toxicity of glyphosate acid, glyphosate IPA salt and its metabolite AMPA to aquatic invertebrates. Tests submitted for the present application for renewal of glyphosate approval as active substance

Species	Substance	Test design	EC_{50} (mg a.s./L)	NOEC (mg a.s./L)	Reference

Daphnia magna	Glyphosate acid	21 d semi-static	-	**12.5 (recalculated by RMS)**	BL6535/B Magor, Shillabeer, 1999; BVL no 2310962
Daphnia magna	AMPA	21 d semi-static	90	**15**	WL-2010-327 Minderhout *et al.* 2011 BVL no 2310965

KIIA 8.3.2.1/01

Author:	Magor, S.E., Shillabeer, N.
Title:	Glyphosate acid: Chronic toxicity to *Daphnia magna*
Date:	29.06.1999
Doc ID:	2310962 /BL6535/B
Guidelines:	OECD 202, Part II, Reproduction Test (1984)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	P30
Purity:	97.6 %
2. Vehicle and/or positive control:	Elendt M4
Species:	*Daphnia magna*
Age:	Neonates (< 24 h old)
Loading:	1 organism per vessel (glass beakers containing 80 mL test solution)
Source:	Continuous laboratory cultures
Temperature:	19.4 to 20.2 °C
pH:	3.67-8.02 (new solutions) ; 3.46-8.00 (old solutions)
Dissolved oxygen:	9.2-9.2 mg O_2/L (dilution water, new); 8.8-9.2 mg O_2/L (test solutions, old)
Conductivity:	572-617 mg/L µS/cm (test solutions)
Hardness:	202.7-218.3 mg $CaCO_3$
Photoperiod:	16 hours light /8 hours dark, 20 minute dawn and dusk transition period; 480 lux
Methods:	The lethal and sub lethal effects of glyphosate acid on *Daphnia magna* were evaluated in a 21-day toxicity test performed under semi-static conditions. Ten replicates of one daphnia per concentration were exposed to 12.5, 25, 50, 100, and 200 mg a.s./L nominal concentrations. In addition, 10 x 1 daphnia were exposed to test medium without test substance (blank control). The daphnia were randomly placed into the test beaker and exposed to the test item for 21 days. The test daphnia were fed daily with cultured algae (*Chlorella vulgaris*). A primary stock solution of 200 mg a.s./L was prepared on day 0 by dissolving 400 mg test item in 2000 mL of dilution water. On days 2, 4, 7, 9, 11, 14, 16, and 18 a primary stock solution of 100 mg a.s./L was prepared by dissolving 200 mg test item in 2000 mL dilution water. The test solutions were prepared by the addition of appropriate aliquots of the stock solutions to dilution water. At each renewal of the test solutions, the surviving P0 generation of daphnia were transferred to the new solutions. The F1 generation of daphnia were removed from each vessel and counted. The numbers of alive and dead F1daphnia were recorded. Mortality of P0 generation of daphnia and observation for the presence of alive and dead offspring (termed F1 generation) were recorded daily in each test vessel. At the

	end of the test, the length of each surviving P0 daphnia was measured. The pH was measured in each newly prepared test solution. The pH and dissolved oxygen concentration of two of the replicates of the old test solutions were measured after transfer of the P0 generation of daphnids. Temperature measurements were recorded daily by means of a thermometer and hourly automatically. The concentration of glyphosate acid in the test solutions was determined on days 0, 2, 7, 9, 14, and 16. Old solutions were analysed on days 2, 7, 9, 14, and 21. The reproduction and length data for each individual P0 generation daphnid were entered into electronic data files and analysed using statistical procedures contained in the Brixham Environmental Laboratory computer programs 'STATS' (version 4.10) and 'EPA' (version1.04).

Results

The validity criteria according to OECD 202 were fulfilled, as immobility of daphnids was < 20 % in control groups and mean offspring number at day 21 was > 60.

The effects of glyphosate acid on *Daphnia magna* mortality and reproduction are shown in the following Table B.9.2-23.

Table B.9.2-23: Offspring per day and female of *Daphnia magna*

Nominal concentration (mg a.s./L)	Mean adult mortality (%)	Total offsprings per parent (No.)	Total offsprings (No.)
Control	10	108± 20	1028
12.5	0	100±21	1003
25	0	84±12*	840
50	0	91±18	912
100	50	105±23	763

* Statistically significant difference

At the nominal concentration of 25 mg/L the total number of offspring per parent was significantly lower when compared to control. Even though the results of this study do not show a classical dose response relation, significant effects were observed and it is proposed to consider these effects. The relevant and accepted long term endpoint for invertebrates established in the EU evaluation of glyphosate in 2001 is in the same order of magnitude.

RMS Conclusions

The study was performed according to OECD 202, Part II. According to current criteria, the OECD 211 would be the relevant directive. Since daphnids were held individually in the test vessel, it is possible to determined the exact number of offspring per parent and therefore a statistical evaluation according to the criteria of OECD 211 is possible. RMS proposes to consider significant effects at 25 mg/L and recommends an NOEC for reproduction 12.5 mg a.s./L based on nominal concentration.

The overall 21-day NOEC for the reproduction of *Daphnia magna* exposed to glyphosate acid is 12.5 mg a.s./L based on nominal concentration.

KIIA 8.3.2.1/02

Author:	Minderhout, T., Kendall, T.Z., Krueger, H.O
Title:	AMPA (Aminomethylphosphonic acid): A semi-static life cycle toxicity

	test with the Cladoceran (*Daphnia magna*)
Date:	17.06.2011
Doc ID:	2310965 / WL-2010-327
Guidelines:	OECD Guideline 211 (1998)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	AMPA (Aminomethylphosphonic acid)
Lot/Batch #:	GLP-0908-199984-A
Purity:	98.7 %
Positive control:	ASTM medium
Species:	*Daphnia magna* Straus
Age:	Neonates (< 24 h old)
Loading:	1 daphnid per 200 mL test medium
Source:	In-house culture
Diet/Food:	Daily; mixture of yeast, cereal grass media and trout chow (YCT) and suspension of *Pseudokirchneriella subcapitata*
Temperature:	19.0 – 20.8 °C
Photoperiod:	16 hours light; Light intensity = 314 lux
pH:	7.1 – 8.6
Dissolved oxygen:	6.8 – 9.1 mg O_2/L
Conductivity:	274 – 391 µS/cm
Hardness:	132 - 140 mg $CaCO_3$/L
Methods:	A 21-day reproductive toxicity test was conducted under semi-static conditions, with renewal of test medium every 2 to 3 days. *Daphnia magna* neonates (<24 hours old) were exposed to nominal concentrations of 7.5, 15, 30, 60 and 120 mg AMPA/L in moderately hard dilution water (ASTM medium). A negative control group was prepared in parallel. Ten glass vessels (250 mL vessels containing 200 mL test medium each) were used per treatment group for the test item and 20 vessels for the control group. One daphnid (neonate < 24 hours old) was exposed per replicate (vessel). The number of living, immobilised and dead parental *Daphnia magna* and the time to gravidity (presence of eggs in brood pouch) were observed daily. Body length and dry weights of surviving parental specimens were measured at the end of the exposure period (21 days). The number of neonate daphnids was counted three days a week and their condition was recorded. The presence of unhatched eggs was recorded, when observed. Incidental mortality was also recorded, when occurred. At the end of the test, body length and dry weight of each surviving parental daphnid was measured. The temperature, pH-values and the oxygen saturation were measured at test initiation, before and after the renewal of the test media in two replicate test chambers and at test termination. Hardness, alkalinity and specific conductance were measured in batch solutions of the negative (dilution water only) control and at the highest test item concentration at test initiation and on one renewal day each week and from pooled replicate solutions at test termination. Analytical measurements were performed by using an HPLC method of analysis using samples taken from all test concentrations for the freshly prepared solutions, at the end of the first renewal cycle (old solution), and at the beginning and end of last renewal cycle. For the aged test media, samples were taken from 2 alternate replicates of each treatment and control group and pooled by treatment group. Data were statistically tested using Chi-square and Fisher's Exact test (discrete-variable data; $\alpha = 0.05$) and Dunnett's t-test (one-tailed, normal distributed data; α =

	0.05). The NOEC was determined by visual interpretation of the results.

Results

The overall mean measured concentrations of AMPA during the test were 7.4, 15, 30, 57 and 120 mg AMPA/L, equivalent to 99, 100, 100, 95 and 100 % of the nominal concentrations, respectively. Since the mean measured test concentrations were within the 80 – 120 % of nominal test concentration, the results of the study are reported as nominal test concentrations. According to the validity criteria of OECD 211, survival in the negative control group should not exceed 20 % at the end of the test. Survival in the control group was below the 80 % validity criterion required in the guideline. However, this deviation is not considered to have had a significant impact on the validity of this study as the surviving daphnids in the control replicates appeared normal and healthy throughout the test and produced an average of 227 live young per surviving adult (CV = 11.6 %), well above the validity criterion of ≥ 60 live young per surviving adult.

Table B.9.2-24: Chronic toxicity of AMPA for *Daphnia magna*

AMPA (mg/L)	Adult mortality after 21 d (%)	Offsprings per adult (No.)
0 (Control)	25	227 ±26.3
7.5	20	229 ±24.8
15	0	213 ±26.6
30	30	189 ±19.7*
60	0	169 ±22.1*
120	10	59.6 ±13.4*

* statistically significant different from the control

Adult daphnids in the 7.5, 15, 30, 60 and 120 mg AMPA/L treatment groups produced an average of 229, 213, 189, 169 and 59.6 live young per surviving adult, respectively. Dunnett's test indicated there was a statistically significant decrease in mean neonate production in the 30, 60 and 120 mg AMPA/L treatment groups (30, 57 and 120 mg AMPA/L as mean measured concentration) in comparison to the control (α = 0.05).Consequently, the NOEC for reproduction was 15mg AMPA/L (15mg AMPA as mean measured concentration).

RMS Conclusions

The study formally does not fulfill the the validity criteria of OECD 211, as survival in the negative control group should not exceed 20 % at the end of the test. However, this deviation is not considered to have had a significant impact on the validity of this study as the surviving daphnids in the control replicates appeared normal and healthy throughout the test and produced an average of 227 live young per surviving adult (CV = 11.6%), well above the validity criterion of ≥ 60 live young per surviving adult. Therefore the study is considered to be acceptable. The overall no observed effect concentration (NOEC) based on reproduction was determined to be 15 mg AMPA/L.

B.9.2.1.6 Algae

In the first evaluation of the active substance glyphosate in the EU peer review in 2001, eight study reports on algae were provided by the notifier showing the toxicity of glyphosate tech-

nical towards algae. Details on the studies for which no update of the former evaluation was necessary, please refer to the initial monograph and its addenda.

Table B.9.2-25: Tests evaluated in the first EU peer review of glyphosate reporting the toxicity of glyphosate technical towards algae

Species	Test substance	Test design	**EC_{50} (mg a.s./L)/ NOEC(mg a.s./L)**	Reference/GLP
Pseudokirchneriella subcapitata	Glyphosate acid	72 h static	E_rC_{50} = 54 E_bC_{50} = 48	141896 Bogers, 1995/ 96-00065
Pseudokirchneriella subcapitata	Glyphosate acid	168 h static	E_bC_{50} (72 h) = 12.31 E_bC_{50} (168 h) = 12.65	95-00722 Hughes, 1987
Desmodesmus subspicatus	Glyphosate acid	96 h static	E_bC_{50} (72 h) = 195.2 E_bC_{50} (96 h) = 70.3	95-00002 Wüthrich, V., 1990
Desmodesmus subspicatus	Glyphosate acid	72 h static	E_rC_{50} (24 h) = 60 E_bC_{50} (72 h) = 46	95-00535 Handley *et al.*, 1995
Anabaena flos-aquae	Glyphosate acid	168 h static	E_bC_{50} (72 h) = 17.5 E_bC_{50} (168 h) = 17.15	95-00738 Hughes, 1987
Nitzschia palea	Glyphosate acid	96 h static	E_rC_{50} = 11.90 E_bC_{50} = 4.47	960606FH Scheerbaum, 1996 97-00013, , **not valid according to current OECD 201**
Skeletonema costatum	Glyphosate acid	168 h static	E_bC_{50} (72 h) = 1.25 **E_bC_{50} (168 h) = 0.64**	1092-02-1100-3 Hughes, 1987 96-00455, **not valid according to current OECD 201**
Navicula pelliculosa	Glyphosate acid	168 h static	E_bC_{50} (72 h)= 43.42 E_bC_{50} (168 h) = 42.40	1092-02-1100-2 Hughes, 1987 96-00454
Desmodesmus subspicatus	Glyphosate-IPA salt	72 h static	E_rC_{50} = 241 E_bC_{50} = 41.1	80-91-2328-01-93 Thun, 1993 93-00002
Desmodesmus subspicatus	Glyphosate-IPA salt	72 h static	E_rC_{50} = 166.0 **E_bC_{50} = 72.9**	95-00554 Dengler, Mende, 1994
Desmodesmus subspicatus	AMPA	72 h static	E_rC_{50} = 452 **E_bC_{50} = 89.8** **NOEC**	IFU93006/01-Ss Dengler, 1994 94-00501

Values in grey: confirmed EU endpoints (SANCO/6511/VI/99-final, or EU Review Monograph

Table B.9.2-26: Toxicity of glyphosate acid, glyphosate IPA salt, K-salt and metabolites AMPA for algae. Newly submitted tests for the present application for renewal of glyphosat approval as active substance

Species	**Test substance**	**Test design**	**EC_{50} (mg a.s./L)** **NOEC(mg a.s./L)**	**Reference/GLP**
Pseudokirchneriella subcapitata	Glyphosate	72 h static	E_rC_{50} = 91.3 (recalculated by RMS) NOEC = 5.6	RF-D2.44/99 Dias Correa Tavares, 2000 BVL no 2310966
Pseudokirchneriella subcapitata	Glyphosate	72 h /120 h static	E_rC_{50} (72 h) =19 E_bC_{50} (72 h) = 18 E_rC_{50} (120 h) =21 E_bC_{50} (120 h) = 17 NOErC = 10	BL5550/B Smyth *et al.*, 1995 BVL no 2310968
Anabaena flos-aquae	Glyphosate	72 h /96 h static	E_rC_{50} (72 h) = 22 **E_bC_{50} (72 h) = 8.5** E_rC_{50} (96h) = 38 E_bC_{50} (96 h) = 15 NOErC = 12	BL5698/B Smyth et al., 1996 BVL no 2310970
Navicula pelliculosa	Glyphosate	72 h /96 h static	E_rC50 (72 h) = 17 E_bC50 (72 h) =16 E_rC50 (96 h) = 17 E_bC50 (96 h) =17	BL5673/B Smyth et al., 1996, BVL no 2310976 **not considered to be valid.**
Skeletonema costatum	Glyphosate	72 h /96 h static	E_rC_{50} (72 h) = 18 **E_bC_{50} (72 h) =11** E_rC_{50} (96 h) = 29 E_bC_{50} (96 h) =11 NOErC = 1.82	BL5684/B Smyth et al., 1996 BVL no 2310972
Pseudokirchneriella subcapitata	Glyphosate isopropylamine salt 62.5%	72 h /96 h static	E_rC_{50} (72 h) =31 (23 a.s.) **E_bC_{50} (72 h) =9.25 (6.85)** E_rC_{50} (96 h) = 32 (23 a.s.) E_bC_{50} (96 h) =10.2 (7.56) NOErC = 4.27	A-99-02-04 Egeler, Baumann, 2002 BVL no 2310979
Pseudokirchneriella subcapitata	MON 78623 Glyphosate K-salt	72 h static	E_rC_{50} = 114 (54 a.s.) E_bC_{50} =74 (35 a.s.)	WL-2002-148 Desjardins *et al.*, 2003; BVL no 2310983 **not considered to be valid.**
Pseudokirchneriella subcapitata	AMPA	72 h static	E_rC_{50} = 200 **E_bC_{50} = 110** NOErC = 46	232458 Bogers, 1998 BVL no 2310985
Pseudokirchneriella subcapitata	HMPA	72 h static	**E_rC_{50} = >115** E_yC_{50} = >115 NOAEC = 60	139A-396A Porch *et al.* 2011 BVL no 2310987

Species	Test substance	Test design	EC_{50} (mg a.s./L) NOEC(mg a.s./L)	Reference/GLP
Pseudokirchneriella subcapitata	MON 52276	72 h static	Cell Counts: E_rC_{50} = 284 (88 a.e. E_bC_{50} = 178 (55 a.e.) Absorbance: E_rC_{50} = 393 (122 a.e. **E_bC_{50} = 150 (47 a.e.)** NOEC = 90, (28 a.e.)	LI-91-389 Neven, B.,1992 BVL no 2317597

KIIA 8.4/01

Author:	Dias Correa Tavares, C.M
Title:	Acute toxicity of glifosate tecnico NUFARM to *Selenastrum capricornutum*
Date:	03.01.2000
Doc ID:	2310966 /RF-D2.44/99
Guidelines:	OECD 201
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	037-919-113
Purity:	954.9 g a.s./kg
2. Vehicle and/or positive control:	Cell growth medium (OECD 201)
Species:	Green algae *Pseudokirchneriella subcapitata*
Source:	UTEX – The culture collection of algae at the University of Texas at Austin, Texas, USA
Initial cell concentration	1.6 x 10^4 cells/mL
Temperature:	24.3-24.4 °C
Photoperiod:	Continuous illumination, 7933 lux
pH:	7.17 – 7.22 at the start of the test, 7.71 – 9.31 at the end of the test
Methods:	The toxicity of glyphosate to the green alga *Pseudokirchneriella subcapitata* was determined in a 96-hour static toxicity test performed at nominal glyphosate concentrations of 5.6, 10, 32, 56, 100, 320, and 560 mg glyphosate/L. A negative control group (algal media only) was also prepared. The test vessels (3 replicates per control and test concentrations) were 250 mL glass Erlenmeyer flasks containing 100 mL of test solution. Single blank vessels were also prepared for the control and at each test concentration which were not inoculated with algal cells. A primary stock solution of nominal concentration of 10000 mg glyphosate/L was prepared by dissolving 1.0 g glyphosate acid in 100 mL distilled and deionised water. The primary stock solution was used to prepare secondary stocks solutions at 10, 100, and 1000 mg glyphosate acid/L. Appropriate aliquots of the secondary solutions were diluted to prepare the test concentrations, of which 100 mL volumes of each were dispensed to each test and blank vessel. Each replicate vessel (except blank vessels) was inoculated with a algal cell density of 1.6 x 10^4 cells/mL, from an exponentially growing pre-culture . The culture vessels were incubated at 24.3 - 24.4°C under continuous illumination for 96 h. During incubation, the algal cells were kept in suspension by continuous shaking (129-130 rpm).

	After 1, 2, 3, and 4 days, volumes of media were removed from all vessels and the algal cell densities were determined by cell counting using a Neubauer improved haemocytometer under and a light microscope (set to phase-contrast). The pH-values were determined in the control and each test medium at the beginning and at the end of the test. No morphological changes in algal cells were observed after 96 hours of exposure to glyphosate acid at any test concentration. The temperature in the incubator was measured daily with a minimum-maximum thermometer. The concentrations of glyphosate acid in the test solutions were measured at the start of the test only and results are based on initial measured concentrations. The computer program used for descriptive statistics and estimating endpoints was STATGRAPHICS – Statistical Graphic System. Toxicity endpoints were calculated based on initial measured means, standard deviations and 95% confidence limits. Fisher's Exact Binomial Test with Bonferroni Correction and Dunnett's test ($\alpha = 0.05$) were used to compare the control with independent test item groups. Abbott's formula was used to correct for control mortality. Toxicity endpoints were recalculated based on cell density/ growth rate for 72h and 96h by RMS via ToXRatPro Version 2.10. Shapiro-Wilk´s Test on Normal Distribution and Cochran´s Test Procedure on Variance Homogeneity were performed. Dunnett`s Multiple t-test Procedure (Alpha = 0.05, one-sided smaller,multiple level) and Probit analysis using linear max. likelihood regression were used to determined NOEC and effective concentration EC_{50}.

Results

The algal growth rate in the control cultures increased by a factor of > 16, the coefficient of variance for section specific growth rates was ≤ 35 %, for the whole test period it was ≤ 7 %. The validity criteria according to guideline OECD 201 are therefore fulfilled.

The effective concentration of glyphosate acid causing 50 % inhibition of growth rate after 72 hours when compared to the control was 91.3 mg glyphosate acid/L (95 % confidence limit 50.1 - 180.1 mg glyphosate acid/L), the no observed effect concentration (NOEC) was 5.6 mg glyphosate acid/L.

The 96 h E_rC_{50} for *Pseudokirchneriella subcapitata* exposed to glyphosate acid was calculated to be 125.3 mg glyphosate acid/L, the no observed effect concentration (NOEC) based on growth rate was 32 mg glyphosate acid/L. All endpoints were recalculated by RMS.

Table B.9.2-27: Mean cell densities and percentage of inhibition of cell growth of *Pseudokirchneriella subcapitata* exposed for 72 and 96 hours to glyphosate acid

Test parameters	Control	Glyphosate acid (mg/L)						
	-	5.6	10	32	56	100	320	560
Mean cell densities (0-72 h) (x 10000 cells/mL)	308	290	248	215	223	173	23.4	23.4
Mean growth rate (0-72 h) (% inhibition)	-	94	81	70	73	57	8	8
Mean cell densities (0-96 h) (x 10000 cells/mL)	740	732	723	723	707	473	48.4	23.4
Mean growth rate (0-96 h) (% inhibition)	-	99	98	98	96	64	7	3

RMS Conclusions

Toxicity endpoints were recalculated based on cell density/growth rate by RMS for 96 h and 72 h. The study is considered to be valid and acceptable.

The 72h E_rC_{50} was calculated to be 91.3 mg glyphosate acid/L (95 % confidence limit 50.1-180.1 mg glyphosate acid/L), the no observed effect concentration (NOEC) was 5.6 mg glyphosate acid/L.

KIIA 8.4/02

Author:	Smyth, D.V., Kent, S.J., Morris, D.S., Morgan, D.J., Magor, S.E.
Title:	Glyphosate acid: Toxicity to the green alga *Selenastrum capricornutum*
Date:	12.12.1995
Doc ID:	2310968 /BL5550/B
Guidelines:	OECD 201 (1984), US EPA Guideline 540/09-82-020 (1982)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	P24
Purity:	95.6 %
Cell growth medium**:**	Cell growth medium acc. to Miller et al. (1978)
Species:	Green algae *Pseudokirchneriella subcapitata* Korshikov
Source:	Brixham Environmental Laboratory culture from strain ATCC 22662
Initial cell concentration	3 x 10^3 cells/mL
Temperature:	24.1-24.2 °C (measured by thermometer). The hourly temperature measured automatically remained within 24±1°C.
Photoperiod:	Continuous illumination
Light intensity	5030 lux
pH:	3.5 – 7.5 at the start of the test ; 3.6 – 8.9 at the end of the test
Methods:	The toxicity of glyphosate acid to the green alga *Pseudokirchneriella subcapitata* was determined in a 120-hour, static toxicity test, performed at six nominal glyphosate acid concentrations of 5.6, 10, 18, 32, 56, and 100 mg glyphosate acid/L. A negative control group (culture medium without test item) was also prepared. The test vessels were conical glass flasks of 250 mL nominal capacity containing 100 mL of test solution. A nominal 100 mg glyphosate acid/L stock solution was prepared by adding glyphosate acid directly to 2000 mL sterile culture medium. Appropriate aliquots of this stock solution were diluted to prepare the lower test concentrations of 5.6, 10, 18, 32, and 56 mg glyphosate acid/L. Then 100 mL volumes of control medium or the appropriate test medium were dispensed to each replicate test and control vessel. A blank vessel was also prepared for each treatment that would not be inoculated with algal cells. The test was performed in 6 replicate vessels for the control group and 3 replicate vessels for each concentration of glyphosate acid. Each replicate test vessel was inoculated with 0.370 mL of the algal inoculum culture to give a nominal cell density of 0.300 x 10^4 cells/mL. The culture vessels were incubated at 24 ± 1°C under continuous illumination for 120 h. During incubation, the algal cells were kept in suspension by continuous shaking. The algal cell densities were determined by electronic particle counting, using a Coulter counter. After 1, 2, 3, 4, and 5 days, samples were removed from all vessels including the blank vessels. The appropriate blank particle count was subtracted from that of the test culture to obtain the corrected cell density. The pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily with a thermometer, and hourly with

	an automatic recording system. The concentrations of glyphosate acid in the test solutions were measured at the start (preparation flasks) and at the end (pooled replicates according to treatment)of the test. One-way analysis of variance and Dunnett's post-hoc test. Median effective concentrations and its 95% confidence limits were determined by linear regression against log concentration.

Results

Deviating from OECD 201guideline, a nominal cell density of 3 x 10^3 cells/mL, which is below the recommended density of 5 x 10^3 – 10^4cells/mL for *P. subcapitata*, was selected at the beginning of the test. The biomass in the control cultures increased by a factor of > 16, the coefficient of variance for section-by-section specific growth rates was ≤ 35%. For the whole test period the coefficient of variance was ≤ 7%.

Table B.9.2-28: Mean cell densities and percentage of inhibition of cell growth of *Pseudokirchneriella subcapitata* exposed to glyphosate acid

Test parameters	Control	Glyphosate acid (mg/L)					
	-	5.6	10	18	32	56	100
Mean cell densities (0-72 h) (x 10000 cells/mL)	73.4	79.1	74.5	2.05	0.143	0.021	0.033
Mean cell densities (0-96 h) (x 10000 cells/mL)	312	314	311	2.60	0.178	0.070	0.045
Mean cell densities (0-120 h) (x 10000 cells/mL)	567	605	568	4.20	0.478	0.138	0.172
Mean area under growth curve (0-72 h) (%)	-	108	104	8	-1	-1	-1
Mean area under growth curve (0-96 h) (%)	-	103	101	2	0	0	0
Mean area under growth curve (0-120 h) (%)	-	104	100	1	0	0	0
Mean growth rate (0-72 h) (%)	-	101	100	35	-13	-48	-40
Mean growth rate (0-96 h) (%)	-	100	100	31	-7	-21	-27
Mean growth rate (0-120 h) (%)	-	101	100	35	6	-10	-7

Table B.9.2-29: End points of cell growth of *Pseudokirchneriella subcapitata* exposed to glyphosate acid

Endpoint	Glyphosate acid (mg/L)
72–h NOE_rC	10
72–h LOE_rC (p=0.05)	18
72-h E_rC_{50}	19 (95% confidence limit 14-25)
72–h NOE_bC	10
72–h LOE_bC (p=0.05)	18
72-h E_bC_{50}	18 (95% confidence limit 13-23)
120–h NOE_rC	10
120–h LOE_rC (p=0.05)	18
120-h E_rC_{50}	21 (95% confidence limit 16-28)
120–h NOE_bC	10
120–h LOE_bC (p=0.05)	18
120-h E_bC_{50}	17 (95% confidence limit 13-22)

RMS Conclusions.

Deviating from OECD 201 guideline, a nominal cell density of 3 x 10^3 cells/mL, which is below the recommended density of 5 x 10^3– 10^4cells/mL for *P. subcapitata*, was selected at test start. Despite this deviation from the guideline, the study is considered to be valid and acceptable. The 72-hour E_bC_{50} and E_rC_{50} were determined to be 18 and 19 mg glyphosate acid/L, respectively. The 72-hour NOE_bC and NOE_rC values were 10 mg/L (nominal), respectively.

KIIA 8.4/03

Author:	Smyth, D.V., Shillabeer, N., Morris, D.S., Wallace, S.J.
Title:	Glyphosate acid: Toxicity to blue-green alga *Anabaena flos-aquae*
Date:	08.11.1996
Doc ID:	2310970 /BL5698/B
Guidelines:	OECD 201 (1984), US EPA Guideline 540/09-82-020 (1982)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	P24
Purity:	95.6 %
Medium	acc. to Miller et al. (1978)
Species:	Blue-green alga *Anabaena flos-aquae*
Source:	Brixham Environmental Laboratory culture from strain CCAP 1403/13A, Culture Centre of Algae and Protozoa, Institute of Freshwater Ecology. Windermere Laboratory, Far Sawrey, Ambleside, Cumbria, UK
Initial cell concentration	2.05 x 10^4 cells/mL
Temperature:	24.1-24.2 °C (measured by thermometer) The hourly temperature measured automatically remained within 24±1°C
Photoperiod:	Continuous illumination
Light intensity:	3600 lux
pH:	3.5 – 7.2 at the start of the test, 3.6 – 8.2at the end of the test
Methods:	The toxicity of glyphosate acid to Anabaena flos-aquae was determined in a 120-hour, static toxicity test. The test incorporated 8 nominal concentrations of glyphosate acid (0.75, 1.5, 3.0, 6.0, 12, 24, 48, 96 mg a.s./L) and a negative control consisting of culture medium without test item. The test vessels were conical glass flasks of 250 mL nominal capacity containing 100 mL of test solution. A stock solution at a nominal concentration of 96 mg glyphosate/L was prepared by adding glyphosate acid directly to 2000 mL sterile culture medium. Appropriate aliquots of this stock solution were diluted to prepare the lower test concentrations of 0.75, 1.5, 3.0, 6.0, 12, 24, and 48 mg a.s./L. 100 mL of the appropriate test solution were dispensed to each test and blank vessel. The test was performed in 6 replicates for the control group and 3 replicates for each concentration of glyphosate acid. Each replicate was inoculated with 1.120 mL of the inoculum culture to give a nominal cell density of 2.05 x 104 cells/mL. Single blank vessels were prepared for the control and each test concentration without algal cells. The culture vessels were incubated at 24±1°C under continuous illumination for 120 h. During incubation, the algal cells were kept in suspension by continuous shaking. The algal cell densities were determined by spectrophotometric absorbance, using a Uvikon 860 UV/visible spectrophotometer. After 1, 2, 3, 4, and 5 days, samples were

	removed from each control, test and blank vessel. The appropriate blank solution absorbance was subtracted from that of the test culture to obtain the algal absorbance reading. The pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily and hourly. The concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test. One-way analysis of variance, and Dunnett's procedure. Median effective concentrations and its 95% confidence limits were determined by linear regression against log concentration.

Results

The biomass in the control cultures increased by a factor of >16, the coefficient of variance for section specific growth rates was ≤ 35 %, for the whole test period it was ≤ 7 %. The validity criteria according to guideline OECD 201 are therefore fulfilled.

The mean measured concentrations of glyphosate acid ranged from 98 to 110 % of the nominal values. On the basis of the analytical results being with 80 and 120 % of the nominal test concentration, ecotoxicological endpoints were evaluated using the nominal concentrations.

Table B.9.2-30: Mean growth rates and mean areas under the growth curve of *Anabaena flos-aquae* exposed for 72 and 96 hours to glyphosate acid

Nominal concentration (mg a.s./L)	Mean growth rates 72h		Mean areas under the growth curve 72h		Mean growth rates 96h		Mean areas under the growth curve 96h	
	Mean growth rate	% of control	Mean areas under the growth curve	% of control	Mean growth rate	% of control	Mean areas under the growth curve	% of control
Control	1.392	-	1.331	-	1.331		1.5	-
0.75	1.365	91	1.357	98	1.357	102	1.5	103
1.5	1.336	85	1.355	96	1.355	102	1.5	99
3.0	1.328	80	1.344	95	1.344	101	1.4	94
6.0	1.321	82	1.342	95	1.342	101	1.4	94
12	1.299	76	1.321	93	1.321	99	1.3	87
24	1.231*	6	0.216*	17	0.216*	16	0.0*	2
48	0.231*	5	0.173*	17	0.173*	13	0.0*	2
96	0.231*	5	0.173*	17	0.173*	13	0.0*	2

* Significant difference from the culture control (α =0.05)

Table B.9.2-31: Ecotoxicological endpoints for *Anabaena flos-aquae* exposed for 72 and 96 hours to glyphosate acid

Endpoint	Glyphosate acid (mg/L)
72–h NOE_rC	12
72-h E_rC_{50}	22 (95% confidence limit 8.8 to >96)
72–h NOE_bC	12
72-h E_bC_{50}	8.5 (95% confidence limit 2.6-28)
96–h NOErC	12
96-h ErC50	34 (95% confidence limit 19 to >92)
96–h NOEbC	12
96-h EbC50	13 (95% confidence limit 7.4-25)

RMS Conclusions

The 72 h E_bC_{50} for *Anabaena flos-aquae* exposed to glyphosate acid was 8.5 mg a.s./L (95 % CL 2.6 to 28 mg a.s./L), the 72 h E_rC_{50} was 22 mg/L (95 % CL 8.8 to >96 mg a.s./L) and the 72-hour NOE_bC and NOE_rC values were 12 mg/L (nominal), respectively.

The study is considered to be valid and acceptable.

KIIA 8.4/04

Author:	Smyth, D.V., Kent, S.J., Morris, D.S., Shearing, J.M., Shillabeer, N.
Title:	Glyphosate acid: Toxicity to the marine alga *Skeletonema costatum*
Date:	08.11.1996
Doc ID:	2310972 /BL5684/B
Guidelines:	OECD 201 (1984), US EPA Guideline 540/09-82-020 (1982)
GLP:	YES
Validity:	YES

Materials and Methods

Test item::	Glyphosate acid
Lot/Batch #:	P24
Purity:	95.6 %
Cell growth medium	Cell growth medium (Walsh & Alexander 1980)
Species:	Marine alga *Skeletonema costatum*, strain CCAP 1077/1C
Source:	Culture centre of algae and protozoa, Dunstaffnage Marine Laboratory, Oban, Argyll, UK
Initial cell concentration	1.00 x 10^4 cells/mL
Temperature:	20.0-20.1°C (measured by thermometer). The hourly temperature measured automatically remained within 20±1 °C.
Photoperiod:	16 h light
Light intensity:	4340 lux
pH:	7.1 – 8.1 at the start of the test, 8.1 – 8.8 at the end of the test
Methods:	The toxicity of glyphosate acid to the marine alga *Skeletonema costatum* was determined in a 120-hour, static test. The test incorporated 8 nominal concentrations of glyphosate acid (1.0, 1.8, 3.2, 5.6, 10, 18, 32, and 56 mg a.s./L) and a control consisting of culture medium without test item. The test vessels were conical glass flasks of 250 mL nominal capacity containing 100 mL of test solution. A stock solution of nominal concentration of 56 mg a.s./L was prepared by adding glyphosate acid directly to 2000 mL sterile culture medium. Appropriate aliquots of this stock solution were diluted to prepare the lower test concentrations of 1.0, 1.8, 3.2, 5.6, 10, 18, and 32 mg a.s./L. 100 mL of the appropriate test solution were dispensed to each test and blank vessel. The test was performed in 6 replicates cultures for control and 3 replicates for each concentration of glyphosate acid. Each replicate was inoculated with 1.250 mL of the inoculum culture to give a nominal cell density of 1.00 x 10^4 cells/mL. The culture vessels were incubated at 20±1°C for 120 h. During incubation, the cells were kept in suspension by continuous shaking. The cell densities were determined by electronic particle counting, using a Coulter counter. After 1, 2, 3, 4, and 5 days, samples were removed from each test and blank vessel. The appropriate blank particle count was subtracted from that of the test culture to obtain the cell density. The pH-values were determined in the test media at the

	beginning and at the end of the test. The temperature in the incubator was measured daily with a thermometer, and hourly with an automatic recording system. The concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test. One-way analysis of variance, and Dunnett's procedure. Median effective concentrations and its 95% confidence limits were determined by linear regression against log concentration.

Results

The biomass in the control cultures increased by a factor of > 16, the coefficient of variance for section specific growth rates was ≤ 35 %, for the whole test period it was ≤ 7 %. The validity criteria according to guideline OECD 201 were therefore fulfilled.

The mean measured concentrations of glyphosate acid ranged from 94 to 106 % of the nominal values. On the basis of the analytical results being with 80 and 120 % of the nominal test concentration, ecotoxicological endpoints were evaluated using the nominal concentrations.

Table B.9.2-32: Mean cell densities and percentage of inhibition of cell growth of *Skeletonema costatum* exposed for 72 an 96 hours to glyphosate

Nominal concentration (mg a.s./L)	Mean growth rates 72h		Mean areas under the growth curve 72h		Mean growth rates 96h		Mean areas under the growth curve 96h	
	Mean growth rate	% of control	Mean areas under the growth curve	% of control	Mean growth rate	% of control	Mean areas under the growth curve	% of control
Control	1.423		37.4		1.113		97.6	
1.0	1.423	101	38.0	102	1.112	100	99.0	101
1.8	1.433	101	38.9	104	1.113	100	100.8	103
3.2	1.443	93	29.5*	79	1.128	101	84.5	87
5.6	1.322*	97	34.2	92	1.121	101	92.6	95
10.0	1.387	78	17.9*	48	1.122	101	62.6	64
18.0	1.111*	25	2.8*	8	0.317*	28	4.6	5
32.0	0.362*	21	2.3*	6	0.190*	17	3.3	3
56.0	0.295*	13	1.5*	4	0.087*	8	1.9	2

* Significant difference from the culture control (α =0.05)

Table B.9.2-33: Ecotoxicological endpoints for *Skeletonema costatum* exposed for 72 and 96 hours to glyphosate acid

Endpoint	Glyphosate acid (mg/L)
72–h NOE_rC	1.8
72-h E_rC_{50}	18 (95% confidence limit 10 to 42)
72–h NOE_bC	1.8
72-h E_bC_{50}	11 (95% confidence limit 7.1-20)
96–h NOErC	10
96-h ErC50	29 (95% confidence limit 16 to >56)
96–h NOEbC	1.8
96-h EbC50	11 (95% confidence limit 7.2-19)

RMS Conclusions

The 72 h E_bC_{50} for *Skeletonema costatum* exposed to glyphosate acid was 11 mg/L (95 % C.I. 7.1 to 20 mg a.s./L) and the 96 h E_bC_{50} was 11 mg/L (95 % C.I. 7.2 to 19 mg a.s./L); the 72 h

E_rC_{50} was 18 mg/L (95 % C.I. 10 to 42 mg a.s./L) and the 96 h E_rC_{50} was 29 mg/L (95 % C.I. 16 to >56 mg a.s./L) (nominal). The 72-hour NOE_bC and NOE_rC values were 1.82 mg/L (nominal), respectively.

The study is considered to be valid and acceptable.

KIIA 8.4/05

The EU review report for glyphosate (6511/VI/99-final) lists an EC_{50}-value for algae of 0.64 mg/L. This was the endpoint used for the assessment of the risk for algae exposed to glyphosate acid. This endpoint was determined in the following study from Hughes, 1987 using *Skeletonema costatum* as a test species. The test validity has to be revaluated, since according to actual guidelines critical criteria are not met.

Author:	Hughes, J. S
Title:	The toxicity of Glyphosate technical to *Skeletonema costatum*
Date:	27.04.1987
Doc ID:	2310975 /1092-02-1100-3
Guidelines:	Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2
GLP:	YES
Validity:	NO

Materials and Methods

Test item::	Glyphosate acid
Lot/Batch #:	NBP-3594465
Purity:	96.6 %
Water solubility	1.2 % at 25 °C
2. Vehicle and/or positive control:	Synthetic sea water containing 30 g/L salt mix (Rila Products, Teaneck, NJ)
Species:	Marine alga *Skeletonema costatum*
Initial cell concentration	1.00 x 104 cells/mL
Source:	Culture collection of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbour, ME
Temperature:	20 ± 2°C
Photoperiod:	14 h light
Light intensity	4306 ± 650 lux
Salinity:	30‰
pH:	Not stated
Methods:	The toxicity of glyphosate acid to the marine alga *Skeletonema costatum* was determined in a 168-hour, static test. The test incorporated 6 nominal concentrations of glyphosate acid (0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg a.s./L) and a control consisting of culture medium without test item. The test vessels were conical glass flasks of 250 mL nominal capacity containing 50 mL of test solution. A stock solution of nominal concentration of 5.0 mg a.s./mL was prepared by adding glyphosate acid to 50 mL sterile deionized water. Stock solutions of 0.5 and 0.05 mg/mL were prepared by serial dilution. 50 mL of the appropriate test solution

	were dispensed to synthetic seawater (prepared by adding approximately 30 grams of a commercial salt mix to 1 L of distilled deionised water). The test was performed in 3 replicate cultures of the culture medium control and of each concentration of glyphosate acid. Each replicate test vessel was inoculated with 0.367 mL of a 7 day old pre-culture of *Skeletonema costatum* to give a nominal cell density of 1.00 x 10^4 cells/mL. The salinity was adjusted to 30‰ at test initiation and the culture vessels were incubated at 20±2 °C for 120 h. During incubation, the flasks were shaken manually each working day. The cell densities were determined by electronic particle counting, using a Coulter counter. After 2, 3, 4, and 7 days, samples were removed from each test vessel. The organic calibration medium was subtracted from that of the test culture to obtain the cell density. The temperature was measured daily and hourly with an automatic recording system. The concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test. Median effective concentrations and its 95% confidence limits were determined by plotting the log of test concentration against percent inhibition expressed as Probit. Toxicity endpoints were recalculated for 72h and 96h by RMS via ToXRatPro Version 2.10. Validity criteria were checked according to OECD 201. Shapiro-Wilk´s Test on Normal Distribution and Cochran´s Test Procedure on Variance Homogeneity were performed. Dunnett`s Multiple t-test Procedure (Alpha = 0,05, one-sided smaller,multiple level) and Probit analysis using linear max. likelihood regression were used to determined NOEC and effective concentration EC_{50}.

Results

According to OECD 201, the increase biomass factor measured in the control between 0 and 72 h must be at least 16. In this test it was 3.6.

Moreover, cell counts were not taken on day 1 of the study. Therfore it is unclear from the test design whether other validity criteria were met (section-by-section specific growth rates in the control < 35% and coefficient of variation of average specific growth rates during the whole test period in the replicate control cultures must not exceed 7 %).

RMS Conclusions

After evaluation of the test according to current OECD 201 test validity criteria, following criteria have to be fullfilled:

- The biomass increase measured in the control vessels between 0 and 72 h must be at least factor 16:
- The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 %.
- The coefficient of variation of average specific growth rates during the whole test period in the replicate control cultures must not exceed 7 %.

In the present study, control cell counts increased by a factor of 3.6 within the initial 72h period, which is below the required 16-fold increase criteria. This indicates that the test culture used in the study suffered major deficiencies that impacted the validity of the study and the response of the test system.

Moreover, other validity critera could not be evaluated because cell counts were not taken on day 1 of the study and consequently no assessment was possible. Based on these points, the study does not meet current OECD 201 validity criteria and therefore is considered not acceptable for use in risk assessment or hazard classification.

KIIA 8.4/06

Author:	Scheerbaum, D.
Title:	Alga, Growth inhibition test to *(Nitzschia palea)*
Date:	04.11.1996
Doc ID:	Project No. 960606FH
Guidelines:	OECD Guideline 201
GLP:	YES
Validity:	NO

Materials and Methods

Test item:	Glyphosate tec.
Lot/Batch #:	Charg: 01/06/96
Purity:	96.7 % glyphosate tech
Vehicle and/or positive control:	Bacillariophycean medium according to SAG
Species:	*Nitzschia palea (*KÜTZING*)* W.SMITH SAG 1052-3b
Initial cell concentration	1.0 - 1.4 $\times 10^4$ cells/mL
Source:	Pflanzenphysiologisches Institut, Göttingen, Germany (Stock No. SAG1052-3b)
Temperature:	21.5 – 23.8 °C
Photoperiod:	24 h light
Light intensity	70-90µE/m^2/s (Fluorescent tube, Radium NL 58W/31, Spectralux Warmton)
Light quality	Universal white light
pH:	7.78-8.72 (control replicates)
Methods:	Based on the results of the preliminary test, a main testwas performed with 7 concentration levels ranging from 0.32 - 320 [mg/L] in a geometrical series with a dilution factor of 10 . A control with test medium (without test substance, three replicates) was tested under the same conditions as the test groups. The highest concentration level of 320 [mg/L] was tested both with and without pH-adjustment. During the study the test container were closed. A three-day-old preculture incubated at study conditions was used for the main study. Chlorophyll-fluorescence was determined at the beginning and after 24, 48, 72 and 96 h. Cell density was measured via Chlorophyll-a-fluorescence (Impulsfluorometer, Kleinfeld company), excitation at 435 [nm], emission at 685 [nm]. Each replicate was measured 6-fold. The cell density was measured at the beginning of the test and every 24 rh]. Filtrated culture medium was used as ground signal. The pH-value at the beginning of the test was measured out of a mixture of two additional replicates of each concentration and control. At the end it was measured from a mixture of all replicates. The temperature was measured continuously by a hygro-thermographe. Out of the fluorescence values 3-6 the mean fluorescence of the replicates of each test concentration and control were calculated The determination of the test substance was performed via HPLC at the beginning and the end of the test. Out of the cell density values the growth rate, the inhibition of biomass and the rate related inhibition after 96 h were calculated according to the formulas listed below. ECo-values (NOEC): the ECo-values were deduced from the dose-response-relationship. There was no mathematical calculation. EC10- and ECso-values: Calculation according to OEeD 201 via probit analysis. Calculations were performed using software SigmaPlot (windows) reI. 3.02 (1995), Jandel Corporation.

Results

The concentrations of the active ingredient were determined in all concentration levels at test start and test end. The recovery rates at the beginning of the test were in the range of 78 - 108 % of the a.s. At the end of the test they were in a range of 68 - 98 %. Low recoveries of 68 and 71 % respectively were found in the lowest test concentration and 76 - 77 % at the test end for 10 mg/L, whereas overall recovery rates of all other test concentration levels were > 80 %. Therefore all effect concentrations are based on nominal concentrations. The temperature during the test was in the range of 21 - 25 °C. The difference from the mean was within ± 2 °C.
The results of the cell densities at each concentration level and the control at each time of observation is shown in the following table.

Table B.9.2-34: Cell density (nominal concetrations of (*Nitzschia palea*)

Nominal concentration of Cell density (N/mL)	Cell density [N/mL]				
	0 h	24 h	48 h	72	96 h
320	1.0	0	0	0	0
100	1.3	0.8	1.0	1.0	1.6
32	1.2	0.7	0.9	1.2	1.8
10	1.4	0.8	1.2	2.3	4.4
3.2	1.3	1.0	1.8	4.8	12.2
1.0	1.3	1.0	2.7	9.0	26.6
0.32	1.4	1.2	2.8	9.5	26.4
control	1.2	1.2	3.0	9.6	23.6

Table B. 9.2-35 Evaluations of biomass inhibition and rate-related inhibition

Nominal concentration of Cell density (N/mL)	Biomass integral	Inhibition of biomass (%)	Growth rate	Rate-related inhibition (%)
320	- 36.67	100.00	0.0	100.00
100	- 11.76	100.00	0.04	94.42
32	- 6.26	100.00	0.09	88.09
10	17.88	91.63	0.29	60.72
3.2	90.75	57.55	0.56	25.43
1.0	215.39	- 0.76	0.76	- 1.03
0.32	219.03	-2.47	0.74	1.07
control	213.76	-	0.75	-

RMS Conclusions
After evaluation of the test according to current OECD 201 test validity criteria, following criteria have to be fullfilled:

- The biomass increase measured in the control vessels between 0 and 72 h must be at least factor 16:
- The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 %.

- The coefficient of variation of average specific growth rates during the whole test period in the replicate control cultures must not exceed 7 %.

In the present study, the test duration was prolonged to 96 h because the diatomeae has a lower growth rate compared to the fast growing green algae listed in the guideline. Therefore the increase of cell density as a validity criteria was evaluated after 96 h. Control cell counts increased by a factor of 19.9 within the 96 h period, and the coefficient of variation of average specific growth rates in the replicate control cultures was 2.0 % . But the mean coefficient of variation for section-by-section specific growth rates in the control cultures was above 70 % which indicates that the test culture used in the study suffered major deficiencies that impacted the validity of the study and the response of the test system.
Finally the study does not meet current OECD 201 validity criteria and therefore is considered not acceptable for use in risk assessment or hazard classification.

KIIA 8.4/07

Author:	Smyth, D.V., Kent, S.J., Morris, D.S., Johnson, P.A., Shillabeer, N
Title:	Glyphosate acid: Toxicity to freshwater diatom *Navicula pelliculosa*
Date:	03.02.1996
Doc ID:	2310976 /BL5673/B
Guidelines:	OECD
GLP:	YES
Validity:	NO

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	P24
Purity:	95.6 %
2. Cell growth medium:	acc. to Miller et al. (1978)
Species:	Freshwater diatom *Navicula pelliculosa* strain UTEX 667
Source:	Brixham Environmental Laboratory culture from strain UTEX 667
Initial cell concentration	0.300 x 10^4 cells/mL
Temperature:	24.0-24.1 °C (measured by thermometer) The hourly temperature measured automatically remained within 24±1°C
Photoperiod:	Continuous illumination, 4560 lux
pH:	3.7 – 8.3 at the start of the test, 3.7 – 8.7 at the end of the test
Methods:	The toxicity of glyphosate acid to the freshwater diatom *Navicula pelliculosa* was determined in a 120-hour, static toxicity test. The test incorporated 8 nominal concentrations (1.8, 3.2, 5.6, 10, 18, 32, 56, and 100 mg a.s./L) and a negative control consisting of culture medium without test item. The test vessels were 250 mL conical glass flasks containing 100 mL of control or test medium. The stock solution of nominal concentration of 100 mg a.s./L was prepared by adding glyphosate acid directly to 2000 mL sterile culture medium. Appropriate aliquots of this stock solution were diluted to prepare the lower test concentrations of 1.8, 3.2, 5.6, 10, 18, 32, and 56 mg a.s./L. 100 mL of the appropriate test solution were dispensed to each control, test and blank vessel. The test was performed in 6 replicate cultures of the culture medium control and 3 replicate cultures of each concentration of glyphosate acid. Each replicate was

	inoculated with 0.915 mL of the inoculum culture to give a nominal cell density of 0.300 x 10^4 cells/mL. A single blank vessel was also prepared for the control and each test concentration without algal cells (blank vessel). The culture vessels were incubated at 24±1°C under continuous illumination for 120 h. During incubation, the cells were kept in suspension by continuous shaking. The cell densities were determined by electronic particle counting, using a Coulter counter. After 1, 2, 3, 4, and 5 days, samples were removed from each control, test and blank vessel. The appropriate blank particle count was subtracted from that of the test culture to obtain the cell density. The % inhibition in growth (biomass and rate) relative to the control group was then determined. The pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily with a thermometer, and hourly with an automatic recording system. The concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test. Probit analysis was used to calculate the EC_{50} values. One-way analysis of variance, and Dunnett's post-hoc test was used to determine significant differences between treatments and the control.

Results

According to OECD 201, the biomass increase, measured in the control between 0 and 72 h, must be at least factor 16. In the current test it was 65. The test fulfills this validity criterion. The mean coefficient of variation, measured in the control from 0 to 72 h, must not be higher than 35 %. The test does not fulfill this validity criterion. High differences between the replicates (curves are not parallel) indicate non-reproducible growth. The coefficient of variation of the mean specific growth rate replicates in the control between 0 and 72 h was 4.4 %. According to OECD 201, the coefficient of variation of the mean specific growth rate, measured in the control from 0 to 72 h, must not exceed 7 %. The test fulfills this validity criterion.

Table B.9.2-36: Mean algal densities of *Navicula pelliculosa* after treatment with glyphosat acid

Glyphosate acid	Algal cell density			
(mg/L)	(x 104 cells ml-1)			
	Day 0	Day 1	Day 3	Day 4
Control	0.321	0.169	18.2	93.2
1.8	0.321	0.109	22.0	165
3.2	0.321	0.271	3.43	171
5.6	0.321	3.38	32.0	190
10	0.321	0.347	29.8	177
18	0.321	0.136	10.9	74.2
32	0.321	0.060	0.071	0.181
56	0.321	0.008	0.005	0.035
100	0.321	0.001*	0.006	0.001*

*Algal densitiy measurement for replicate was lower than the blank solution

Table B.9.2-37: Mean growth of *Navicula pelliculosa* after treatment with glyphosat acid

Nominal concentration (mg a.s./L)	Mean growth rates 72h		Mean areas under the growth curve 72h		Mean growth rates 96h		Mean areas under the growth curve 96h	
	Mean growth rate	% of control	Mean areas under the growth curve	% of control	Mean growth rate	% of control	Mean areas under the growth curve	% of control
Control	1.346		11.0		1.418		66.3	
1.0	1.409	105	12.1	111	1.560	110	105.1*	159
1.8	1.485	110	16.7	153	1.569*	111	115.5*	174
3.2	1.534	114	22.6*	206	1.578	113	133.4*	201
5.6	1.510	112	17.9*	163	1.578	111	120.8*	182
10.0	1.175	87	5.8	53	1.361	96	48.0	72
18.0	-0.504*	-37	-0.7*	-6	-0.143*	-10	-0.9*	-1
32.0	-1.366*	-102	-0.8*	-7	-0.552*	-39	-1.1*	-2
56.0	-1.309*	-97	-0.8*	-7	-1.443*	-102	-1.1*	-2

* Significant difference from control (p=0.05)

RMS Conclusions

No clear concentration/response relationship was found. Due to the lacking concentration/ response relationship, the shown ECx are not valid. The NOEC appears to be higher than or equal to 32 mg/L.

The mean coefficient of variation, measured in the control from 0 to 72 h, must not be higher than 35 %. The test does not fulfill this validity criterion.

The study is not considerd to be valid and is not acceptable.

KIIA 8.4/08

Author:	Egeler, P., Baumann, J.
Title:	A study on the toxicity of glyphosate isopropylamine salt 62.5 % to algae (*Pseudokirchneriella subcapitata*)
Date:	27.11.2002
Doc ID:	2310979 /A-99-02-04
Guidelines:	OECD Guideline 201, EEC Directive 92/69 C.3
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate isopropylamine salt
Lot/Batch #:	Tech L 020131
Purity:	62.66 % glyphosate isopropylamine salt
Vehicle and/or positive control:	SAG medium, Potassium dichromate
Species:	*Pseudokirchneriella subcapitata*
Initial cell concentration	10^4 cells/mL
Source:	Pflanzenphysiologisches Institut, Göttingen, Germany (Stock No. SAG 61.81)

Temperature:	21.7 – 25.0 °C
Photoperiod:	24 h light
Light intensity	8082 lux
Light quality	Universal white light
pH:	5.7 – 6.2
Methods:	On the basis of a range finding test, the test was performed with 5 concentrations, 4.27, 9.39, 20.66, 45.45 and 100 mg test item/L. A negative control (culture medium only) was prepared in parallel. For each concentration and the control, four vessels were prepared using 300 ml Erlenmeyer flasks each containing 100 mL of control or test medium. The initial cell concentration was 10^4 cells/mL. The concentrations of glyphosate IPA salt in the test solutions were measured by HPLC as concentrations of glyphosate acid at the start and at the end of the test in the 4.27, 20.66 and 100 mg test item/L treatments. A stability sample was analysed from a test vessel without algae with the highest test item concentration at the end of the exposure period. To maintain the algae in the suspension, all flasks were shaken continuously over the entire test period (100 ± 5 oscillations/min). After 24, 48, 72 and 96 hours of growth, the algal cell densities in the control and test concentration vessels were determined using a Thoma counting chamber with a light microscope and the % growth inhibition (biomass and rate) relative to the control group was determined. This was achieved by plotting the mean value of the cell concentration (converted in log values) against the percentage growth inhibition to generate dose-response curves for each concentration. The concentrations resulting in 50 % inhibition (E_rC_{50}, E_bC_{50}), were determined, as well as the NOEC. The pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily with a thermometer, and continuously with an automatic recording system. The concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test. Probit analysis was used to calculate the EC_{50} values. One-way ANOVA, Cochran´s Test and subsequent Dunnett´s t-test was used to calculate whether there were significant differences between the growth of algae in the controls and the algae exposed to the various test item concentrations to establish NOE_rC and NOE_bC values.

Results

The pH-values of the algal medium recommended by "Schlösser (1982): Sammlung von Algenkulturen, Pflanzenphysiologisches Institut der Universität Göttingen (SAG) - List of Strains", were lower than reported in OECD 201. In addition to the slightly lower pH values measured in the vessels allocated to concentration 100.0 mg/L, there could be an effect of the parameter pH on the growth rate of the algae.

For the reference item, the 96-hour E_bC_{50} was 0.497 mg/L and the 96-hour E_rC_{50} was 1.721 mg/L. These results were in agreement with what was expected on the basis of data shown in EEC Directive 92/69 method C.3.

The biomass in the control cultures increased by a factor of > 16 (153x), the coefficient of variance for section-by-section specific growth rates was ≤ 35 % (< 19 %), and for the whole test period ≤ 7 % (< 2 %). The validity criteria according to guideline OECD 201 are therefore fulfilled.

In stock solutions prepared at test start, measured concentrations were 81.9 % of nominal glyphosate concentrations .In test media at the beginning of the test, mean concentrations were 75.9 % of nominal concentrations and at the end of the test (96 h), mean concentrations were 64.2 %, of nominal with 45.2 % found in the stability sample without algae (see table below). The average recovery in all water samples containing algae was 70.1 % for glyphosate-IPA salt. Therefore, result are based on mean measured concentrations.

The results of the definitive test show that algal growth rates were significantly inhibited after 72 hours at nominal concentrations of 9.39 mg test item/L and higher. After 96 hours, significant inhibition was observed at 20.66 mg test item/L and higher.
For biomass, there were significant effects observed at nominal concentrations of 9.39 mg test item/L and higher after 72 and 96 hours.
In contrast, no inhibition of the algae growth was found at or below a nominal concentration of 4.27 mg test item/L.

Table B.9.2-38: Inhibition of growth rate and biomass of *Pseudokirchneriella subcapitata* exposed for 72 and 96 hours to glyphosate isopropylamine salt

Glyphosate isopropylamine salt formulation (nominal) (mg/L)	Control	4.27	9.39	20.66	45.45	100.0
Glyphosate isopropylamine salt (mean measured) (mg/L)		**1.95**	**4.12**	**9.19**	**19.96**	**41.56**
Glyphosate acid (mean measured) (mg/L)		1.45	3.05	6.81	14.79	30.79
Inhibition growth rate (0-72 h) (%)	-	1.6	6.6*	25.2*	64.8*	61.4*
Inhibition growth rate (0-96 h) (%)	-	-0.9	4.2	17.5*	53.4*	77.1*
Inhibition biomass (0-72 h) (%)	-	11.4	33.5*	70.7*	92.4*	91.9*
Inhibition biomass (0-96 h) (%)	-	3.2	27.1*	68.0*	94.9*	95.9*

RMS Conclusions

The 72 h and 96 h E_rC_{50} values for *Pseudokirchneriella subcapitata* exposed to glyphosate isopropylamine salt were calculated to be 31.7 and 32.0 mg test item/L, equivalent to 23.5 and 23.7 mg glyphosate acid/L (mean measured). The 72 h and 96 h E_bC_{50} for *P. subcapitata* exposed to glyphosate isopropylamine salt were calculated to be 9.3 and 10.2 mg test item/L, equivalent to 6.9 and 7.6 mg glyphosate acid/L (mean measured). No growth inhibition was observed at or below 4.27 mg test item/L.

The study is considered to be valid and acceptable.

KIIA 8.4/09

Author:	Desjardins, D., Kendall, T. Z., Krueger, H.O.
Title:	MON 78623: A 72-hour toxicity test with the freshwater alga (*Selenastrum capricornutum*)
Date:	11.02.2003
Doc ID:	2310983 /WL-2002-148
Guidelines:	OECD 201, EU Directive C3
GLP:	YES
Validity:	NO

Materials and Methods

Test item::	MON 78623 (potassium salt of glyphosate)

Lot/Batch #:	GLP-0108-11688-F
Purity:	47.7 % glyphosate
2. Vehicle and/or positive control:	OECD 201 medium
Species:	*Pseudokirchneriella subcapitata*
Initial cell concentration:	10^4 cells/mL
Source:	In-house culture
Acclimatisation period:	The pre-culture, which was used for the inoculation of the test cultures, was incubated 2 weeks under the conditions of the test.
Temperature:	22.0 – 22.3 °C
Photoperiod:	24 h light
Light intensity	6500 – 8550 lux
pH:	6.9 – 8.1 (test initiation), 7.8 – 8.0 (test termination)
Methods:	Based on the range finding test, the definitive algal growth inhibition test was performed with five concentrations of 7.5, 15, 30, 60 and 120 mg test item/L (achieving mean measured concentrations of 7.1, 15, 30, 61, 122 mg test item/L). In addition, algae were exposed to test medium without test substance (negative control). The algal medium recommended in OECD Guideline 201 was used. For each concentration, three parallel cultures were prepared in 250 mL Erlenmeyer flasks plugged with foam stoppers. To each test vessel, 100 mL of the algal suspension containing the test item preparation were added, with an initial cell density adjusted to 10^4 cells/mL. For the control group, six parallel test vessels were prepared with 100 mL volumes of culture media only. The culture vessels were incubated on a mechanical shaker in an environmental chamber for 72 h. During the incubation, the algal cells were kept in suspension by continuous shaking. After 24, 48, and 72 hours, samples from all vessels were taken and algal cell counts were performed using an electronic particle counter. Use of the particle counter was validated / verified using a haemocytometer and a microscope. Cell densities, areas under the growth curve, growth rates and percent inhibition were calculated using the SAS System for Windows v 8.02. Temperature was recorded twice daily during the test, while the pH was measured in each test concentration and the control at test initiation and test termination. Light intensity (lux) was measured at test initiation. EC_{50} values were calculated by non-linear regression Cell density, area under the growth curve and growth rate were evaluated for normality and homogeneity of variance (p = 0.05) using Shapiro-Wilks and Levene's test. Data were compared to the negative control using ANOVA and Dunnett's test (α = 0.05). These data were used to determine the NOEC values.

Results

Chemical analyses were performed on samples of the test solutions to quantify glyphosate. The recoveries at test initiation were between 83 and 99 % and at test termination 103 – 107 %, confirming the test substance to be stable. The ecotoxicological endpoints are based on the mean measured concentrations of 7.1, 15, 30, 61, 122 mg test item/L.

The biomass in the control cultures increased by a factor of > 16, the coefficient of variance for section specific growth rates was > 39 %, for the whole test period it was ≤ 7 %. The validity criteria according to guideline OECD 201 are therefore not fulfilled.

At and above mean measured concentrations of 61 mg test item/L, cell density, biomass and growth were significantly inhibited by the test item compared to the control see table below.

Table B.9.2-39: Reduction of growth rate and inhibition of cell growth of *Pseudokirchneriella subcapitata* exposed for 72 hours to glyphosate K-salt

Glyphosate K-salt (mg/L)	**Control**	**7.1**	**15**	**30**	**61**	**122**
Glyphosate acid (mg/L)	**-**	**3.4**	**7.2**	**14.3**	**29.1**	**58.2**
Cell density inhibition (%)	-	-14	-20	-6.8	33*	91*
Cell biomass inhibition (%)	-	-12	-17	-6.1	26*	88*
Cell growth inhibition (%)	-	-3.1	-4.3	-1.7	9.0*	54*

RMS Conclusions

The study is not considered to be valid and acceptable, as the coefficient of variance for section specific growth rates was > 39 %.

The 72 h E_rC_{50} and the 72 h E_bC_{50} for *Pseudokirchneriella subcapitata* exposed to glyphosate K-salt were calculated to be 114 and 74 mg glyphosate K-salt/L, equivalent to 54.4 and 35.3 mg glyphosate acid/L, respectively. The NOEC was determined to be 30 mg glyphosate K-salt/L equivalent to 14.3 mg glyphosate acid/L. Based on cell density the 72 h EC_{50} was calculated to be 69 mg glyphosate K-salt/L, equivalent to 32.9 mg glyphosate acid/L. The NOEC was determined to be 30 mg glyphosate K-salt/L equivalent to 14.3 mg glyphosate acid/L.

KIIA 8.4/10

Author:	Bogers, M.
Title:	Fresh Water Algal Growth Inhibition Test with (Aminomethyl)-Phosphonic Acid
Date:	30.06.1998
Doc ID:	2310985 /232458
Guidelines:	OECD Guideline 201 (1984) EEC Directive 92/69, Part C.3 (1992)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	(Aminomethyl)phosphonic acid (AMPA)
Lot/Batch #:	A010047101
Purity:	99 %
Control:	ISO-medium; Reference item: Potassium dichromate ($K_2Cr_2O_7$)
Species:	*Pseudokirchneriella subcapitata*, strain: CCAP 278/4
Initial cell concentration:	10^4 cells/mL
Source:	In-house culture
Acclimatisation:	4 days
Temperature:	22.5 – 23.0 °C
Photoperiod:	24 h light

Light intensity	6000 - 7500 lux
Light quality	TLD-lamps of 18 Watt
pH:	8.5 (control), 6.0 – 6.8 (highest test concentration)
Hardness:	24 mg $CaCO_3$/L
Methods:	On the basis of preliminary test results, the main test was performed with five concentrations: 10, 22, 46, 100 and 220 mg AMPA/L. A negative control group (culture medium only) was prepared in parallel. The test solutions were prepared using ISO-medium.The culture vessels were incubated on a shaking plate for 72 hours. For each concentration, three parallel cultures were prepared in 100 ml all-glass vessels each containing 50 mL of control or test item preparation. All vessels were inoculated with 10^4 algal cells/mL. Additionally, for the highest test concentration one replicate without algae was provided (blank vessel). For the control group, six parallel test vessels were prepared. After 24, 48, and 72 hours, mean algal cell densities for each test concentration and control were determined based on spectrophotometrical absorbance measurements from all test vessels following correction for the blank vessel and a linear calibration curve relating absorbance and cell density. The concentrations resulting in 50% reduction in growth rates (E_rC_{50}) and 50% inhibition of growth in terms of biomass (E_bC_{50}) were determined, and the associated NOEC values. The pH-values of test solutions were measured at test initiation and test termination. Temperature was recorded daily in a temperature-control vessel incubated alongside all other control and test vessels.Concentrations of AMPA were determined using an HPLC method of analysis, in samples taken from three representative concentrations, 10, 46 and 220 mg test item/L at the start and end of the test. The calculation of the E_rC_{50} and E_bC_{50} values were based on linear regression analysis of the percentages of growth inhibition of growth (biomass and rate) versus the logarithms of the corresponding nominal concentrations of the test substance. Data were compared to the negative control using ANOVA and Tukey's multiple comparisons test, Williams' test ($\alpha = 0.05$). These data were used to determine the NOEC values.

Results

The biomass in the control cultures increased by a factor of > 16 (68 in the test), the coefficient of variance for section specific growth rates was ≤ 35 % (29 % in the test), for the whole test period it was ≤ 7% (1 % in the test). All validity criteria according to guideline OECD 201 are fulfilled.

Measured concentrations of AMPA at test initiation were 99 %, 100 % and 102 % of the nominal 10, 46 and 220 mg AMPA/L concentrations, respectively. At test termination, concentrations were 98 %, 98 % and 96 % of the nominal concentrations, respectively. As the mean measured content of the test item ranged between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

The 72-hour E_bC_{50} of the reference item was 0.69 mg/L, the 72-hour E_rC_{50} was 1.7 mg/L (95% CI: 1.1 - 2.8 mg/L).

Cell densities were comparable to blank at nominal concentrations up to 46 mg/L, while cell densities at 100 mg/L and 220 mg/L were increasingly reduced. At 220 mg/L, almost no increase in cell densities were observed during the 72 hour test period.

Inhibition of cell growth increased with increasing concentration of AMPA from a nominal concentration of 22 mg/L upwards. Statistically significant inhibition of cell growth was found at test concentrations of 100 mg/L and higher.

Growth rates were in the range of the controls at the concentrations from 10 to 46 mg/L during the 72-hour test period, whereas the growth rate of algae exposed to 100 and 220 mg/L

were increasingly reduced. Statistically significant reduction of growth rate was found at test concentrations of 100 mg/L and higher.

Table B.9.2-40: Reduction of growth rate and inhibition of cell growth of *Pseudokirchneriella subcapitata* exposed for 72 hours to AMPA

Test parameters	Control	AMPA (mg/L)				
	-	10	22	46	100	220
Mean cell densities (0-72 h) (x 10000 cells/mL)	67.8	73.0	67.6	64.5	41.5	5.4
Inhibition of biomass (0-72 h) (%)		-1.7	0.1	1.2	12.0	59.8
Inhibition of cell growth rate (0-72 h) (%)		-3.5	3.0	6.6	35.4	87.8

RMS Conclusions

The 72 h $E_rC_{50}$and E_bC_{50} toxicity values for *Pseudokirchneriella subcapitata* exposed to aminomethylphosphonic acid (AMPA) were calculated to be 200 mg AMPA/L and 110 mg AMPA/L, respectively. The NOE_rC and NOE_bC values were both determined to be 46 mg AMPA/L.

The study is considered to be valid and acceptable.

KIIA 8.4/11

Author:	Porch, J.R. Kendall, T.Z., Krueger, H.O.
Title:	HMPA (hydroxymethylphosphonic acid): A 72-hour toxicity test with the freshwater alga (*Pseudokirchneriella subcapitata*)
Date:	11.10.2011
Doc ID:	2310987 /WL-2010-330 ! 139A-396A
Guidelines:	OECD
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	HMPA (hydroxymethylphosphonic acid)
Description:	solid
Lot/Batch #:	GLP-1003-20448-A
Purity:	97.0 %.
Control:	OECD medium
Species:	*Pseudokirchneriella subcapitata*
Initial cell concentration	1×10^4 cells/mL
Source:	In-house, original culture obtained from University of Toronto Culture Collection
Acclimatisation period:	Not stated
Temperature:	24 ± 2 °C
Light intensity	Continuous illumination, 6030 - 7040 lux

pH:	7.0 – 7.2 at test start; 7.5 – 9.3 at test termination
Hardness:	1.044 mg (K_2HPO_4/L)
Methods:	On the basis of the results of a range finding test, a main test was performed exposing actively growing *Pseudokirchneriella subcapitata* to HMPA at concentrations of 7.5, 15, 30, 60, and 120 mg HMPA/L. In addition, a negative control (culture medium only) was also prepared with algae exposed without test substance or other additives (negative control). The control and test concentration vessels were incubated at 24 ±2ºC for 72 h on a mechanical shaker at 100 rpm. The replicate test vessels were 250 mL Erlenmeyer conical flasks, each containing 100 mL of control or test medium, with 6 control vessels and 3 test concentrations vessels prepared. Test algae were taken from a 3-day old stock culture and were aseptically added to the test medium to obtain a nominal initial concentration of 3000 cells/mL. After 24, 48, and 72 hours, mean algal cell densities in samples of control or treated test media were determined using an electronic particle counter (Coulter Electronics, Inc.). Three counts per replicate were made. On the basis of the mean cell count, the percentage inhibition in algal cell growth relative to the control was determined. Prior to performing cell counts, the linearity of the instrument response was determined at settings previously established for *P. subcapitata*. The concentrations resulting in 50% reduction of growth rate (E_rC_{50}) and growth (biomass) inhibition (E_bC_{50}) were determined, as well as the associated NOAEC value. The pH-value of the test item treatments and the controls was measured at test initiation and test termination. At test initiation, pH was adjusted to 7.4 - 7.5 with 10 % HCl and 0.1N NaOH. The temperature was monitored continuously and recorded twice daily. Samples of test media were taken at test initiation and test termination for analysis of the active ingredient content. At test initiation, samples were collected for each treatment and control group prior to distribution of test solution into test chambers. At 72 hours, samples were collected from the pooled biological replicates from each respective treatment and control group. Samples were analysed using an HPLC method of analysis with mass detection (MS). The calculation of the EC_{50} values was based on the percentages of growth (biomass) inhibition and the percentages of growth rate reduction versus (log) concentration using the linear regression method. The treatment groups were also compared to the negative control using Dunnett's test (α = 0.05).

Results

Measured concentrations of HMPA at test initiation were 106 %, 94.1 %, 99.4 %, 104 %, and 91.7 % of the nominal concentrations (7.5, 15, 30, 60, and 120 mg HMPA/L) respectively. At test termination concentrations were, 88.0, 92.8, 92.4, 95.8, and 100% of the nominal concentrations, respectively. The overall mean measured test concentrations were 7.3, 14, 29, 60, and 115 mg HMPA/L, representing 97, 93, 97, 100, and 96 % of the nominal concentrations, respectively. The results of the study were based on mean measured test concentrations.

The biomass in the control cultures increased by a factor of > 16 (299 in the test), the coefficient of variance for section specific growth rates was ≤ 35 % (23.4 % in the test), for the whole test period it was ≤ 7 % (0.96 % in the test). All validity criteria according to guideline OECD 201 are therefore fulfilled.

The pH tended to increase relative to increases in algal densities, which is typical for tests conducted with *Pseudokirchneriella subcapitata*.

HMPA inhibited cell growth of the fresh water algae *P. subcapitata* increasingly at increasing concentrations, resulting in a reduction in cell growth rate of 8 % and an inhibition of cell growth (biomass) of 38 % at 120 mg HMPA/L.

After 72 hours of exposure, inhibition of cell density in the 7.3, 14, 29, 60, and 115 mg HMPA/L treatment groups was -7, 1, 4, 9, and 38 %, respectively, relative to the

negative control. Inhibition of growth rate in the 7.3, 14, 29, 60, and 115 mg HMPA/L treatment groups was -1, 0, 1, 2, and 8 %, respectively, relative to the negative control. Dunnett's test indicated that cell density and growth rate were significantly reduced (α = 0.05) in the 115 mg HMPA/L treatment level relative to the negative control. Consequently, the 72-hour NOAEC determined in this study was 60 mg HMPA/L.

Table B.9.2-41: Reduction of growth rate and inhibition of cell growth (biomass) of *Pseudokirchneriella subcapitata* exposed for 72 hours to HMPA

Test parameters	Control	HMPA (mg/L)				
	-	7.3	14	29	60	115
Mean number of algae cells after 72 hours (x 1000 Cells/mL)	106.5	108.9	96.4	89.1	81.2	65.8
Cell growth rate reduction (0-72 h) (%)		-1	0	1	2	8
Cell growth inhibition (0-72 h) (%)		-7	1	4	9	38

RMS Conclusions

The 72 h E_rC_{50} and E_bC_{50} toxicity values for *P. subcapitata* exposed to HMPA were both determined to be >115 mg HMPA/L. The NOEC was determined to be 60 mg HMPA/L.

The study is considered to be valid and acceptable.

KIIIA 10.2.2.3./01

Author:	Neven, B.,
Title:	A1ga, Growth Inhibition Test Effect of MON 52276 on The Growth Of *Selenastrum capricornutum*
Date:	1992
Doc ID:	2317597 /LI-91-389
Guidelines:	OECD Guideline 201 (2006)
GLP:	YES
Validity:	YES

Materials and Methods

Test item::	MON 52276
Lot/Batch #:	LLN 260491 B
Purity:	31 % glyphosate acid equivalent, as 41 % isopropylamine salt of glyphosate
Density:	1.16 mg/cm3
Vehicle and/or positive control:	None
Species:	*Pseudokirchneriella subcapitata*
Initial cell concentration	104 cells/mL
Source:	In-house culture
Acclimatisation period:	The pre-culture, which was used for the inoculation of the test cultures, was incubated 4 days under the conditions of the test.
Temperature:	20.9 – 23.1°C

Photoperiod:	24 h light
Light intensity	8875 ± 125 Lux
pH:	8.31 – 8.97 (control), 5.88 – 5.98 (highest test concentration)
Conductivity/ Hardness:	Not stated
Methods:	Based on the range finding test, the definitive algal growth inhibition test was performed with five concentrations (50, 90, 160, 290 and 500 mg test item/L). In addition, algae were exposed to test medium without test substance (blank control). The algal medium recommended in OECD Guideline 201 (1981) was used. For each concentration, three parallel cultures were prepared in 150 mL Erlenmeyer glass flasks covered with cotton wool. To each test vessel, 100 mL of the algal suspension containing the test item preparation were added, with an initial cell density adjusted to 104 cells/mL. For the control group, six parallel test vessels were prepared. The culture vessels were incubated on a shaking plate over several generations for 72 h. During the incubation, the algal cells were kept in suspension by continuous shaking. After 24, 48, and 72 hours, aliquots of media from each culture were taken and mean cell densities for each test concentration and control were determined based on spectrophotometrical measurements (absorbance measurement). Algal cell concentrations were also determined by microscopic counting at 48 h and 72 h. The endpoints were calculated for the absorbance and cell counting method. Temperature and the light intensity were recorded daily during the test, while the pH was measured in one replicate of each test concentration at test initiation and test termination. Inhibition of cell growth and reduction of cell growth rate were derived from growth curves obtained by plotting the average algal cell concentrations for each test concentration against time. Concentrations resulting in 50% reduction of growth rate (ErC50) and 50% inhibition of cell growth (EbC50) were determined, with corresponding NOEC values. The median effect concentrations (EC50 values) were determined using the Logit model of Chou and Chou (1985).

Results

The effects of the test item MON 52276 on *Pseudokirchneriella subcapitata* (formerly named *Selenastrum capricornutum*) were evaluated in a 72-hour static toxicity test. After a range-finding test, suspensions of *Pseudokirchneriella subcapitata* were exposed to five nominal concentrations (50, 90, 160, 290 and 500 mg test item/L). In addition, algae were exposed to test medium without test substance (negative control).

Based on absorbance, the 72 h E_rC_{50} and the 72 h E_bC_{50} for *Pseudokirchneriella subcapitata* exposed to MON 52276 were calculated to be 393 mg test item/L and 150 mg test item/L, equivalent to 121.8 mg glyphosate acid/L and 46.5 mg glyphosate acid/L, respectively. The NOEC was determined to be 90 mg test item/L equivalent to 27.9 mg glyphosate acid/L. For the cell counting method, the 72 h E_rC_{50} and the 72 h E_bC_{50} for *Pseudokirchneriella subcapitata* exposed to MON 52276 were calculated to be 284 mg test item/L and 178 mg test item/L, equivalent to 88 mg glyphosate acid/L and 55.2 mg glyphosate acid/L, respectively. The NOEC was determined to be 90 mg test item/L equivalent to 27.9 mg glyphosate acid/L.

Table B.9.2-42: Reduction of growth rate and inhibition of cell growth of *Pseudo-kirchneriella subcapitata* exposed for 72 hours to MON 52276

MON 52276 (mg/L)	**Control**	**50**	**90**	**160**	**290**	**500**
Glyphosate acid (mg a.s./L)	**-**	**15.5**	**27.9**	**49.6**	**89.9**	**155**
Mean cell densities (x 1000 cells/mL)	644	741	663	315	45	33
Cell growth rate reduction (%)	--	-13.6	-8.4	10.9	42.8	58.2
Cell growth inhibition (%)	--	-36.9	-27.7	50.3	81.5	89.6

The cell density decrease continuously as the test concentrations increase, reaching 33 x 10^3 cells/mL at the highest test concentration, against 644 x 10^3 cells/mL observed in blank control. The mean cell numbers observed for the two lowest test concentrations were numerically higher that those observed in control.
Inhibition of cell growth increased with increasing concentration of MON 52276, from a nominal concentration of 160 mg test item/L upwards. For the two lowest test concentration of 50 mg test item/L and 90 mg test item/L, increases in cells growth of 36.9 % and 27.7 %, respectively, compared to the control were observed.
Reduction of algal growth rate increased with increasing concentration of MON 52276, from a nominal concentration of 160 mg test item/L upwards. For the two lowest test concentration of 50 mg test item/L and 90 mg test item/L, increases of algal growth rate of 13.6 % and 8.4 %, respectively, compared to the control were observed.

Table B.9.2-43: End points determined for *Pseudokirchneriella subcapitata* exposed to MON 52276

	MON 52276 (mg/L)		Glyphosate acid (mg a.s./L)	
Endpoint	absorbance	cell counting	absorbance	cell counting
E_rC_{50} (72 hours)	393	284	121.8	88.0
E_bC_{50} (72 hours)	150	178	46.5	55.2
NOEC (72 hours)	90		27.9	

RMS Conclusions
No analysis of glyphosate in the test system was conducted. However, no degradation of glyphosate is expected to have occurred over the course of the study, since glyphosate concentrations have been demonstrated to be stable over a 72-hour period in other formulation studies.
The pH of the test system was influenced by MON 52276 concentrations, and varied by more than 1.5 units across the 5 dose levels. Within each test concentration, the pH variation was less than 1.5 units. These deviations were not considered to have affected the validity of the study. Based on nominal concentrations, the 72-hour E_rC_{50} (growth rate) and E_bC_{50} (biomass) of MON 52276 to *Pseudokirchneriella subcapitata* under static test conditions were determined to be 284 mg/L (88 mg a.s./L) and 178 mg/L (55 mg a.s./L), respectively. The corresponding 72-hour no observed effect concentration (NOEC) for both parameters was 90 mg/L (28 mg a.s./L).

The test is considered to be acceptable and valid.

B.9.2.1.7 Aquatic plants

For aquatic macrophytes, two studies conducted with *Lemna gibba* (1092-02-1100-5, Hughes, 1987 and BL5662/B, Smyth *et al.* 1996) were included in the first EU-Evaluation of glyphosate in 2001. Regarding those studies for which no amendment of the former evaluation was necessary, please refer to the glyphosate monograph of 2001 and its addenda.
A summary of all relevant data (including data already reviewed during the 2001 EU evaluation of glyphosate) for glyphosate, glyphosate salts and the aquatic metabolites AMPA and HMPA is presented in the table below.

Table B.9.2-44: Toxicity of glyphosate acid, glyphosate IPA-salt, K-salt and metabolites AMPA and HMPA for aquatic plants

Species	Test substance	Test design	EC_{50} (mg a.s./L)	Reference/GLP
Lemna gibba	Glyphosate acid	14 d semi-static	$EC_{50,\ frond\ count}$ = **12** $EC_{50,\ dry\ weight}$= 20 $NOEC_{empiric}$ = 1.5	Confirmed EU endpoint (SANCO/6511/VI/99-final and glyphosate Monograph 2001) BL5662/B Smyth *et al.*, 1996
Lemna gibba	Glyphosate acid	14 d static	$EC_{50,\ frond\ count}$ = 25.5	1092-02-1100-5 Hughes, 1987

Species	Test substance	Test design	EC_{50} (mg a.s./L)	Reference/GLP
				98-00103
Myriophyllum aquaticum	MON77973 Glyphosate acid	14 d static	$EC_{50,\ shoot\ length,\ relative\ increase}$ = 278.7 $EC_{50,\ shoot\ length,\ growth\ rate}$ = 276 $EC_{50,\ fresh\ weight,\ relative\ increase}$ = **12.3** $EC_{50,\ fresh\ weight,\ growth\ rate}$ = 23.4 $EC_{50,\ dry\ weight,\ relative\ increase}$ = 25.2 $EC_{50,\ dry\ weight,\ growth\ rate}$ = 18.0 $EC_{50,\ root\ length,\ relative\ increase}$ = 18.0 $EC_{50,\ root\ length,\ growth\ rate}$ > 500 NOEC << 5	CHE-015/4-80/A Wenzel, 2012 BVL no 2310995
Lemna gibba	Glyphosate-IPA salt	14 d static	$EC_{50,\ frond\ count}$ = 53.56 (33 a.e.) $EC_{50,\ dry\ weight}$= 62.59 (39 a.e.) NOEC = 25 (16 a.e.)	980909FH Scheerbaum, 1999 BVL no 2310990
Lemna minor	Glyphosate-IPA salt	7 d semi-static	$EC_{50,\ frond\ count}$ = **25.5** $EC_{50,\ dry\ weight}$= 46.2 $EC_{50,\ growth\ rate}$= 42.6 NOEC = 12 (9 a.e.)	CEMR-1873 Mallett, 2002 BVL no 2310992
Myriophyllum aquaticum	AMPA	14 d static	$EC_{50,\ shoot\ length,\ relative\ increase}$ = 103.3 $EC_{50,\ shoot\ length,\ growth\ rate}$ > 94.6 $EC_{50,\ fresh\ weight,\ relative\ increase}$ = **70.8** $EC_{50,\ fresh\ weight,\ growth\ rate}$ = 97.3 $EC_{50,\ dry\ weight,\ relative\ increase}$ = 63.2 $EC_{50,\ dry\ weight,\ growth\ rate}$ = 72.0 $EC_{50,\ root\ length,\ relative\ increase}$ = 31.1 $EC_{50,\ root\ length,\ growth\ rate}$ = 150.1 NOEC < 5.4	CHE-022/4-80/A Wenzel, 2012 BVL no 2310997
Lemna gibba	HMPA	7 d semi-static	$EC_{50,\ frond\ count}$ >123 $EC_{50,\ dry\ weight}$ >123 NOEC = 123	139A-397 Porch *et al.* 2011/yes BVL no 2310999
Lemna gibba	MON 52276	7d semi-static	$EC_{50,\ frond\ count}$ = 67 (21) NOEC= 0.9 (0.3)	GA-2002-051 Dengler, 2002/yes BVL no 2316015
Myriophyllum aquaticum	MON 52276	14 d	$EC_{50,\ shoot\ length,\ relative\ increase}$ = 13.44 $EC_{50,\ shoot\ length,\ growth\ rate}$ = 42.79 $EC_{50,\ fresh\ weight,\ relative\ increase}$ = **4.44** $EC_{50,\ fresh\ weight,\ growth\ rate}$ = 10.33 $EC_{50,\ dry\ weight,\ relative\ increase}$ = nd $EC_{50,\ dry\ weight,\ growth\ rate}$ = 143.34 $EC_{50,\ root\ length,\ relative\ increase}$ = 5.84 $NOEC_{Fresh\ weight/relative\ increas}$ <0.30	CHE-016/4-80/A Wenzel, 2012 BVL no 2316017

Values in grey: confirmed EU endpoints (SANCO/6511/VI/99-final, or EU Review Monograph

KIIA 8.6/01

The following study by Smyth et al. (1996, BVL no 2310988) gives the confirmed EU endpoint employed in the risk assessment for aquatic plants (SANCO/6511/VI/99-final, or EU Review Monograph):

Author:	Smyth, D.V., Kent, S.J., Morris, D.S., Cornish, S.K., Shillabeer, N
Title:	GLYPHOSATE ACID: Toxicity to duckweed (*Lemna gibba*)

Date:	31.01.1996
Doc ID:	2310988 /BL5662/B
Guidelines:	EPA FIFRA Subdivision J Guideline 123-2
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid	
Description:	White solid	
Lot/Batch #:	P24	
Purity:	95.6 %	
2. Vehicle and/or positive control:	Hoaglands M medium	
Species:	*Lemna gibba*, Strain G3	
Source:	In-house culture originally obtained from University of Waterloo, Canada	
Temperature:	24.6 – 25.0 °C	
Photoperiod:	24 h illumination	
Light intensity	5000 lux	
pH:	Freshly prepared test media: Control:4.7 – 4.9 0.75 mg/L: 4.7 – 4.8 1.5 mg/: 4.6 – 4.7 3.0 mg/L: 4.6 6.0 mg/L: 4.5 12 mg/L: 4.4 24 mg/L: 4.2 – 4.3 48 mg/L: 3.9 – 4.0 96 mg/: 3.5 – 3.6	Old test media: Control:5.3 – 5.7 0.75 mg/L: 5.3 – 5.8 1.5 mg/: 5.2 – 5.8 3.0 mg/L: 5.2 – 5.8 6.0 mg/L: 5.1 – 5.7 12 mg/L: 4.8 – 5.6 24 mg/L: 4.6 – 5.0 48 mg/L: 4.0 – 4.2 96 mg/: 3.6 – 3.7
Methods	The toxicity test on *Lemna gibba* was performed with eight concentration levels, 0.75, 1.5, 3.0, 6.0, 12, 24, 48 and 96 mg glyphosate acid/L with 3 replicates per test concentration. Three control replicates (without test substance) were tested under the same conditions as the test groups. The plants were placed in 400 mL beakers (test vessels), containing 160 mL of Hoagland's M-medium prepared according to Hillman (1961). The test was conducted under semi-static conditions with renewal of the test medium after 5 and 9 days. Three uniform healthy-looking plants with 4 fronds each were added to each control and test vessel. The number of plants and fronds were counted after 2, 5, 7, 9, 12 and 14 days. Also symptoms of toxicity were recorded on these dates. At test end the weight of the dried plant tissue (at 60 °C) was recorded. The pH was measured in the old and the new test medium (new= day 0, 5 and 9, old = day 5, 9 and 14). Temperature in the test chamber was recorded daily and light intensity was recorded once a week. Analytical measurements of glyphosate acid were performed by means of HPLC analysis at test start and after 5 and 9 d (after test medium renewal). Fresh media was analysed on days 0, 5 and 9. Old media were analysed on days 5, 9 and 14. The EC_{50} and its 95% confidence interval were calculated by moving average angle method. The NOEC values were determined by calculation of statistical significance using one-way analysis of variance (ANOVA) and Dunnett's test for inhibition of frond number and biomass dry weight, respectively, at p = 0.05.	

Results

Analytical measurements were performed in the freshly prepared (day 0, 5 and 9) and the old (day 5, 9 and 14) test media. The measured concentrations in the fresh media ranged from 90

– 108 % of nominal and in the old media from 87 – 102 % of nominal (overall mean measured: 93 – 100 % of nominal).
All validity criteria according to OECD 221 were fulfilled, as the doubling time of frond numbers in the control were less than 2.4/d. According to EPA FIFRA Subdivision J Guideline 123-2, endpoints were determined after 14 days.
The increase in frond number was significantly inhibited at nominal test concentration of 6.0 mg test item/L and higher, when compared to the control. The growth of the plant in terms of tissue dry weight was significantly reduced at 12 mg test item/L and higher. At 24, 48 and 96 mg test item/L dose related symptoms like pale frond colouration, emergence of stunted new frond growth, reduced root growth and unnatural floating on the solution surface were observed from day 2 onwards. Visually observed effects were apparent at concentrations of 3.0 mg/L and above. Therefore, the overall NOEC was 1.5 mg a.s./L.

Table B.9.2-45: Frond numbers, increase in frond numbers and inhibition compared to the control

Test item rate (mg a.s./L)	Number of fronds						Increase in frond numbers	Inhibition (%)
	Day 2	Day 5	Day 7	Day 9	Day 12	Day 14	(Day 0 – 14)	
Control	21	48	85	134	222	327	315	-
0.75	23	47	79	125	232	343	331	0
1.5	23	45	78	113	220	323	311	1
3.0	21	48	78	120	206	300	288	9
6.0	21	49	81	116	198	269	257	18*
12	20	44	74	105	148	173	161	49*
24	16	28	44	59	82	91	79	75*
48	15	21	24	28	28	30	18	94*
96	13	14	15	16	18	17	5	98*

* significant at p = 0.05

Table B.9.2-46: Mean dry weight of plant tissue after 14 d, main increase in dry weight and inhibition compared to the control

Test item rate (mg a.s./L)	Mean tissue dry weight after 14 day (mg)	Mean increase (mg)	Inhibition (%)
Control	40.7	39.2	-
0.75	51.3	49.8	0
1.5	49.8	48.3	0
3.0	44.0	42.5	0
6.0	40.3	38.8	1
12	29.8	28.3	28*
24	16.5	15.0	62*
48	6.0	4.5	89*
96	1.4	> 0.1	100*

* significant at p = 0.05

RMS Conclusions

Glyphosate acid was found to significantly inhibit the growth of *Lemna gibba* after 14 days at or above a nominal concentration of 6 mg a.s./L. The 14-d EC_{50} value for inhibition of front number was 12 mg a.s./L (95% CL = 11 - 14 mg a.s./L) and for tissue dry weight 20 mg a.s./L (95% CL = 18 - 22mg a.s./L).The NOEC was determined to be 3.0 and 6.0 mg a.s./L for frond number and weight increase, respectively.
The study is considered to be valid and acceptable.

KIIA 8.6/02

Author:	Scheerbaum, D.
Title:	Glyphosate 62 % IPA-Salt, aquatic plant toxicity test using *Lemna gibba*
Date:	12.02.1999
Doc ID:	2310990 /980909FH
Guidelines:	Guideline ASTM E 1415- 91 (June 1991)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate 62% IPA-Salt
name	Glyphosate Isopropylamine Salt
Lot/Batch #:	22-9754
Purity:	62.4 % glyphosate IPA-Salt
Density:	1.2355 g/mL
2. Vehicle and/or positive control:	20X-AAP media, zinc chloride
Species:	*Lemna gibba*

Source:	Bundesanstalt für Gewässerkunde, Koblenz, Germany
Temperature:	23.9 – 26.9 °C
Photoperiod:	24 h fluorescence light
Light intensity	from 4941 – 5629 lux
pH:	7.40 – 7.56 in new test media, 7.81-9.08 in old test media
Conductivity /Hardness	not stated
Methods:	On the basis of the results of a range finding test, the definitive test was performed with five concentration levels, 6.25, 12.5, 25, 50 and 100 mg test item/L with 3 replicates per test concentration. Three negative control replicates (20X-AAP media only) were tested under the same conditions. Three plants per replicate were used. The plants were placed in 500 mL beakers (test vessels), which already contained 300 mL 20X-AAP dilution media prepared according to the guideline. The pH of the test medium was adjusted to 7.5 ±1 prior to the test. Three uniformly healthy-looking plants with 4 fronds each were used in each test vessel. The test was conducted under semi-static conditions with renewal of test media on day 2, 4, 7, 9 and 11. Glyphosate isopropylamine salt concentrations were measured via HPLC as glyphosate acid on days 4 and 11 (fresh media) and on days 7 and 14 (old media). The reference substance (zinc chloride) was equally tested at 1.0, 3.2 and 10 mg/L resulting in 7 d E_bC_{50} of 4.67 mg/L and E_rC_{50} of 5.47 mg/L. The number of plants and fronds in all test vessels was determined on day 0, 7 and 14. Observation of change in colour, break-up of plants and destruction of roots were made on day 7 and 14. Dry biomass weight was determined on day 14. The pH values were measured on day 0, 2, 4, 7, 9, 11 and 14. The room temperature in the test chamber was measured and recorded continuously. Sampling and analysis of the test concentration were carried out on day 4 and day 11 (freshly prepared media) and on day 7 and 14 (3 day old test media). All test concentrations and control replicates were analysed. EC_{50} and EC_{90} values of frond number inhibition after day 7 and 14 were calculated by Probit analysis. The NOEC values were determined by calculation of statistical significance using one-way analysis of variance (ANOVA) and Dunnett's test for inhibition of frond number and biomass dray weight, respectively, at $\alpha = 0.05$.

Results

In freshly prepared test media the mean recoveries of the active substance varied between 81 % and 85 % for day 4 and 107 % to 111 % for day 11. In the aged test media (3 days old), 97 % to 109 % of the active substance were recovered for day 7 and 89 % to 103 % for day 14. No active substance was found in the control samples.

The doubling time of frond numbers in the control was less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days. The validity criteria according to the current guideline OECD 221 are therefore fulfilled.

Increase of growth similar to control vessels was found at nominal concentrations of 6.25, 12.5 and 25 mg test item/L. Glyphosate isopropylamine salt was found to significantly inhibit the growth of *Lemna gibba* after 14 days at or above a concentration of 50 mg test item/L. Front number inhibition values after day 14 as well as biomass dry weight inhibition are presented in the tables below.

Visual observations: After 7 days smaller roots and fronds partially with more pigmentation were observed at 6.25, 12.5 and 25 mg test item/L. Smaller fronds and roots as well as fronds partially without pigmentation were observed at 50 and 100 mg glyphosate IPA/L. After 14 days fronds partially without pigmentation were observed at 12.5 and 25 mg glyphosate

IPA/L. Smaller fronds and roots as well as fronds partially without pigmentation were observed at 50 and 100 mg test item/L.

Table B.9.2-47: Frond numbers and inhibition values of *Lemna gibba* exposed to glyphosate IPA salt

		Control	Test item (mg/L)				
Glyphosate IPA			**6.25**	**12.5**	**25**	**50**	**100**
Glyphosate acid			**3.90**	**7.80**	**15.60**	**31.20**	**62.40**
Mean frond number	Day 0	12.0	12.0	12.0	12.0	12.0	12.0
	Day 7	91.0	127.7	113.7	120.3	56.3	12.7
	Day 14	535.0	776.7	757.3	875.7	119.3	20.7
Mean increase of frond number	Day 7	79.0	115.7	101.7	108.3	44.3	0.7
Mean inhibition (%)		-	-46.0± 17.6	-29.0± 11.4	-37.0± 6.4	-44.0± 18.1	-99± 1.5
Mean increase of frond number	Day 14	523.0	764.7	745.3	863.7	107.3	8.7
Mean inhibition (%)		-	-46.0± 14.0	-43.0± 12.7	-65.0± 15.4	79.0± 7.5	98.0± 1.1

Table B.9.2-48: Dry weight of *Lemna gibba* exposed for 14 days to glyphosate IPA salt

		Control	Test item (mg/L)				
Glyphosate IPA			**6.25**	**12.5**	**25**	**50**	**100**
Glyphosate acid			**3.90**	**7.80**	**15.60**	**31.20**	**62.40**
Mean biomass dry weight (mg)	Day 14	48.9	65.2	66.0	69.8	18.7	6.6
Mean inhibition (%)		-	-33.0 ± 10.8	-35.0 ± 9.8	-43.0± 12.8	62.0± 15.4	86.0± 2.7

RMS Conclusions

Glyphosate isopropylamine salt was found to significantly inhibit the growth of *Lemna gibba* after 14 days at or above a nominal concentration of 50 mg a.s./L. The EC_{50} values for inhibition of front number and dry weight after 14 days were 53.6 mg glyphosate isopropylamine salt /L (equivalent to 33.4 mg glyphosate acid/L) and 62.6 mg glyphosate isopropylamine salt/L (equivalent to 39.1 mg glyphosate acid/L) respectively. The NOEC was determined to be 25 mg glyphosate isopropylamine salt/L, equivalent to 15.6 mg glyphosate acid/L.The study is considered to be valid and acceptable.

KIIA 8.6/03

Author:	Mallett, M.J./ G. Turnbull
Title:	IPA Salt of Glyphosate: Effects on *Lemna minor*
Date:	05.12.2002
Doc ID:	2310992 /CEMR-1873
Guidelines:	OECD OECD Guideline 221 (2006)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate isopropylamine (IPA) salt
Lot/Batch #:	1002B
Purity:	97.1 % as IPA salt
2. Vehicle and/or positive control:	Swedish Standard (SIS) *Lemna* growth medium
Species:	*Lemna minor*
Source:	Pond in Marlow, Buckinghamshire, UK
Temperature:	20.5 – 22.8 °C
Photoperiod:	24 h fluorescence light
Light intensity	6600 - 8100 Lux
pH:	6.06 – 6.96
Methods:	On the basis of the results of a range finding test, the definitive test was performed with six concentration levels, 2.92, 5.83, 11.7, 48.6 and 97.2 mg glyphosate IPA salt/L, equivalent to 2.16, 4.32, 8.64, 18.0, 36.0 and 72.0 mg glyphosate/L. A negative control group with *Lemna minor* exposed to culture medium (SIS) only was run in parallel. The medium in each of the test vessels was renewed on day 2 and 4.Three replicates were prepared with 9-10 fronds (in 3-4 colonies) were used for each test concentration and control. Temperatures and pH values were measured in the test media at the start of tests and at the end. In addition, temperature was monitored continuously. Analytical samples for analysis of glyphosate were collected at the start of the tests and at the end and following each media renewal. Samples were analysed using HPLC with fluorescence detection. The numbers of fronds and colonies were counted on days 0 (start), 2, 4 and 7 during the definitive test. Dry weights of the fronds were determined at the end of the tests. The fronds from each vessel were collected, rinsed with de-ionised water and dried at 60°C to a constant weight. The dry weights of fronds from each vessel were measured to ±0.1 mg. EC_{50} values were calculated using the LC_{50} program of Stephan *et al.*, 1986. The no-observed-effect concentration (NOEC) and the lowest- observed-effect concentration (LOEC) were based on statistical analysis of *L. minor* final frond numbers, growth rate and area under growth curve values, as well as the final biomass, for the definitive test. Data were first tested for compliance with the assumptions of ANOVA in terms of normality of distribution and homogeneity. The treatment means were tested for significant difference from the control mean at α=0.05 using the Dunnett's test.

Results

Chemical analyses were performed on samples of the test media to quantify glyphosate in the test solution. The mean measured content of the test item always ranged between 80 and 120 % of nominal concentrations.

Concerning the compliance with validity criteria given in OECD 221, the doubling time in the control ranged between 1.63 and 1.83 days for the parameter growth rate and was less than 2.5h; after seven days number of *Lemna* fronds increased by a factor of 16 in control treatment. The validity criteria according to guideline OECD 221 are fulfilled.

The results of the definitive test showed no effect on frond growth at 11.7 mg glyphosate isopropylamine salt/L and partial and statistically significant inhibition at 24.3 mg/L. Nevertheless, visible effects were noted like chlorosis and elogation of fronds at concentrations of 11.7 mg glyphosate isopropylamine salt/L and 24.3 mg/L mg glyphosate isopropylamine salt/L.

At 48.6 and 97.2 mg glyphosate IPA salt/L the inhibition of frond growth was greater at 81% and 87% inhibition for final frond numbers.

RMS Conclusions

Based on nominal concentrations if IPA salt of glyphosat was significantly inhibited at 24.3 mg glyphosate IPA salt/L, equivalent to 18.0 mg glyphosate acid/L. The overall no-observed effect concentration (NOEC) of the IPA salt of glyphosate to *Lemna minor* measured over a 7 day exposure period was 11.7 mg/L, equivalent to 8.64 mg glyphosate acid/L. The lowest 7 day EC_{50} was 25.5 mg/L with 95 % confidence limits of 11.1 to 73.4 mg glyphosate IPA salt/L measured from final frond numbers at 7 days, equivalent to 18.9 mg glyphosate acid/L (8.22 – 54.37 mg a.s./L).The study is considered to be valid and acceptable.

KIIA 8.6/04

Author:	Wenzel, A
Title:	Effect of MON77973 (Glyphosate acid) on the Growth of *Myriophyllum aquaticum* in the Presence of Sediment. Test with a subsequent Recovery Period
Date:	21.02.2012
Doc ID:	2310995 /CHE-015/4-80/A
Guidelines:	Maltby, L., *et al.* (2008): Aquatic Macrophyte Risk Assessment for Pesticides, SETAC AMRAP
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid (MON77973)
Lot/Batch #:	GLP-0807-19475-T
Purity:	85.2 % glyphosate
Control:	None
Species:	*Myriophyllum aquaticum*
Source:	Institut für Gewässerschutz, MESOCOSM GmbH, Neu-Ulrichstein 5, D-35315 Homberg (Ohm), Germany
Growth medium:	Smart & Bako medium
Artificial sediment:	4-5% peat 20% kaolin clay 75-76% quartz sand $CaCO_3$ (if needed to adjust pH to 7.0 ± 0.5) Based on artificial soil used in OECD guideline 219 Moistening of sediment up to 30% with deionised water or nutrient medium (ammonium chloride and sodium phosphate)
Temperature:	18.0-20.5 °C
Photoperiod:	16 h light/ 8 h dark
Light intensity	6541-7097 lux
pH:	Values recorded at test start and end (in brackets) of 14 day exposure period: Controls = 7.99 (8.14-9.06) 5 mg/L = 8.06 (8.77-10.0) 15.8 mg/L: = 7.99 (8.96-9.96)

	50.0 mg/L = 7.36 (7.35-9.13) 158 mg/L = 3.84 (4.88-5.28) 500 mg/L = 2.80 (3.29-3.43)
Oxygen saturation	14 day exposure period: 92 – 94% at the start of the test 114 – 193% at the end of the test
Methods:	The toxicity test on Myriophyllum aquaticum was performed with 6 concentration levels of 5.0, 15.8, 50, 158 and 500 mg glyphosate/L, equivalent to 5.87, 18.5, 58.7, 185.4 and 587 mg Glyphosate acid/L, with 3 replicates per test concentration, including 6 control replicates (Two sets of vessels (exposure and recovery) were prepared at the start of the test. The plants were planted in plastic plant pots into sediment and placed in glass beakers (test vessels), containing 2 L Smart & Bako medium. The test was conducted under static conditions. Five plants were added to each test and control replicate. After 14 days exposure plants in the recovery set of *Myriophyllum aquaticum* replicates, exposed to the same concentration levels, were transferred into freshly prepared test medium without test item to determine the potential recovery after an exposure event. Plant length, fresh weight, dry weight and root length were determined in all vessels. Plant length was recorded at test start and after 3, 7, 10 and 14 days. At test start and test end, fresh weight of each plant was determined. Dry weight was determined at test initiation using 25 additional plants and at test end on the tested plants (dried at 105 °C for 24 h). At the end of the test all plants were harvested and the root length was assessed semi-quantitatively in terms of length of the main root. Temperature in the test chamber was recorded continuously. Oxygen content, pH and light intensity was at test start and after 14 days. Analytical control measurements of the actual concentration of the glyphosate acid were performed by means of LC/MS-MS analysis at test start, after 14 (after exposure phase) and 21 days (after recovery phase). The EC10, EC20 and EC50 and its 95% confidence interval were calculated by Probit analysis modified for continuous data. The NOEC values were determined by calculation of statistical significance using one-way analysis of variance (ANOVA), followed by Williams' t-test, Dunnett's t-test or Welch's t-test (α = 0.05).

Results

The measured concentrations in the test media (aqueous solution) were between 94.3 and 99.6 % of nominal at test start and between 92.0 and 100.6 % at test end (after 14days). Due to deviations from the nominal concentrations < 20 %, the analysis of the results is based on the nominal concentrations.

Specific criteria for macrophyte growth tests have not been set yet. The study fulfils the validity criteria proposed by the AMRAP working group (Maltby, L., *et al.* (2008): Aquatic Macrophyte Risk Assessment for Pesticides, SETAC AMRAP) -with an increase of biomass (length) in controls ≥ 50%, indicating that continuous growth was supported throughout the test duration. Furthermore, constant maintenance of temperature (20 ± 2 °C) was also achieved.

There was a concentration dependent effect on the growth of *Myriophyllum aquaticum* based on increase in shoot length and growth rate of length increase, on weight increase and weight growth rate. Fresh weight was found to be the most sensitive parameter with significant inhibition relative to the control at the lowest test concentration (5.0 mg a.s./L).

Table B.9.2-49: Percentage of inhibition on growth parameters of *Myriophyllum aquaticum* exposed for 14 days to glyphosate acid

Test parameters	Glyphosate acid (mg/L)				
	5.0	15.8	50.0	158	500
Inhibition of shoot length increase (%)	19.2	29.9	55.9	50.3	70.8
Inhibition of shoot length growth rate (%)	11.8	19.5	41.9	36.7	57.9
Inhibition of fresh weight increase (%)	34.2	57.5	69.2	83.7	109
Inhibition of fresh weight growth rate (%)	24.6	46.5	59.0	76.7	115
Inhibition of dry weight increase (%)	-11.8	46.5	26.8	92.7	108
Inhibition of dry weight growth rate (%)	-10.2	40.8	40.4	92.4	114
Inhibition of root length increase (%)	19.4	52.3	76.0	79.7	88.8
Inhibition of root length growth rate (%)	2.0	7.0	13.5	15.1	21.1

At 500 mg glyphosate/L, there was 100 % inhibition in fresh weight increase and growth rate, with 70.8 and 57.9 % inhibition in shoot length increase and growth rate, respectively. It is considered that toxic effects at the two highest concentrations will have been exacerbated by the low starting pH values of the test media (pH 3.84 and 2.80). There was a concentration dependent effect on growth, root length, fresh and dry weight of *Myriophyllum aquaticum*. Growth was significantly reduced at 5.00 mg glyphosate/L.

Table B.9.2-50: EC_{50} and NOEC values for *Myriophyllum aquaticum* after 14 day exposure to glyphosate acid

Endpoint	Glyphosate acid (mg/L)	
	14 Day EC_{50}	14 Day NOEC
Shoot length/relative increase	78.7 (46.1- -146)	5.0
Shoot length/growth rate	276 (159-664)	<5.0
Fresh weight/relative increase	12.3 (9.19-15.8)	<5.0
Fresh weight/ growth rate	23.4 (17.2-30.9)	<5.0
Dry weight/relative increase	25.2 (2.61-151)	50.0
Dry weight/ growth rate	18.0 (5.19-43.0)	50.0
Root length/relative increase	18.0 (5.19-43.0)	>5.0
Root length/growth rate	>500	<5.0

RMS Conclusions

Glyphosate acid significantly inhibited the fresh weight of *Myriophyllum aquaticum* after 14 days at a nominal concentration of <5.0 mg glyphosate acid/L. Therfore the NOEC for both fresh weight parameters was < 5.0 mg a.s./L. The EC_{50} values for fresh weight increase and growth rate for fresh weight were 12.3 mg a.s./L and 23.4 mg a.s./L, respectively.
The extrapolated EC_{20} values were calculated to be 1.72 and 3.60 mg a.s./L, respectively.

The study is considered to be valid and acceptable.

KIIA 8.6/05

Author:	Wenzel, A.
Title:	Effect of AMPA (Aminomethylphosphonic acid) on the Growth of *Myriophyllum aquaticum* in the Presence of Sediment, with a subsequent Recovery Period
Date:	21.02.2012
Doc ID:	2310997 /CHE-022/4-80/A
Guidelines:	Maltby, L., *et al.* (2008): Aquatic Macrophyte Risk Assessment for Pesticides, SETAC AMRAP
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	AMPA (Aminomethylphosphonic acid)
Lot/Batch #:	GLP-0905-19864A (recertified as GLP-110521446-A)
Purity:	98.5 %
Control:	None
Species:	*Myriophyllum aquaticum*
Source:	Institut für Gewässerschutz, MESOCOSM GmbH, Neu-Ulrichstein 5, D-35315 Homberg (Ohm), Germany
Growth medium:	Smart & Bako medium
Artificial sediment:	4-5% peat, 20% kaolin clay, 75-76% quartz sand $CaCO_3$ (if needed to adjust pH to 7.0 ± 0.5) Based on artificial soil used in OECD guideline 219 Moistening of sediment up to 30% with deionised water or nutrient medium (ammonium chloride and sodium phosphate)
Temperature:	20.5 – 21.0 °C
Photoperiod:	16 h light/ 8 h dark
Light intensity	7571 - 7903 lux
pH:	Values recorded at test start and end (in brackets) of 14 day exposure period: Controls = 7.91 (8.54–8.91) 0.88 mg/L = 8.06 (8.04-8.08) 2.23 mg/L: = 7.99 (8.05-8.11) 5.43 mg/L = 7.36 (8.05-8.07) 14.3 mg/L = 3.84 (7.90-7.99) 37.1 mg/L = 2.80 (7.75-7.79) 94.6 mg/l = 6.60 (7.23-7.33) Values at start and end of 7 day recovery period: Recovery period start = 7.97-9.04 Recovery period end = 8.18 – 9.28
Oxygen saturation	14 day exposure period: 95 – 97% at the start of the test 101 – 138% at the end of the test 7 day recovery period: 96 – 138% at the start of the test 90 – 114% at the end of the test
Methods:	The toxicity test on Myriophyllum aquaticum was performed with six concentration levels of 1.0, 2.6, 6.4, 16, 40 and 100 mg AMPA/L with 3 replicates per test concentration. Six control replicates (without test substance) were tested under the same conditions as the test groups. The plants were planted in small plastic plant pots

	into sediment and placed in glass beakers (test vessels), containing 2 L Smart & Bako medium. The test was conducted under static conditions. Five plants were added to each test and control replicate. After 14 days exposure another set of Myriophyllum aquaticum replicates, exposed to the same concentration levels, was transferred into freshly prepared test medium without test item to determine the potential recovery after an exposure event. Plant length, fresh weight, dry weight and root length were determined in all vessels. Plant length was recorded at test start and after 5, 8 and 14 days. At test start and test end, fresh weight of each plant was determined. Dry weight was determined at test initiation using 25 additional plants and at test end on the tested plants (dried at 105 °C for 24 h). At the end of the test all plants were harvested and the root length was assessed semi-quantitatively in terms of length of the main root. Temperature in the test chamber was recorded continuously. Oxygen content, pH and light intensity was at test start and after 14 days. Analytical control measurements of the actual concentration of AMPA were performed by means of LC/MS-MS analysis at test start, after 14 and 21 days (after recovery phase). The EC10, EC20 and EC50 and its 95% confidence interval were calculated by Probit analysis modified for continuous data. The NOEC values were determined by calculation of statistical significance using one-way analysis of variance (ANOVA), followed by Dunnett's t-test or Welch's t-test (p = 0.05).

Results

Analytical control measurements of the actual concentration of AMPA were performed at test start, after 14 and 21 days (after recovery phase). The measured concentrations ranged from 75.5 – 102 % of nominal. Therefore the test was evaluated using the geometric mean measured concentrations.

Table B.9.2-51: Measured concentrations of AMPA in the macrophyte growth inhibition test

Nominal (mg/L)	Test start 14 d growth test		End of test 14 d growth test		Mean measured (mg/L)
	Measured (mg/L)	% of nominal	Measured (mg/L)	% of nominal	
Control	< LOQ -	-	<LOQ	-	< LOQ
1.0	1.02	101.7	0.76	76.4	0.88
2.6	2.49	95.8	1.99	76.6	2.23
6.4	6.09	95.2	4.85	75.7	5.43
16	15.5	96.6	13.2	82.2	14.26
40	40.0	100.0	34.4	86.1	37.13
100	98.3	98.3	91.1	91.1	94.61

The study fulfils the validity criteria as stated in the study plan which follows the criteria established by the AMRAP working group (Maltby, L., *et al.* (2008): Aquatic Macrophyte Risk Assessment for Pesticides, SETAC AMRAP) with an increase of biomass (shoot length) in controls was > 50 %, indicating that continuous growth was supported throughout the test duration. Furthermore, constant maintenance of temperature (20 ± 2 °C) was also achieved.
Increase and growth rate of both shoot length and fresh weight were significantly affected at concentrations above 14.3 mg AMPA/L.

Table B.9.2-52: Percentage of inhibition of shoot length of *Myriophyllum aquaticum* exposed for 14 days to AMPA

Test parameters	AMPA (mg/L)					
	0.88	2.23	5.43	14.26	37.13	94.61
Inhibition of shoot length increase (%)	20.8	16.8	12.5	16.7	40.8	54.3
Inhibition of shoot length growth rate (%)	11.7	9.2	6.4	9.0	26.4	38.0
Inhibition of fresh weight increase (%)	-14.1	-15.2	-7.0	-10.9	29.0	60.2
Inhibition of fresh weight growth rate (%)	-9.0	-9.4	-3.9	-6.9	20.8	48.3
Inhibition of dry weight increase (%)	-47.5	-45.6	-7.1	1.1	-4.6	79.9
Inhibition of dry weight growth rate (%)	-28.9	-26.5	-4.9	1.6	-2.1	71.2
Inhibition of root length increase (%)	-13.1	-8.8	15.7	26.4	55.0	79.3
Inhibition of root length growth rate (%)	-3.5	-2.5	4.2	7.7	20.4	39.5

Fresh weight was found to be the most sensitive parameter, with EC_{50} values for fresh weight increase and growth rate of 70.8 and 97.3 mg/L, respectively. The EC_{50} values for increase and growth rate related to shoot length were 103.3 and > 94.6 mg/L, respectively. The EC_{50} values for growth rate of fresh weight and increase of shoot length were extrapolated since they were only slightly higher than the highest treatment.

Table B.9.2-53: EC_{50} and NOEC values for *Myriophyllum aquaticum* exposed to AMPA for 14 days (based on geometric mean measured concentrations)

Endpoint	AMPA (mg/L)[#]	
	14 Day EC_{50}	14 Day NOEC
Shoot length/relative increase	103.3 (54.8-337)	14.3
Shoot length/growth rate	> 94.6	14.3
Fresh weight/relative increase	70.8 (59.4-87.7)	14.3
Fresh weight/ growth rate	97.3 (81.8-126)	14.3
Dry weight/relative increase	63.2 (49.0-79.2)	37.1
Dry weight/ growth rate	72.0 (59.4-83.6)	37.1
Root length/relative increase	31.1 (28.1-34.6)	5.4
Root length/growth rate	150.1*(136.1-168.1)	5.4

* extrapolated, highest test concentration was 94.6 mg AMPA/L

[#] 95% confidence intervals presented in brackets.

RMS Conclusions

AMPA significantly inhibited the fresh weight and shoot length of *Myriophyllum aquaticum* after 14 days at a nominal concentration of >14.3 mg AMPA/L. The 14-d EC_{50} value for fresh weight inhibition was 70.8 mg AMPA/L and for shoot length > 94.6 mg AMPA/L. *Myriophyllum aquaticum* pre-exposed for 14 day to up to 5.4 mg AMPA/L were able to recover in untreated culture medium after a 7 day recovery period.

The study is considered to be valid and acceptable.

KIIA 8.6/06

Author:	Porch, J.R., Kendall, T.Z., Krueger, H.O.
Title:	HMPA (hydroxymethylphosphonic acid): A 7-day static-renewal toxicity test with Duckweed (*Lemna gibba* G3)
Date:	11.10.2011
Doc ID:	2310999 /139A-397
Guidelines:	OECD221
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	HMPA (hydroxymethylphosphonic acid)
Lot/Batch #:	GLP-1003-20448-A
Purity:	97.0 %.
Control:	20X AAP medium
Species:	*Lemna gibba* G3, up to 7 days old
Source:	In-house culture
Temperature:	23.7 – 25.4 °C
Light intensity:	Continuous illumination, 4410 - 5250 lux
pH:	7.1 – 8.0 at test start; 8.8 – 9.0 at test termination
Hardness:	20.88 mg (K_2HPO_4/L)
Methods:	On the basis of of a range finding test, the definitive test was performed at five concentration levels, 7.5, 15, 30, 60, and 120 mg HMPA/L with 3 replicates per test concentration, including three control replicates. Four plants totalling 12 fronds were added to each replicate test chamber. The plants were placed in 250 mL test vessels containing 100 mL 20X-AAP test media. The pH of the test medium was adjusted with 0.1N NaOH prior to the test. The test was conducted under a 7-day static-renewal test conditions. The renewal of the test media was performed on day 3 after test initiation. The toxicity of HMPA to duckweed was determined by direct counts of frond numbers and observations for chlorosis, necrosis, dead fronds and frond appearance were made on Days 3, 5 and 7. Dry weight was measured at the beginning of the test on a representative sample from the culture used to initiate the test. At the end of the test, dry weight was determined from each test vessel. The pH values were measured on day 0, 3, and 7. Temperature was measured continuously and recorded twice daily. Samples of the test solutions were collected from new solution of each experimental group at the beginning of the test, from new solutions and pooled old solutions at the end of the renewal period on Day 3, and from pooled test solutions at test termination to determine test substance concentrations. Samples were processed immediately for analysis. All test concentrations and control replicates were analysed using HPLC with mass selective detection. The 7-day EC_{50} value for frond counts; biomass and growth rates based on frond counts and biomass are based on descriptive analysis of the data. The NOEC values were determined by calculation of statistical significance using one-way analysis of variance (ANOVA) and Dunnett's test for inhibition of frond number and biomass dry weight, respectively, at $\alpha = 0.05$.

Results

In freshly prepared test media, the recovery of the active substance ranged between 92.5 % and 103 %. In the aged test media (7 days old), 104 % to 110 % of the active substance was recovered. Samples from new and old test solution at Day 3 renewal ranged from 90.1 to

101 % and 96.9 to 107 %, respectively. The doubling time of frond numbers in the control was less than 2.5 days (1.96 days), corresponding to approximately a twelve-fold increase after seven days. The validity criteria according to the current guideline OECD 221 are therefore fulfilled.
After 7 days of exposure, there were no apparent treatment related effects upon growth at any of the concentrations tested and there were nostatistically significant differences (Dunnett´s test, p>0.05) between negative control and any treatment.

Table B.9.2-54: Frond numbers and growth inhibition of *Lemna gibba* G3 after 7 days of exposure to HMPA

Test item	Control	HMPA (mg/L)				
Nominal concentrations (mg HMPA/L)	-	7.5	15	30	60	120
Mean measured concentrations (mg HMPA/L)	-	7.4	15	30	60	123
Mean frond number	145	158	166	147	156	174
Mean inhibition (%)	-	-9	-15	-1	-7	-20
Mean biomass (mg)	16.73	18.90	20.93	19.17	20.10	22.20
Mean inhibition (%)		-13	-25	-15	-20	-33
Mean growth rate based on frond number	0.3531	0.3681	0.3751	0.3564	0.3656	0.3818
Mean inhibition (%)	-	-4	-6	-1	-4	-8
Mean growth rate based on biomass	0.3494	0.3679	0.3821	0.3699	0.3763	0.3909
Mean inhibition (%)	-	-5	-9	-6	-8	-12

RMS Conclusions
Since no inhibition effects of HMPA was observed on the frond number, frond number growth rate, biomass and biomass growth rate of *Lemna gibba* G3 after 7 days at all concentrations tested, the EC_{50} values for frond number, frond number growth rate, biomass and biomass growth rate were all >123 mg HMPA/L, the highest concentration tested. The NOEC was determined to be 123 mg HMPA/L.

The study is considered to be valid and acceptable.

KIIIA 10.8.2.1/01

Author:	Dengler, D.
Title:	Assessment of toxic effects of MON 52276 on aquatic plants using the duckweed *Lemna gibba*
Date:	18.11.2002
Doc ID:	2316015 /GA-2002-051
Guidelines:	OECD 221 (draft of October 2000)
GLP:	YES
Validity:	YES, please refer to comment by RMS

Materials and Methods

Test item::	MON 52276
Lot/Batch #:	A1C1204104
Purity:	30.9 % glyphosate acid equivalent, as 41.5% isopropylamine salt of glyphosate
Control:	none
Species:	Young *Lemna gibba* G3, 2 – 5 fronds
Source:	Institut für Pflanzenökologie und Ökotoxikologie, University of Hohenheim, Stuttgart, Germany
Temperature:	22 - 25 °C
Light intensity:	Continuous illumination, 7000 lux
pH:	7.49 – 9.42 (adjusted to 7.5)
Conductivity/Hardness:	not stated
Methods:	On the basis of the results of a range finding test, the definitive test was performed with six concentration levels, 0.9, 2.4, 6.8, 19.1, 53.6 and 150 mg MON 52276/L, with 3 replicates per test concentration. Three control replicates (without test substance) were tested under the same conditions. Colonies consisting of 2-5 fronds totalling 12 fronds per replicate were added to each replicate test chamber. The plants were placed in 100 mL test vessels containing 50 mL 20X-AAP test media. The pH of the test medium was adjusted at each test media renewal to 7.5, to avoid extreme pH values. The test was conducted under a 7-day semistatic test conditions. The renewal of the test media was performed on day 3 and 5 after test initiation. Observations were made on the number and the condition of the fronds on Days 3, 5 and 7. The growth rate inhibition was determined by counting the number of fronds produced for each test concentration and control group. The effect on biomass production was evaluated by determining the final dry weights of the plants. pH and temperature of the test vessels were measured on days 0, 3, 5 and 7. Samples from all the test concentrations were collected for analysis of glyphosate by HPLC on Days 0, 3, 5 and 7. The 7-day EC_{50} value for frond counts and growth rates based on frond counts and biomass were determined by calculation of statistical significance using one-way analysis of variance (ANOVA) and Dunnett's test ($\alpha = 0.05$).

Results

The mean measured glyphosate concentrations were between 83 and 104 % of nominal concentrations. Results were based on nominal MON 52276 concentrations.

The doubling time of frond numbers in the control was less than 2.5 days (37.4 hours). The validity criteria according to the current guideline OECD 221 are therefore fulfilled.

Although significant differences were calculated at 2.4 and 6.8 mg/L for growth rate and biomass, as well as at 6.8 mg/l for frond numbers, these effects were not considered by the authors of the study, because root decay was observed in these concentrations. It was assumed by the authors that in the concentration at 2.4 and 6.8 mg/L, bacterial contamination occurred, which may distort the resuslts of the study resulting in the inclusion of only 3 test concentration with monotonic repsonse, The E_bC_{50} calculation by the authores is based on the distribution of the data at the concentration levels of only 19.1, 53.6 and 150 mg/L.

Table B.9.2-55: Percentage of inhibition of *Lemna gibba* exposed for 7 days to MON 52276

MON 52276 concentration (mg/L) (nom.)	Inhibtion of frond numbers (%)	Inhibtion of growth rate (%)	Inhibtion of biomass (%)
0 (control)	0.0	0	0
0.9	14.0	4.7	12.4
2.4	25.6	9.8 *	14.7 *
6.8	29.8 *	10.9 *	19.4 *
19.1	10.9 *	3.6	13.0
53.6	43.8	17.5 *	24.9 *
150.0	77.1 *	43.0 *	58.7 *

*significant (multiple test procedure according to Dunnett, t=7d)

There were inhibitory effects at the highest test concentration of 150 mg/L of 43 % for growth rate, 58.7 % for biomass and 77.1 % for frond numbers. Since the percentage inhibition compared to control was only 43 % at the highest MON 52276 concentrations tested, the E_rC_{50} was estimated to be > 150 mg MON 52276/L, equivalent to 46.35 mg a.e./L. Based on nominal concentrations, the E_bC_{50} was 118.16 mg MON 52276/L (95% confidence limits of 91.37 and 171.37mg MON 52276/L), equivalent to 36.51 mg a.e./L. Based on nominal concentrations, the EC_{50} for frond count of *Lemna gibba* exposed to MON 52276 under semi-static test conditions for 7 days were calculated to be 66.58 mg/L (95 % confidence limits of 56.30 and 79.66 mg MON 52276/L), equivalent to 20.57 mg a.s./L.
The no-observed-effect-concentration (NOEC) was set to 0.9 mg MON 52276/L, equivalent to 0.3 mg a.e./L.

RMS Conclusions
The study is considered to be valid and will be used as additional information, as the study shows deficiencies: It was assumed by the authors that in the concentration at 2.4 and 6.8 mg/L, bacterial contamination occurred, which may distort the results of the study resulting in the inclusion of only 3 test concentration with monotonic response. Therefore results might understimate EC_{50} values. Based on nominal concentrations, the lowest EC_{50} for frond count of *Lemna gibba* exposed to MON 52276 under semi-static test conditions for 7 days was calculated to be 66.58 mg/L (95 % confidence limits of 56.30 and 79.66 mg MON 52276/L), equivalent to 20.57 mg a.s./L.

KIIIA 10.8.2.1/01

Author:	Wenzel, A.
Title:	Effect of MON 52276 (Glyphosate formulation) on the growth of *Myriophyllum aquaticum* in the presence of sediment, with a subsequent recovery period
Date:	24.02.2012
Doc ID:	2316017 /CHE-016/4-80/A
Guidelines:	OECD
GLP:	Maltby, L., et al. (2008): Aquatic Macrophyte Risk Assessment for Pesticides, SETAC AMRAP
Validity:	Specific criteria for macrophyte growth tests have not been set yet.

Materials and Methods

Test item:	Glyphosate SL formulation (MON 52276)
Lot/Batch #:	A9K0106104
Purity:	358.8 ± 4.0 g glyphosate acid equivalent/L (30.68% w/w)
Species:	*Myriophyllum aquaticum*
Source:	Institut für Gewässerschutz, MESOCOSM GmbH, Neu-Ulrichstein 5, D-35315 Homberg (Ohm), Germany
Growth medium:	Smart & Bako medium
Artificial sediment:	4-5% peat 20% kaolin clay 75-76% quartz sand $CaCO_3$ (if needed to adjust pH to 7.0 ± 0.5) Based on artificial soil used in OECD guideline 219 Moistening of sediment up to 30% with deionised water or nutrient medium (ammonium chloride and sodium phosphate)
Temperature:	20.0 °C
Photoperiod:	16 h light
Light intensity	7295-7518 lux
pH:	Values recorded at test start and end (in brackets) of 14 day exposure period: Controls = 7.97 (8.78-8.82) 0.3 mg/= 8.25 (8.82) 1.1 mg/L = 8.01 (8.82) 5.16 mg/L = 8.15 (8.82) 26.8 mg/L = 7.79 (8.81-8.82) 145 mg/L = 7.26 (6.11-8.82) 723 mg/L = 5.86 (6.09-6.82) Values at start and end of 7 day recovery period: Recovery period start = 6.0 – 9.2 Recovery period end = 8.3 – 9.8
Oxygen saturation	Values recorded at test start and end (in brackets) of 14 day exposure period: Controls = 96% (102-108%) 0.3 mg/L = 90% (107-108%) 1.1 mg/L = 96% (107-111%) 5.16 mg/L = 91% (114-132%) 26.8 mg/L = 95% (100-104%) 145 mg/L = 90% (116-122%) 723 mg/L = 96% (4-9%) Values at start and end of 7 day recovery period: Controls = 103-110% (99-109%) 0.3 mg/L not included in the recovery period 1.1 mg/L = 108-114% (103-110%) 5.16 mg/L = 111-113% (115-121%) 26.8 mg/L = 123-130% (123-126%) 145 mg/L = 127-137% (104-143%) 723 mg/L = 6-33% (107-111%)
Methods:	The toxicity test on *Myriophyllum aquaticum* was performed with six concentration levels of 0.24, 1.2, 6.0, 30, 150 and 750 mg glyphosate/L, equivalent to 0.78, 3.91, 19.6, 97.8, 489 and 2445 mg MON 52276/L, with 3 replicates per test concentration and Six control replicates. Two sets of vessels (exposure and recovery) were prepared at the start of the test. The plants were planted in small plastic plant pots into sediment and placed in glass beakers (test vessels), containing 2 L Smart & Bako medium. The test was conducted under static conditions. Five plants were added to each test and control replicate. Plant length, fresh weight, dry weight and root length were determined in all vessels. Plant length was recorded at test start and after 5, 8, 11 and 14 days. At test start and test end, fresh weight of each plant was determined. Dry weight was determined at test

	initiation using 25 additional plants and at test end on the tested plants (dried at 105 °C for 24 h). At the end of the test all plants were harvested and the root length was assessed semi-quantitatively in terms of length of the main root. Temperature in the test chamber was recorded continuously. Oxygen content, pH and light intensity was recorded at test start and after 14 days. Analytical control measurements of the actual concentration of the glyphosate acid were performed by means of LC/MS-MS analysis at test start, after 14 (after exposure phase) and 21 days (after recovery phase). The EC_{10}, EC_{20} and EC_{50} and its 95% confidence interval were calculated by probit analysis modified for continuous data. The NOEC values were determined by calculation of statistical significance using one-way analysis of variance (ANOVA), followed by Williams' t-test, Dunnett's t-test or Welch's t-test (p = 0.05).

Results

Analytical control measurements of the actual glyphosate concentration were performed at test start, after 14 and 21 days (after recovery phase). The measured concentrations ranged from 83.9 - 145 % of nominal. Therefore, the test was evaluated using the geometric mean measured concentrations.

The study fulfils the validity criteria as stated in the study plan which follows the criteria established by the AMRAP working group (Maltby, L., *et al.* (2008): Aquatic Macrophyte Risk Assessment for Pesticides, SETAC AMRAP); with an increase of biomass (shoot length) in controls > 50 %, indicating that continuous growth was supported throughout the test duration. Furthermore, constant maintenance of temperature (20 ± 2 °C) was also achieved.

Table B.9.2-56: Percentage of inhibition of *Myriophyllum aquaticum* exposed for 14 days to MON 52276

Test parameters	Glyphosate Acid (mg/L) (mean measured)					
	0.3	1.1	5.12	26.8	145	723
Inhibition of shoot length increase (%)	-3.5	5.1	30.5	74.1	70.3	94.1
Inhibition of shoot length growth rate (%)	-2.6	2.0	17.5	58.1	53.6	88.3
Inhibition of fresh weight increase (%)	20.7	19.2	61.2	80.1	77.6	93.8
Inhibition of fresh weight growth rate (%)	14.6	13.3	49.4	70.9	67.8	90.2
Inhibition of dry weight increase (%)	14.7	18.2	34.3	15.8	-6.9	106.6
Inhibition of dry weight growth rate (%)	11.1	14.4	29.6	19.6	-4.7	112.3
Inhibition of root length increase (%)	-6.8	-3.9	52.0	82.9	94.5	98.3
Inhibition of root length growth rate (%)	-1.7	-0.9	18.3	43.9	66.7	86.8

There was a concentration dependent effect on shoot length, and growth of length increase as well as on fresh and dry weight and root length of *Myriophyllum aquaticum*. Over 14 days fresh weight was found to be the most sensitive parameter of the relevant valid measured parameters shoot length and fresh weight. Dry weight and main root were documented as additional information.

Table B.9.2-57: EC_{50} and NOEC for *Myriophyllum aquaticum* after 14 day exposure to MON 52276 based on geometric mean measured concentrations.

Endpoint	Glyphosate Acid (mg/L)	
	14 Day EC_{50} (CI*)	14 Day NOEC
Shoot length/relative increase	13.44 (7.72 – 23.74)	5.16
Shoot length/growth rate	42.79 (24.74 – 76.48)	5.16
Fresh weight/relative increase	4.44 (2.28 – 8.51)	< 0.30
Fresh weight/ growth rate	10.33 (5.59 – 19.21)	< 0.30
Dry weight/relative increase	n.d.	145
Dry weight/ growth rate	143.34 (10.06 – n.d.)	145
Root length/relative increase	5.84 (4.65 – 7.37)	1.10
Root length/growth rate	46.50 (34.75 – 62.52)	1.10

***** 95% confidence intervals presented in brackets

RMS Conclusions

MON 52276 significantly inhibited the fresh weight of *Myriophyllum aquaticum* after 14 days at a mean measured concentration of <0.3 mg glyphosate acid equivalents/L. The NOEC for both fresh weight parameters is <0.3 mg glyphosate acid equivalents/L.

Shoot length was inhibited at or above mean measured concentrations of 5.16 mg glyphosate acid equivalents/L. The 14-d EC_{50} value for fresh weight inhibition was 4.4 mg glyphosate acid equivalents/L and for shoot length it was 13.44 mg glyphosate acid equivalents/L.

The study is considered to be valid and acceptable.

B.9.2.1.8 Sediment-dwelling organisms

As described in Section B.8 (Fate and Behaviour), the principal metabolite of glyphosate in water/sediment system is AMPA. The maximum amounts of AMPA detected were 16 % (water phase), 19 % (sediment) and up to 27 % (total system) of the total glyphosate applied. This indicates that there is a potential for exposure to glyphosate for sediment-dwelling organisms. However, the NOEC values from the long-term *Daphnia* test with glyphosate acid are well above 0.1 mg/L, indicating low toxicity to aquatic invertebrates. According to the Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001 rev.4), specific toxicity studies on sediment-dwellers should therefore not be necessary.

B.9.2.1.9 Microcosm or mesocosm study

No microcosm or mesocosm study was performed with MON 52276, glyphosate acid, glyphosate-IPA salt and its major aquatic metabolites AMPA and HMPA.

B.9.2.2 Summary of aquatic toxicity data

A summary of the relevant acute and long-term endpoints, representing the worst case for key species are presented in the tables below: Full details of the tests on the active substance and the metabolites AMPA and HMPA are provided in chapters B 9.2.1.1 to B 9.2.1.8 above.

Table B.9.2-58: Summary of the relevant acute and long-term endpoints for fish exposed to glyphosate acid, glyphosate salts, glyphosate metabolites and MON 52276

Species	Substance	Test design	EC_{50} (mg a.s./L)	NOEC (mg a.s./L)	Reference
Fish, acute toxicity					
Oncorhynchus mykiss	Glyphosate acid	96 h static	**38**	10	Review Report for the active substance Glyphosate (SANCO/6511/VI/99-final), [redacted]., 1972; 95-00016
Lepomis macrochirus	Glyphosate acid	96 h static	47	32	[redacted]., 1995; BL5553/B BVL no 2310926
Danio rerio	Glyphosate acid	96 h semi-static	123	-	[redacted], 2000, RF-D61.47/99 BVL no 2310928
Cyprinus carpio	Glyphosate acid	96 h semi-static	>100	100	[redacted], 2006; 2060/015 BVL no 2310930
Lepomis macrochirus	Glyphosate IPA-salt	96 h static	**> 1000 741 a.e.**	560 415 a.s.	[redacted], 1981; 95-00712
Oncorhynchus mykiss	Glyphosate IPA-salt	96 h static	> 1000 741 **a.e.**	1000 741 a.s.	[redacted], 1981; 94-01161
Leuciscus idus	Glyphosate IPA-salt	96 h static	> 5000 3704 **a.e.**	5000 3704 a.s.	[redacted] 1993; 94-00156 80-91-2328-02-93
Oncorhynchus mykiss	Glyphosate K-salt	96 h static	**> 2573 1227 a.e.**	157	[redacted]., 2003; WL-2002-149 BVL no 2310937
Oncorhynchus mykiss	AMPA	96 h static	**520**	32	[redacted] 1991; 94-01162; AB-90-402
Oncorhynchus mykiss	MON 52276	96 h static	**> 989 > 306 a.e.**	989 306 a.s.	[redacted] 1992; TO-91-296 BVL no 2317591
Cyprinus carpio	MON 52276	96 h static	> 895 >277 a.e.	895 277 a.s.	[redacted] 1992; TO-91-298 BVL no 2317595

Species	**Substance**	**Test design**	**EC_{50} (mg a.s./L)**	**NOEC (mg a.s./L)**	**Reference**
Fish, long-term toxicity					
Pimephales promelas	Glyphosate acid	255d	.	25.7	Review Report for the active substance Glyphosate (SANCO/6511/VI/99-final), Anonym, 95-00020
Brachydanio rerio	Glyphosate acid	168 h	25	**1**	[redacted], C.M.,2000; RF-D62.16/99 BVL no 2310938
Oncorhynchus mykiss	Glyphosate acid	85-day (60 days post-hatch)	-	**9.63**	[redacted] 2010; 1005.029.321 BVL no 2310941
Pimephales promelas	AMPA	33-day (7days post-hatch)	-	**12**	[redacted] 2011; WL-2010-328 BVL no 2310943

a.e.: acid equivalents

Values in grey: confirmed EU endpoints (SANCO/6511/VI/99-final, or EU Review Monograph)

Values in bold : Endpoints which are used in the risk assessment.

Table B.9.2-59: Summary of the relevant acute and long-term endpoints for aquatic invertebrates exposed to glyphosate acid, glyphosate salts, glyphosate metabolites and MON 52276

Species	Substance	Test design	EC_{50} (mg a.s./L)	NOEC (mg a.s./L)	Reference
Aquatic invertebrates, acute toxicity					
Daphnia magna	Glyphosate acid	48 h	**40**	18	95-00537 Handley *et al.*, 1995
Daphnia magna	Glyphosate IPA salt	48 h	**930**	320	94-01160 Forbis, Boudreau, 1981
Daphnia magna	Glyphosate K-salt	48 h static	**592 (278 a.s.) (recalculated by RMS)**	312 (149 a.s.)	Palmer et al., 2003; WL-2002-150 BVL no 2310957
Daphnia magna	AMPA	48 h static	**690**	320	Burgess, Hicks, 1991; 94-01163; AB-90-401
Daphnia magna	HMPA	48 h static	**>100**	100	Palmer et al., 2011; WL-2010-329 BVL no 2310961
Daphnia magna	MON 52276	48 h flow-through	**676 (209 a.s.)**	356 (110 a.s.)	Lintott, D.R.1992; TO-91-295 BVL no 2317597
Aquatic invertebrates, long-term toxicity					
Daphnia magna	Glyphosate acid	21 d semi-static	-	30 mg/L	Wüthrich, 1990; 250795
Daphnia magna	Glyphosate-IPA salt	21 d semi-static	-	**58** [1)] (43 a.s.)	Thun, 1993; 95-00549; 89-91-2328-05-93
Daphnia magna	Glyphosate acid	21 d semi-static	-	**12.5 (recalculated by RMS)**	Magor, Shillabeer, 1999; BL6535/B BVL no 2310962
Daphnia magna	AMPA	21 d semi-static	90	**15**	Minderhout et al., 2011 WL-2010-327 BVL no 2310965

Values in grey: confirmed EU endpoints (SANCO/6511/VI/99-final, or EU Review Monograph)

Value in bold : Endpoints which are used in the risk assessment.

1) in SANCO/6511/VI/99-final, a NOEC of 455 mg/L was proposed. The NOEC value for reproduction was re-determined to be 94 mg test item/L, equivalent to 57.90 mg glyphosate isopropylamine salt/L and 42.90 mg glyphosate acid/L (nominal)

Table B.9.2-60: Summary of the relevant endpoints for algae exposed to glyphosate acid, glyphosate salts, glyphosate metabolites and MON 52276

Species	**Substance**	**Test design**	**EC_{50} (mg a.s./L)**	**Reference**
Skeletonema costatum	Glyphosate acid	168 h static	E_bC_{50} (72 h) = 1.25 E_bC_{50} (168 h) = 0.64	1092-02-1100-3 Hughes, 1987 96-00455, **not considered to be valid according to current OECD 201**
Nitzschia palea	Glyphosate acid	96 h static	E_rC_{50} = 11.90 **E_bC_{50} = 4.47**	960606FH Scheerbaum, 1996 97-00013; , **not considered to be valid according to current OECD 201**
Skeletonema costatum	Glyphosate acid	72 h /96 h static	E_rC_{50} (72 h) = 18 **E_bC_{50} (72 h) =11** E_rC_{50} (96 h) = 29 E_bC_{50} (96 h) =11	Smyth et al., 1996; BL5684/B BVL no 2310972
Pseudokirchneriella subcapitata	Glyphosate acid	72 h /120 h static	E_rC_{50} (72 h) =19 E_bC_{50} (72 h) = 18 E_rC_{50} (120 h) =21 E_bC_{50} (120 h) = 17 NOErC = 10	Smyth *et al.*, 1995; BL5550/B BVL no 2310968
Anabaena flos-aquae	Glyphosate acid	72 h /96 h static	E_rC_{50} (72 h) = 22 **E_bC_{50} (72 h) = 8.5** E_rC_{50} (96h) = 38 E_bC_{50} (96 h) = 15 NOErC = 12	Smyth et al., 1996; BL5698/B BVL no 2310970
Pseudokirchneriella subcapitata	Glyphosate isopropyl-amine salt 62.5%	72 h /96 h static	E_rC_{50} (72 h) =31 (23 a.s.) **E_bC_{50} (72 h) =9.25 (6.9 a.s.)** E_rC_{50} (96 h) = 32 (23 a.s.) E_bC_{50} (96 h) =10.2 (7.6) NOErC = 4.27	Egeler, Baumann, 2002; A-99-02-04 BVL no 2310979
Desmodesmus subspicatus	AMPA	72 h static	ErC_{50} = 452 **EbC_{50} = 89.8**	Dengler, 1994 94-00501, IFU93006/01-Ss
Pseudokirchneriella subcapitata	AMPA	72 h static	E_rC_{50} = 200 E_bC_{50} = 110 NOErC = 46	Bogers, 1998 232458 BVL no 2310985
Pseudokirchneriella subcapitata	HMPA	72 h static	**E_rC_{50} = >115** E_yC_{50} = >115 NOAEC = 60	Porch et al. 2011; 139A-396A BVL no 2310987
Pseudokirchneriella subcapitata	MON 52276	72 h static	Cell Counts: E_rC_{50} = 284 (88 a.s.) **E_bC_{50} = 178 (55 a.s.)** Absorbance: E_rC_{50} = 393 (122 a.s.) E_bC_{50} = 150 (47 a.s.) NOEC = 90 (28 a.e.)	Neven, B.,1992; LI-91-389 BVL no 2317597

Table B.9.2-61: Summary of the relevant endpoints for aquatic macrophytes exposed to glyphosate acid, glyphosate salts, glyphosate metabolites and MON 52276

Species	Substance	Test design	EC_{50} (mg a.s./L)	Reference
Lemna gibba	Glyphosate acid	14 d semi-static	EC_{50}, frond count = 12 EC_{50}, dry weight = 20	Smyth et al., 1996; BL5662/B (SANCO/6511 /VI/99-final, or EU Review Monograph)
Lemna minor	Glyphosate-IPA salt	7 d semi-static	EC_{50}, frond count = 25.5 EC_{50}, dry weight = 46.2 EC_{50}, growth rate = 42.6	Mallett, 2002; CEMR-1873 BVL no 2310992
Lemna gibba	HMPA	7 d semi-static	EC_{50}, frond count >123 EC_{50}, dry weight >123	Porch et al. 2011; 139A-397 BVL no 2310999
Lemna gibba	MON 52276	7d semi-static	EC_{50}, frond count = 20.57 mg a.s./L	Dengler, 2000; GA-2002-051 BVL no 2316015
Myriophyllum aquaticum	MON 77973 Glyphosate acid	14 d static	EC_{50}, shoot length, relative increase = 278.7 EC_{50}, shoot length, growth rate = 276 EC_{50}, fresh weight, relative increase = 12.3 EC_{50}, fresh weight, growth rate = 23.4 EC_{50}, dry weight, relative increase = 25.2 EC_{50}, dry weight, growth rate = 18.0 EC_{50}, root length, relative increase = 18.0 EC_{50}, root length, growth rate > 500	Wenzel, 2012; CHE-015/4-80/A BVL no 2310995
Myriophyllum aquaticum	MON 52276	14 d static	EC_{50}, shoot length, relative increase = 13.44 EC_{50}, shoot length, growth rate = 42.79 **EC_{50}, fresh weight, relative increase = 4.44** EC_{50}, fresh weight, growth rate = 10.33 EC_{50}, dry weight, relative increase = nd EC_{50}, dry weight, growth rate = 143.34 EC_{50}, root length, relative increase = 5.84 NOEC Fresh weight/relative increas <0.30	Wenzel, 2012; CHE-016/4-80/A BVL no 2316017
Myriophyllum aquaticum	AMPA	14 d static	EC_{50}, shoot length, relative increase = 103.3 EC_{50}, shoot length, growth rate > 94.6 EC_{50}, fresh weight, relative increase = 70.8 EC_{50}, fresh weight, growth rate = 97.3 EC_{50}, dry weight, relative increase = 63.2 EC_{50}, dry weight, growth rate = 72.0 EC_{50}, root length, relative increase = 31.1 EC_{50}, root length, growth rate = 150.1	CHE-022/4-80/A Wenzel, 2012 BVL no 2310997

B.9.2.3 Literature Review

Aquatic organisms are considered to be exposed to glyphosate containing plant protection products via spray drift, runoff and drainage as a consequence of use near aquatic environments. Aquatic algae and macrophytes are especially vulnerable to the impact of glyphosate due to their physiological similarity to terrestrial plants.
For the group of algae, a comprehensive database of nearly 30 peer-reviewed papers was submitted by the notifier. The notifier considered five publications (Sobrero et al. 2007; Sanchez et al. 2007; Turgut & Fomin, 2002 and Vera et al. 2010) and considered to be rated in category "Klimisch 2" (Klimisch 1997) and annotated with minimal remarks, whereas the remaining were considered as not acceptable for risk assessment. The submitted publications were also evaluated by RMS and have been assigned according to an UBA screening. Out of the submitted publications, 15 studies were recognised as supporting information (category UBA2) and are reviewed here. Endpoints derived out of these publications are listed in the table below.

The peer reviewed open literature about toxicity on algae provides a wide range of EC_{50} and IC_{50} values for algae treated with glyphosate (technical grade). The EC_{50} values range from 2.3 mg/l for *Skeletonema costatum* (Tsui, 2003) to 70 mg/L for *Scenedesmus quadricauda* (Ma, 2006) and the marine diatom *Skeletonema costatum* seems to be the most sensitive species towards glyphosate. Regarding macrophytes, similar EC_{50} values compared to algae are reported in the peer reviewed open literature. IC_{50} and EC_{50} values ranged from 0.22 mg a.s./L for *Myriophyllum aquaticum* (Turgut & Fomin, 2002) to 46.9 mg/L for *Lemna minor* (Cedergreen & Streibig, 2005).

Beside single species tests, a few studies were performed focusing on the natural aquatic community in order to assess indirect effects towards algae. Mesocosm studies showed differences at 6 mg glyphosate containing product/L in the structure of phytoplankton and periphyton assemblages in treated mesocosms compared to controls. Total micro- and nanophytoplankton decreased in abundance, whereas the abundance of picocyanobacteria increased (Perez, 2007). Similar effects were observed by Vera et al. (2010), who could also show that despite the mortality of algae, mainly diatoms, cyanobacteria were favored in treated mesocosms. However, it must be considered that in both studies commercial products containing surfactants were used, and therefore the toxicity is determined by the joint effect of both glyphosate and the surfactants of the commercial formulations. Commercial products containing specific formulation ingredients additionally to the active ingredient were shown to be more toxic towards algae than glyphosate acid (Cedergreen & Streibig, 2005; Tsui, 2003).
There was no critical data in the open literature that could be directly included in an environmental risk assessment for the active substance glyphosate. Endpoints reported have been detected in the same magnitude or it was not possible to distinguish between the effects of the technical glyphosate and the surface-active substances added to the commercial formulations in the experimental designs used.

Table B.9.2-62: Effects values of algae and aquatic plants in per-reviewed literature

Species	Substance	Study type	EC50 (mg/L)	Reference (internal tag)
Algae				
Chlorella vulgaris	Glyphosate 95 %, technical product	96h	5	Ma, J.,2002; glyphecotox_476
Raphidocelis subcapitata	Glyphosate 95 %, technical product	96h	5.5	Ma, J.,2006; glyphecotox_474
Scenedesmus obliquus	Glyphosate 95 %, technical product	96h	56	Ma, J., 2002; glyphecotox_471
Scenedesmus quadricauda	Glyphosate 95 %, technical product	96h	70	Ma, J.,2006; glyphecotox_473
Chlorella pyrenoidosa	Glyphosate 95 %, technical product.	96h	3.5	Ma. J.,2001; glyphecotox_477
Chlorella kessleri	ATANOR	96h	55.62	Romero, et al., 2011 glyphecotox_578
Pseudokirchneriella subcapitata	Glyphosate 95 %, technical product.	48h	270	Cedergreen, N., Streibig, J.C., 2005; glyphecotox_319
	Roundup 360 g/L		64.7	
Periphyton	Commercial product with surfactant	Mesocsm, 42days	8mg/l Changes in community structure	Vera, M.S.,, 2010; glyphecotox_129
Periphyton, Phytoplancton	Roundup®	Mesocsm, 11days	6 mg/L Changes in community structure	Perez, G.L.,2007; glyphecotox_539
Selenastrum capricornutum	Glyphosate acid	96h	24.7 mg a.e./L	Tsui, M.T.K2003 glyphecotox_195
	IPA salt of glyphosate		41.0 mg a.e./L	
	POEA		3.92 mg a.e./L	
	Roundup®		1.85 mg a.e./L	
Skeletonema costatum	Glyphosate acid	96h	**2.27 mg a.e./L**	**Tsui, M.T.K., 2003 glyphecotox_195**
	IPA salt of glyphosate		5.89 mg a.e./L	
	POEA		3.35 mg a.e./L	
	Roundup®		1.85 mg a.e./L	
Macrophytes				
Myriophyllum aquaticum	Commercial product, 36 % a.s.	14 days	**2.0 (fresh weight) 0.22 (chl a)**	**Turgut & Fomin, 2002, glyphecotox_128**
Lemna minor L.	Glyphosate 95 %, technical product	7 days	46.9	Cedergreen, N., Streibig, J.C., 2005; glyphecotox_319
	Roundup 360 g/L		11.2	
Lemna minor L.	Glyphosate 95 %, technical product	10 days	20.5	Sobrero, M.C.,2007; glyphecotox_125
	Roundup1Max, 70.7 % w/w a.s. as acid		11.6	

For the group of aquatic invertebrates, a comprehensive database of 42 peer-reviewed papers was submitted by the notifier. The notifier considered three publications (Bringolf *et al.* 2007; Chen et al. 2004 and Mensah et al. 2011) to be rated in category "Klimisch 2" (Klimisch 1997) and annotated with minimal remarks, whereas the remaining were considered as not acceptable for risk assessment. The submitted publications were also evaluated by RMS and

have been assigned according to an UBA screening. Out of the submitted publications, 18 studies were recognised as supporting information (category UBA2) and are reviewed here. Endpoints derived out of these publications are listed in the table below.

Most of the cited studies were performed with formulated products and not with the active ingredient alone. Those studies, which investigated the effect of glyphosate itsef or the glyphosate IPA-salt obtained LC_{50} values ranging from 49.3 mg acid equivalents /L for the marine copepod *Acartia tonsa* to 415 mg acid equivalents /L for the cladoceran *Ceriodaphnia dubia* (Tsui, 2003; Le, 2010; Tsui et al., 2004; Dominguez-Cortinas et al., 2008; Bringolf et al., 2007; Mottiera et al., 2013; Frontera, 2011). However, more sensitive species like the coelenterate *Hydra attenuata* showed lower sensibility and LC_{50} values were determined to be 18.2 mg/L for the active ingredient glyphosate. These organisms are generally not considered in Tier 1 risk assessment, but is was shown that they are exposed to toxicants to a higher extent due their anatomical and physiological structure (Demetrio, 2012). Moreover, sublethal effects were observed at much lower concentrations of glyphosate in comparison to lethal effects (Mottiera, 2013).

In general, the formulations are of higher ecotoxicological relevance than the active ingredient glyphosate itself. One of the main commercial formulations is Roundup ®, which in addition to the active ingredient glyphosate contains polyoxyethoxylated alkylamines (POEA) as a surfactant. A few studies investigate the effects of the formulation versus the surfactant POEA. These studies have shown that formulations containing POEA are several times more toxic (3 to 5 fold more toxic than Roundup®) to aquatic invertebrates than the active ingredient glyphosate acid or formulations without POEA. For more details concerning surfactant ingredients and their toxicity to aquatic organisms please refer to chapter B.9.11.

There were no critical data that could directly be included in the environmental risk assessment for the active substance glyphosate.

Table B.9.2-63: Effects values for aquatic invertebrates exposed to glyphosate acid or formulated products with glyphosate. Enpoints published in per-reviewed open literature

Species	**Test item**	**Study type**	**LC_{50} (mg a.e./L)**	**Reference (internal tag)**
		Crustaceans		
Daphnia magna	glyphosate acid	48h (mortality)	234	**Le**, T.H.,2010; glyphecotox_122
C. quadricarinatus	glyphosate acid	50days	>33	Frontera, J.L.,2011; glyphecotox_378
Daphnia magna	glyphosate acid	48h (mortality)	146	Dominguez-Cortinas, G.,2008; glyphecotox_347
	Faena®		7.9	
Lecane quadridentata	glyphosate acid	48h (mortality)	150	
	Faena®		13.1	
Hyalella castroi	Roundup®	7days (survival estimated)	2.16	Dutra, B.K.,, 2011; glyphecotox_121
Chordodes nobilii	Roundup®	96h (mortality)	1.76	Achiorno, C.L.,2008; glyphecotox_110

Species	**Test item**	**Study type**	**LC_{50} (mg a.e./L)**	**Reference (internal tag)**
Caridina nilotica	Roundup®	48h (mortality)	neonates = 4.45	Mensah, P.K.,2011; glyphecotox_123
			juvenile = 9.39	
			adults=37.12	
		96h (mortality)	neonates = 2.54	
			juvenile = 6.96	
			96h adults=25.507	
Daphnia magna	Roundup®	48h (mortality)	0.019	Sarigül Z.,2009; glyphecotox_124
Simocephalus vetulus	Vision®	48h (mortality)	0.75 to 1.5 a.e.	Chen, C.Y.,2004; glyphecotox_120
Ceriodaphnia affinis	Fakel herbicide	48	13.6	Melnichuk, S.D.,2007; glyphecotox_501
Thamnocephalus platyurus	POEA 15:1	48h (mortality)	2.01	Brausch, J.M.,2007, glyphecotox_113
	POEA 10:1		2.70	
	POEA 5:1		5.17	
Daphnia magna	POEA 15:1	48h (mortality)	0.85	Brausch, J.M.,2007; glyphecotox_114
	POEA 10:1		0.097	
	POEA 5:1		0.18	
Ceriodaphnia dubia	Glyphosate IPA-salt	48h (mortality)	415	Tsui, M.T.K., 2003 glyphecotox_195
	Roundup®		5.4	
	POEA		1.2	
Acartia tonsa	Glyphosate IPA-salt	48h (mortality)	49.3	Tsui, M.T.K., 2003 glyphecotox_195
	Roundup®		1.77	
	POEA		0.57	
Ceriodaphnia dubia	Rodeo®	48h (mortality)	415 a.e.	Tsui, M.T.K., 2004; glyphecotox_018
	Roundup Bioactive®		81.5 a.e.	
	Roundup®		5.7 a.e.	
Hyalella azteca	Rodeo®	48h (mortality)	347a.e.	
	Roundup Bioactive®		120 a.e.	
	Roundup®		1.5 a.e.	
Nonarthropoda				
Lampsilis siliquoidea	Glyphosate tech.	48h (mortality)	>200	Bringolf, R.B.,, 2007; glyphecotox_119
	Glpyhosate IPA		5	
	Aquastar®		>148	
	Roundup®		2.9	
	MON0818		0.5	
Utterbackia imbecillis	Roundup®	24h (mortality)	18.3	Conners, D.E.,2004; glyphecotox_325
Hydra attenuata	glyphosate (as acid)	96h (mortality)	18.2	Demetrio, P.M.,,2012; glyphecotox_342
	RoundupMax® (74.4% glyphosate)		21.8	
Mature oysters	Glyphosate technical		[>0.005] (larval development)	Akcha, F.,2012; glyphecotox_273
Limnoperna fortunei	Glyphosate technical	26d	1 (Glutathione-S-transferase activity)	Immanuto, M.M. et al. 2013
Crassostrea gigas	Glyphosate tech.	48h (mortality)	>100	Mottiera, A., 2013
	AMPA		>100	

Species	**Test item**	**Study type**	**LC_{50} (mg a.e./L)**	**Reference (internal tag)**
	Roundup Express®		8.5	
	Roundup Allées et Terrasses®		7.9	
Crassostrea gigas	Glyphosate tech.	48h (larval development)	27.1	
	AMPA		46.1	
	Roundup Express®		1.1	
	Roundup Allées et Terrasses®		2.0	

For the group of aquatic vertebrates, a database of more than 60 peer-reviewed publications was submitted by the notifier. The notifier considered seven publications (Filizadeh et al. 2011; Guilherme et al. 2012; Hued et al. 2012; Kelly et al. 2010; Salbego et al. 2010; Soso et al. 2007 and Tierney et al. 2006) and all seven were rated in category "Klimisch 3" (Klimisch 1997). The submitted publications were also evaluated by RMS and have been assigned according to an UBA screening. Out of the submitted publications, 24 studies were recognized as supporting information (category UBA2) and are reviewed here.

In the environmental risk assessment of pesticides, the group of aquatic vertebrates is mainly assessed by the results of acute, early life stage or full-life cycle effect studies on laboratory level with the choice of survival, growth and reproduction of individuals as endpoints. This is mainly due to the fact that on higher tiers of the aquatic assessment procedure in semi-field mesocosm studies plankton-dominated aquatic communities are tested that would be strongly disturbed by the presence of fish.

Nevertheless, recent research is focused on endpoints on sub-organismic level, such as indicators of metabolic, haematological and reproduction alterations caused by glyphosate formulations. Various studies deal with sub-lethal endpoints such as histological alterations of gill, liver and further organ tissues, such as neurotoxic endpoints and genetic biomarkers (Guilherme et al., 2010, Salbego et al., 2010; Soso et al., 2007; De Menezes et al., 2011; Kreutz et al., 2011; Cavalcante et al., 2008; Ferreira et al. 2010; Cattaneo et al., 2011; Modesto et al., 2010).

In a few studies (Evrard et al., 2010; Langiano et al., 2008) histological alterations in the gills and liver or in liver gene expressions or in methionine metabolism, lipid transport and metabolisms related to oxidative stress were observed. Most of these endpoints measured can be taken as early warning indicators of genotoxic and oxidative stress at the individual level but could not be used in traditional environmental risk assessment, which takes into account the population levels. Moreover, a few alterations like the enhancement of stress related genes and enzymes are of general character since linked to the metaboolic response towards abiotic and biotic factors of the experimental environment. In most cases they are not considered to be life-threatening or have evident effects on population level. In cases where strong histological changes were observed, which might lead to impaired organ functioning (e.g Zhidenko et al., 2007; Ortiz-Ordoñez et al., 2011), the commercial formulation tested was likely to contain POEA as surfactant. The toxicological studies testing the the commercial formulation Roundup® are of limited validity regarding effects of glyphosate-based formulations that do not contain POEA. Although Roundup as the most important herbicide formulation world-wide has been tested frequently, most of the authors have not stated exactly the contents of acid equivalents, POEA or other surfactants in the formulation used. Concerns on side-effects of glyphosate formulations containing POEA as surfactants raised in

particular early studies (Folmar et al., 1979, Smith et al., 2004, Haller et al., 2003), wheras testing on technical grade glyphosate have seldom been conducted. One example for a test with glyphosate technical is the study by Tierney et al. (2006), who evaluated the effect of relatively low doses of glyphosate on the olfactorial sense of salmons.

None of the studies that were evaluated in detail reported the statistical power of the respective test design. This poses a common difficulty in classifying the validity of tests of highly variable biological systems, even conducted under formally unified laboratory conditions regarding the influence of the environment. The minimal detectable difference between a treatment and a control group depends on the number of replicates and the variability amongst them. There were no acute mortality endpoints on fish reported in the peer-reviewed open literature that raise particular new concerns compared to the standard studies submitted with the notification of the active substance glyphosate. Most studies were conducted with commercially available formulations that did not allow for keeping apart the effects of the parent active substance glyphosate, its metabolites and the surfactants.

Newly published literature was identified, mainly for the time period 2012- 2014 and is included in the RAR.

Several studies investigated changes in the metabolic and enzymatic state in aquatic organsims (Fan, et al. 2013, Sandrini, et al 2013, Syedkolaei, et al. 2013, Gholami-Seyedkolaei, et al. 2013). It seems that theses changes in biochemical parameters could be used as biomarkers, because a dose-response association between commercial formulation treatment and enzymtic activity was found in the different tissues. For an adequate appreciation of the ecological relevance of biochemical, metabolic and histopathological effects, their impact on population structure and function remains to be elucidated further. All studies have been supporting character for traditional environmntal risk assessment, because the concentrations tested are exceeding the environmental concentrations of the active ingredient and endpoints are covered by the risk assessment. Nevertheless, an additional risk assessment was performed to integrate endpoint derived from peer-reviewed literture as well. The toxicological studies testing the commercial formulation Roundup® are of limited validity regarding effects of glyphosate-based formulations that do not contain POEA.

Many tests using fishes were conducted in order to investigate the genotoxic and cytotoxic potential of glyphosate towards different aquatic organisms (Nwani et al. 2013, Moreno,et al. 2014, De Souza Filho, et al. 2013, de Castilhos Ghisi, et al. 2012, Vera-Candioti, et al. 2013, Guilherme, et al. 2012 and 2014). Most of the studies were performed with ecologically realistic concentration of the herbicide. Nevertheless, in most cases, again commercial formulations have been used which do not allow to discriminate which compound of the commercial formulation could be responsible for the observed effects. It has also been reported that glyphosate itself caused oxidative DNA damage in cells of *A. anguilla* exposed under laboratory conditions (Guilherme et al., 2012). At present, it seems evident that more information is required to understand and clarify the risk of genotoxicity of glyphosate containing herbicides.Taking together, these results revealed that both glyphosate itself as well as the formulated products should be carefully monitored considering their potential impact on aquatic biota. Moreover, it seems that a transition from traditional ecotoxicological methods determining acute toxicity with endpoints on mortality and reproduction can be complemented by far subtler methods taking into account biochemical parameters. It is not clear at the moment, if alterations might impair normal organ functioning and therefore, the studies have limited value to conclude on the relevance on the population level. Nevertheless,

biochemcical biomarkers allow the examination of specific target organs, including gills, kidney and liver and blood that are responsible for vital functions.

Effects values for aquatic vertebrates exposed to glyphosate acid or formulated products. Endpoints published in peer-reviewed open literature

Species	**Substance**	**Study type**	**EC_{50} (mg/L)**	**Reference**
Fish				
Chlorella vulgaris	Roundup®	96h	20-26	Filizadeh, et al. (2011)
Jenynsia multidentata	Roundup Max®	96h	19.2	Hued, A.C. et al. (2012)
Rhamdia quelen	Roundup®	96h	7.3	Kreutz et al. (2008)
Pimephales promelas	Glyphosate a.s,	96h	97	Folmar et al. (1979)
Prochilodus lineatus	Roundup®	96h	14	Langiano et al. (2008)
Goodea Atripinnis	Yerbimat	96h	39	Ortiz-Ordoñez et al. (2003)
Channa punctatus	Roundup®	96h	32	Nwani et a. (2013)
Cyprinus carpio	Roundup®	96h	22	Syedkolaei, et al. (2013)
Danio rerio	glyphosate®	21d	0.5	Webster, et al. (2013)
Clarias gariepinus	Dizensate®	96h	43	Akinsorotan, et al. (2013)
Cnesterodon decemmaculatus	Panzer®	96h	16	Vera-Candioti, et al (2013)
	Credite®		92	

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B.9.2.4 Risk assessment

The main routes of exposure to aquatic organisms considered in this aquatic risk assessment are spray drift, runoff and drainage.
As described in Section B.8 (Fate and Behaviour), predicted environmental concentrations in surface water (PEC_{sw}) and sediment (PEC_{sed}) of the active substance glyphosate, the metabolites AMPA and HMPA were estimated using the programs FOCUS Steps 1-2.

The toxicity of glyphosate acid, the isopropylamine (IPA) salt and the potassium (K)-salt as well as its metabolites AMPA and HMPA, as well as the representative formulation MON 52276 were investigated to aquatic organisms in laboratory studies with representative species of fish, aquatic invertebrates, algae and aquatic plants according to current guidance (SANCO/3268/2001). Full summaries of these studies are provided in chapters B 9.2.1.1 to B 9.2.1.7.
The first assessment of glyphosate has shown that the risk to aquatic organisms is determined by the toxicity of the active substance towards algae *Skeletonema costatum*. However, the study delivering the relevant endpoint in the risk assessment needed to be reevaluated under current validity criteria. Newly submitted studies were performed with algae *Skeletonema costatum* and *Myriophyllum aquaticum*. The results of the study by Wenzel (2012, BVL no 2316017) with *Myriophyllum aquaticum* and the representative formulation MON 52276 delivered the most sensitive endpoint regarding aquatic organisms. The results of the study conducted with *Myriophyllum aquaticum* and the active substance (Wenzel, 2012, BVL no 2310995) show that aquatic macrophytes were more sensitive towards the representaive formulation than to glyphosate.
Studies performed with glyphosate-IPA salt and glyphosate-K salt indicate that toxicity from the salts of glyphosate is either substantially lower or at least in the same order of magnitude as for glyphosate acid itself.

Ecotoxicological data for the two major metabolites of glyphosate AMPA and HMPA indicate that the toxicity to *Daphnia magna*, algae and also to aquatic plants is less or at least at the same order of magnitude than the parent material. As algae and aquatic plants are considered more sensitive to the metabolites of glyphosate no tests on aquatic vertebrates were conducted on HMPA for animal welfare reasons.

The following endpoints and effect values have been identified as relevant for the quantitative risk assessment.

Table B.9.2-64: Endpoints for aquatic species (most sensitive species of each group) relevant for the quantitative risk assessment

Species	Substance/ Test item	Test design	Toxicity (mg test item/L)	Endpoint	Safety factor
Oncorhynchus mykiss	Glyphosate acid	acute	38	mortality	100
Lepomis macrochirus	Glyphosate IPA-salt	acute	>1000 741 a.s.	mortality	100
Oncorhynchus mykiss	Glyphosate K-salt	acute	>2573 1227 a.s	mortality	100
Oncorhynchus mykiss	AMPA	acute	520	mortality	100
Oncorhynchus mykiss	MON 52276	acute	> 989 > 306 a.s.	mortality	100
Brachydanio rerio	Glyphosate acid	long-term	1 (recalculated by RMS)	mortality	10
Pimephales promelas	AMPA	long-term	12	hatching success, fry survival, length and weight	10
Daphnia magna	Glyphosate acid	acute	40	immobilization	100
Daphnia magna	Glyphosate IPA salt	acute	930	immobilization	100
Daphnia magna	Glyphosate K-salt	acute	592 278 a.s. (recalculated by RMS)	immobilization	100
Daphnia magna	AMPA	acute	690	immobilization	100
Daphnia magna	HMPA	acute	>100	immobilization	100
Daphnia magna	MON 52276	acute	676 209 a.s.	immobilization	100

Species	**Substance/ Test item**	**Test design**	**Toxicity (mg test item/L)**	**Endpoint**	**Safety factor**
Daphnia magna	Glyphosate acid	long-term	12.5 (recalculated by RMS)	reproduction	10
Daphnia magna	AMPA	long-term	15	reproduction	10
Skeletonema costatum	Glyphosate acid	chronic	11	biomass	10
Anabaena flos-aquae	Glyphosate acid	chronic	8.5	biomass	10
Pseudokirchneriella subcapitata	Glyphosate IPA salt	chronic	9.25	biomass	10
Desmodesmus subspicatus	AMPA	chronic	89.8	biomass	10
Pseudokirchneriella subcapitata	HMPA	chronic	>115	growth rate	10
Pseudokirchneriella subcapitata	MON 52276	chronic	178	biomass	10
Lemna gibba	Glyphosate acid	chronic	12	frond count	10
Lemna minor	Glyphosate-IPA salt	chronic	25.5	frond count	10
Lemna gibba	HMPA	chronic	>123	frond count	10
Lemna gibba	MON 52276	chronic	21	frond count	10
Myriophyllum aquaticum	MON77973 Glyphosate acid	chronic	12.3	fresh weight, relative increase	10
Myriophyllum aquaticum	MON 52276	chronic	4.4	fresh weight, relative increase	10
Myriophyllum aquaticum	AMPA	chronic	31.1	root length, relative increase	10

Comparison of calculated TER values with the respective acceptability criteria shows that the risk for aquatic organisms is triggered by the possible long-term effects on macrophytes and acute effects on fish.

The predicted environmental concentrations and further details of calculation for glyphosate acid in surface water (PECsw), arising as a consequence of drift, drainage and run-off, are calculated and are provided in Section 8. PECsw values for glyphosate acid and the major metabolites were calculated using the FOCUS (2000) surface water models.

As worst case covering all intended uses, PEC_{sw} and PEC_{sed} were derived for pre-emergence application of glyphosate to various field crops and for post-weed emergence use of glyphosate to the soil and trunks of pome/ stone fruit trees representing the intended use in orchard crops, vines including citrus & nut trees (= perennial crops).

Calculated TER values referring to FOCUS Step 1 and 2 are provided in the following tables:

Table B.9.2-65: Maximum PEC_{sw} values and TER values for glyphosate, Focus Step 1 & 2

Scenario	Applic. rate (g/ha)	PEC_{sw} (µg/L)	PEC_{sed} (µg/kg)	Fish acute	Fish prolonged	Fish prolonged	Inverte-brates acute	Inverte-brates prolonged	Algae	Aquatic plants	Sediment dwelling org.
				O. mykiss	*B. rerio*	*P. promelas*	*D. magna*	*D. magna*	*A. flos-aquae*	*M. aquaticum* (MON 52276)	-
				LC_{50} (µg/L)	NOEC (µg/L)	NOEC (µg/L)	EC_{50} (µg/L)	NOEC (µg/L)	E_bC_{50} (µg/L)	E_bC_{50} (µg a.e./L)	-
				38000	1000	25700	40000	12500	8500	4400	-
						Calculated TER					
FOCUS Step 1 Not crop specific	1 x4320	104.81	10300	363	~~53~~ 9.5	245	382	119	81	42	-
FOCUS Step 2 North Europe (Oct-Feb)	2 x 2160	~~23.58~~ 23.38	-	~~1612~~ 1625	~~237~~ 42	~~1090~~ 1099	~~1696~~ 1711	~~530~~ 535	~~360~~ 364	188	-
North Europe (Mar – May) and (Jun-Sep)	2 x 2160	18.49	-	2055	~~303~~ 54	1390	2163	676	460	240	-
South Europe	2 x 2160	~~19.30~~ 19.14	-	~~1969~~ 1985	~~290~~ 52	~~1332~~ 1343	~~2073~~ 2090	~~648~~ 653	~~440~~ 444	230	-
TER criterion				100	10	10	100	10	10	10	-

Risk assessment with endpoints derived from peer- reviewed literature:

Scenario	**Applic. rate (g/ha)**	**PEC_{sw} (µg/L)**	**Fish prolonged (Webster et al. 2013)**	**Invertebrate chronic (Immanuto et al. (2013)**	**Invertebrate chronic: (Cuhra et al. 2013)**	**Algae (Tsui et al. 2003)**	**Aquatic plants (Turgut & Formin, 2002)**
			B. rerio	*Limnoperna fortunei*	*D. magna*	*S. costatum*	*M. aquaticum*
			NOEC (µg/L)	**EC_{50} (estimated) (µg/L)**	**NOEC (µg/L)**	**E_bC_{50} (µg/L)**	**EC_{50} (µg a.e./L)**
			500	1000 (Glutathione-S-transferase activity)	450 /(150) (fecundity/ juvenile size)	2270	220 (commercial unknown formulation)
FOCUS Step 1 Not crop specific	1 x4320	104.81	5	10	4.3 / (1)	22	[2]
FOCUS Step 2 North Europe (Oct-Feb)	2 x 2160	23.58	21	42	19 / (6)	96	[9.3]
North Europe (Mar – May) and (Jun-Sep)	2 x 2160	18.49	27	54	24 / (8)	123	[11]
South Europe	2 x 2160	19.30	26	52	23 / (8)	118	[11]
FOCUS Step 3	2 x 2160	Max. 14.17	35	71	32 / (11)	162	[16]
TER criterion			10	10	10	10	10

Table B.9.2-66: Maximum PEC_{sw} values and TER values for AMPA, Focus Step 1

Scenario	Application rate (g/ha)	PEC_{sw} (µg/L)	PEC_{sed} (µg/kg)	Fish acute	Fish prolonged	Inverte-brates acute	Inverte-brates prolonged	Algae	Aquatic plants	Sediment dwelling org.
				O. mykiss	P. *promelas*	*D.magna*	*D.magna*	*D. subspicatus*	*M. aquaticum*	-
				LC_{50} (µg/L)	NOEC (µg/L)	EC_{50} (µg/L)	NOEC (µg/L)	E_bC_{50} (µg/L)	E_bC_{50} (µg/L)	-
				520000	12000	690000	15000	89900	31100	-
				Calculated TER						
FOCUS Step 1 Not crop specific	1 x 4320	40.93	3300	12705	293	16858	366	2196	760	-
TER criterion				100	10	100	10	10	10	-

Table B.9.2-67: Maximum PEC_{sw} values and TER values for HMPA, Focus Step 1

Scenario	**Application rate (g/ha)**	**PEC_{sw} (µg/L)**	**PEC_{sed} (µg/kg)**	**Fish acute**	**Fish prolonged**	**Inverte-brates acute**	**Inverte-brates prolonged**	**Algae**	**Aquatic plants**	**Sediment dwelling org.**
				-/-	*-/-*	*D.magna*	*-/-*	*P.subcapitat*	*L. gibba*	*-/-*
				LC_{50} (µg/L)	**NOEC (µg/L)**	**EC_{50} (µg/L)**	**NOEC (µg/L)**	**E_bC_{50} (µg/L)**	**E_bC_{50} (µg/L)**	**-**
				-	-	>100000	-	>115000	>123000	-
				Calculated TER						
FOCUS Step 1 Not crop specific	1 x 4320	6.71	696	-	-	14903	-	17139	18331	-
TER criterion				100	10	100	10	10	10	-

Calculated TER values referring to FOCUS Step 1-3 are provided. Comparison of calculated TER values with the respective acceptability criteria shows that the risk for aquatic organisms is acceptable. Concerning the risk assement with endpoints from open literature it must be taken into account that there are many uncertainties in transferring results relatedto biochemical or developmental endpoints to populations of organsims which is the relevant organisational level for an environmental risk assessment.

B.9.2.5 Summary of results and TER-calculation for the formulated product

The TER values for glyphosate acid, glyphosate IPA-salt, glyphosate K-salt AMPA and HMPA exposure to aquatic macrophytes all exceed the Annex VI trigger of 10, indicating that glyphosate acid, IPA salt, AMPA and HMPA do not pose an unacceptable risk to aquatic macrophytes following application according to the proposed uses of MON 52276.

B.9.2.6 Ecotoxicological relevance of monitoring data

For the assessment of the risk for aquatic organisms exposed to glyphosate, the toxicity values are also compared to results from surface water monitoring. Horth (2012, BVL no 2310291) provided a review that covers glyphosate and AMPA monitoring results for surface (fresh) waters and groundwater across Europe, i.e. all 27 Member States of the EU. Analyses to detect glyphosate were performed in almost 75000 surface water samples from about 4000 sites (from 1993-2011). Glyphosate was detected in 33 % of samples, with 23 % above 0.1 µg/L.

The maximum concentrations of glyphosate acid found in surface water reached from 1.3 to 370 µg/L. The highest glyphosate values in surface water were detected in Sweden (370 µg/L), Ireland (186 µg/L) and Belgium (139 µg/L).

The main metabolite AMPA has been analysed in about 56700 samples from nearly 3000 sites (1997-2011) and was detected in 54 % of samples, with 46 % above 0.1 µg/L and maximum concentrations reaching from 0.22- >200 µg/L.

Glyphosate and AMPA monitored in this study exceeded the predicted environmental concentrations for glyphosate acid and AMPA in surface water (PECsw) calculated using the FOCUS (2000) surface water models, even though worst case applications was assumed. Nevertheless, when calculating TER values with the concentrations monitored in the study by Horth (2012, BVL no 2310291), the outcome of the assessment still that the risk for aquatic organisms from the intended uses of glyphosate is acceptable.

B.9.3 Effects on other terrestrial vertebrates (Annex IIIA 10.3)

B.9.3.1 Toxicity data

B.9.3.1.1 Acute Toxicity

A detailed summary of acute oral toxicity studies with glyphosate acid is given in Volume 3, chapter B.6.2.1, Table B.6.2-1. In the current dossier, a variety of additional studies in rats

with administration of glyphosate acid and two more studies in rats and mice with the IPA salt were provided and mentioned in the tables below. All results and RMS Conclusions of the evaluation are summarised in RAR, glyphosate, Volume 3, chapter B.6.2.1.

KIIA 5.2.1/01

Author:	[redacted]
Title:	Glyphosate: Acute Oral Toxicity Study (UDP) In Rats.
Date:	11.03.2009
Doc ID:	2309084/ASB2012-11381
Guidelines:	US EPA OPPTS 870.1100 equivalent to OECD 425 (2008).
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.2.1.

KIIA 5.2.1/02

Author:	[redacted]
Title:	Acute Oral Toxicity Study In Rats.
Date:	20.02.1995
Doc ID:	2309086/ASB2012-11382
Guidelines:	OECD 401 (1987), JMAFF 59 NohSan 4200 (1995), US EPA (1984)
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.2.1.

KIIA 5.2.1/03

Author:	[redacted]
Title:	Acute Oral Toxicity Study In Mice.
Date:	20.02.1995
Doc ID:	2309088/ASB2012-11383
Guidelines:	OECD 401 (1987), JMAFF 59 NohSan 4200 (1995), US EPA (1984)
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.2.1.

KIIA 5.2.1/04

Author:	[redacted]
Title:	Glyphosate Technical: Acute Oral Toxicity Study in Rats.
Date:	2009
Doc ID:	2309090/ASB2012-11384
Guidelines:	OECD 423 (2001) Commission Regulation (EC) No 440/2008 (2008), method B.1 tris
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.2.1.

KIIA 5.2.1/05

Author:	[redacted]
Title:	Acute Oral Toxicity Study of Glyphosate TC in Rats.
Date:	16.06.2009
Doc ID:	2309092/ASB2012-11385
Guidelines:	EC method B.1 tris (2004/73/EC), OECD 423 (ATC method) and OPPTS 870.1100.
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.2.1.

KIIA 5.2.1/06

Author:	[redacted]
Title:	Acute Oral Toxicity Study of Glyphosate TC in Rats.
Date:	6.01.2010
Doc ID:	2309094/ASB201211386
Guidelines:	EC method B.1 tris (2004/73/EC), OECD 423 (ATC method) and OPPTS 870.1100.
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.2.1.

KIIA 5.2.1/07

Author:	[redacted]
Title:	Acute Oral Toxicity Study of Glyphosate TC in Rats
Date:	19.02.2010
Doc ID:	2309096/ASB2012-11387
Guidelines:	EC method B.1 tris (2004/73/EC), OECD 423 (ATC method) and OPPTS 870.1100.
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.2.1.

KIIA 5.2.1/08

Author:	[redacted]
Title:	Glyphosate Acid Technical – Acute Oral Toxicity Up and Down Procedure in Rats.
Date:	2005
Doc ID:	2309098/ASB2012-11388
Guidelines:	US EPA OPPTS 870.1100 (2002) OECD 425 (2001).
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.2.1.

KIIA 5.2.1/09

Author:	[redacted]
Title:	Acute Oral Toxicity Study in Wistar Hannover Rats for Glyphosate Technical
Date:	16.09.2008
Doc ID:	2309100/ASB2012-11389
Guidelines:	OECD guideline 423 (2001).
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.2.1.

KIIA 5.2.1/10

Author:	[redacted]
Title:	GLYPHOSATE TECHNICAL (NUP05068) : Acute oral toxicity study in rats
Date:	1.03.2007
Doc ID:	2309103/ASB2012-11390
Guidelines:	Japanese guideline Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Preparation of Study Results, Acute oral toxicity studies. Guideline 2"1-1 Notification 12 NohSan No. 8147, as partly revised in 16-Shouan-9260, on 16 March 2005. English translation by ACIS on 17 Oct 2005. Directive 2004173/EC, 8.1 tris "Acute Oral Toxicity-Acute Toxic Class Method", April 29, 2004. OECD Guidelines for the Testing of Chemicals, Number 423 "Acute Oral Toxicity – Acute Toxic Class Method", adopted 17 December 2001.
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.2.1.

KIIA 5.2.1/11

Author:	[redacted]
Title:	Acute Oral Toxicity Study of Glyphosate Batch/Lot/NBR No. XLI-55 in Sprague-Dawley Rats
Date:	8.06.1988
Doc ID:	2309105/Z35389
Guidelines:	US EPA 81-1
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.2.1.

KIIA 5.2.1/12

Author:	[redacted]
Title:	Acute Oral Toxicity Study In Rats.
Date:	6.08.1979
Doc ID:	2309107/Z35541
Guidelines:	None (pre-guideline)
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.2.1.

KIIA 5.2.1/13

Author:	[redacted]
Title:	Glyphosate Acid: Acute Oral Toxicity Study In Rats.
Date:	23.08.1996
Doc ID:	2309109/CTL/P/4660/TOX2000-1982
Guidelines:	OECD 425 (2001) US EPA OPPTS 870.1100 (2002)
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.2.1.

KIIA 5.2.1/14

Author:	[redacted]
Title:	Glyphosate technical material: Acute oral toxicity study in the rat (up and down procedure).
Date:	8.02.2007
Doc ID:	2309111/ASB2012-11391
Guidelines:	OECD 425 (2001) US EPA OPPTS 870.1100 (2002) Japanese MAFF 12 NohSan No. 8147
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.2.1.

KIIA 5.2.1/15

Author:	[redacted]
Title:	Glyphosate technical: Acute oral toxicity study in the rat (up and down procedure)
Date:	15.04.2011
Doc ID:	2309113/ASB2012-11392
Guidelines:	OECD 425 (2008), OPPTS 870.1100 (2002)
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.2.1.

KIIA 5.2.1/16

Author:	[redacted]
Title:	Acute Toxicity Study of MON 0139 by Oral Administration in Mice.
Date:	5.10.1995
Doc ID:	2309115/ASB2012-11393
Guidelines:	JMAFF 59 NohSan No. 4200 (January 28, 1985)
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.2.1.

KIIA 5.2.1/17

Author:	[redacted]
Title:	NUP5a99 62% glyphosate MUP: Acute oral toxicity study in rats – Limit test
Date:	16.09.1999
Doc ID:	2309117/ASB2012-11394
Guidelines:	US EPA Health Effects Test Guidelines, OPPTS 870.1100 (1998)
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.2.1.

B.9.3.1.2 Reproductive toxicity

The reproductive toxicity of glyphosate was tested in several of multi-generation studies in rats. For the first EU evaluation of glyphosate in 2001, a total of 8 studies in rats had been submitted, of which four were still considered acceptable or, in case of a single one-generation study, at least supplementary upon re-evaluation (please refer to Vol.3, B.6.6).

The overall assessment of reproductive toxicity of glyphosate by the RMS is provided in RAR, Vol. 1, chapter 2.6.6 and RAR, Vol.3, B.6.6. A summary is given below under point B.9.3.2. Please refer to table Table B.9.3 3: Reproductive toxicity studies with rats provided for the present EU peer review assessment of glyphosate together with valid reproductive toxicity study endpoints reviewed in the first EU evaluation in 2001.

KIIA 5.6.1/01

Author:	[redacted].
Title:	Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat
Date:	31.10.2007
Doc ID:	2309418/ASB2012-11494
Guidelines:	OECD 416 (2001), JMAFF 2-1-17 (2001), US-EPA OPPTS 870.3800 (1998)
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.6.
Comment in Vol3, Part B6.	*At highest dose level of 15000 ppm increased organ weights in liver (F0 & F1 females) and kidneys (F0 females) were observed. The Notifiers stated that there is no toxicological concern regarding the significant increased liver weights due to the absence of any histopathological changes in the liver. In fact, in the present study no evidence for histopathological examination of the liver was given.*

	*At this high dose level a significant decrease in homogenisation resistant spermatids (HRS, cauda epididymis) was counted in F0 males (Control: 399.9 million/gram; 15000 ppm: 309.0 million/gram**). No remarkable effects were seen at lower dose levels.* *Furthermore, in F1 male offsprings sexual maturation (preputial separation) was delayed at 15000 ppm without any additional developmental retardation (e.g. body weight, please see* Table B.6.6-7 *below). The authors of the study considered this finding in F1 males (45.9 d versus control 43.0 d) to be unrelated to treatment, because no effects on sexual maturation were evident for females and there were no differences in mating performance. Sperm changes and histopathological examinations did not reveal any changes in the testis or epididymes. Although, the later onset of preputial separation in male offsprings at 15000 ppm had obviously no impact on reproductive performance in week 29, a treatment related effect on sexual maturation at parental toxic dose cannot be excluded. All in all, the NOAEL of 5000 ppm (ca. 351 mg/kg bw/d) is considered to be more appropriate regarding parental, reproductive and offspring toxicity.*

KIIA 5.6.1/02

Author:	[redacted]
Title:	Glyphosate acid: Multigeneration reproduction toxicity study in rats
Date:	16.06.2000
Doc ID:	2309423/CTL/P/6332/TOX2000-2000
Guidelines:	OECD 416 (2001), Annex V 67/548/EEC, 9.ATP 87/302/EEC OJEC, L133, 47-50 (1988), US-EPA OPPTS 870.3800 (1998)
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.6.
Comment in Vol3, Part B6.	*The NOAEL for parental and offspring toxicity was considered to be 3000 ppm, which has to be converted into 293 mg/kg bw/d on the basis of a reduction in body weight of F1 pups and a subsequent reduction in body weight of in F1 males.*

KIIA 5.6.1/03

Author:	[redacted]
Title:	A two-generation reproduction study in rats
Date:	19.06.1997
Doc ID:	2309425/ASB2012-11495
Guidelines:	OECD 416 (1981), US-EPA FIFREA Guidelines Subdivision F (1984), Japan MAFF Guideline 59 NohSan No. 4200 (1985)
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.6..
Comment in Vol3, Part B6.	*Offspring toxicity was observed at highest dose level only and confined to reduced body weight and caecum distension in both sexes. Sexual maturation (preputial separation, vaginal opening) was not examined in this study. Based on the results the NOAEL for parental and offspring toxicity was considered to be 6000 ppm and for reproductive toxicity to be 30000 ppm (>2000 mg/kg bw/d).*

KIIA 5.6.1/04

Author:	[redacted]
Title:	Two Generation Reproduction Study in Wistar Rats.
Date:	27.08.1993
Doc ID:	2309427/TOXI 885-RP-G2/TOX9300009
Guidelines:	OECD 416 (1983)
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.6.
Comment in Vol3, Part B6.	*The previous evaluation in 2001 regarding the NOAELs is confirmed. However, this study is now considered supplementary, because an effect dose was lacking. The highest dose of 10000 ppm is considered to be the NOAEL for parental, reproductive and offspring toxicitiy. This dietary level would correspond to a mean daily compound intake of 700-800 mg/kg bw/d.*

KIIA 5.6.1/05

Author:	[redacted]
Title:	A three generation reproduction study in rats with glyphosate
Date:	31.03.1981
Doc ID:	2309434/BDN 77-417/TOX9552385
Guidelines:	None (pre-guideline)
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.6.
Comment in Vol3, Part B6.	*This study is now considered not acceptable due to the selected dose levels that were much too low. Accordingly, an effect dose was not reached (Evaluation in 2001 not confirmed).*

KIIA 5.6.1/06

Author:	[redacted]
Title:	The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat.
Date:	14.05.1992
Doc ID:	2309436/CHV 47/911129/TOX9552389
Guidelines:	OECD 416 (1983), US-EPA FIFRA 83-4 (1982)
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.6.
Comment in Vol3, Part B6.	*In conclusion, no evidence of reproductive effects was observed and therefore, the NOAEL on reproductive toxicity is considered 10000 ppm (668 mg/kg bw/d). The NOAEL for parental and offspring toxicity is calculated to be 3000 ppm (197 mg/kg bw/d) based on increased food and water consumption in F1 females, lower body weight of F1 males and an increased incidence of cellular alteration of the parotid (males and females) and submaxillary (females) salivary gland in both F0 and F1 adults at 10000 ppm (Evaluation in 2001 confirmed).*

KIIA 5.6.1/07

Author:	[redacted]
Title:	Two Generation Reproduction Feeding Study with Glyphosate in Sprague-Dawley Rats
Date:	27.08.1990
Doc ID:	2309439/ASB2012-11496
Guidelines:	Not stated, but in general accordance with OECD 416 (1983)
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.6.
Comment in Vol3, Part B6.	*The mid dose of 10000 ppm is considered the NOAEL for parental, offspring and reproductive toxicity. This assessment is in accordance with the evaluation in the previous DAR (2001, ASB2010-10302) and based on reduced body weight gain and soft stools in high dose adults, decreased pup body weight gain and equivocally reduced litter size at highest dose level of 30000 ppm. The intermediate dietary concentration of 10000 ppm was calculated to be 722 for male rats and 757 mg/kg bw/d for females.*

B.9.3.1.3 Teratogenicity test by the oral route in the rabbit

For the current EU evaluation of glyphosate, two studies in rats were submitted ([redacted] 2002 and [redacted] 1995) and additional seven studies in rabbits ([redacted] 1994, [redacted] 2002, [redacted] 1995, [redacted] 1989, [redacted] 1980, [redacted] 1993 and [redacted] 1996).

KIIA 5.6.11/01

Author:	[redacted]
Title:	Glyphosate acid: Developmental toxicity study in the rabbit
Date:	2.07.1996
Doc ID:	2309450/CTL/P/5009/TOX2000-2002
Guidelines:	OECD 414 (1981), EEC B.31 (1988),US-EPA 83-3
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.6.
Comment in Vol3, Part B6.	*The dose level of 1000 mg glyphosate acid/kg/day was the no observed effect level in this study for both maternal and developmental effects.*

KIIA 5.6.11/02

Author:	[redacted]
Title:	Technical Glyphosate: Teratology study in rabbits
Date:	29.02.1980
Doc ID:	2309452/IR-79-018/TOX9552392
Guidelines:	Not stated. (pre-guideline; satisfies in general the requirements of OECD 414 (1981), but not of OECD 414 (2001))
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.6.
Comment in Vol3, Part B6.	*The study is considered supplementary, because the highest dose level revealed only 6 does with litters for examination due to the high maternal mortality. The NOAEL of 75 mg/kg bw/d for maternal toxicity is*

	agreed. However, the previously established NOAEL for developmental toxicity of 350 mg/kg bw/day was lowered to 175 mg/kg bw/day because of the low number of available foetuses in the high dose group avoiding meaningful evaluation.

KIIA 5.6.11/03

Author:	[redacted]
Title:	The Effect of Glyphosate on Pregnancy of the Rabbit (Incorporates Preliminary Investigations)
Date:	14.10.1991
Doc ID:	2309454/CHV 45 & 39 & 40/901303/TOX9552391
Guidelines:	OECD 414, US EPA 83-3
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.6.
Comment in Vol3, Part B6.	*In this study there was a significant increase in embryonic death and post-implantation loss in treated groups compared to controls, however without a clear dose-relationship. Regarding the post-implantation losss, values for the low and high dose groups are outside the historical control range. The cardiac malformation observed with greatest frequency in this study was the interventricular septal defect. At 450 mg/kg bw/d this effect was outside the historical control range (4.2 % compared to 0.66 % in historical controls). Taken into account the high postimplantation loss at the same dose level, the incidence of additionally cardiac malformation may be covered, therefore. At mid dose level foetuses with an higher incidende of retrooesophageal right subclavian artery were reported. However, this effect has to be considered equivocal, because no clear-dose relationship could be established.* *In conclusion, the NOAEL for maternal toxicity is considered 50 mg/kg bw/d based on slightly restricted inappetence, slightly reduced body weight gain and soft/liquid faeces at 150 mg/kg bw/d. The NOAEL for developmental toxicity is considered to be 150 mg/kg bw/d based on the post-implantation loss, late embryonic death and an increase in cardiac malformations at 450 mg/kg bw/d.*

KIIA 5.6.11/04

Author:	[redacted]
Title:	Teratogenicity study in rabbits – Test compound: Glyphosate technical (FSG 03090 H/05 March 1990)
Date:	17.04.1993
Doc ID:	2309457/TOXI: 884-TER-RB /TOX9551106
Guidelines:	OECD 414 (1981)
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.6.
Comment in Vol3, Part B6.	*The study is considered supplementary due to several weaknesses including a small number of litters for examination (low pregnancy rate at all dose levels, lethality in mid and high dose dams) and reporting deficiencies. The percentage of foetuses with 'dilated heart' was significantly increased at all dose levels. However, the absolute num-*

	ber of affected foetuses and litters is quite small and did not show a marked difference between the treated groups. Furthermore, the diagnosis 'dilated heart' was not defined in this study report and neither criteria used to this diagnosis nor measurements of the heart were provided. The NOAEL for maternal toxicity is still considered 20 mg/kg bw/d, because it cannot be excluded, that the intercurrent four deaths at 100 mg/kg bw/d were substance-related. Therefore, the previous evaluation in 2001 is confirmed: the maternal NOAEL is considered 20 mg/kg bw/d and the developmental NOAEL 100 mg/kg bw/d.

KIIA 5.6.11/05

Author:	[redacted]
Title:	Rabbit Teratology Study with Glyphosate Technical
Date:	3.11.1989
Doc ID:	2309462/Proj.No. 1086/TOX9551960
Guidelines:	OECD 414 (1981)
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.6.
Comment in Vol3, Part B6.	*The study is considered supplementary due to serious reporting deficiencies (e.g. no individual data, no statistical analysis, no uterine weights, no results of maternal necropsy).* *The previous NOAEL for maternal and developmental toxicity is still considered to be 250 mg/kg bw/d based on reduced food consumption and body weight gain at 500 mg/kg bw/d in does* *Developmental effects were visible as foetolethality and several malformations (external, visceral, skeletal) at high dose levels: The previous evaluation did not mentioned the external malformation in rabbits which are now reported in the present RAR (abnormal tails). Total number of foetuses per litter with malformations was higher in the groups receiving the mid and high dose lever, but without statistical significance. However, it remains unclear, whether statistical analysis of the data had been performed at all. Ventricular septal defects were noted in 2 out of 78 foetuses in the high dose group (control incidence 0/109). The higher number of further visceral malformations at the top dose level was due to absent kidneys and postcaval lung lobes. Because no individual data are provided it is not identifiable, whether the malformations described were confined to single foetuses or if the foetuses were multiple malformed.*

KIIA 5.6.11/06

Author:	[redacted]
Title:	HR-001: Teratogenicity Study in Rats
Date:	21.07.1995
Doc ID:	2309444 // IET 94-0152
Guidelines:	Japan MAFF Guidelines 59 NohSan No.4200, 1985 U.S. EPA FIFRA Guidelines Subdivision F, 1984
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.6.
Comment in Vol3, Part B6.	*The study is considered acceptable. The evaluation regarding maternal toxicity is agreed, which was confined to loose stool at highest dose level. The NOAEL for developmental toxicity is not supported due to the slight increase in skeletal variations at highest dose level:lumbar ribs were observed in 11 fetuses out of 7 litters compared to only 4 fetuses out of 2litters in control animals. Teratogenic effects were not observed. Based on findings in dams and foetuses, the NOAEL for both maternal and developmental toxicity is considered to be 300 mg/kg bw/d.*

KIIA 5.6.11/07

Author:	[redacted]
Title:	Glyphosate acid: Developmental Toxicity Study in the Rat
Date:	20.11.2002
Doc ID:	2309442 /CTL/P/4819 Amend.
Guidelines:	OECD 414 (2001): OPPTS 870.3700 (1998): 2004/73/EC B.31 (2004)
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.6.
Comment in Vol3, Part B6.	*The dose level of 1000 mg glyphosate acid/kg/day was the no observed effect level in thi sstudy for both maternal and developmental effects*

KIIA 5.6.11/08

Author:	[redacted]
Title:	HR-001: A Teratogenicity Study in Rabbits
Date:	21.07.1995
Doc ID:	2309446 /IET 94-0153
Guidelines:	OECD 414 (1981), JMAFF 59 NohSan 4200 (1985), US-EPA 83-3 (1984)
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.6.
Comment in Vol3, Part B6.	*The NOAEL for both maternal and developmental toxicity in this study conducted with artificial inseminated rabbits is considered to be 100 mg/kg bw/day for maternal toxicity. The NOAEL for offspring is considered to be 300 mg/kg bw/day.*

KIIA 5.6.11/09

Author:	[redacted]
Title:	Glyphosate technical: Oral gavage teratology study in the rabbit
Date:	4.07.1996
Doc ID:	2309448 //434/020/ASB2012-11499
Guidelines:	OECD 414 (1981), JMAFF 59 NohSan 4200 (1985), US-EPA 83-3 (1984)
GLP and Validity	Please refer to comment by RMS in the RAR, Vol.3, B.6.6.
Comment in Vol3, Part B6.	*The NOAEL is considered to be 50 mg/kg bw/d due to slight reduction in body weight gain at 200 mg/kg bw/d. The NOAEL is considered to be 50 mg/kg bw/d due to significantly increased post-implantation loss at 200 mg/kg bw/d. The statement, that this increase was caused by a slight rise in early foetal deaths and not in late foetal deaths, as seen at the high dose level and therefore considered not to be treatment-related, cannot be followed, because there is no information given regarding the mechanism behind this foetal deaths.* .

B.9.3.2 Summary of terrestrial vertebrate toxicity data

For the first EU peer-reviewed evaluation of glyphosate in 2001, a large number of oral toxicity studies in rats and mice were submitted that had been conducted with either glyphosate acid or its salts. For the current re-evaluation of glyphosate, several additional studies in rats with administration of glyphosate acid and two more studies in rats and mice with the IPA salt were provided (see Vol.3, B.6.2.1).

The reproductive toxicity of glyphosate was tested in several multi-generation studies in rats. For the first EU evaluation of glyphosate, a total of eight studies in rats had been submitted of which four were still considered acceptable or, in case of a single one-generation study, at least supplementary upon re-evaluation (please refer to Vol.3, B. 6.6).

The developmental toxicity and teratogenicity of glyphosate were tested in several studies in rats and rabbits. For the first EU evaluationof glyphosate, a total of five studies in rats were reported in the DAR, of which four were still considered acceptable or at least supplementary for the current evaluation. Five developmental studies in rabbits had been submitted for the first EU evaluation of glyphosate, of which four may are still acceptable following re-evaluation by the RMS. However, it has become necessary to revise the enpoint determination (NOAELs and LOAELs) in some cases and also to downgrade the reliability of individual studies (for further details please refer to Vol.1, chapter B. 2.6.7.2).

Table B.9.3-1: Summary of acute glyphosate acid oral toxicity for mammals

Species	**Number of animals / Dose levels (mg/kg bw)**	**Main effects**	**LD_{50} (mg/kg bw)**	**Reference/GLP**
Rat, Sprague Dawley	5/sex/5000	decreased activity, diarrhoea, piloerection, polyuria, salivation	> 5000 (limit test)	[redacted] 2009 (HAG) ASB2012-11381
Rat, Sprague Dawley	5/sex/5000	decreased spontaneous motor activity and salivation	> 5000 (limit test)	[redacted] 1995 (ALS) ASB2012-11382

Species	**Number of animals / Dose levels (mg/kg bw)**	**Main effects**	**LD_{50} (mg/kg bw)**	**Reference/GLP**
Mice, ICR	5/sex/5000	decreased spontaneous motor activity, sedation and crouching position	> 5000 (limit test)	[redacted] 1995 ASB2012-11383
Rat, Wistar	3 females/2000 (step 1) 3 females/2000 (step 2)	No findings	> 2000	[redacted] 2009 (EXC) ASB2012-11384
Rat, CD	3 females/2000 (step 1) 3 females/2000 (step 2)	No findings	> 2000 (limit test)	[redacted] 2009 (HAG) ASB2012-11385
Rat, CD	3 females/2000 (step 1) 3 females/2000 (step 2)	No findings	> 2000 (limit test)	[redacted] 2010 (HAG) ASB2012-11386
Rat, CD	3 females/2000 (step 1) 3 females/2000 (step 2)	No findings	> 2000 (limit test)	[redacted] 2010 (HAG) ASB2012-11387
Rat, Sprague-Dawley	3 females/5000	Diarrhea, ano-genital & facial staining, reduced faecal volume	> 5000 (limit test)	[redacted] 2005 (HAG) ASB2012-11388
Rat, Wistar	3 females/2000 (step 1) 3 females/2000 (step 2)	No findings	> 2000 (limit test)	[redacted] [redacted] 2008 (HAG) ASB2012-11389
Rat, HanRcc:WIST	2 x 3 ♀/2000	Slightly ruffled fur	> 2000 (limit test)	[redacted], 2007 (NUF) ASB2012-11390
Rat, Sprague Dawley	5/sex/5000	diarrhea, apparent urinary incontinence and hair loss on the abdomen	> 5000	[redacted], 1988 (MON) Z35389
Rat, Wistar	5/sex/2500 5/sex/3500 5/sex/5000 5/sex/7000 5/sex/9900	Mortalities: 1/10 1/10, 3/10,7/10, 10/10 at 2500, 3500, 5000, 7000 and 9900 mg/kg bw; clinical signs: ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy, and fecal staining of the abdomen	> 5000	[redacted]., 1979 (MON) Z35541, *supplementary*
Rat	5/sex/5000	No findings	> 5000	[redacted] 1996 (SYN) TOX2000-1982
Rat	3 females /5000	Ruffled fur, hunched posture	> 5000	[redacted] 2007 (SYN) ASB2012-11391
Rat	3 females /5000	No findings	>5000	[redacted] 2011 (SYN) ASB2012-11392

The acute toxicity of glyphosate is low in all species tested. The oral and dermal LD_{50} was above 2000 mg/kg bw. General signs of oral intoxication were breathing difficulties, reduced

activity, ataxia, piloerection, convulsions and hunched posture. For further details please refer to Volume 3, B.6.2.1.

RMS proposes to base risk assessment on the lowest limit LD_{50} of >2000 mg/kg bw/d in order to take into account several aspects: In all acute test performed including the higher test rat of 5000 mg/kg /bw strong clinical signs were observed including ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, diarrhoea, piloerection, lethargy, and fecal staining of the abdomen, decreased spontaneous motor activity and sedation and crouching position.
A few acute tests were performed and the majority resulted in a LD_{50} >2000 mg/kg bw, see Summary of acute glyphosate acid oral toxicity for mammals table 9.3.1. As for animal welfare reason , the assessment of product toxicity can be based on the toxicity of the active substance. Considering the extent of glyphosate use in the EU and being aware of the loss of diversity e.g. in farmland bird species (please refer to Vol. 3, B.9.1.7.7) it is strongly proposed to follow the precautionary principle and base risk assessment on the lowest limit LD_{50} of >2000 mg/kg bw.

Table B.9.3-2: Summary of acute oral glyphosate IPA-salt toxicity for mammals (62 % glyphosate)

Species	**Number of animals / Dose levels (mg/kg bw)**	**Main effects**	**LD50 (mg/kg bw)**	**Reference**
Mice CD-1	5/sex/5000	Slight reduction in body weight gain in males at 5000 mg/kg/day versus controls	>5000	[redacted] 1995 (MON) ASB2012-11393
Rat, Sprague Dawley	5/sex/5000	n♀: anogenital staining, soft faeces or diarrhoea	> 5000 (limit test)	[redacted] 1999 (NUF) ASB2012-11394

Table B.9.3-3: Reproductive toxicity studies with rats provided for the present EU peer review assessment of glyphosate together with valid reproductive toxicity study endpoints reviewed in the first EU evaluation in 2001

Study type, strain, duration, route	**Dose levels**	**NO(A)EL (mg/kg bw/d)**	**LO(A)EL (mg/kg bw/d)**	**Targets/ Main effects**	**Reference**
Two-gen., Sprague-Dawley, diet	0, 1500, 5000, 15000 ppm	Parental, reproductive, offspring: 5000 ppm **(351 mg/kg bw/d)**	Parental, reproductive, off-spring: 15000 ppm (1000-1600 mg/kg bw/d)	Parental.: liver, kidney wt↑; Repro: homogenisation resistant spermatid count↓; Offspring: delay in preputial separation in F1 males	[redacted] 2007; ASB2012-11494
Two-gen., Wistar-derived AlpK, diet	0, 1000, 3000, 10000 ppm	Parental, offspring: 3000 ppm **(293 mg/kg bw/d)** Reproductive: 10000 ppm **(985 mg/kg bw/d)**	Parental, offspring: 10000 ppm (985 mg/kg bw/d), reproductive: not established	Parental, offspring: bw↓ F1 pups & F1-adults	[redacted] 2000; TOX2000-2000
Two-gen., Sprague-Dawley, diet	0, 1200, 6000, 30000 ppm	Parental, offspring: 6000 ppm (417 mg/kg bw/d), reproductive: 30000 ppm **(> 2000 mg/kg bw/d)**	Parental, offspring: 30000 ppm (> 2000 mg/kg bw/d), reproductive: not established	Parental: loose stool, bw↓, caecum distention, organ wt changes; offspring: bw↓, caecum distention	[redacted] 1997; ASB2012-11495
Two-gen., Wistar rat, diet	0, 10, 100, 1000, 10000 ppm	Parental, offspring & reproductive: 10000 ppm **(700-800 mg/kg bw/d)**	-	No treatment related effects	[redacted] 1993; TOX9300009; ADM *
Two-gen., Sprague-Dawley, diet	0, 1000, 3000, 10000 ppm	Parental, offspring: 3000 ppm **(197 mg/kg bw/d);** reproductive: 10000 ppm **(668 mg/kg bw/d)**	Parental, offspring: 10000 ppm (668 mg/kg bw/d)	Parental: bw↓, food & water ↑, cellular alterations of salivary glands in F0/F1 m/f	[redacted] 1992; TOX9552389
Two-gen., Sprague – Dawley rat, diet	0, 2000, 10000, 30000 ppm	Parental, offspring & reproductive: 10000 ppm **(720-760 mg/kg bw/d)**	Parental, offspring & reproductive: 30000 ppm (~2000 mg/kg bw/d)	Parental: bw gain↓, soft stool, reproductive: litter size ↓; (equivocal) offspring: bw gain↓	[redacted] 1990; TOX9552387

*Study now considered supplementary no effect dose, NOAELs

The overall assessment of reproductive toxicity of glyphosate by the RMS is provided in the RAR, Vol. 1, chapter 2.6.7 and Vol. 3, B.6.6. A summary is given below under point B.9.3.2. The reproductive mammalian toxicity of glyphosate was tested in several multi-generation studies in rats only.

The study of Brooker et al. (1992) (TOX9552389) showed the lowest NOAEL for parental and offspring toxicity. The NOAEL for parental and offspring toxicity is calculated to be 3000 ppm (197 mg/kg bw/d) based on increased food and water consumption in F1 females, lower body weight of F1 males and an increased incidence of cellular alteration of the parotid (males and females) and submaxillary (females) salivary gland in both F0 and F1 adults at 10000 ppm (Evaluation in 2001 confirmed, for more details please refer to Vol.3, chapter B6.6). For these effects a population relevance can not be excluded. Recently submitted reproductive toxicity studies show an ‘overall’ parental NOAEL in the magnitude of 300-400

mg/kg bw/day ([redacted] 1997, ASB2012-11495; [redacted] 2000, TOX2000-2000; [redacted], 2007, ASB2012-11494).

No reproductive toxicity studies in rabbits were provided nor in the present EU evaluation of glyphosate nor with the first submission in 2001. For further details please refer to Vol.1, 2.6.7.2 and Vol. 3, B.6.6.

Table B.9.3-4: Developmental toxicity studies in rats and rabbits reviewed in the first EU evaluation of glyphosate in 2001 and new studies in rats submitted by the notifier for the present re-evaluation

Study type, strain, duration, route	Dose levels	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Targets/ Main effects	Reference
rabbit, Alpk (Wi-star derived), gavage, d 7-16 p.c.	0, 250, 500, 1000 mg/kg bw/d	Maternal & dev.: 1000 mg/kg bw/d	Not established	None	[redacted] 1996; TOX2000-2001 and 2002 ASB2012-10080;
rat, CD (SD),, gavage, d 6-15 p.c.	0, 30, 300, 1000 mg/kg bw/d	Materna l & dev.: 300 mg/kg bw/d	Maternal & dev.: 1000 mg/kg bw/d	Maternal: Loose stool Dev.: skeletal anomalies↑	[redacted] 1995 ASB2012-11497; 940908-1
rabbit, Charles River, gavage, d 6-19 p.c.	0, 300, 1000, 3500 mg/kg bw/d	Maternal & dev.: 1000 mg/kg bw/d	Maternal & dev. 3500 mg/kg bw/d	Maternal:mortality, soft stool, diarrhea, Dev.: bw↓, post implantation losses	[redacted], 1980; TOX9552392
Developmental, Dutch Belted rabbit, d 6-27 p.c.,	350 mg/kg bw/d	mg/kg bw/d, dev.: 175 mg/kg bw/d	Maternal: 175 mg/kg bw/d, dev.: not established due to low number of foetuses	Maternal: mortality, soft stool, Diarrhea; dev.: none	[redacted] 1980; TOX9552390; Lot XHJ-64, 98.7%; Monsanto
rat, CD, gavage, d 6-15 p.c.	0, 300, 1000, 3500 mg/kg bw/d	Maternal & developmental: 300 mg/kg bw/d	Maternal & dev.: 1000 mg/kg bw/d	Maternal: slight bw gain↓, noisy respiration (2/25); Dev.: ossification↓, skeletal anomalies	[redacted] 1991, TOX9552393
rabbit, Wistar, gavage, d 6-15 p.c.	0, 1000 mg/kg bw/d	Maternal: 1000 mg/kg bw/d Dev. < 1000mg/kg bw/d	Maternal: not established Dev.: 1000 mg/kg bw/d	Maternal: no effects; Dev.: ossification↓	[redacted] 1991, TOX9551105; FSG 03090 H/05.
rabbit, NZW rabbit, d 6-18 p.c., gavage	0, 125, 250, 500 mg/kg bw/d	Maternal & dev.: 250 mg/kg bw/d	Maternal & dev. 500 mg/kg bw/d	Maternal: food, bw↓, abortion; Dev.: dead foetuses, malformations (external, visceral & skeletal)	[redacted] 1989; TOX9551960 *
rabbit, NZW rabbit, d 8-20 p.c., gavage	0, 100, 175, 300 mg/kg bw/d	Maternal: 100 mg/kg bw/d, Dev.: 175 mg/kg bw/d	Maternal: 175 mg/kg bw/d Dev.: 300 mg/kg bw/d	Maternal: food, bw gain ↓, clinical signs, dev.: foetal wt & ossification ↓	[redacted] 1996; TOX2000-2002
Developmental, Japanese White rabbits (Kbl:JW), d 6-18 p.c., gavage	0, 10, 100, 300 mg/kg bw/d	Maternal: 100 mg/kg bw/d, Developmental: 300 mg/kg bw/d	Maternal: 300 mg/kg bw/d, Developmental: not Established	Maternal: Loose stool, abortion, 1 doe died; Dev.: none	[redacted] 1995, ASB2012-11498

Study type, strain, duration, route	**Dose levels**	**NO(A)EL (mg/kg bw/d)**	**LO(A)EL (mg/kg bw/d)**	**Targets/ Main effects**	**Reference**
Developmental, NZW rabbit, d 7-19 p.c., gavage	0, 50, 200, 400 mg/kg bw/d	Maternal & dev.: 50 mg/kg bw/d	Maternal & dev.: 200 mg/kg bw/d	Maternal: bw gain ↓, Dev.: post-implantation loss	[redacted], 1996; ASB2012-11499; H95D161A

*Study supplementary due ot severe reporting deficiencies,; please refer to RAR, Vol 1, 2.6.7

The developmental toxicity and teratogenicity of glyphosate were tested in several studies in rats and rabbits.

In rats, the lowest NOAEL for both maternal and developmental effects was 300 mg/kg bw/day based on the study by [redacted]. (1991) (TOX9552393). Evidence of delayed ossification and increased incidence of foetuses with skeletal anomalies was observed at 1000 mg/kg bw/day. In addition, two new studies have been performed and were provided . At least the study of [redacted] (1995, ASB2012-11497) supported the previously established overall NOAEL of 300 mg/kg bw/day for both maternal and developmental effects. For further details please refer to Vol.1, 2.6.7.2 and Vol. 3, B.6.6.

For the test species rabbit, five developmental studies submitted and evaluated in the first EU evaluation of glyphosate in 2001 have been re-evaluated (please refer to Vol. 3, B.6.6).

The study by [redacted] (1993, TOX9551106) and the study by [redacted] (1980, TOX2552390) were considered supplementary, mainly because the outcome was compromised by high maternal mortality. The study by [redacted] (1989, TOX9551960) is now considered supplementary due to serious reporting deficiencies (*e.g.* no individual data, no results of maternal necropsy).

By contrast, the study by [redacted]. (1991, TOX9552391) is still considered acceptable and the NOAELs for maternal toxicity of 50 mg/kg bw/day as well as for developmental toxicity of 150 mg/kg bw/day were confirmed. The developmental NOAEL was established because of a higher frequency of late embryonic death at the highest dose level that was significantly elevated over the control value and was just at the upper edge of the historical control range. Furthermore, total embryonic losses were increased in all treated groups.

In addition, three new studies in rabbits were provided.

In the study by [redacted] (1995, ASB2012-11498), the NOAEL for maternal toxicity was 100 mg/kg bw/day, based on obviously gastrointestinal disturbances and a single death at 300 mg/kg bw/day. No effects on development were reported and, therefore, the highest dose of 300 mg/kg bw/day is considered the foetal NOAEL.

The study by [redacted] (1996, TOX2000-2002) showed a NOAEL for maternal toxicity of 100 mg/kg bw/day was based on clinical signs, reduced food consumption and body weight gain at the mid dose level of 175 mg/kg bw/day and above. Developmental toxicity comprised reduced foetal body weight and reduced ossification at 300 mg/kg bw/day, resulting in a foetal NOAEL of 175 mg/kg bw/day.

[redacted] (1996, ASB2012-11499) determined a maternal NOAEL of 50 mg/kg bw/day, based on decreased body weight gain at 200 mg/kg bw/day and above. The NOAEL for developmental toxicity was set at the same dose because of increased post-implantation losses at the two upper dose levels. Due to some reporting deficiencies, it remains unclear whether the heart was part of visceral examination.

B.9.3.3 Risk assessment

The wild mammal risk assessment was carried out according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009), which follows a tiered approach to assess the effects of plant protection products based on the requirements of Regulation 544/2011 and Regulation 545/2011 for active substances and plant protection products, respectively.The mammalian toxicity studies with the active substance glyphosate acid, the formulation MON 52276 and the metabolite AMPA are summarised in the table below. Detailed descriptions of toxicological studies with mammals are given in part RAR, Vol. 1 chapter 2.6.7.1.
The acute mammalian toxicity of glyphosate is low in the species tested. The oral LD_{50} was above 2000 mg/kg bw. General signs of oral intoxication were breathing difficulties, reduced activity, ataxia, piloerection, convulsions and hunched posture. For further details please refer to Volume 3, B.6.2.1. For risk assessment an LD_{50} >2000 mg/kg bw is proposed.

The reproductive toxicity of glyphosate was tested in several multi-generation studies in rats. The study of [redacted]. (1992) (TOX9552389) showed the lowest NOAEL for parental and offspring toxicity. The NOAEL for parental and offspring toxicity is calculated to be 3000 ppm (197 mg/kg bw/d) based on increased food and water consumption in F1 females, lower body weight of F1 males and an increased incidence of cellular alteration of the parotid (males and females) and submaxillary (females) salivary gland in both F0 and F1 adults at 10000 ppm (Evaluation in 2001 confirmed, for more details please refer to Vol.3, chapter B6.6). For these effects a population relevance can not be excluded.

No reproductive toxicity studies in rabbits were provided. The developmental toxicity and teratogenicity of glyphosate were tested in several studies in rats and rabbits. The overview of the studies shows a particular vulnerability of pregnant female rabbits .

Overview of reproductive, maternal and developmental toxicity of glyphosate

Study type	**Dose levels**	**NO(A)EL (mg/kg bw/d)**	**LO(A)EL (mg/kg bw/d)**	**Targets/ Main effects**	**Reference**
Develop-mental, NZW rabbit, gavage	0, 20, 100, 500 mg/kg bw/d	Maternal: **20 mg/kg bw/d.** dev.: **100 mg/kg bw/d**	Maternal: 100 mg/kg bw/d; dev.: not established due to low number of foetuses	Maternal: mortality, soft/liquid stool; dev.: no clear-cut effects up to 100 mg/kg bw/d, high dose group excluded due to low number of foetuses/ litters	[redacted] 1993; TOX9551106; **Study sup-plementary;**
Develop-mental, rabbit, gavage	0, 50, 150, 450 mg/kg bw/d	Maternal: **50 mg/kg bw/d,** dev.: **150 mg/kg bw/d**	Maternal: 150 mg/kg bw/d, dev.: 450 mg/kg bw/d	NOAEL for maternal toxicity is considered 50 mg/kg bw/d based on slightly restricted inappetence, slightly reduced body weight gain and soft/liquid faeces at 150 mg/kg bw/d. The NOAEL for developmental toxicity is considered to be 150 mg/kg bw/d based on the post-implantation loss, late embryonic death and an increase in cardiac malformations at 450 mg/kg bw/d. Maternal: GI-tract, food & bw gain ↓: dev.: late embryonic death, post implanta-tion loss, cardiac malforma-tions*	[redacted] 1991; TOX9552391;
Develop-mental, rabbit, gavage	0, 50, 200, 400	Maternal & dev.: **50 mg/kg bw/d**	Maternal: 175 mg/kg bw/d Dev.: 300 mg/kg bw/d	Maternal: bw gain ↓, Dev.: post-implanta-tion loss	[redacted] 1996; ASB2012-11499;

Study type	**Dose levels**	**NO(A)EL**	**LO(A)EL**	**Targets/ Main effects**	**Reference**
	mg/kg bw/d				
Develop-mental, rabbit, gavage	0, 100, 175, 300 mg/kg bw/d	Maternal: **100 mg/kg bw/d**, Dev.: **175 mg/kg bw/d**	Maternal: 175 mg/kg bw/d Dev.: 300 mg/kg bw/d	Maternal: food, bw gain ↓, clinical signs, dev.: foetal wt & ossification ↓	[redacted] 1996; TOX2000-2002
Devleop-mental, rabbit, gavage	0, 125, 250, 500 mg/kg bw/d	Maternal& dev.: **250 mg/kg bw/d**	Materna l & dev. 500 mg/kg bw/d	Maternal: food, bw↓, abortion; Dev.: dead foetuses, malforma-tions (external, visceral & skeletal)	[redacted] 1989; TOX9551960; **Study supple-mentary due ot severe reporting deficiencies,**
Develop-mental, CD (SD),, gavage, .	0, 30, 300, 1000 mg/kg bw/d	Maternal & dev.: **300 mg/kg bw/d**	Maternal& dev: 1000 mg/kg bw/d	At the highest dose level, maternal toxicity was confined to loose stool and in the foetuses a slight increase in lumbar ribs were observed (11 out of 7 litters compared to 4 out of 2 litters in control animals). Therefore, the NOAEL for maternal and developmental toxicity is considered to be 300 mg/kg bw/d, despite absent statistical significance for the findings in foetuses.	[redacted] 1995 ASB2012-11497;
Two-gen., rat; diet	0, 1000, 3000, 10000 ppm	Parental, offspring: 3000 ppm **(197 mg/kg bw/d);** reproductive: 10000 ppm **(668 mg/kg bw/d)**	Parental, offspring: 10000 ppm (668 mg/kg bw/d)	The NOAEL for parental and offspring toxicity is calculated to be 3000 ppm (197 mg/kg bw/d) based on increased food and water consumption in F1 females, lower body weight of F1 males and an increasedincidence of cellular alteration of the parotid (males and females) and submaxillary (females)salivary gland in both F0 and F1 adults at 10000 ppm Parental: bw↓, food & water ↑, cellular alterations of salivary glands in F0/F1 m/f	[redacted] 1992; TOX9552389
Two-gen., Rat; diet	0, 1000, 3000, 10000 ppm	Parental, offspring: 3000 ppm **(293 mg/kg bw/d)** Reproductive: 10000 ppm **(985 mg/kg bw/d)**	Parental, offspring: 10000 ppm (985 mg/kg bw/d), reproductive: not established	The NOAEL 3000 ppm (293 mg/kg bw/d) was set for both offspring and parents (F1) on the basis of a reduction in body weight of F1 pups and a subsequent reduction in body weight of in F1 males. Parental, offspring: bw↓ F1 pups & F1-adults	[redacted] 2000; TOX2000-2000
Two-gen., rat; diet	0, 1500, 5000, 15000 ppm	Parental, reproductive, offspring: 5000 ppm **(351 mg/kg bw/d)**	Parental, reproductive, off-spring: 15000 ppm (1000-1600 mg/kg bw/d)	At highest dose level of15000 ppm increased organ weights in liver (F0 & F1 females) and kidneys (F0 females), a significant decrease in homogenisation resistant spermatids were observed and in F1 male offsprings sexual maturation (preputial separation) was delayed. Parental.: liver, kidney wt↑; Repro: homogenisa-tion resistant spermatid count↓; Offspring: delay in preputial separation in F1 males	[redacted] 2007; ASB2012-11494
Two-gen., rat, diet	0, 2000, 10000 30000 ppm	Parental, offspring & reproductive: 10000 ppm **(720-760 mg/kg bw/d)**	Parental, offspring & reproductive: 30000 ppm (~2000 mg/kg bw/d)	Parental: bw gain↓, soft stool, reproductive: litter size ↓; (equivocal) offspring: bw gain↓	[redacted] 1990; TOX9552387
Two-gen., rat, diet	0, 10, 100, 1000,	Parental, offspring & reproductive: 10000 ppm	-	No treatment related effects	[redacted] 1993; TOX9300009; FSG *

Study type	Dose levels	NO(A)EL	LO(A)EL	Targets/ Main effects	Reference
	10000 ppm	**(700-800 mg/kg bw/d)**			
Develop-mental; rabbit, gavage,	0, 250, 500, 1000 mg/kg bw/d	Maternal & dev.: **1000 mg/kg bw/d**	Not established	None	[redacted] 1996; TOX2000-2001 ASB2012-10080;
Two-gen., rat; diet	0, 1200, 6000, 30000 ppm	Parental, offspring: 6000 ppm (417 mg/kg bw/d), reproductive: 30000 ppm **(> 2000 mg/kg bw/d)**	Parental, offspring: 30000 ppm (> 2000 mg/kg bw/d), reproductive: not established	Parental: loose stool, bw↓, caecum distention, organ wt changes; offspring: bw↓, caecum distention	[redacted] 1997; ASB2012-11495

The overall NOAEL for maternal toxicity was 50 mg/kg bw/d as found by [redacted] (1996, ASB2012-11499) and by [redacted] (1991, TOX9552391). It is further supported by the (supplementary) study of [redacted] (1980, TOX2552390) who had established 75 mg/kg bw/day and is well between the NOAEL of 20 mg/kg bw/day and the LOAEL in the (supplementary) study by [redacted]. (1993, TOX9551106). The lowest NOAEL for developmental effects was 50 mg/kg bw/day, based on post-implantation loss at 200 mg/kg bw/day in the study by [redacted] (1996, ASB2012-11499). The NOAEL for developmental toxicity was the same because of increased post-implantation losses at the two upper dose levels. Due to dose spacing, the NOAELs in other studies were higher [redacted] 1980, TOX2552390; [redacted] 1991, TOX9552391; [redacted] 1996, TOX2000-2002) but consistently below 200 mg/kg bw/day. Beside post-implantation losses and late embryonic death, which can be pointed out to display effects related to population level ([redacted], 1989, TOX9551960; [redacted] 1991, TOX9552391), developmental findings at higher dose levels included a lower foetal weight and delayed ossification.

~~The lowest NOAEL for developmental effects was 50 mg/kg bw/day, based post-implantation loss at 200 mg/kg bw/day in the study by [redacted] (1996, ASB2012-11499). Due to dose spacing, the NOAELs in other studies were higher ([redacted] 1980, TOX2552390; [redacted] 1991, TOX9552391; [redacted], 1996, TOX2000-2002) but consistently below 200 mg/kg bw/day. Beside post-implantation losses and late embryonic death ([redacted] 1989, TOX9551960; [redacted] 1991, TOX9552391), developmental findings at higher dose levels included a lower foetal weight and delayed ossification (please refer to RAR, Vol. 1, chapter 2.6.7.2.2.).~~

This endpoint is proposed for risk assessment, because the (pregnant) rabbit proved to be more sensitive than rats (factor 6 between the lowest NOAEL in rats and rabbits) and the importance of the observed effects at population level cannot be ignored.

It is assumed that a 12-day exposure in the study by [redacted] (1996, ASB2012-11499) mimics a realistic worse case scenario in the field for small herbivorous mammals. Information from reproductive or subchronic toxicity studies show that no avoidance of glyphosate acid on food consumption in rats and mice is observed ([redacted] 2007, ASB2012-11494, [redacted] 1996, TOX2000-1990, [redacted] 1995, ASB2012-11453). It is considered a worse case as the glyphosate was administered by gavage, probably not representing the normal feeding behaviour. Under realistic conditions feeding would occur in

lower doses averaged over the day. One the other hand information from reproductive or subchronic toxicity studies show that no avoidance of glyphosate acid on food consumption in rats and mice is observed ([redacted]., 2007, ASB2012-11494, [redacted] 1996, TOX2000-1990, [redacted] 1995, ASB2012-11453) and therfore high consumption levels might be possible. Besides it should be taken into account that alternative natural food sources are not abundant in all conditions when glyphosate is supposed to be implemented in large areas, as well as repeatedly during the season. Therefore the consumtion of tested doses of contaminated food for a period of 12 days might generally understandable and mimic a worse case scenario during rabbit pregnancy. Due to a significant increase in foetal deaths a population relevance can not be excluded.

RMS Conclusions

An overall NOAEL of 50 mg/kg bw/day is proposed and considered for risk assessment for the following reasons:

- The endpoint is in line with the endpoint on human long-term risk assessment.
- The endpoint mirrors effects relevant at population level (e.g. post implantation losses).
- Information from reproductive and subchronic toxicity studies show that no avoidance of food treated with glyphosate acid is observed in rats, mice and rabbits.
- High consumption levels are therefore not excluded in a field situation.
- Alternative natural food sources might not always be available in the field, considering that intended uses of glyphosate are to be expected in all crops and over large areas, as well as repeatedly during the season.
- Consumption of contaminated food for a period of 12 days as in the considered studies mimics a realistic worst case scenario during e.g. rabbit pregnancy.

B.9.3.3.1 Risk from dietary exposure

The first-tier risk assessment for mammals is performed by calculating TER values based on dietary exposure according to the scenarios as provided by the current EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009). The relevant crop group for the intended use in all crops of glyphosate as well as the corresponding shortcut values for estimating the daily dietary dose of glyphosate from uptake of contaminated food and, finally, the resulting TER values are summarised in tables below.

According to GAP, in the intended use ‘Orchard, Vine’ the glyphosate containing product is applied round the base of trunk and to the intra-rows (inner strips between two trees within a row) and strips between the rows are left untreated. Over-sprayed grasses and herbs as well as untreated plant material might thus be available for small herbivorous mammals. We consider the long-term exposure of small herbivorous mammals to contaminated material to be unlikely, because plant material in the treated parts will degrade due to the herbicidal activity glyphosate and alternative food sources is available. As a conservative estimate, we assume that application rates per ha are expressed per ‘unit of treated surface area’ and the actual application rate per ha orchard or vineyard will be roughly only 50 %. Consequently, the daily dietary doses were calculated with 50 % application rate.

Table B.9.3-5: Mammalian screening risk assessment for dietary exposure to glyphosate (acute toxicity, indicator species)

Crop and application timing	**Applic. Rate / treated area (ARTreated) (g a.s. /ha)**	**Applic. rate /cropped area (ARCropped) (50 % of (ARTreated) (g a.s. /ha)**	**Scenario**	**Generic focal species**	**Shortcut value × MAF**	**Daily dietary dose (mg a.s./kg bw/d)**	**TER**
Acute toxicity: LD_{50} > 2000 mg a.s./kg bw							
All crops (all seeded or transplanted crops) Preplanting of crop	Max. 2 × 2160 g a.s./ha min 21 d interval	-	Crops, cereals, oilseed rape,	Small herbivorous mammal	118.4 × 1.2	306.9	> **6**
			Bare soil	Small granivorous mammal	14.4 ×1.2	37.32	> 54
All crops (all seeded crops) Post planting/pre emergence of crop	1 × max. 1080 g a.s./ha	-	Crops, cereals, oilseed rape	Small herbivorous mammal	118.4	127.9	> 15
Cereals (pre harvest) wheat, rye, triticale, barley and oats Crop maturity	1 × max. 2160 g a.s./ha	-	Cereals	Small herbivorous mammal	118.4	255.7	> **7**
Oilseed (pre harvest) rapeseed, mustard seed, linseed Crop maturity	1 × max. 2160 g a.s./ha	-	Oilseed rape	Small herbivorous mammal	118.4	255.7	> **7**
Orchard crops (vines including citrus and tree nuts) Post emergence of weeds 28 days interval bet.applic. **Intrarow & Spot treatment (50% applic. rate)** *	1 × max. 2880 g a.s./ha	1 × max. 1440 g a.s./ha	Orchards, Vineyard	Small herbivorous mammal	136.4	196.4*	> 10
	1- 3 applic e.g. 2 × max. 720 g a.i/ha +1 × max. 2880 g a.s./ha	1- 3 applic e.g. 2 × max. 360 g a.i/ha +1 × max. 1440 g a.s./ha			136.4 × 1.2 + 136.4	59.0 + 196.4 =254.4*	> **7**
	2 × max. 2160 g a.s./ha	2 × max. 1080 g a.s./ha			136.4 × 1.2	176.8*	> 11
	3 × max. 1440 g a.s./ha	3 × max. 720 g a.s./ha			136.4 × 1.3	127.7*	> 15
TER in bold do not meet the acceptability criteria.							

***** Because applications are made round base of trunk and to the intra-rows , (inner strips between two trees within a row), application rates per ha are expressed per 'unit of treated surface area' the actual application rate per ha orchard or vineyard will only be 50% of the reported rate. For application in orchards it is recommend that only the strip along the tree line will be treated (approx 33% to max. 50% of the cropped area) to create a weed free area. Consequently the alleys (inter-rows) are left as untreated permanent grassy strips with no exposure to glyphosate. In case the whole area is treated the full dose should be taken into account.

Table B.9.3-6: Mammalian screening risk assessment for dietary exposure to glyphosate (long-term/reproductive toxicity, indicator species)

Crop and application timing	Applic. Rate / treated area (ARTreated) (g a.s. /ha)	Applic. rate /cropped area (ARCropped) (50 % of (ARTreated) (g a.s. /ha)	Scenario	Generic focal species	Shortcut value × MAF x TWA	Daily dietary dose (mg a.s./kg b w/d)	TER
Long-term/reproductive toxicity: NOAEL = 50 mg a.s./kg bw/d							
All crops (all seeded or transplanted crops) Pre-planting of crop	Max. 2 × 2160 g a.s./ha min. 21 d interval	-	Crops, cereals, oilseed rape	Small herbivorous mammal	48.3 × 1.4 × 0.53	77.41	**0.7**
			Bare soil	Small granivorous mammal	6.6 × 1.4 × 0.53	10.58	**4.7**
All crops (all seeded crops) Post planting/pre-emergence of crop	1 × max. 1080 g a.s./ha	-	Crops, cereals, oilseed rape	Small herbivorous mammal	48.3 × 0.53	27.65	**1.8**
Cereals (pre-harvest) wheat, rye, triticale, barley and oats Crop maturity	1 × max. 2160 g a.s./ha	-	Cereals	Small herbivorous mammal	48.3 × 0.53	55.29	**0.9**
Oilseed (pre-harvest) rapeseed, mustard seed, linseed Crop maturity	1 × max. 2160 g a.s./ha	-	Oilseed rape	Small herbivorous Sammal	48.3 × 0.53	55.29	**0.9**
Orchard crops (vines including citrus and tree nuts) Post emergence of weeds 28 d. interval bet. applic. **Intrarow & Spot treatment (50% applic. rate) ***	1 × max. 2880 g a.s./ha	1 × max. 1440 g a.s./ha	Orchards Vineyard	Small herbivorous mammal	72.3 × 0.53	55.18*	**1**
	1- 3 applic. e.g. 2 × max. 720 g a.s/ha +1 × max. 2880 g a.s./ha	1- 3 applic. e.g. 2 × max. 360 g a.s/ha +1 × max. 1440 g a.s./ha			72.3 × 1.4 × 0.53 + 72.3 × 0.53	19.31 + 55.18 = 74.5*	**0.7**
	2 × max. 2160 g a.s./ha	2 × max. 1080 g a.s./ha			72.3 × 1.4 × 0.53	57.9*	**0.9**
	3 × max. 1440 g a.s./ha	3 × max. 720 g a.s./ha			72.3 × 1.5 × 0.53	41.4*	**1.2**
TER in bold do not meet the acceptability criteria.							

***** Because applications are made round base of trunk and to the intra-rows , (inner strips between two trees within a row), application rates per ha are expressed per 'unit of treated surface area' the actual application rate per ha orchard or vineyard will only be 50% of the reported rate. For application in orchards it is recommend that only the strip along the tree line will be treated (approx 33% to max. 50% of the cropped area) to create a weed free area. Consequently the alleys (inter-rows) are left as untreated permanent grassy strips with no exposure to glyphosate. In case the whole area is treated the full dose should be taken into account.

The screening steps showed an unacceptable acute and long-term risk for the indicator species small herbivorous mammal, indicating a need for further assessment.

A Tier 1 risk assessment is required, if the TER values calculated in the screening assessment are below the Annex VI trigger of concern of 10 for acute and 5 for long-term exposure. Tier 1 adresses more realistic exposure estimates along with a more representative 'generic focal species'.

B.9.3.3.2 Tier 1 acute risk assessment

Generic Focal species:
The small herbivorous mammal "vole" should be considered in the risk assessment for mammals as 'generic focal species' for the intended use in crops, cereals and orchards (EFSA, 2009). This species has the worst case exposure and the assessment covers other species with lower exposure risk. Since voles are not required as focal species in all EU-member states, a more detailed risk assessment with additional generic focal species is added for a possible risk envelope approach.

MAF
For the assessment of the acute risk, multiple application factors for 90th percentile residue data (MAF_{90}) for an application intervals of 21 days and and 2 applications (considering a default DT_{50} of 10 days on foliage) were calculated accorging to EFSA Guidance Document on Risk Assessment for Birds and Mammals, Appendix H (2009).

Table B.9.3-7: Mammalian Tier 1 risk assessment for dietary exposure to glyphosate (acute toxicity, worst-case generic focal species)

Crop and application timing	Applic. Rate / treated area (ARTreated)	Applic. rate /cropped area (ARCropped) (50 % of (ARTreated)	Generic focal species	Scenario	Shortcut value × MAF	Daily dietary dose	TER
Acute toxicity: LD_{50} > 2000 mg a.s./kg bw							
All crops (all seeded or transplanted crops) Pre planting of crop	Max. 2 × 2160 g a.s./ha min. 21 d interval	-	Small herbiv. "vole"	Cereals	40.9 × 1.14	100.40	> 20
			Large herbivorous "lagomorph"		42.1× 1.14	103.35	>19
			Large herbiv. "lagomorph"	Oilseed rape (all season) Sunflower, Potatoes	35.1 × 1.14	86.17	>23
			Small herbiv. "vole"	Hop, Root & stem vegetables	40.9 × 1.14	100.40	> 19
			Small omniv. "mouse"		17.2× 1.14	42.224	>47

Crop and application timing	**Applic. Rate / treated area (ARTreated)**	**Applic. rate /cropped area (ARCropped) (50 % of (ARTreated)**	**Generic focal species**	**Scenario**	**Shortcut value × MAF**	**Daily dietary dose**	**TER**
Grassland	Max. 1× 2160 g a.s./ha	-	Small herbiv.s "vole"	Grass-land	136.4	294.62	**>7**
			Large herbiv. "lagomorph"		32.6	70.41	>28
	1 × max. 1440 g a.s./ha		Small herbiv. "vole"		136.4	197.23	>10
			Large herbiv. "lagomorph"		32.6	46.94	>42
Cereals (pre harvest) wheat, rye, triticale, barley and oats Crop maturity	1 × max. 2160 g a.s./ha	-	Small herbiv."vole"		40.9	88.34	> 23
			Small omnivorous "mouse"		5.2	11.23	>178
Oilseed (pre harvest) rapeseed, mustard seed, linseed Crop maturity	1 × max. 2160 g a.s./ha	-	Small herbivorous "vole"	Oilseedrape	34.1	73.66	> 27
			Large herbiv. "lagomorph"		35.1	75.82	> 26
Orchard crops (vines including citrus & tree nuts) Post emergence of weeds 28 d.interval bet.applic. **Intrarow & Spot treatment (50% applic. rate) ***	1 × max. 2880 g a.s./ha	1 × max. 1440 g a.s./ha	Small herbivorous "vole"	Orchards	136.4	196.42*	> 10
	1- 3 applic. e.g. 2 × max. 720 g a.i/ha +1 × max. 2880 g a.s./ha	1- 3 applic. e.g. 2 × max. 360 g a.i/ha +1 × max. 1440 g a.s./ha			136.4 × 1.08 + 136.4	52.9 + 196.42 =249.3*	**> 8**
	2 × max. 2160 g a.s./ha	2 × max. 1080 g a.s./ha			136.4 × 1.08	159.09*	> 12
	3 × max. 1440 g a.s./ha	3 × max. 720 g a.s./ha			136.4 × 1.09	107.05*	> 18
	1 × max. 2880 g a.s./ha	1 × max. 1440 g a.s./ha	Large herbiv. "lagomorph"		35.1	50.54*	>39
	1- 3 applic. e.g. 2 × max. 720 g a.i/ha +1 × max. 2880 g a.s./ha	1- 3 applic. e.g. 2 × max. 360 g a.i/ha +1 × max. 1440 g a.s./ha			35.1× 1.08 + 35.1	13.66 + 50.54*= 64.20*	>13
	2 × max. 2160 g a.s./ha	2 × max. 1080 g			35.1× 1.08	40.98*	>48

Crop and application timing	**Applic. Rate / treated area (ARTreated)**	**Applic. rate /cropped area (ARCropped)** **(50 % of (ARTreated)**	**Generic focal species**	**Scenario**	**Shortcut value × MAF**	**Daily dietary dose**	**TER**
		a.s./ha					
	3 × max. 1440 g a.s./ha	3 × max. 720 g a.s./ha			35.1 × 1.09	27.60	>72
TER in bold do not meet the acceptability criteria.							

***** Because applications are made round base of trunk and to the intra-rows (inner strips between two trees within a row), application rates per ha are expressed per 'unit of treated surface area' the actual application rate per ha orchard or vineyard will only be 50% of the reported rate. For application in orchards it is recommend that only the strip along the tree line will be treated (approx 33% to max. 50% of the cropped area) to create a weed free area. Consequently the alleys (inter-rows) are left as untreated permanent grassy strips with no exposure to glyphosate. In case the whole area is treated the full dose should be taken into account.

Based on the presumptions of Tier 1, the calculated TER values for the risk resulting from an exposure of mammals to the active substance glyphosate according to the GAP of the formulation MON 52276 reach the acceptability criteria TER ≥ 10, according to Regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2. The results of the assessment indicate an acceptable risk for mammals.

In the orchard scenario a serial application of 2×max. 720 g a.s./ha and 1×max. 2880 g a.s./ha indicates a risk (TER<10) for the genereic focal species "small herbivorous mammals" due to uptake of contaminated food. The TER is >10 for the following usage patterns: 1×2880 kg/ha, 2×2160 g/ha and 3×1440 g/ha.
Nevertheless, it can be asumed that the calculation of TER is in that case too conservative and the DDD is overestimated. Taking into consideration that the third application differs from the others the use of MAF for the calculation of DDD can be modified. The MAF values for 3 applications with an interval of 28 days was calculated using the formula in Appendix H, which results in a MAF of 1.09. The value 1 corresponds to the residues from last application whereas the value 0.09 to the residues level from the first and the second application at time of the third application. The DDD can be calculated as a sum of DDD resulting from the two first applications 0.36 x 136.4 x 0.09 and the third one 1.44 x 136.4 x 1. For the usage scenario 2x720 g a.s./ha + 2880 g a.s./ha the following DDD can be calculated:
DDD = (0.72/2) x 136.4 x 0.09 + (2.88/2) x 136.4 = 4.4 + 196.4 =200.8
The resulting TER is > 10.

In the grassland scenario the application of 2160 g a.s./ha indicates a risk (TER<10) for small herbivorous mammals due to uptake of contaminated food. The TER is >10 for the usage pattern of max. 1440 g a.s./ha.

B.9.3.3.3 Tier 1 long-term risk assessment

Generic Focal species:
For the use in crops, cereals and orchards, the small herbivorous mammal "mouse" should be considered as respective 'generic focal species'in the risk assessment according to EFSA

(2009). This species has the worst case exposure and the assessment covers other species with lower exposure risk.

Table B.9.3-8: Mammalian Tier 1 risk assessment for dietary exposure to glyphosate (long-term toxicity, worst-case generic focal species)

Crop and application timing	**Applic. Rate / treated area (ARTreated)**	**Applic. rate /cropped area (ARCropped)** (50% of **(ARTreated)**	**Generic focal species**	**Scenario**	**Shortcut value × (MAF × TWA)**	**Daily dietary dose**	**TER**
Long-term/reproductive toxicity: NOAEL = 50 mg a.s./kg bw/d							
All crops (all seeded or transplanted crops) Pre planting of crop	Max. 2 × 2160 g a.s./ha min. 21 d interval	-	Small herbiv. "vole"	Cereals	21.7 × 0.65	30.45	**1.6**
			Large herbivorous "lagomorph"		22.3× 0.65	31.29	**1.6**
			Large herbiv. "lagomorph"	Oilseed rape (all season) Sunflower, Potatoes	14.3 × 0.65	20.07	**2.5**
			Small herbiv. "vole"	Hop, Root & stem vegetables	21.7 × 0.65	30.45	**1.6**
			Small omniv. "mouse"		7.8× 0.65	10.94	**4.6**
Grassland	Max. 1× 2160 g a.s./ha	-	Small herbiv."vole"	Grass-land	72.3 x0,53	82.26	**0.6**
			Large herbiv. "lagomorph"		17.3 x 0,53	19.80	**2.5**
	1 × max. 1440 g a.s./ha		Small herbiv. "vole"		72.3 x 0,53	54.84	**0.9**
			Large herbiv. "lagomorph"		17.3 x 0,53	13.20	**3.8**
Cereals (pre harvest) wheat, rye, triticale, barley and oats Crop maturity	1 × max. 2160 g a.s./ha	-	Small herbiv."vole"		21.7 x 0,53	24.69	**2.0**
			Small omnivorous "mouse"		7.8 x 0,53	8.88	5.6
Oilseed (pre harvest) rapeseed, mustard seed,	1 × max. 2160 g a.s./ha	-	Small herbivorous "vole"	Oilseedrape	18.1 x 0,53	20.60	**2.4**

Crop and application timing	**Applic. Rate / treated area (ARTreated)**	**Applic. rate /cropped area (ARCropped)** (50% of **(ARTreated)**	**Generic focal species**	**Scenario**	**Shortcut value × (MAF × TWA)**	**Daily dietary dose**	**TER**
linseed Crop maturity			Large herbiv. "lagomorph"		14.3 x 0,53	16.37	**3.1**
Orchard crops (vines including citrus & tree nuts) Post emergence of weeds 28 d.interval bet.applic. **Intrarow & Spot treatment (50% applic. rate) ***	1 × max. 2880 g a.s./ha	1 × max. 1440 g a.s./ha	Small herbivorous "vole"	Orchards	72.3 × 0.53	55.17*	**0.9**
	1- 3 applic. e.g. 2 × max. 720 g a.i/ha +1 × max. 2880 g a.s./ha	1- 3 applic. e.g. 2 × max. 360 g a.i/ha +1 × max. 1440 g a.s./ha			72.3 × 0.60 + 72.3 × 0.53	15.62 + 55.17 = 70.79*	**0.7**
	2 × max. 2160 g a.s./ha	2 × max. 1080 g a.s./ha			72.3 × 0.60	46.85*	**1.0**
	3 × max. 1440 g a.s./ha	3 × max. 720 g a.s./ha			72.3 × 0.61	31.75*	**1.6**
	1 × max. 2880 g a.s./ha	1 × max. 1440 g a.s./ha	Large herbiv. "lagomorph"		14.3	10.88*	**4.6**
	1- 3 applic. e.g. 2 × max. 720 g a.i/ha +1 × max. 2880 g a.s./ha	1- 3 applic. e.g. 2 × max. 360 g a.i/ha +1 × max. 1440 g a.s./ha			14.3× 0.60 + 14.3	3.44 + 10.88*= 14.24*	**3.5**
	2 × max. 2160 g a.s./ha	2 × max. 1080 g a.s./ha			14.3× 0.60	9.30*	5.4
	3 × max. 1440 g a.s./ha	3 × max. 720 g a.s./ha			14.3× 0.61	6.31*	7.9
TER in bold do not meet the acceptability criteria.							

***** Because applications are made round base of trunk and to the intra-rows (inner strips between two trees within a row), application rates per ha are expressed per 'unit of treated surface area' the actual application rate per ha orchard or vineyard will only be 50% of the reported rate. For application in orchards it is recommend that only the strip along the tree line will be treated (approx 33% to max. 50% of the cropped area) to create a weed free area. Consequently the alleys (inter-rows) are left as untreated permanent grassy strips with no exposure to glyphosate. In case the whole area is treated the full dose should be taken into account.

Based on Tier 1 assessment step, the calculated TER values for the long-term risk resulting from an exposure of mammals to glyphosate do not achieve the acceptability criteria TER ≥ 5. The results of the assessment indicate an unacceptable risk for small herbivorous mammals due to the intended uses according to the label.

A refined risk assessment is considered necessary.

B.9.3.3.4 Refined risk assessment

A refinement for the long-term risk is presented via the decay of glyphosate in plant material over time. Please refer to chapter B.9.13 for details on the calculation of the 21day time-weighted–average (twa) for glyphosate in grass foliage used in the mammalian risk assessment.

f_{twa}

The methodology used to calculate the Time Weighted Average (twa) for glyphosate in grass foliage for the long-term mammalian risk assessment follows the procedure described in the Guidance Document on Terrestrial Ecotoxicology (91/414/EEC, September 2002, SANCO/4145/2000).The decline of glyphosate residue in grass was characterised using data from 22 residue trials each of which had a Day 0 value, corresponding to the day of application. **The average DT_{50} of glyphosate on grass in the 22 trials was 2.8 days.** The 21-day time weighted average (twa) for glyphosate in grass foliage has been used to calculate a refined f_{twa}. The 21-Day twa is calculated to be **0.19** for the active substance glyphosate acid.The RMS still considers this acceptable. A f_{twa} of 0.19 will be used in the risk assessment for grass foliage.

Table B.9.3-9: Mammalian refined risk assessment for dietary exposure to glyphosate (long-term toxicity, worst-case generic focal species)

Crop and application timing	**Applic. Rate / treated area (ARTreated)**	**Generic focal species**	**Scenario**	**Shortcut × (MAF × TWA)**	**Daily dietary dose (mg a.s./kg bw/d)**	**TER**
Long-term/reproductive toxicity: NOAEL = 50 mg a.s./kg bw/d						
All crops (all seeded or transplanted crops) Pre- planting of crop	Max. 2 × 2160 g a.s./ha min. 21 d interval	Small herbivorous mammal "vole"	Cereals, Hop, Potatoes; Root & stem vegetables, sunflower	21.7 × 0.19	9.01	6
			Oilseed rape, Sunflower	18.1 × 0.19	7.52	7
		Large herbiv. "lagomorph"	Cereals, Hop, Potatoes; Root & stem vegetables, sunflower	22.3 × 0.19	9.21	5.4
			Oilseed rape, Sunflower	14.3× 0.19	5.9	8.5
Cereals (pre-harvest) wheat, rye, triticale, barley and oats Crop maturity	1 × max. 2160 g a.s./ha	Small herbivorous mammal "vole"	Cereals	21.7 × 0.19	9.02	6
		Large herbiv. "lagomorph"		22.3 × 0.19	9.21	5.4
Oilseed (pre-harvest) rapeseed, mustard seed, linseed Crop maturity	1 × max. 2160 g a.s./ha	Small herbivorous mammal "vole"	Oilseedrape	18.1 × 0.19	7.520	7
		Large herbiv. "lagomorph"		14.3 × 0.19	5.90	8.5

Crop and application timing	**Applic. Rate / treated area (ARTreated)**	**Generic focal species**	**Scenario**	**Shortcut × (MAF × TWA)**	**Daily dietary dose (mg a.s./kg bw/d)**	**TER**
Grassland	1 × max. 2160 g a.s./ha	Small herbivorous mammal "vole"	Grassland	72.3× 0.19	29.87	**1.7**
		Large herbiv. "lagomorph"		17.3 × 0.19	7.15	7.0
	1 × max. 1440 g a.s./ha	Small herbivorous mammal "vole"		72.3× 0.19	15.77	**3.2**
		Large herbiv. "lagomorph"		17.3 × 0.19	3.77	13
	1× max. 1080 g a.s./ha	Small herbivorous mammal "vole"		72.3× 0.19	14.9	**3.3**
Orchard crops (vines include citrus and tree nuts) Post-emergence of weeds 28 d interval bet. applic. **Intrarow & Spot treatment (50 % applic. rate) ***	1 × max. 2880 g a.s./ha	Small herbivorous mammal "vole"	Orchards, Vineyards	72.3 × 0.19	19.78*	**3**
	1- 3 applic. e.g. 2 × max. 720 g a.i/ha +1 × 2880 g a.s./ha			72.3 × 0.19 + 72.3 × 0.19	4.94 + 19.78 = 24.72*	**2**
	2 × max. 2160 g a.s./ha			72.3 × 0.19	14.83*	**3**
	1 × max. 2160 g a.s./ha			72.3 × 0.19	14.83*	**3**
	3 × max. 1440 g a.s./ha			72.3 × 0.19	9.89*	5
	1 × max. 2880 g a.s./ha	Large herbiv. "lagomorph"	Orchards, Vineyards	14.3 × 0.19	3.91*	13
	1- 3 applic. e.g. 2 × max. 720 g a.i/ha +1 × 2880 g a.s./ha			14.3 × 0.19 + 14.3 × 0.19	0,98 +3.91* =4.89	10
	2 × max. 2160 g a.s./ha			14.3 × 0.19	2.95*	17
	1 × max. 2160 g a.s./ha			14.3 × 0.19	2.95*	17

Crop and application timing	Applic. Rate / treated area (ARTreated)	Generic focal species	Scenario	Shortcut × (MAF × TWA)	Daily dietary dose (mg a.s./kg bw/d)	TER
	3 × max. 1440 g a.s./ha			14.3 × 0.19	1.97*	25
TER in bold do not meet the acceptability criteria.						

***** Because applications are made round base of trunk and to the intra-rows (inner strips between two trees within a row), application rates per ha are expressed per 'unit of treated surface area' the actual application rate per ha orchard or vineyard will only be 50% of the reported rate. For application in orchards it is recommend that only the strip along the tree line will be treated (approx 33% to max. 50% of the cropped area) to create a weed free area. Consequently the alleys (inter-rows) are left as untreated permanent grassy strips with no exposure to glyphosate. In case the whole area is treated the full dose should be taken into account.

Based on refined assessment step, the calculated TER values for the long-term risk resulting from an exposure of mammals to glyphosate with

- a serial of three applications with 2 × max.720 g a.s./ha and once max. 2880 g a.s./ha in orchards (application not crop directed, spot treatment and intra-row treatment with an reduced actual application rate of 50 %) as well as in the intended uses with
- a serial of two applications max. 2160 g a.s./ha and single application of 1× max. 2880 g a.s/ha
- one application of 1× max. 2160 g a.s./ha
- the application in the scenario grassland with 1× max. 2160 g a.s./ha, 1 × max. 1440 g a.s./ha and 1 × max. 1080 g a.s./ha

do not achieve the acceptability criteria TER ≥ 5. The results of the assessment indicate an unacceptable risk for small herbivorous mammals.

For the a serial application of 3 × max. 1440 g a.s./ha the results of the assessment indicate a low risk for small herbivorous mammals due to uptake of contaminated food.

B.9.3.3.5 Bioaccumulation and food chain behaviour

According to the EFSA Guidance Document, substances with a $\log_{pow} \geq 3$ are likely to possess a potential for bioaccumulation that might result in unacceptable risks for organisms at higher trophic levels. Hence, a specific risk assessment ('secondary poisoning') must be performed for these substances. Since the log K_{ow} values of glyphosate is log P < –3.2 (pH 2–5, 20 °C), the active substance is deemed to have a negligible potential to bioaccumulate in animal tissues. No formal risk assessment from secondary poisoning is therefore required. The primary metabolite of glyphosate is aminomethylphosphonic acid (AMPA). Most of the parent glyphosate is eliminated unchanged and only a small amount (less than 1 % of the applied dose) is transformed to aminomethylphosphonic acid (AMPA). The metabolite AMPA has been tested in several toxicity studies which demonstrated that it is of lower toxicity than glyphosate acid (see Volume1, chapter 2.6.8).
Furthermore, the log Kow for AMPA - estimated via EpisuiteProgramm and SMILES code (C(N)P(=O)(O)O) - is -2.47 and does not indicate a potential for bioaccumulation.

B.9.3.3.6 Risk assessment for metabolites

The primary metabolite of glyphosate is aminomethylphosphonic acid (AMPA). Most of the parent glyphosate is eliminated unchanged and only a small amount (less than 1 % of the applied dose) is transformed to aminomethylphosphonic acid (AMPA). The metabolite AMPA has been tested in several toxicity studies which demonstrated that it is of lower toxicity than glyphosate acid (see Volume1, chapter 2.6.8).

Following direct treatment via foliar, trunk, stem or hydroponic treatment unchanged glyphosate was the only significant residue. In presence of soil as a substrate the active substance is quickly degraded, leaving AMPA at rates comparable or even higher than parent glyphosate. However, the uptake via the roots and the translocation in the plants was very low, not resulting in significant residue levels as confirmed by plant metabolism and confined rotational crop studies. A major part of the glyphosate was degraded into CO_2 (for details please refer to Volume 1, chapter 2.7.2). Therfore it can be concluded that the risk to mammals will be acceptably low and no further quantitative risk assessment is conducted.

B.9.3.3.7 Risk from exposure to contaminated drinking water

In addition to their diet, birds may also be exposed to glyphosate via drinking water.
As pointed out in the EFSA Guidance document (EFSA, 2009), specific calculations of exposure and TER values are only necessary when the ratio of effective application rate (in g a.s./ha) to relevant endpoint (in mg a.s./kg bw/d) exceeds 50 in the case of less sorptive (K_{oc} < 500 L/kg) or 3000 in the case of more sorptive ($K_{oc} \geq 500$ L/kg) substances. For glyphosate, the ratio of highest application rate (4320 g a.s./ha) to lowest relevant endpoint (NOAEL = 50 mg a.s./kg bw/d) is 86.4; therefore, the risk can be considered acceptable without the need for further calculations.

B.9.3.3.8 Indirect effects via trophic interactions

In chapter B.9.1.7.7 we discuss the evidence of an impact of herbicide use on the biodiversity of terrestrial non-target species with an emphasis on farmland bird species. Although the impact of indirect effects of herbicides is probably similarly relevant for mammals and farmland birds, they are much less well understoold for mammals as thorough studies on effects on the population level are lacking. Therefore the RMS focussed the discussion primarily on farmland birds for which the current state of knowledge on the impact of indirect effects allows for a more profound discussion. See chapter B.9.1.7.7 for details on options for risk assessment and mitigation.

B.9.3.3.9 Literature Review

Please refer to sections regarding amphibians and to the summary of the relevant literature on surface active substances in glyphosate-based formulations (chapters B.9.9.2 and B.9.11 324). Please refer also to the human toxicity sections of this report.

B.9.4 Effects on bees (Annex IIA 8.3.1; Annex IIIA 10.4)

In order to reevaluate glyphosate a total of seven laboratory studies with technical or formulated glyphosate, a tunnel test and a study about the honeybee brood development with technical glyphosate were submitted.

B.9.4.1 Acute toxicity (OECD IIA 8.7.1, OECD IIA 8.7.2, OECD IIIA 10.4.2)

Laboratory data reviewed in the 2001 EU evaluation of glyphosate acid and new laboratory studies are summarized in Table B.9.4-1. The data of the new laboratory studies presented below were generated in accordance with OECD, EPPO 170 or other appropriate test guidelines and GLP requirements.

Table B.9.4-1: Results of laboratory toxicity studies of glyphosate acid and its salts to bees

No.	Test substance	Exposure route	LD_{50} [µg a.s./bee]	GLP	Reference	2001 EU evaluation monograph reference
Glyphosate acid						
2001 EU review of glyphosate	glyphosate acid	oral 48 h	**100**	no	Fraser and Jenkins (1972)HU85X094	BIE 96-00071
		contact 48 h	**>100**			
IIA 8.7.1/01	glyphosate	oral 48 h	**>200**	yes	Kleiner (1995) 95 10 48 065 BVL no 2311000	
		contact 48 h	**>200**			
IIA 8.7.1/02	glyphosate	oral 48 h	**>40**	yes	Weyman (1996) 1413/3-1018 BVL no 2311002	
		contact 48 h	**>20**			
IIA 8.7.1/03	glyphosate	oral 48 h	**>182**	yes	Thompson (1998) FN9700 BVL no 2311004	
		contact 48 h	**>103**			
IIA 8.7.1/04	glyphosate	oral 72 h	**>116.67**	yes	Van der Steen (1995) 141907 BVL no 2311007	
		contact 72 h	**-**			
IIA 8.7.1/05	glyphosate	oral 72 h	**-**	yes	Van der Steen (1995) 142335 BVL no 2311009	
		contact 72 h	**>100**			

No.	Test substance	Exposure route	LD_{50} [µg a.s./bee]	GLP	Reference	2001 EU evaluation monograph reference
Glyphosate-IPA salt						
2001 EU review of glyphosate	MON 2139	oral 48 h	**>100**	yes	Fraser and Jenkins (1972) HU85X094	BIE 96-00071
		contact 48 h	**>100**			
KIIIA1 10.4.2	MON 52276	oral 48 h	**>77**	yes	Baxter (2001) MON-00-2-version 2 BVL no 2316011	
		contact 48 h	**>100**			
IIA 8.7.2/06	Glyphosate IPA 62%	contact 48 h	**>61.3**	yes	Franco Perina (2000) RF-D4.0177/00 BVL no 2311010	
Glyphosate K-salt						
IIA 8.7.1/07	MON 78623	oral 48 h	**>104**	yes	Halsall (2003) MT-2002-108 BVL no 2311012	
		contact 48 h	**>100**			

Bee studies on the active substance and formulated products which were not included in the EU review of glyphosate (2001) are presented below.

B.9.4.1.1 Technical glyphosate

IIA 8.7.1/01 Kleiner, 1995

Annex point	Author(s)	Year	Study title
IIA 8.7.1/01	Kleiner, R.	1995	Testing Toxicity to Honeybee - *Apis mellifera* L. (laboratory) accordeing to EPPO Guideline No 170. Glyphosate (tec.) BioChem GmbH Karlsruhe, Labor Cunnersdorf, Cunnersdorf, Germany Report No: 2311000/ 95 10 48 065 Date: 1995-09-11 GLP: yes not published

Guideline: EPPO Guideline No. 170
Deviations: None
Dates of experimental work: 1995-08-21 to 1995-09-01

Executive Summary

In an acute laboratory study, the oral and contact toxicity of glyphosate acid to the honey bee, *Apis mellifera* L. was tested. Adult worker bees were exposed to two nominal test doses of 100 and 200 µg glyphosate acid/bee. In the test, three replicate cages, each containing 10 bees were used for the test item treatment, control and reference treatment. Mortality, poisoning symptoms and behavioural abnormalities were recorded 24 and 48 hours after treatment initiation.
Results showed no mortality of bees during the 48 hours test period for test concentrations of up to 200 µg test item/bee (the highest test concentration) in oral and contact toxicity tests. In addition, no behavioural abnormalities were observed in test item and control groups during the whole test period.
All validity criteria according to the OECD guidelines 213 and 214 were fulfilled.
No effects of glyphosate acid on mortality and behaviour of honey bees were observed at concentrations of up to 200 µg glyphosate acid/bee in oral and contact toxicity tests. Therefore, oral and contact LD_{50} of glyphosate acid were determined to be >200 µg glyphosate acid/bee.

Materials

Test material

Test item: Glyphosate acid
Description: Not stated
Lot/Batch #: 01/07/95
Purity: 98.2% a.s.

Vehicle and/or positive control

Dimethoate EC 400, containing 411.14 g a.s./L Extravon (surfactant)

Test organisms

Species: Honey bee (*Apis mellifera* L.)
Age: Adult worker bees
Diet/Food: 50 % aqueous sucrose solution *ad libitum* (except for 1 - 2 hours prior to oral test initiation)

Environmental conditions

Temperature: 25 – 26 °C
Humidity: 53 – 70 %
Photoperiod: 8 hours diffuse light / 16 hours darkness

Study design and methods

Experimental treatments

Oral and contact toxicity tests were conducted with two nominal test doses of 100 and 200 µg glyphosate acid/bee. In addition, for the oral test a control group was fed with 50 % sucrose solution. For the contact test, as a watery solution of 0.1 % Extravon was used as a negative control. In both tests, dimethoate was used a toxic reference, at test doses ranging from 0.20 to 0.40 µg/bee and 0.0313 to 1.0 µg/bee for oral and contact tests respectively. Both toxicity tests were conducted in triplicate using 10 bees per replicate (30 bees). For oral toxicity test, bees were fed with 50% aqueous sucrose solutions, containing appropriate concentrations of the test item. For contact toxicity test, test solutions containing appropriate concentrations of the test item were dosed to bees by thorax application. After administration of the test substance, the bees were provided with sucrose solution 50 % *ad libitum*.

Observations
Mortality, poisoning symptoms and behavioural abnormalities were recorded 24 and 48 hours after test start.

Results and discussion
Observations and findings
No biologically relevant mortality of bees was observed during the 48-hour test period for test concentrations of up to 200 µg test item/bee, which was the highest concentration tested. In addition, no behavioural abnormalities were observed at any test item concentration and in the control groups. For the toxic reference dimethoate, the LD_{50} was 0.35 µg test substance/bee for the oral toxicity test and 0.41 µg test substance/bee for the contact toxicity test.

Table B.9.4-2: Toxicity of glyphosate acid to honey bees in oral and contact toxicity tests from Kleiner

Test	Time [h]	Mortality [%]			
		Control	Glyphosate Acid [µg/bee]		Toxic reference
		--	100	200	Highest test dose*
Oral	24	0	3	0	83
	48	0	3	0	83
Contact	24	0	0	0	97
	48	0	0	0	97

* 0.40 µg/bee for oral toxicity test and 1.0 µg/bee for contact toxicity test

The validity criteria according to the OECD guidelines 213 and 214 were fulfilled as the mortality in the control was < 10% at test termination.

Conclusion
No effects of glyphosate acid on mortality and behaviour of honey bees were observed at concentrations of up to 200 µg glyphosate acid/bee in oral and contact toxicity tests.
The LD_{50} (48 h) was determined as > 200 µg glyphosate acid /bee in the oral toxicity test and in the contact toxicity test.

IIA 8.7.1/02 Weyman, 1996

Annex point	Author(s)	Year	Study title
IIA 8.7.1/02	Weyman, G. S.	1996	Glyphosate: Acute contact and oral toxicity to honey bees. Corning Hazleton (Europe). Harrogate, North Yorkshire, UK Report No: 2311002/ 1413/3-1018 Date: 1996-08-14 GLP: yes not published

Guideline: EPPO Guideline No. 170

Deviations: None
Dates of experimental work: 1996-06-27 to 1996-07-06

Executive Summary

In an acute laboratory study, the oral and contact toxicity of glyphosate acid to honeybee, *Apis mellifera* was tested. After a preliminary dose range-finding test, adult worker bees were treated with 0, 0.625, 1.25, 2.5, 5, 10 and 20 µg a.s./bee in the contact test and with 1.25, 2.5, 5, 10, 20 and 40 µg a.s./bee in the oral test. Three replicate cages, containing 10 bees each, were used. Mortalities and sub-lethal effects were made 1, 4, 24 and 48 h after treatment.
No mortalities or sub-lethal effects were seen in any treatment or controls over the 48 h definitive test period.
The validity criteria according to current OECD guidelines 213 and 214 are fulfilled.

In conclusion the 24 and 48-hour oral LD_{50} values for glyphosate acid were >20 µg a.s./bee for contact exposure (nominal) and >40 µg a.s./bee for oral exposure (nominal).

Materials

Test material
Test item: Glyphosate acid
Description: White powder
Lot/Batch #: H95 D161A
Purity: 95.3 %

Vehicle and/or positive control
Reference item: formulated Dimethoate (BASF Dimethoate 40 EC)

Test organisms
Species: Honey bee (*Apis mellifera*)
Age: Adult worker bees
Diet/Food: 50% sucrose solution *ad libitum*
Environmental conditions
Temperature: 24.5 - 25.8°C
Humidity: 49.1 - 86.0%
Photoperiod: darkness

Study design and methods

Experimental treatments
To determine the test concentrations of glyphosate acid for the definitive study a range-finding test was performed. The nominal doses of glyphosate used for the range-finding test were 0, 0.1, 1, 10 and 20 µg a.s./bee for contact dosing and 0, 0.04, 0.4, 4 and 40 µg a.s./bee for oral dosing.

Contact test:
In a dose response test, three replicates of 10 bees were exposed to Glyphosate acid, administered topically in a small droplet (1 µL) to the thorax of each bee. The nominal doses of glyphosate was 0, 0.625, 1.25, 2.5, 5, 10 and 20 µg a.s./bee. The nominal dose of 20 µg a.s./bee was given as a double droplet application (2 x 1 µL).

Oral test:

The nominal doses of glyphosate used for the definitive oral test were 0, 1.25, 2.5, 5, 10, 20 and 40 µg a.s./bee. Three replicate cages, containing 10 bees each, were used.
Reference (dimethoate):
The reference substance was prepared and dosed in the same media and manner as the test substance doses. The toxic standard test was run in concurrently with the range-finding test and shared the controls. The nominal doses of dimethoate was 0, 0.2, 0.4 and 0.8 µg a.s./bee in the contact test and 0, 0.1, 0.15 and 0.2 µg a.s./bee in the oral test.

Observations
Assessment of mortalities and sub-lethal effects were made 1, 4, 24 and 48 h after treatment.

Results and discussion
Observations and findings
No mortalities or sub-lethal effects were seen in any treatment or controls during the 48 h test period. The 48 h LD_{50}-values for dimethoate were calculated to be 0.452 µg a.s./bee for contact exposure and 0.146 µg a.s./bee for oral exposure.

Table B.9.4-3: Toxicity of glyphosate acid to honey bees in oral and contact toxicity tests from Weyman

Test	Time [h]	Mortality [%]					
		Control	Dimethoate [µg a.s./bee]				Toxic reference
			0.1	0.15	0.2	0.4	Highest test dose*
Oral	24	0	2	15	-	-	23
	48	0	3	18	-	-	24
Contact	24	0	-	-	3	11	24
	48	0	-	-	3	12	25

* 0.20 µg a.s./bee for oral toxicity test and 0.80 µg a.s./bee for contact toxicity test, - not applicable

Conclusion
No effects of glyphosate acid on mortality and behaviour of honey bees were observed at the highest concentration tested (contact: 20 µg a.s./bee, oral: 40 µg a.s./bee).
The 48-hour LD_{50} values for glyphosate acid were >20 µg a.s./bee for contact exposure (nominal) and >40 µg a.s./bee for oral exposure (nominal).

IIA 8.7.1/03 Thompson, 1998

Annex point	Author(s)	Year	Study title
IIA 8.7.1/03	Thompson, H.M.	1998	Glyphosate Acid: Acute Contact and Oral Toxicity to Honey Bees (*Apis mellifera*) National Bee Unit, Central Science Laboratory, Sand Hutton, York, UK Report No: 2311004/ FN9700 Date: 1998-12-04 GLP: yes not published

Guideline: EPPO 170 guideline (1992) OPPTS 850.3020
Draft OECD 213 (1997) and Draft OECD 214 (1997)

Deviations: The starvation of bees before test initiation was 2 h and 10 min, instead of 1-2 h. This is not supposed to have an effect on the reliability of the study.

Dates of experimental work: 1998-08-24 to 1998-09-04

Executive Summary

In an acute laboratory study the contact and oral toxicity of glyphosate acid to the honey bee, *Apis mellifera* L., were tested. Following a range finding test, a definitive test was conducted exposing female worker bees to nominal doses of 0.0984, 0.984, 9.84 and 103 µg test item/bee. In the oral toxicity test, 206 µg a.s./bee was tested additionally. In both test setups, three replicate cages, each containing 10 bees, were used for the test item treatments, controls and reference treatments. Mortality and sub-lethal effects were assessed 4, 24 and 48 h after test initiation for contact and oral toxicity.
No mortality of bees was observed after 72 hours of exposure. In addition, no sub-lethal effects were observed in the test item and the control groups during the 72 hours test period. All validity criteria according to OECD 213 and OECD 214 were fulfilled.
In conclusion, the toxicity of glyphosate acid was tested in an acute contact and an oral toxicity test on honey bees. The LD_{50} (48 h) was >103 µg a.s./bee in the contact toxicity test, in the oral toxicity test it was > 182 µg a.s./bee.

Materials

Test material:
Test item: Glyphosate acid
Description: White powder
Lot/Batch #: TSC 0521/05148
Purity: 97.6 %

Vehicle and/or positive control:
Vehicle for test item: Agral 90
Vehicle for positive control: Triton X100
Positive control: Dimethoate (BASF 40 lot 083.10/96)

Test organisms:
Species: Honey bee (*Apis mellifera* L.)
Age: Adult worker bees
Diet/Food: Not stated

Environmental conditions:
Temperature: 25 ± 1 °C
Humidity: 65 ± 5 %
Photoperiod: 24 hours darkness (except during observation)

Study design and methods:

Experimental treatments

Contact test: The definitive test was conducted with 0.0984, 0.984, 9.84 and 103 µg glyphosate acid/bee prepared in an appropriate carrier (deionised water containing 500 mg/L of the wetting agent Agral 90) and administered as a 1.0 µL droplet per bee (dorsal thorax) to each of ten bees in each of three cages per treatment. A control with 500 mg Agral 90/L and a toxic reference solution containing 1g Triton X100/L were run in parallel. During the observation method a 50 % w/v aqueous sucrose solution was provided.

Oral test: The definitive test was conducted with 0.0984, 0.984, 9.84,103 and 206 µg glyphosate acid/bee, in 50% w/v aqueous sucrose solution, the formulation having first been dissolved in deionised water containing 500 mg/L Agral 90. A control of 0.5 mL water and the toxic reference solution containing 1 g Triton X100/L were prepared analogically. The treated food was offered in glass test feeders, which were weighed before and after introduction into the three cages per treatment. Each replicate cage contained ten bees. Duration of uptake was 4 hours for the test item treatments. At the highest treatment level the mean dose consumed was 182 µg glyphosate acid/bee.

Observations
Mortality and sub-lethal effects were assessed 4, 24 and 48 h after test initiation for contact and oral toxicity.

Results and discussion
Observations and findings
In both test setups, no mortality of bees was observed in the 72 hours test period. In the oral toxicity test the maximum nominal test level of 206 µg a.s./bee corresponded to an actual intake of 182 µg a.s./bee. In addition, no sub-lethal effects were observed in the test item group and the control group during the 72 hours test period.

Table B.9.4-4: Toxicity of glyphosate acid to honey bees in oral and contact toxicity tests from Thomson

Dose [µg a.s./bee]	Mean intake of test item [µg a.s./bee]	Mortality [%]		
		24 h	48 h	72 h
Contact toxicity test				
Control	--	0	0	0
0.0984	--	0	0	0
0.984	--	0	0	0
9.84	--	0	0	0
103	--	0	0	0
Oral toxicity test				
Control	--	0	0	0
0.0984	0.0947	0	0	0
0.984	0.937	0	0	0
9.84	9.7	0	0	0
103	80	0	0	0
206	182	0	0	0

All validity criteria according to OECD guideline 213 and 214 were fulfilled, since the average mortality in the control group did not exceed 10% and the LD_{50} of the toxic standard meets the specified range.

Conclusion

The toxicity of glyphosate acid was tested in an acute contact and an oral toxicity test on honey bees. No effects of glyphosate acid on mortality and behaviour of honey bees were observed.
The LD_{50} (48 h) was >103 µg a.s./bee in the contact toxicity test, in the oral toxicity test it was >182 µg a.s./bee.

IIA 8.7.1/04 van der Steen, 1995

Annex point	Author(s)	Year	Study title
IIA 8.7.1/04	van der Steen, J.J.M.	1995	Honey Bees (*Apis mellifera* L.), oral toxicity study in the laboratory with Glyphosate NOTOX B.V, Hertogenbosch, The Netherlands Report No: 2311007/ 141907 Date: 1995-09-22 GLP: yes not published

Guideline: EPPO 22, 203-215 (1992)
Deviations: None
Dates of experimental work: 1995-03-08 to 1995-03-16

Executive Summary
In an acute laboratory study the oral toxicity of glyphosate acid to the honey bee, *Apis mellifera* L., was tested. Following a range finding test, a definitive test was conducted exposing worker bees to a single nominal dose of 121 µg test item/bee.
In the test, three replicate cages, each containing 10 bees, were used for the test item treatments, controls and reference treatments. Paralysis and mortality effects were recorded at least the following approximate time intervals: 30, 60, 90 and 120 minutes after treatment and 24, 48 and 72 hours after treatment.
No mortality of bees was observed after 72 hours of exposure. In addition, no paralysis was observed in the test item and the control groups during the 72 hours test period.
The validity criteria according to guideline OECD 213 are fulfilled.
In an oral toxicity test, glyphosate acid had no effects on mortality of honey bees at concentrations of up to 116.67 µg a.s./bee. Therefore, the oral LD_{50} of glyphosate acid was determined to be >116.67 µg a.s./bee.

Materials and methods
Test material
Test item: Glyphosate acid
Description: White powder
Lot/Batch #: 22021
Purity: 96 %

Vehicle and/or positive control
Parathion 25% liquid

Test organisms
Species: Honeybee (*Apis mellifera* L.)

Age: Adult worker bees
Diet/Food: 50 % aqueous sucrose solution *ad libitum* (except during oral dosing and prior starvation)

Environmental conditions
Temperature: 24 – 25 °C
Humidity: 34 – 37 %
Photoperiod: 24 hours darkness (except during observation)

Study design and methods
Experimental treatments
Prior to the main test, a range-finding test was performed exposing bees to nominal glyphosate acid concentrations of 1, 10, 51 and 101 µg a.s./10 µL sucrose solution.
The definitive test was conducted as a limit test with a single nominal concentration of 121 µg test item/10 µL sucrose solution. All test solutions were prepared in a 50% sucrose solution. In addition a water-treated control and a reference substance (Parathion 25% liquid) were tested.
For the test, 10 bees per cage were exposed in triplicate and fed with the test substance suspension. Per group of 10 bees 100 µL test substance suspension was administered (10 µL test solution/bee).

Observations
Mortality, paralysis and any other abnormalities were recorded at least the following approximate time intervals: 30, 60, 90 and 120 minutes after treatment and 24, 48 and 72 hours after treatment start.

Results and discussion
Oral toxicity test: No mortality of bees was observed at the in the 72 hours test period for the test concentration of 121 µg test item/bee, which is the highest test concentration. In addition, no paralysis was observed in the test item group and the control group during the 72 hours test period.

Table B.9.4-5: Toxicity of glyphosate acid to honey bees in an oral toxicity test from van der Steen

Dose [µg test item/bee]	**Intake of test item [µg a.s./bee]**	**Mortality [%]**		
		24 h*	**48 h***	**72 h***
Control (sugar solution)	--	0.00	3.33	3.33
Definitive test glyphosate				
121	116.67	0.00	0.00	0.00

* Corrected for mortality in the negative control

The test is considered to be valid according to OECD guideline 213 as mortality in the negative control did not exceed 15 % after 48 hours. In addition the LD_{50} for the reference item met the specified range.

Conclusion

In an oral toxicity test, glyphosate acid had no effects on mortality of honey bees at concentrations of up to 116.67 µg a.s./bee. Therefore, the oral LD_{50} of glyphosate was determined to be >116.67 µg a.s./bee.

IIA 8.7.1/05 van der Steen, 1995

Annex point	Author(s)	Year	Study title
IIA 8.7.1/05	van der Steen, J.J.M.	1995	Honey Bees (*Apis mellifera* L.), contact toxicity study in the laboratory with Glyphosate NOTOX B.V, 5231 DD 's-Hertogenbosch, The Netherlands Report No: 2311009/ 142335 Date: 1995-09-22 GLP: yes not published

Guideline: EPPO 22, 203-215 (1992)
Deviations: None
Dates of experimental work: 1995-03-20 to 1995-03-25

Executive Summary

In an acute laboratory study the contact toxicity of glyphosate acid to the honey bee, *Apis mellifera* L. was tested. Following a range finding test, adult worker bees were exposed to a single nominal dose of 100 µg test item/bee. In the test, three replicate cages, each containing 10 bees, were used for the test item treatment, control and reference treatment. Mortality and paralysis effects were recorded at least at the following approximate time intervals: 30, 60, 90 and 120 minutes after treatment and 24, 48 and 72 hours after treatment.
No mortality of bees was observed during the 72 hours of exposure. In addition, no paralysis was observed in the test item groups and the control groups during the 72 hours test period. The validity criteria according to guideline OECD 214 are fulfilled.
In a contact toxicity test, no effects of glyphosate acid on the mortality and the paralysis of honey bees were observed at concentrations up to and including 100 µg a.s./bee. The contact LD_{50} of glyphosate was therefore determined to be>100 µg a.s./bee.

Materials and methods

Test material
Test item: Glyphosate acid
Description: White powder
Lot/Batch #: 22021
Purity: 96 %

Vehicle and/or positive control
Acetone (vehicle), Parathion 25 % liquid (positive control)

Test organisms
Species: Honey bee (*Apis mellifera* L.)
Age: Adult worker bees
Diet/Food: 50 % aqueous sucrose solution *ad libitum* (except during the treatment)

Environmental conditions
Temperature: 24 – 25 °C
Humidity: 34 – 40 %
Photoperiod: 24 hours darkness (except during observation)

Study design and methods
Experimental treatments
Prior to the main test, a range-finding test was conducted (1, 10, 50, 99 µg a.s. item/bee).
The definitive test was conducted as a limit test with a single nominal dose of 100 µg test item/bee. In addition, a negative control constituted of acetone and the reference substance Parathion 25% liquid was tested.
For the definite test, adult worker bees were exposed in triplicates (10 bees/test cage) to the test item, control and reference item. After administration of the test substance, the bees were provided with sucrose solution 50 % *ad libitum*.

Observations
Mortality, paralysis and any other abnormalities were recorded at least the following approximate time intervals: 30, 60, 90 and 120 minutes after treatment and 24, 48 and 72 hours after treatment start.

Results and discussion
1. Observations and findings
Contact toxicity test: No mortality of bees was observed during the 72 hours test period for the test concentration of 100 µg test item/bee. In addition, no paralysis was observed in test item groups and control groups during the 72 hours test period.

Table B.9.4-6: Toxicity of glyphosate acid to honey bees in a contact toxicity test from van der Steen

Dose	**Mortality [%]**		
[µg a.s./bee]	**24 h**	**48 h**	**72 h**
Control (Acetone)	0.00	0.00	0.00
100	0.00	0.00	0.00

The test is considered to be valid according to OECD 214 as mortality in the negative control did not exceed 15% and the LD_{50} of the toxic standard met the range specified.

Conclusion
In a contact toxicity test, no effects of glyphosate acid on mortality of honey bees were observed at concentrations up to 100 µg a.s./bee.
The contact LD_{50} of glyphosate was determined to be >100 µg a.s./bee.

B.9.4.1.2 Formulated glyphosate

KIIIA1 10.4.2 Baxter, 2001

Annex point	Author(s)	Year	Study title
IIA 8.7.1/06	Baxter, I.	2001	Laboratory bioassays to determine acute oral and contact toxicity of MON 52276 to the Honeybee, *Apis mellifera* Mambo-Tox Ltd., SO16 7PX, Southampton, UK Report No: 2316011/ MON-00-2 version 2 Date: 2001-01-08 (original issue date = 2000-12-04) GLP: yes Not published

Guideline: EPPO Guideline No. 170
Deviations to OECD 213/214: None
Dates of experimental work: 2001-02-09

Executive Summary
In an acute laboratory study the oral and contact toxicity of MON 52276 (glyphosate isopropylamine (IPA)) to the honey bee, *Apis mellifera* was tested. Following an initial range finding test for both methods of application, definitive tests were conducted as limit tests, with a single dose rate for the test item (norminally 100 µg a.e./bee), a control treatment and three rates of the toxic reference item (dimethoate).
In the definitive tests, five replicate cages, each containing 10 bees, were used for the test item and the control and three replicate cages for each rate of the toxic reference item. Mortality and sub-lethal effects were recorded at 1, 3, 24 and 48 hours after treatment.

No significant mortality of bees was observed during the 48 hours observation period. In addition, no sub-lethal effects were observed. The validity criteria according to guideline OECD 213/214 are fulfilled.

In conclusion, under the conditions of the present test, the 48 h oral LD_{50} of MON 52276 was determined to be >77 µg MON 52276/bee, and for the 48 h contact LD_{50} >100 µg MON 52276/bee.

Materials and methods

Test material

Test item: MON 52276 (41.5 % w/w glyphosate isopropylamine; 31 % w/w glyphosate acid equivalent (a.e.)
Description: Soluble concentrate
Lot/Batch #: 100399

Vehicle and/or positive control

Farmon Blue (vehicle), dimethoate (positive control)

Test organisms

Species: Honey bee (*Apis mellifera* L.)
Age: Adult worker bees
Diet/Food: 50 % aqueous sucrose solution *ad libitum* (except during the treatment)

Environmental conditions

Temperature: 24 – 26 °C
Humidity: 46 – 83 %
Photoperiod: 24 hours darkness (except during observation)

Study design and methods

Experimental treatments

Both topical and oral routes of exposure were assessed.
For the topical bioassays, solutions of the test item (nominally 100 µg a.e./bee MON 52276) and toxic reference item (dimethoate) were prepared in a 0.01 % v/v solution of wetting agent (Farmon Blue). In addition a 0.01 % v/v solution of Farmon Blue was applied as a control treatment.
For the oral bioassay, worker bees were exposed to solutions of the test and toxic reference items via feeding tubes placed in each cage. The treated feeding tubes were replaced with ones containing untreated sugar solution after approximately 5 h. The mean amount of test item solution consumed per bee was determined by weighing the feeding tubes before and after being placed in the cages. For the control group an untreated sugar solution was provided.

Observations

Mortality and sub-lethal effects were recorded at 1, 3, 24 and 48 hours after treatment.

Results and discussion

Observations and findings

At the end of the oral toxicity test (48 hours after application), there was 4 % mortality at 77 µg MON 52276/bee, compared with a control mortality of 4 %.

The mortality in the contact toxicity test was 6 % for the test item (100 µg MON 52276/bee) and 2 % for the control.
In addition, no paralysis was observed in test item groups and control groups during the 48 hours test period.

Table B.9.4-7: Toxicity of glyphosate acid to honey bees in oral and contact toxicity tests from Baxter

Test	Time [h]	Mortality [%]		
		Mean % mortality of bees		
		Control	Dose offered orally µg a.e./bee (µg a.s./bee)	Dose applied topically µg a.e./bee (µg a.s./bee)
			77* (103)	100 (134)
Oral	1	0	0	-
	3	0	0	-
	24	0	0	-
	48	4	4	-
Contact	1	0	-	0
	3	0	-	0
	24	0	-	0
	48	2	-	6

* based on mean weight of test solution of 5 µg a.e./µL consumed per cage of 10 bees, corrected for the density of the 50% w/v sugar solution

The tests are considered to be valid according to OECD 213/214 as mortality in the negative control did not exceed 15 % and the LD_{50} of the toxic standard met the range specified.

Conclusion
Under the conditions of the present test, the 48 h oral LD_{50} of MON 52276 was determined to be >77 µg MON 52276/bee, and for the 48 h contact LD_{50} >100 µg MON 52276/bee.

IIA 8.7.1/06 Franco Perina, 2000

Annex point	Author(s)	Year	Study title
IIA 8.7.1/06	Franco Perina, V.C.	2000	Acute Contact Toxicity of GLIFOSATO IPA TECHNICO NUFARM to Honey Bees (*Apis mellifera* L.) BIOAGRI Laboratorios Ltda., Piracicaba, Brasil Report No: 2311010/ RF-D4.017/00 Date: 2000-07-24 GLP: yes not published

Guideline: OECD Draft Proposal for a New Guideline: Honey bees, Acute Contact Toxicity Test (1996).
Deviations: None
Dates of experimental work: 2000-06-05 to 2000-06-14

Executive Summary
In an acute laboratory study the contact toxicity of the isopropylamine (IPA) salt of glyphosate to the honey bee, *Apis mellifera* was tested. Following a range finding test, adult worker bees were exposed to nominal dose rates of 10, 12.5, 24, 62.5 and 100 µg test item/bee. In addition, an undosed control was tested. Technical dimethoate was used as a reference item.
In the test, three replicate cages, each containing 10 bees, were used for the test item treatment, control and reference treatment. Mortality and sub-lethal effects were recorded at 24 and 48 hours after the treatment.
No significant mortality of bees was observed during the 48 hours observation period. In addition, no sub-lethal effects were observed.
The validity criteria according to guideline OECD 214 are fulfilled.

In conclusion, under the conditions of the present test, the 48 h contact LD50 of glyphosate IPA salt was determined to be >100 µg glyphosate IPA salt/bee, equivalent to >30.44 µg a.e./bee.

Materials and methods
Test material
Test item: Glyphosate isopropylamine salt (technical)
Description: Not stated
Lot/Batch #: MJRT 02 S 201 04
Purity: 612.7 g/kg salt equivalent (analysed), equivalent to 304.4 g a.e./kg
Density: Not stated

Vehicle and/or positive control
technical dimethoate (vehicle)

Test organisms
Species: Honey bee (*Apis mellifera*)
Age: Adult worker bees
Diet/Food: Sucrose solution *ad libitum*
Acclimatisation: At 25 ± 2 °C and 65 ± 5 % relative humidity between collection of worker bees and test initiation (time span not stated)

Environmental conditions
Temperature: 27 – 31 °C
Relative humidity: 40 – 67 %
Photoperiod: 24 hours darkness

Study design and methods
Experimental treatments
Based on the results of a range-finder test, bees in the main test were exposed to the nominal dose rates of 10, 12.5, 24, 62.5 and 100 µg test item/bee. In addition, an undosed control was tested. Technical dimethoate was used as a reference item. The test was conducted with 3 replicates (cages) per test concentration/control and 10 bees per cage. Bees were exposed to the test item by administering 1.0 µL of the test substance or reference item on the ventral surface of the thorax with a micro syringe. After dosing the cages were kept in darkness for 48 hours. Sucrose solution was available *ad libitum* throughout the whole test period.

Observations
Mortality and sub-lethal effects were recorded at 24 and 48 hours after treatment.

Statistical calculations
Descriptive statistics (test item)
Spearman-Karber (dimethoate)

Results and discussion
Observations and findings
Analytical determination of the test concentrations showed that the deviation from the nominal concentrations was not higher than 20 %. Therefore, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item. The determined 48h LD_{50} for the reference item dimethoate was 0.12 mg/L. No sub-lethal effects were observed up to a dose of 100 µg test item/bee, equivalent to 30.44 µg a.e./bee.

Table B.9.4-8: Toxicity of glyphosate acid to honey bees in contact toxicity tests from Franco Perina

Dose	**Mortality (mean of 3 replicates) [%]**	
[µg test item/bee]	**24 h**	**48 h**
Control (undosed)	0.00	0.00
10.0	0.00	0.00
10.0	0.00	0.00
12.5	0.00	0.00
24.0	0.00	0.00
62.5	0.00	0.00
100.0	33.3	33.3

The test is considered to be valid according to OECD 214 as mortality in the negative control did not exceed 15 % and the LD_{50} of the toxic standard met the range specified.

Conclusion
Under the conditions of the present test, the 48 h contact LD_{50} of glyphosate IPA salt was determined to be >100 µg glyphosate IPA salt/bee, equivalent to >30.44 µg a.e./bee.

IIA 8.7.1/07 Halsall, 2003

Annex point	**Author(s)**	**Year**	**Study title**
IIA 8.7.1/07	Halsall, N.	2003	Laboratory bioassays to determine acute oral and contact toxicity of MON 78623 to the honey bee, *Apis mellifera* Mambo-Tox Ltd., Biomedical Sciences Building, Bassett Crescent East, Southamton SO16 7PX, UK Report No: 2311012/ MT-2002-108 Date: 2003-04-11

			GLP: yes not published

Guideline: EPPO guideline 170 (1992)
Deviations: None
Dates of experimental work: Not stated

Executive Summary

In an acute laboratory study the contact and oral toxicity of MON 7862 (glyphosate K-salt) to the honey bee, *Apis mellifera* L., were tested. Following a range finding test, a definitive test was conducted exposing worker bees to nominal doses of 100 µg glyphosate acid equivalent/bee. In both test setups, five replicate cages, each containing 10 bees, were used for the test item treatments, controls and three for the reference treatments. Mortality and sub-lethal effects were assessed 1, 3, 24 and 48 h after test initiation for contact and oral toxicity.
Corrected mortality for contact toxicity was 0 % and for oral toxicity 4 %. No sub-lethal effects were observed except for one bee one hour after test item application.
All validity criteria according to OECD 213 and OECD 214 were fulfilled.
In conclusion, the toxicity of MON 7862 (glyphosate K-salt) was tested in an acute contact and an oral toxicity test on honey bees. The LD_{50} (48 h) was >100 µg glyphosate acid equivalent/bee in the contact toxicity test, in the oral toxicity test it was >104 µg glyphosate acid equivalent/bee.

Materials and methods

Test material
Test item: MON 78623 (58% potassium salt of glyphosate)
Description: Amber liquid
Lot/Batch #: GLP-0108-11688-F
Purity: 47.3 % (w/w nominal content of glyphosate a.e)

Vehicle and/or positive control
Vehicle for test item: wetting agent Farmon Blue (87.3 % w/w alkyl phenol ethylene oxide)
Positive control: Dimethoate technical grade

Test organisms
Species: Honey bee (*Apis mellifera* L.)
Age: Adult worker bees
Diet/Food: 50 % w/v aqueous sucrose solution

Environmental conditions
Temperature: 25 – 26 °C
Humidity: 64 – 79 %
Photoperiod: 24 hours darkness (except during observation)

Study design and methods

Experimental treatments
Contact test: Following an initial range-finding test, the definitive test was conducted as a limit test with 100 µg glyphosate acid equivalent/bee, prepared in an appropriate carrier (0.05 % solution of the wetting agent Farmon Blue) and administered as a 1.0 µL droplet per

bee (dorsal thorax) to each of ten bees in each of five cages per treatment. A vehicle control containing 0.05 w/v solution of Farmon Blue and deionised water and a toxic reference solution containing dimethoate were run in parallel. During the observation method a 50% w/v aqueous sucrose solution was provided ad libitum.
Oral test: The definitive test was conducted with 100 µg test item/bee, in 50 % w/v aqueous sucrose solution. A control of deionised water and the toxic reference solution containing dimethoate were prepared analogically. The treated food was offered in narrow glass vials, which were weighed before and after introduction into the three cages per treatment. Each replicate cage contained ten bees.

Observations
Mortality and sub-lethal effects were assessed 1, 3, 24 and 48 h after test initiation for contact and oral toxicity.

Statistical calculations
Corrected mortality was calculated according to Abbott (1925).
LC_{50} values were determined by Probit analysis and the 95 % confidence interval by Chi-square goodness of fit test.

Results and discussion
Observations and findings

Corrected mortality at 48 h was 0 %. No sub-lethal effects were observed except for one bee one hour after test start, but it recovered by 3 h. Oral toxicity test: No sub-lethal effects of bees were observed during the 48 hour test period for the test concentration of 104 µg glyphosate acid equivalent/bee and the sucrose control. The corrected mortality after 48 h was 4 %.
At the highest treatment level the mean dose consumed was 104 µg a.e./bee.
The determined contact 48h LD_{50} for the reference item dimethoate was 0.123 µg/bee and 0.126 µg/bee for oral toxicity. These results are in line with published values, indicating that the test insects were of suitable sensitivity.

Table B.9.4-9: Toxicity of glyphosate K-salt to honey bees in oral and contact toxicity tests from Halsall

Dose [µg a.s./bee]	**Mean intake of test item [µg a.s./bee]**	**Mortality [%]**			
		1	**2**	**24 h**	**48 h**
Contact toxicity test					
Control	--	**0**	0	2	4
Farmon Blue control	--	**0**	0	2	4
100	--	**0**	0	2	2 (0)
Oral toxicity test					
Control	--				6
100	104				10 (4)

In brackets the Abbot corrected mortality is given

All validity criteria according to OECD 213 and OECD 214 were fulfilled, since the average mortality in the control group did not exceed 10 % and the LD_{50} of the toxic standard meets the specified range.

Conclusion
The toxicity of MON 7862 (glyphosate K-salt) was tested in an acute contact and an oral toxicity test on honey bees. The LD_{50} (48 h) was >100 µg glyphosate acid equivalent/bee in the contact toxicity test, in the oral toxicity test it was >104 µg glyphosate acid equivalent/bee.

B.9.4.2 Bee brood feeding test (OECD IIA 8.7.4)

KIIA 8.7.4 Thompson, 2012

Annex point	Author(s)	Year	Study title
IIA 8.7.4/01	Thompson, H.M.	2012	Glyphosate: Evaluating potential effects on honey bee brood (*Apis mellifera*) development The Food and Environmental Research Agency, Sand Hutton, York, U.K. Report No: 2311020/ V7YH1001 Date: 20012-01-27 GLP: yes not published

Guideline: Oomen *et al.*, 1992
Deviations: Some colonies used in the study were slightly smaller in terms of the number of brood frames, but this was not considered to have a significant impact on the study as all were viable colonies at the start of the study and a sufficient number of brood cells were available for detailed observations.
Feeding period was extended up to 5 days.
This extension of the feeding period is not considered to have had an impact on the validity of the study.
Dates of experimental work: 2011-06-21 to 2011-08-23

Executive Summary
A field study was undertaken to determine the potential for toxicity to developing honey bee larvae and pupae to glyphosate (tested as the IPA salt) when fed directly to honey bee colonies. The IPA salt was selected as the test substance because it is representative of the active substance in glyphosate formulations and the appropriate for this terrestrial study. Three groups of four colonies were treated with 75, 150 and 301 mg a.e./L of glyphosate in 1 litre of 50 % w/v sucrose. One group of four colonies was fed with 1 litre 50 % w/v sucrose solution only and one group of four colonies was fed with the toxic reference fenoxycarb dispersed in 1 litre of 50 % w/v sucrose. Brood cells were marked in each colony (100 cells containing eggs, 100 cells containing 1-2 day old larvae, and 100 cells containing 3-4 day old larvae) up to 24 hours, to dosing using the standard acetate overlay method. On day 7 and just

prior to expected emergence, the marked brood cells (eggs, young and old larvae) were assessed for mortality and appearance in each test colony. The content of the dead bee traps attached to the colonies was counted daily during brood assessment period. All colonies were assessed within one week prior to the dosing and within week 1, 2 and 3 after dosing. Samples of each concentration of test item treated sucrose solution were taken on the day of dosing. Four to five day old larvae were sampled 4 and 7 days following start of dosing.
Measured glyphosate (a.e.) concentrations in the dosing solutions were within 11 % of the nominal doses. Mean measured glyphosate (a.e.) residues in larvae on 4 days were 13, 37 and 53 mg a.e./kg for the nominal dose levels of 75, 150, and 301 mg a.e./L. Mean measured residues after 7 days were reduced with values of 1.7, 3.2 and 4.1 mg a.e./kg for the nominal dose levels of 75, 150, and 301 mg a.e./L. Glyphosate acid was not detected in the control group.
No biologically significant adult mortality was observed in any treatment group. Over a 16 day observation period after dosing, 2 dead pupae/colony were observed in the control and 1.3 - 1.8 dead pupae/colony were observed in the glyphosate treated colonies. Overall survival was 85 % for marked eggs, 96 % for marked young larvae and 96% for marked old larvae in controls and 82-87 % for marked eggs, 87-94 % for marked young larvae and 94-95 % for marked old larvae in the glyphosate treated colonies.

The overall NOAEL for brood development of honey bees was the highest dose tested – 301 mg glyphosate acid equivalent/L (nominal) corresponding to 245 mg glyphosate acid equivalent/kg (considering the density of the sucrose solution) and 266 mg glyphosate acid equivalent/kg actually measured.

Materials and methods

Test material

Test item:	MON 0139
Active substance:	Glyphosate isopropylamine salt
Active substance content:	62.27 % Glyphosate isopropylamine salt 46.14 % glyphosate acid equivalents/L (measured)
Description:	Clear light yellow liquid
Lot/Batch #:	GLP-1104-21370-T

Vehicle and/or positive control
Fenoxycarb (750 mg a.s./L)

Test organism

Species:	*Apis mellifera*
Age:	not stated
Acclimatisation:	not required

Test system
Twenty standardized field colonies housed in a single chamber wooden Smith hive with British standard frames each and headed by queens of similar age. The honey bee colonies contained 12000 - 22500 adult bees and consisted of 3-6 frames of brood (with few exceptions), 0.5 - 2 frames of honey and 0 - 1 frame of pollen.
Crop cultivated: Not applicable; the test site with no nearby flowering crops and few flowering weeds, Dunnington, York, U.K.
Replication: 4 colonies/treatment and control

Environmental conditions
Temperature: 3.4 – 46.3 °C
Relative humidity: 0 – 100 %
Average wind speed: 4.0 - 13.1 mph
Precipitation: 0.0 - 9.71 mm

Study design and methods
Experimental treatments
Test system:
Twenty standardized honey bee colonies, each equipped with a dead bee trap fitted to the front were used in this study. All colonies were placed on varroa floors and sticky inserts were placed on the trays to trap any fallen mites. Colonies were located on a test site at Dunnington, York and allowed to fly freely, there were no nearby flowering crops and few flowering weeds (clover). Colonies were placed in groups according to treatment and placed at least 20 m apart from each other.

Experimental design:
Up to 24 hours prior to dosing, 100 brood cells containing eggs, 100 cells containing 1-2 day old larvae and 100 cells containing 3-4 day old larvae were selected in each colony and marked using the Oomen *et al.* (1992) 6 acetate overlay sheet method.

Test doses:
Dose setting was based on measured residues achieved in a glasshouse residues study after spray application onto *Phacelia* plants at 2.88 kg glyphosate a.e./ha. Considering that bee colonies used in the brood study may be up to 50 % bigger than those used in the residue study, an additional calculation for the expected total daily intake of glyphosate residues was undertaken assuming that such colonies would collect 9 g pollen and 1944 mL nectar. Furthermore the determined residue content based on application of 2.88 kg a.e./ha was adjusted to reflect the lower application rate of 2.16 kg a.e./ha.

Table B.9.4-10: Exposure assessment of a brood study colony to glyphosate under two scenarios used to establish test doses for use in the brood study

Scenario	Daily intake of glyphosate residues in nectar (1944 g nectar/d) [mg]	Daily intake of glyphosate residues in pollen (9 g pollen/d) [mg]	Total daily intake of glyphosate residues [mg]	Uptake over 3 days [mg]	Adjustment from 2.88 kg a.e./ha to 2.16 kg a.e./ha [mg] [7]
Day 1 maximum mean residues (31.3 µg a.e./g in nectar, 574 µg a.e./g in pollen)	68.8 [1]	5.2 [2]	66.0	198	148.5 [3]
Mean residues over days 1-3 (15.5 µg a.e./g in nectar, 310 µg a.e./g in pollen)	30.3 [4]	2.8 [5]	33.1	99.3	74.5 [6]

[1] Derived from 1.944 kg nectar consumed/day * 31.3 mg/kg = 60.8 mg glyphosate a.e.

[2] Derived from 0.009 kg pollen consumed/day * 573.5 mg/kg = 5.2 mg glyphosate a.e.
[3] Value of 148.5 mg was rounded to 150 mg to achieve the nominal mid-dose concentration in brood study
[4] Derived from 1.944 kg nectar consumed/day * 15.6 mg/kg = 30.3 mg glyphosate a.e.
[5] Derived from 0.009 kg pollen consumed/day * 310.1 mg/kg = 2.8 mg glyphosate a.e.
[6] Value of 74.5 mg was rounded to 75 mg to achieve the nominal low-dose concentration in brood study
[7] The determined residue content based on application of 2.88 kg a.e./ha was adjusted to reflect the lower application rate of

Test item application: Three groups of colonies (i.e. four colonies per group) were treated with glyphosate isopropylamine salt added to 1 litre of 50 % sucrose solution to achieve doses of 75, 150, and 301 mg a.e./L and one group was an untreated control, i.e. fed 1 litre 50 % sucrose solution, only. In addition, one group was treated with the toxic reference fenoxycarb, dispersed in 1 L of 50 % sucrose (750 mg a.s./L).
Doses were administered by removing frames of stores from the colonies and placing a 1 litre glass container containing the treatment solution within the brood chamber.

Observations
The content of dead bee traps was counted daily during the brood assessment period. All colonies were assessed within one week prior to dosing and within weeks 1, 2 and 3 after dosing, including counts of the number of combs of adults, brood, stores and pollen as well as behavioural or physical abnormalities. The uptake of each sucrose solution was checked daily and the container removed when empty or after 5 days. On day 7 the marked brood cells (eggs, young and old larvae) were assessed for mortality and appearance. On day 13 brood cells marked as containing old larvae, on day 15 cells previously containing young larvae and on day 16 cells previously containing eggs, were assessed. Cells were uncapped; the bee removed carefully with forceps and the age of bee was assessed, weighed and observed for deformities. The temperature and humidity were recorded continuously using a data logger; local (within 10 km) weather data was also collected.

Residues analysis
Analysis of glyphosate acid in larvae samples was conducted following extraction with acetonitrile:water (1:4, v/v), clean up by solid phase extraction on C18 and derivatisation as FMOC glyphosate and a second clean up (solid phase extraction on Oasis HLB, methanolic elution) by HPLCMS/MS. Analysis of glyphosate acid in treated sugar solution samples was conducted following extraction with acetonitrile:water (1:4, v/v), solid phase extraction on Oasis HLB, methanolic elution and derivatisation as FMOC-glyphosate by HPLC-MS/MS. Limit of quantification (LoQ) and limit of detection (LoD) were 1.0 and 0.3 mg/kg, respectively. Freshly prepared test treated sucrose solution samples were retained for analysis. On day 4 and 7, samples of ten 4-5 day old larvae were collected from each colony for residue analysis.

Data analysis
Brood mortality was analysed using a generalised linear model (Logit distribution) and an ANOVA for pupae weight data to determine NOAEL statistically.

Results and discussion
Observations and findings
Analytical data:
Residues in samples of sucrose treatment solutions were within 11 % of nominal doses. The nominal dose of 75 mg glyphosate a.e./L (corresponding to 61 mg glyphosate a.e./kg) was confirmed to be 65.7 mg glyphosate a.e./kg. The nominal dose of 150 mg glyphosate a.e./L (corresponding to 122 mg glyphosate a.e./kg) was confirmed to be 135 mg glyphosate a.e./kg.

The nominal dose of 301 mg glyphosate a.e./L (corresponding to 245 mg glyphosate a.e./kg) was confirmed to be 266 mg glyphosate a.e./kg (conversion from nominal dose rate in mg a.e./L to nominal dose rate in mg/kg was based on a density of 50% w/v sucrose solution of 1.23 kg/L).
Residues in larvae sampled from the hive on day 4 and day 7 ranged from 7.9 to 18.4 and below LoQ to 3 mg glyphosate a.e./kg, respectively on the dose 75 mg a.e./L, from 26.3 to 53.2 and 1.9 to 4.9 mg glyphosate a.e./kg, respectively on the dose 150 mg a.e./L and from 33.1 to 82.1 and 3.2 to 6.3 mg glyphosate a.e./kg, respectively on the dose 301 mg a.e./L, confirming that larvae were exposed to the test item provided in the sugar solution and consumed it.
Consumption of treated sucrose solution: The control colonies consumed between 0.625 and 1.0 L of untreated sucrose. In the glyphosate treated colonies at least 3 of 4 colonies consumed the total volume of treated sucrose.

Bee brood assessments:
No significant statistical difference in brood development (eggs, young larvae, old larvae) was observed for all glyphosate treatment groups compared to control ($p<0.05$).

Table B.9.4-11: Survival of marked brood exposed to glyphosate isopropylamine salt

Dose rate [mg/L]	**Control**	**75**	**150**	**301**
Mean dose consumed [mg]		**73±2**	**138±12**	**255±46**
7-d old cells marked as eggs [%]	87.3±1.9	84.8±4.0	87.5±2.7	86.2±3.3
16-d old cells marked as eggs [%]	85.0±2.0	82.3±3.3	86.8±2.7	84.2±3.9
7-d old cells marked as young larvae [%]	96.4±3.0	93.5±1.8	91.5±4.3	95.0±1.8
16-d old cells marked as young larvae [%]	95.9±3.1	93.5±1.8	86.5±4.3	90.0±5.4
7-d old cells marked as old larvae [%]	97.0±0.4	96.8±0.5	96.8±1.7	95.3±2.9
16-d old cells marked as old larvae [%]	95.8±1.3	94.8±1.1	94.3±1.0	95.3±2.9

There were no significant effects of the treatment on the mean weight of the exposed pupae. No biologically significant adult mortality was observed in any treatment group. No adverse effects on colonies were observed in any treatment group apart from an apparent decline in the number of bees and brood in the fenoxycarb treated colonies in the later stages of the study.

Table B.9.4-12: Pupae weight at final assessment

Dose rate [mg/L]	**Control**	**75**	**150**	**301**
Mean dose consumed [mg]		**73±2**	**138±12**	**255±46**

Pupae marked as eggs [mg]	127.5±0.7	124.7±0.8	126.7±0.6	135.7±0.6
Pupae marked as young larvae [mg]	128.4±0.6	126.3±1.0	124.4±0.8	125.4±0.6
Pupae marked as old larvae [mg]	128.9±0.4	121.2±0.5	122.6±0.5	125.6±0.4

In the fenoxycarb toxic reference treated colonies, the overall survival of marked cells was 20 % for marked eggs, 0 % for marked young larvae and 12 % for marked old larvae, meeting the validity criterion for the toxic reference (>40 % effect on all stages).

Conclusion

The overall NOAEL for brood development of honey bees was the highest dose tested – 301 mg glyphosate acid equivalent/L (nominal) equivalent to 245 mg glyphosate acid equivalent/kg nominal when considering the density of the sucrose solution and 266 mg glyphosate acid equivalent/kg actually measured.

B.9.4.3 Cage and tunnel tests (OECD IIIA 10.4.4, OECD IIIA 10.4.7)

The exposure study does not evaluate the toxicity to bees. It was performed in support of the experimental design and dose setting of the bee brood study of Thompson, 2012, BVL no 2311020 presented under 9.4.2.

KIIA 8.7.3/01 Thompson, 2011

Annex point	Author(s)	Year	Study title
IIA 8.7.3/01	Thompson, H.M.	2011	Glyphosate: Study to determine potential exposure of honeybee colonies to residues under semi-field conditions The Food and Environmental Research Agency, Sand Hutton, York, U.K. Report No: 2311016/ V7YH1002 Date: 20011-12-21 GLP: yes not published

Guideline: None; tailor made study
Deviations: None
Dates of experimental work: 2011-05-20 to 2011-05-27

Executive Summary

A semi-field study was undertaken to determine the potential exposure of honeybee colonies to glyphosate by quantifying residues in relevant food matrices, i.e. pollen and nectar, when the formulation MON 52276 was applied to flowering *Phacelia* grown in two large (180 m²) glasshouses. Following treatment of nominal 8 L/ha, equivalent to 2.88 kg a.e./ha, two honey bee colonies per glasshouse were exposed. Foraging activity in the crop and activity at each hive was assessed daily for 7 days. On days 0, 1, 2, 3, 4 and 7, forager bees were taken to get hold of the nectar from the honey stomach of the bees after foraging in the treated crop. On

days -1, 1, 2, 3, 4 and 7, samples of pollen were collected from the pollen traps fitted to each hive. Samples of nectar were also collected from the combs in each hive on day 7. Furthermore, samples of larvae were collected from the combs in each hive on days 4 and 7. Daily assessments were made of the percentage of plants with wilted leaves or flowers.
Foraging assessment showed foraging activity on the crop from start of study throughout the exposure period in glasshouse 1 with a peak on day 4. The lowest foraging activity was observed on day 5 at 38 % of the mean pre-spray activity. In glasshouse 2 the activity declined throughout the assessment period to reach less than 10 % of mean spray activity on days 5-7. In line with the decreased foraging activity in glasshouse 2, the crop started to show significant effects of the treatment from day 4 onwards.
Residues in nectar samples taken from forager bees at various time points after application ranged from 2.78 to 31.3 mg a.e./kg; residues in nectar samples taken from the colonies ranged from below LoQ (1.0 mg a.e./kg) to 1.30 mg a.e./kg. Residues in pollen samples taken from the pollen trap at various time points after application ranged from 87.2 to 629 mg a.e./kg. Residues in larvae samples ranged from 1.23 to 19.50 mg a.e./kg.
The residue data can be used to assess the approximate exposure level of brood within colonies exposed under worst-case conditions.
The maximum pollen collected per colony was 2.9 g on day 1 and the traps are estimated to be about 50 % efficient so about 6 g of pollen per day was returned to the hive (the colony is using about 4.5 g of this based on the Rortais *et al.* 2005).
The nectar can be assessed using a mean of 18 foragers returning to the hive per 30 seconds and approximately 50 µL per load (max), which gives 18 trips/30 sec * 60 sec/min * 60 min/hour * 12 hours max foraging/day, equal to 25,920 trips/day * 0.050 mL, resulting in 1296 mL/day (of which the colony is using 135 g based on Rortais *et al.* 2005).

As a worst case example considering the colony size of the present study, a honey bee colony collects 6 g pollen and 1296 mL nectar and of this the brood consumes 4.5 g pollen and 135 g nectar, which allows the excess to be stored for later consumption. As simulated in this study, for honeybee colonies foraging on the model crop *Phacelia* treated with 8 L MON 52276/ha, a total daily intake of glyphosate residues of 44.0 mg a.e. (based on day 1 maximum mean residues) and of 22 mg a.e. (based on mean residues over days 1-3) can be estimated.

Materials and methods

Test material

Test item:	MON 52276 (Soluble concentrate)
Active substance:	Glyphosate acid
Active substance content:	360 g glyphosate acid equivalents/L (nominal) 358.8 g glyphosate acid equivalents/L (according to the Certificate of Analysis)
Proposed use:	Herbicide
Description:	Clear brown liquid
Lot/Batch #:	A9K0106104
Density:	1.1693 g/mL at 20 °C (according to the Certificate of Analysis)

Vehicle and/or positive control

None

Test organism

Species:	*Apis mellifera*; 4 honey bee colonies containing 4 - 6 frames of brood, containing 6000 - 12000 adult bees
Age:	Not stated
Source:	UK national Bee Unit
Acclimatisation:	3 days

Test system

Two 180 m² glasshouses at Stockbridge Technology Centre, Selby, North Yorkshire, U.K.

Crop cultivated:	*Phacelia* (sown directly into soil of the glasshouse, no pesticide use during cultivation) Replication: 2 glasshouses, each containing 2 bee colonies

Environmental conditions

Temperature:

Glasshouse 1= 7.7 – 39.9 °C, temperatures of >35 °C were recorded on day 6 and 7 for 10 and 30 min.

Glasshouse 2= 8.3– 47.4 °C, temperatures of >35 °C were recorded on days -1, 1, 2, 4, 6 and 7 for up to 30 min until day 4, for 1.5 h on day 4, 50 min on day 6 and 40 min on day 7.

High temperatures occurred primarily between 11:30 and 14:00 and exhibited no obvious effects on crop or foraging bees

Humidity:

Glasshouse 1= 19.5 to 93.4 %

Glasshouse 2= 13.9 to 100 %

Study design and methods

Experimental treatments

Study site:

The study was conducted in two 180 m² glasshouses situated at Stockbridge Technology Centre, Cawood, Selby, North Yorkshire. The glasshouses were well ventilated (but equipped with insect proof) to be as representative as possible of the outdoor situation but without direct precipitation. *Phacelia* was planted directly into the soil inside the glasshouse and no pesticides were applied during cultivation. The timing of the start of test i.e. transfer of colonies into the glasshouse was determined by the flowering of the crops. Temperature and humidity in the glasshouses was recorded continuously.

Experimental design:

Four colonies of bees and brood comprising each of 4 to 6 frames of brood and containing 6000 to 12000 adult bees were used. Hives were fitted with a pollen trap. Three days prior to application two colonies each were located on opposite sides of each glasshouse and allowed to fly freely within the glasshouse. Colonies A and B were placed in glasshouse 1, colonies C and D were placed in glasshouse 2.

Test item application:

The test item MON 52276 (nominal content: 360 g glyphosate acid equivalent/L) was applied onto the crop grown in the glasshouse on day 0 during a period when bees were actively foraging using a 3 nozzle lunch box sprayer unit with a hand-held boom fitted with Lurmark 03 F110 nozzles. The sprayer was pre-calibrated to deliver a known application rate of 400 L/ha. The colonies were protected from direct overspray and spray drift during the application.

Observations

Foraging assessments were performed each day during times peak foraging activity. The assessments were performed by counting the number of bees foraging in a marked area (5 times in 1 m transects) during a 1 minute period during peak activity. In addition, the number of bees returning to each hive and the number carrying pollen loads were counted during a 30 second period.

Visual assessment of the crop was performed daily by determination of the proportion of plants with wilted flowers and wilted leaves.

The contents of the pollen traps were collected on days -1, 1, 2, 3, 4 and 7 after application. Samples of forager bees were collected on days 0, 1, 2, 3, 4 and 7 after application. The nectar was collected from the bees honey stomachs. On days 4 and 7 samples of ten 4-5 day old larvae were taken from each colony, on day 7 an additional sample of nectar was collected from the combs of each colony.

Residues analysis

Analysis of glyphosate acid in samples was conducted following extraction with acetonitrile:water (1:4, v/v), clean up by solid phase extraction on C18 and derivatisation as FMOCglyphosate and a second clean up (solid phase extraction on Oasis HLB, methanolic elution) by HPLCMS/MS. Limit of quantification (LoQ) and limit of detection (LoD) were 1.0 and 0.3 mg/kg, respectively.

Data analysis

Considering residue levels determined in nectar and pollen after treatment of a model crop, possible exposure scenarios of honeybee brood are estimated based on information available from literature and the present study.

Results and discussion

Observations and findings

Verification of test item application:

The actual application rates were 8.19 L MON 52276/ha (2.94 kg a.e./ha) in glasshouse 1 and 8.30 L MON 52276/ha (2.98 kg a.e./ha) in glasshouse 2. The application rate was 102 – 104% of the nominal application rate of 8 L MON 52276/ha and 102-103 % of the nominal application rate of 2.88 kg a.e./ha.

Residue analysis: Residues in nectar samples taken from forager bees at various time points after application ranged from 2.78 to 31.3 mg a.e./kg; residues in nectar samples taken from the colonies ranged from below LoQ (1.0 mg a.e./kg) to 1.30 mg a.e./kg. Residues in pollen samples taken from the pollen trap various times after application ranged from 87.2 to 629 mg a.e./kg. Residues in larvae samples ranged from 1.23 to 19.50 mg a.e./kg.

Table B.9.4-13: Summary of residue analysis of pollen, nectar and larvae samples

		Days after treatment [mg glyphosate acid equivalent/kg]					
	Hive	**-1**	**1**	**2**	**3**	**4**	**7**
Nectar (honey stomachs)	A + B	n.d.	25.5	9.24	4.90 (samples combined DAT 3, 4, 7)		
	C + D	n.d.	31.3	15.2	7.18 (samples combined DAT 3, 4)		2.78
	Overall mean	**n.d.**	**28.4**	**12.2**	**6.0**		
Nectar (hive)	A	--	--	--	--	--	<LoQ
	B	--	--	--	--	--	1.30
	C	--	--	--	--	--	1.06

		Days after treatment [mg glyphosate acid equivalent/kg]					
	Hive	**-1**	**1**	**2**	**3**	**4**	**7**
	D	--	--	--	--	--	1.00
Mean							**0.99**
Larvae (comb)	A	--	--	--	--	8.32	2.54
	B	--	--	--	--	16.70	10.6
	C	--	--	--	--	19.50	6.72
	D	--	--	--	--	2.88	1.23
Mean						**11.9**	**5.3**
Pollen (pollen trap)	A	n.d.	325	255	119 (samples combined)	134 (samples combined)	87.2 (samples combined)
	B	n.d.	405	213			
	Mean A + B	--	**365**	**234**	**119**	**134**	**87.2**
	C	n.d.	518	333	181	176	130 (samples combined)
	D	n.d.	629	477	147	180	
	Mean C + D	--	**573.5**	**405**	**164**	**178**	**130**
	Overall mean	--	**469**	**320**	**142**	**156**	**109**

DAT day after treatment
n.d. not detected
LoQ 0.6 mg/kg
LoD 0.3 mg/kg

Foraging activity:
Foraging assessment showed foraging activity on the crop from start of study throughout the exposure period in glasshouse 1 with a peak on day 4. The lowest foraging activity was observed on day 5 at 38% of the mean pre-spray activity. In glasshouse 2 the activity declined throughout the assessment period to reach less than 10% of mean spray activity on days 5-7. In line with the decreased foraging activity in glasshouse 2, the crop started to show significant effects of the treatment from day 4 onwards.
Data analysis:
The residue data can be used to assess the approximate exposure level of brood within colonies exposed under worst-case conditions.

Table B.9.4-14: Assessment of possible exposure of honey bee colonies to glyphosate residues under two scenarios is depicted below

Scenario	**Daily intake of glyphosate residues in nectar (1296 g nectar/d) [mg]**	**Daily intake of glyphosate residues in pollen (6 g pollen/d) [mg]**	**Total daily intake of glyphosate residues [mg a.e.]**
Day 1 maximum mean residues	40.6	3.4	44.0

(31.3 µg a.e./g in nectar, 574 µg a.e./g in pollen, glasshouse 2)			
Mean residues over days 1-3 (15.5 µg a.e./g in nectar, 310 µg a.e./g in pollen, both glasshouses)	20.1	1.9	22.0

Two approaches can be made to assessing exposure - one based on generic published data on the requirements for nectar and pollen by larvae (generic data) and the other based on the observations made in this study (study data).

Generic data:

The calculations are based on a daily brood requirement of 30 mg nectar (based on 40% sugar in nectar) and 1 mg pollen for worker brood (Rortais *et al.* 2005). Based on a brood frame being 3600 cells and 25% of the time is as unsealed brood (hatch day 3 to sealed day 8 with emergence day 21) then five frames of brood (4-6 were used in this study) is 18,000 brood cells therefore for 4500 larvae with a requirement of 135 g/day nectar and 4.5 g/day pollen for the colony.

Study data:

The second approach is to assess the amount of pollen and nectar returning to the hive over the time course of exposure using the data on the numbers of returning foragers in the study and the amounts of pollen and nectar collected from bees by using the pollen trap and individual bee samples. The maximum pollen collected per colony was 2.9 g on day 1 and the traps are estimated to be about 50% efficient so about 6 g of pollen per day was returned to the hive (the colony is using about 4.5 g of this based on the Rortais *et al.* 2005).

The nectar can be assessed using a mean of 18 foragers returning to the hive per 30 seconds and approximately 50 µL per load (max), which gives 18 trips/30 sec * 60 sec/min * 60 min/hour * 12 hours max foraging/day, equal to 25,920 trips/day * 0.050 mL, resulting in 1296 mL/day (of which the colony is using 135 g based on Rortais *et al.* 2005).

Conclusion

As a worst case example considering the colony size of the present study, a honey bee colony collects 6 g pollen and 1296 mL nectar per day and of this the brood consumes 4.5 g pollen and 135 g nectar, which allows the excess to be stored for later consumption. As simulated in this study, for honeybee colonies foraging on the model crop *Phacelia* treated with 8 L MON 52276/ha, a total daily intake of glyphosate residues of 44.0 mg a.e. (based on day 1 maximum mean residues) and of 22 mg a.e. (based on mean residues over days 1-3) can be estimated.

B.9.4.4 Field tests (OECD IIIA 10.4.5)

Not required.

B.9.4.5 Investigation of special effects (OECD IIIA 10.4.6)

Not required.

B.9.4.6 Summary of effects on honeybees

The 2001 EU glyphosate evaluation concluded that the hazard quotient values for intended uses of glyphosate are well below 50, indicating a low risk to honeybees according to the EPPO risk assessment scheme. The results from new laboratory toxicity studies demonstrate that glyphosate, glyphosate salts and MON 52276 have very low acute contact and oral toxicity to honeybees (see table 9.4.7-1 below). Additionally a bee brood study was performed following established methodology which demonstrates that glyphosate poses no chronic risk to bee brood as well at worst case field exposure levels (see B 9.4.2.1).

Table B.9.4-15: Summery of all laboratory studies submitted for the EU evaluation of glyphosate acid

No.	Test substance	Exposure route	LD_{50} [µg a.s./bee]	GLP	Reference	2001 EU evaluation monograph reference
Glyphosate acid						
2001 EU review of glyphosate	glyphosate	oral 48 h	**100 ***	no	Fraser and Jenkins (1972)HU85X094	BIE 96-00071
		contact 48 h	**>100 ***			
IIA 8.7.1/01	glyphosate	oral 48 h	**>200**	yes	Kleiner (1995) 95 10 48 065 BVL no 2311000	
		contact 48 h	**>200**			
IIA 8.7.1/02	glyphosate	oral 48 h	**>40**	yes	Weyman (1996) 1413/3-1018 BVL no 2311002	
		contact 48 h	**>20**			
IIA 8.7.1/03	glyphosate	oral 48 h	**>182**	yes	Thompson (1998) FN9700 BVL no 2311004	
		contact 48 h	**>103**			
IIA 8.7.1/04	glyphosate	oral 72 h	**>116.67**	yes	van der Steen (1995) 141907 BVL no 2311007	
		contact 72 h	**-**			
IIA 8.7.1/05	glyphosate	oral 72 h	**-**	yes	van der Steen (1995) 142335 BVL no 2311009	
		contact 72 h	**>100**			
Glyphosate-IPA salt						
2001 EU review of glyphosate	MON 2139	oral 48 h	**>100**	no	Fraser and Jenkins (1972) HU85X094	BIE 96-00071
		contact 48 h	**>100**			
KIIIA1 10.4.2	**MON 52276**	oral 48 h	**>77 ***	yes	Baxter (2001) MON-00-2-version 2 BVL no 2316011	
		contact 48 h	**>100 ***			
IIA 8.7.2/06	Glyphosate IPA 62%	contact 48 h	**>61.3**	yes	Franco Perina (2000) RF-D4.0177/00 BVL no 2311010	

Glyphosate-K salt						
IIA 8.7.1/07	MON 78623	oral 48 h	>**104**	yes	Halsall (2003) MT-2002-108 BVL no 2311012	
		contact 48 h	>**100**			

* LD_{50} values used for risk assessment

B.9.4.7 Risk assessment for honeybees

The 2001 EU glyphosate evaluation concluded that glyphosate is of low risk to bees. In order to reevaluate glyphosate a total of seven laboratory toxicity studies with technical or formulated glyphosate, a tunnel test and a study about the honeybee brood development with technical glyphosate were submitted. The risk assessment is based on the intended uses and nominal field rates.

Exposure
The recommended use pattern for glyphosate includes application in a wide range of application areas (e.g. agriculture, viticulture, ornamental plants) at a maximum application rate of up to 2880 g a.s./ha.
Bees may be exposed to glyphosate by direct spraying while they are foraging on flowers and weeds, through contact with fresh or dried residues or by oral uptake of contaminated pollen, nectar and honey dew.

Toxicity
There is an extensive regulatory database assessing the acute contact and oral toxicity of glyphosate, glyphosate salts, MON 52276 and other glyphosate formulations. Results of all laboratory studies with technical and formulated material demonstrate that glyphosate is of very low toxicity to honeybees with LD_{50} values around or higher than 100 µg a.s./bee.

Hazard quotients
Hazard quotients for oral and contact exposure according to EPPO (2003) Environmental risk assessment scheme for plant protection products (Chapter 10: Honeybees (PP 3/10(2)). Bulletin OEPP/EPPO Bulletin 33: 141-145) were calculated as follows:

Hazard Quotient = max. application rate [g a.s./ha] / LD_{50} [µg a.s./bee]

Table B.9.4-16: Hazard quotients for honeybees

Test substance	Max. single application rate [g a.s./ha]	Exposure route	LD_{50} [µg a.s./bee]	Hazard quotient (HQ)	HQ trigger
glyphosate tech.	2880	oral	= 100	29	50
		contact	> 100	< 29	
MON 52276	2880	oral	> 77	< 38	50
		contact	> 100	< 29	

Honey bee brood

A semi-field study (Thompson, 2011, BVL no 2311016) was undertaken to determine the potential exposure of honeybee colonies to glyphosate by quantifying residues in relevant food matrices, i.e. pollen and nectar, when the formulation MON 52276 was applied to flowering *Phacelia* grown in two large (180 m²) glasshouses. Following treatment of nominal 8 L/ha, equivalent to 2.88 kg a.e./ha, two honey bee colonies per glasshouse were exposed. As simulated in this study, for honeybee colonies with 6000 - 12000 adult honeybees foraging on the model crop *Phacelia* treated with 8 L MON 52276/ha, a total daily intake of glyphosate residues of 44.0 mg a.e. (based on day 1 maximum mean residues) and of 22 mg a.e. (based on mean residues over days 1-3) can be estimated.

A field study (Thompson, 2012, BVL no 2311020) was undertaken to determine the potential for toxicity to developing honey bee larvae and pupae to glyphosate (tested as the IPA salt) when fed directly to honey bee colonies. In this study the overall NOAEL (No Observed Adverse Effect Level) for brood development of honey bee colonies was 301 mg glyphosate a.e./L sucrose solution, the highest dose tested.

Since glyphosate residues levels derived from semi-field tests under absolute worst case conditions are far below the NOAEL of 301 mg glyphosate a.e./L no negative effects on brood development are expected when glyphosate is used under practical conditions.

Overall conclusion

It is concluded that glyphosate will not adversely affect bees or bee colonies when used as recommended.

B.9.5 Effects on other arthropod species (Annex IIA 8.3.2; Annex IIIA 10.5)

Several studies on the effect of glyphosate formulations on non-target arthropods were assessed during the first EU evaluation of glyphosate in 2001 and are summarised in SANCO/6511/VI/99-final. These studies were evaluated in the monograph but were not submitted with the renewal dossier and are not documented in detail in this Review Assessment Report.

Due to the significant developments and changes in the risk assessment approach and strategy for terrestrial non-target arthropods since the evaluation of glyphosate in 2001, those older studies are no longer considered appropriate for a quantitative risk assessment according to current standards. Nevertheless, the results from the laboratory tests on inert substrates are useful as additional information, also due to the fact that the spectrum of tested species partly differs from that of the newly submitted studies. The following table contains the summary data from studies of the first monograph on glyphosate from 2001 amended with new studies with the lead formulation MON 52276 for the current evaluation of glyphosate.

Additional studies with the lead formulation MON 52276 have been conducted with *Aphidius rhopalosiphi*, *Typhlodromus pyri* and *Aleochara bilineata* to meet the data requirements that are acceptable for an updated non-target arthropod risk assessment.

Table B.9.5-1: Toxicity of MON 52276 to non-target arthropods submitted for the present application for renewal of approval for glyphosate

Species	Substance	System	Results	Reference
Aphidius rhopalosiphi	MON 52276	Extended laboratory (whole plant), 3D	LR_{50} > 16.0 L product/ha (5760 g a.s./ha)	Stevens, 2010 MT-2009-405 BVL no 2316022
Typhlodromus pyri	MON 52276	Extended laboratory (leaf discs), 2D	ER_{50} ≥ 12.0 L product /ha (4320 g a.s./ha)	Fallowfield, 2010 MT-2009-404 BVL no 2316024
Aleochara bilineata	MON 52276	Extended Laboratory (soil)	ER_{50} > 12.0 L product /ha (4320 g a.s./ha)	Spincer, 2010 MT-2009-403 BVL no 2316026

Table B.9.5-2: Studies on the toxicity of MON 52276 for non-target arthropods already reviewed during the first EU evaluation of glyphosate in 2001

Species	Substance	System	Results	Reference
Typhlodromus pyri	MON 52276	Laboratory	LC_{100}= 10 L/ha	Kleiner, 1995; 95 10 48 056
Typhlodromus pyri	MON 52276	Extended laboratory (leaf discs)	> 75% mortality at 6 and 12 L/ha; 36% mortality at 3 L/ha; No effects on fecundity at 3 L/ha after 16 d	Kleiner, 1998; XX-98-195
Typhlodromus pyri	MON 52276	Extended laboratory (whole leaf)	36% and 30% mortality at 6 and 12 L/ha, respectively; 21% mortality at 3 L/ha; No mortality at 0.6 L/ha; No effects on fecundity at 12 L/ha or less	Vinall, 1999; US-99-092
Aphidius rhopalosiphi	MON 52276	Laboratory	LC_{100}= 10 L/ha	Kleiner, 1995; 95 10 48 054
Aphidius rhopalosiphi	MON 52276	Extended laboratory (whole plant)	No effect on mortality at 12 L/ha No effects on fecundity at 12 L/ha	Kleiner, 1999; 98 10 48 066
Chrysoperla carnea	MON 52276	Laboratory	59% mortality and 20% reduction on fecundity at 12 L/ha (no dose-response effect on fecundity); No effects on survival or fecundity at 0.6 and 6 L/ha	Barton, 1999; MON-99-093
Poecilus cupreus	MON 52276	Laboratory	LC_{50} > 10 L/ha	Kleiner, 1995; BI-2000-203
Pardosa sp.	MON 52276	Laboratory	LC_{50} > 10 L/ha	Kleiner, 1995; BI-2000-204

B.9.5.1 Acute Toxicity data

See above.

B.9.5.1.1 Laboratory studies with glas plates

Please refer to the first assessment of the active substance glyphosate (SANCO/6511/VI/99-final, or EU Review Monograph).

B.9.5.1.2 Extended laboratory studies

KIIIA 10.5.2/01

Author:	Stevens, J.
Title:	A rate-response extended laboratory test to determine the effects of MON 52276 on the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae)
Date:	27.04.2010
Doc ID:	2311022 /MON-09-2 ! MT- 2009-405
Guidelines:	OECD/ Mead-Briggs *et al.* (2010).
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	MON 52276
Lot/Batch #:	A9B1207115
Purity:	Glyphosate (isopropylamine salt) 360 g/L
Density:	1.1683 g/cm^3 (at 20 °C ± 0.5 °C)
2. Positive control:	Perfekthion - BAS 152 11 I (dimethoate: 400 g/L)
Species:	Parasitic wasp (*Aphidius rhopalosiphi*)
Age:	Adults approximately 48 h old
Source:	In-house culture originally obtained from PK Nützlingszuchten, Welzheim, Germany
Diet/Food:	Solution of honey in water (1 : 3 v/v)
Temperature:	20 °C
Relative humidity:	69 – 72 %
Photoperiod:	16 hours light / 8 hours darkness
Methods:	Following a preliminary range-finding test, MON 52276 was evaluated in a definitive rate-response test at five application rates, equivalent to 16000, 12000, 8000, 6000 and 4000 mL product/ha. These variants were compared to a control treatment of purified water and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate) applied at a rate of 10 mL product/ha (nominally 4 g a.s./ha).Treatments were applied at a volume rate equivalent to 400 L spray solution/ha to pots of seedlings barley. Once dry, the barley plants were enclosed within cylindrical, ventilated collars. Five female wasps were then confined in each arena, with six replicates (i.e. a total of 30 wasps) prepared for each treatment. To determine any significant sub-lethal effects on wasp reproduction, assessments were then carried out using the surviving insects from the control and the three highest treatment rates of the test item that resulted in < 60% corrected mortality. Fifteen wasps from each treatment were confined individually over pots of untreated barley plants that had previously been infested with cereal aphids (*Metopolophium dirhodum* and *Rhopalosiphum padi*). The wasps were then removed from the plants after 24 h and the aphids and plants left for a further 10 days before the number of 'mummies'(parasitized aphids containing wasp pupae) that had developed was recorded.

	Mortality of the wasps was recorded approximately 2, 24 and 48 h after treatment. The behaviour of the wasps was assessed during the first 3 h after treatment and also at 24 and 48 h after treatment, to determine whether there was any apparent repellence from the treated plants, and wasp survival was assessed over a period of 48 h. ANOVA followed by Fisher's Exact test (α = 0.05) for mortality. One-way ANOVA and Dunnett's Test as post hoc (α = 0.05) for reproduction.

Results

The mortality in the control treatments did not exceed 10%, the corrected mortality in the reference treatment was >50 %. In the control treatments, more than a minimum mean value of 5.0 mummies was produced per female. Not more than two of the surviving wasps of the control treatments did not reproduce. The validity criteria according to Mead-Briggs *et al.* (2010) are fulfilled.

Treatment with the reference item Perfekthion at a concentration of 10 mL/ha resulted in significant effects on reproduction after 48 h of exposure.

Table B.9.5-3: Toxicity of MON 52267 to *Aphidius rhopalosiphi* in a 48 h extended laboratory test

Test rate (mL/ha)	Mortality (%)	Corrected mortality[1] (%)	Mummies/female (No.)	Effect on reproduction (%)[2]
Control	0	-	21.4	
4000	0	0	nd	
6000	0	0	nd	
8000	0	0	28.4	-32.3
12000	3.3	3.3	30.6	-43.0
16000	0	0	31.5	-46.8

[1] :Derived using Abbott's formula.; nd: not determined

[2] Percentage effect on reproduction, relative to the control. A negative value indicates an increase.

Reproduction assessments showed that the mean number of mummies produced per surviving female was 21.4 in the control, compared with 31.5, 30.6 and 28.4 in the 16000, 12000 and 8000 mL/ha treatment rates of MON 52276, respectively. Therefore, relative to the control, there was an increase in reproduction of 46.8 %, 43.0 % and 32. 3% in the 16000, 12000 and 8000 mL/ha treatment rates, respectively.

Table B.9.5-4: Repellent assessment of MON 52276 to parasitic wasps (*Aphidius rhopalosiphi*) in a 48 h extended laboratory test

Test rate (mL/ha)	% observations where wasps recorded to be settled on the treated plants	
	Initial 3 h [1)]	24 h & 48 h [2)]
Control	32.7	40.0
4000	22.0	28.3
6000	24.7	28.3
8000	26.0	25.0
12000	20.7 *	27.5
16000	20.0 *	28.3

[1] Data from assessments made during the initial 3 h after wasp introduction. Results for the individual test item treatments were compared by one-way ANOVA and Dunnett's Test. Values marked with asterisks differed significantly from the control (* $P < 0.05$).

[2] Data from assessments made at 24 h and 48 h after wasp introduction. Results for the individual test item treatments were compared by one-way ANOVA ($\alpha = 0.05$), but values for the test item treatments did not differ significantly from the control.

* Significantly different from control

RMS Conclusions

In an extended laboratory test to determine the effects of MON 52276 on the parasitic wasp *Aphidius rhopalosiphi*, the 48-h LR_{50} was higher than 16000 mL product/ha. MON 52276 stimulated the reproductive performance of surviving wasps up to and including a treatment rate of 16000 mL product/ha.

The study is valid and acceptable.

KIIIA 10.5.2/02

Author:	Fallowfield, L.
Title:	An extended laboratory bioassay of the effects of MON 52276 on the predatory mite, *Typhlodromus pyri* (Acari: Phytoseiidae)
Date:	27.04.2010
Doc ID:	2311024 /MON-09-3 ! MT- 2009-404
Guidelines:	OECD/ Blümel et al., 2000
GLP:	Yes
Validity:	Yes

Materials and Methods

Test item:	MON 52276 (glyphosate in the form of its isopropylamine salt)
Lot/Batch #:	A9B1207115
Purity:	360 g/L glyphosate acid equivalent
Positive control:	BASF Dimethoate 40 (400 g/L dimethoate)
Species:	Predacious mite (*Typhlodromus pyri*)
Age:	< 24 hours
Source:	P.K. Nützlingszuchten, Germany
Diet/Food:	Almond and apple pollen

Acclimatisation:	Not stated
Temperature:	Mortality test: 25– 26 °C Reproductive test: 25- 27 °C
Relative humidity:	Mortality test: 49.6 – 79 % Reproductive test: 63 – 79 %
Photoperiod:	16 hours light / 8 hours darkness
Light intensity	660 – 1230 lux
Methods:	Leaf discs (5-cm diameter) cut from detached primary leaves of bean plants (*Phaseolus vulgaris L.*) were sprayed with MON 52276 at rates equivalent to 16000, 12000, 8000, 6000, and 3000 mL/ha (5760, 4320, 2880, 2160, and 1080 g a.s./ha). Additional leaf discs were treated with the reference toxicant Perfekthion (414.8 g dimethoate/L) applied at a rate of 12 g a.s./ha, or with deionised water as a negative control. All treatments were sprayed in an application volume equivalent to 200L/ha. After the sprayed discs were air-dried for approximately 1 hour, protonymphal mites were placed on the leaves, inside petri dishes, and observed for seven days. There were three replicates, each containing twenty mites, per treatment. On Day 7, any eggs present were removed, and the number of male and female mites in each of the replicates was recorded to verify the male:female ratio was at least 1:5. Between days 7 and 14, a fecundity assessment was carried out for the control and the treatments. The nymphs were fed with a mixture of almond and apple pollen provided daily, and were provided water ad libitum. Test units were kept under a 16/8h light:dark regime. The number of living, dead, stuck, drowned, or missing mites was recorded on day 1 and 7. The number of eggs laid, and live or dead juvenile states per female were recorded on days 10, 13, and 14. The mortality was corrected with the control mortality using Abbot's correction (1925).The percentage mortality in each treatment in the bioassay was compared to the control using Fisher's Exact Test (α = 0.05). Reproduction results were compared by one-way ANOVA and Dunnett's Test.

Results

Treatment with the reference item resulted in significant effects on reproduction (87 %).

Table B.9.5-5: Toxicity of MON 52267 to predatory mites (*Typhlodromus pyri)* in a 7 d extended laboratory test

Test rate (mL/ha)	Mortality after 7 days (%)	Abbott Corrected mortality [1] (%)	Mean egg number/ female after 14 days (No.)	Effects on Reproduction[3] (%)
Control	15	-	6.9	-
3000	13	0	8.1	-17.4
6000	18	4	4.2	39.1
8000	23	9	5.9	14.5
12000	32	20	3.8*	44.9
16000	40*	29	3.0*	56.5
Perfekthion (12 g a.s./ha)	87*	85	-	-

[1] Including dead, stuck, drowned, or missing mites.

[2] According to Abbott (1925)

[3] Change in numbers of eggs per female, relative to control (after Blümel et al., 2000). A positive value indicates a decrease and a negative value indicates an increase.

* Significantly different from control (α = 0.05)

Validity criteria according to Candolfi et al. (2000) were fulfilled; mortality in control group did not exceed 20 %, the mortality in the toxic reference treatment was between 50-100 %, and the mean cumulative number of eggs produced between 7 and 14 days exceeded 4.0 per female in the control treatment.

RMS Conclusions

The effects of MON 52276 on the predatory mite, (*Typhlodromus pyri*) were evaluated under extended laboratory test conditions. The study is valid and acceptable. The 7-day LR_{50} is found to be higher than the maximum rate tested >16000 mL MON 52276 /ha (nominally 5760 g a.s./ha) and the surrogate endpoint for reproduction was set to be $ER_{50} \geq 12000$ mL MON 52276 /ha.

MON 52276 induced a reduction of reproductive capacity by 39.1 % in mites at treatment rates of 6000 mL formulation/ha. At treatment rates of 12000 mL formulation/ha, reproductive capacity of mites was also significantly reduced. Since sublethal endpoints are considered to be relevant for higher tier risk assessment steps, the lowest dose tested (12000 ml a.s./ha) with significant effects on reproduction was employed in the risk assessment (surrogate endpoint reproduction = $ER_{50} \geq 12000$ mL MON 52276 /ha).

KIIIA 10.5.2/03

Author:	Spincer, D.
Title:	An extended laboratory test to determine the effects of MON 52276 on the ground-active beetle, *Aleochara bilineata* (Coleoptera, Staphylinidae)
Date:	27.04.2010
Doc ID:	2311026 /MON-09-4 ! MT- 2009-403
Guidelines:	OECD/ Grimm *et al* .(2000)
GLP:	Yes
Validity:	Yes

Materials and Methods

Test item:	MON 52276
Lot/Batch #:	A9B1207115
Purity:	Glyphosate (glyphosate acid equivalent) 360 g/L
Density:	1.1683 g/cm^3 (at 20 °C ± 0.5 °C)
2. Positive control:	Reference item: Cyren (chlorpyrifos: 480 g/L)
Species:	Rove beetle (*Aleochara bilineata*)
Age:	Physiologically 3 - 4 days old
Source:	Commercial supplier (De Groene Vlieg, Nieuwe Tonge, The Netherlands)
Diet/Food:	Pellets (approximately 0.2-0.5 g) of raw minced beef for food every 1-3 days, until the adult beetles were removed 28 days after treatment (DAT)
Acclimatisation:	Not stated
Temperature:	19 – 21 °C
Relative humidity:	51 – 86 %
Photoperiod:	16 hours light / 8 hours darkness

Methods:	MON 52276 was evaluated at three rates, equivalent to 6000, 8000 and 12000 mL product/ha. These were compared to a water-treated control (negative control) and a toxic reference treatment of chlorpyrifos (a 480 g/L EC formulation applied at a rate equivalent to 240 g a.s./ha). All treatments were applied to boxes of a standard sandy soil (LUFA 2.1), using a track sprayer calibrated to 400 L spray solution/ha. Applications were made to four replicate arenas per treatment and, immediately following spraying, twenty adult *Aleochara bilineata* (10 males: 10 females) were introduced into each replicate. Beetles were fed with raw minced beef, initially an hour after treatment and then every 2 to 3 days thereafter. The parasitic success of their larval offspring was assessed by the provision of ca. 500 onion fly pupae (*Delia antiqua*) in each replicate box on three weekly occasions, i.e. 7, 14 and 21 DAT. The original adult beetles were removed from the arenas 28 DAT and the number of new adults (F1 progeny) that subsequently developed from the parasitized fly pupae was recorded over a further 46-day period. Assessments of the condition of the beetles were made at 1, 7 and 28 days after treatment (DAT). Assessment of reproduction was conducted from 28 DAT for 46 days. Fisher's Exact test (α = 0.05) for mortality, ANOVA (α = 0.05) for reproduction.

Results

Treatment with the reference item Cyren at a concentration of 240 g a.s./ha resulted in 100 % effects on reproduction.

Table B.9.5-6: Sublethal effects of MON 52276 on rove beetles (*Aleochara bilineata*) in an extended laboratory test (mean number of F1 progeny)

Test rate (mL/ha)	Mortality after 28 days (%)	Corrected mortality [1] (%)	Mean number of F_1 progeny per arena [1] (No.)	Effect on reproduction (%) [2]
Control	32.5	-	862.5	
6000	38.8	9.3	706.3	18.1
8000	47.5	22.2	846.0	1.9
12000	38.8	37.0	778.0	9.7

[1] The numbers of progeny emerging in the control and test item treatments were compared by ANOVA, but treatment means did not differ significantly (P > 0.05). For the toxic reference treatment (% effect), no statistical comparisons were made.

[2] The percentage change in numbers of F1 progeny, relative to the control was calculated using the formula: R = (1-(Rt/Rc) x 100, where Rt and Rc are the numbers of offspring observed in the treatment and control groups, respectively. Positive values indicate a decrease, relative to the control.

An extended laboratory study was conducted with MON 52276 to investigate the reproduction capacity of the staphylinid beetle *Aleochara bilineata*. The beetles were exposed to rates up to 12.0 L MON 52276/ha applied on a soil substrate.

The protocol by Grimm et al. (2000) states that the bioassay should take place in a cabinet maintained at 60-90 % RH and only minor fluctuations outside of these parameters for periods of <2 h are to be considered not to results in major disturbances. However, in the present bioassay, the ambient conditions actually recorded were 51.9%-61.4 % RH for one week. These deviations were due to inadequate control being achieved by the cabinet. Even though all treatments were exposed to similar conditions, it was considered that this deviation did not support the integrity of the study.

At 28 days, mortality in the control treatment was 32.5 %, compared with 35.0 %, 47.5 % and 38.8 % in the 12000, 80000 and 6000 ml/ha tratment rates of MON 52276, respectively. Mortality rates for the adult beetles are not considered a key assessment criterion in the guideline of Grimm et al. (2000), but can provide an early indication of acute harmful treatment effects. The average number of beetles emerging from parasitized fly pupae in the control treatment was >400 per replicate, and a minimum reduction of 50% reproductive capacity was achieved in the reference item treatment when compared to the control.

RMS Conclusions

In the extended laboratory test to determine the effects of MON 52276 on the rove beetle (*Aleochara bilineata*), no significant effects on the parasitation success of the beetles were observed up to and including the highest treatment rate of 12000 mL/ha. The test is considered as valid and acceptable, despite the above mentioned deficiencies.

B.9.5.2 Field tests

The results presented on non-target arthropods other than bees indicate an acceptable in –field risk of glyphosate and the formulation MON 52276 when applied at maximum recommended application rate and according to good agricultural practices.

B.9.5.3 Metabolite testing

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B.9.5.4 Literature Review

Indirect effects on beneficial arthropod communities take place within treated areas and are principally due to vegetation changes subsequent to herbicide application. These vegetation changes, mainly decompostion of plant cover, might result in a drastic reduction of the habitats of beneficial and other non-target arthropod communities and a loss of their refuges from predators.In a multiyear study using pitfall trapping to collect mobile arthropod species on the soil surface, the combination of conservation tillage and herbicide treatment had less impact on biodiversity than conventional ploughing (Schier, 2006). However, conservation tillage without the use of glyphosate is not practiced, due to the upcoming weed pressure on culture crops. It is not possible to identify the effects of glyphosate applications in the perfomed studies, When collembolan populations were assessed in field plot experiments in Mediterranean vineyards (Renaud et al., 2004) the result suggested apparently that plant protection products containing glyphosate favored the occurrence of epigeic and hemiedaphic species due to the preservation of decaying organic material on the soil surface compared to tillage practice. RMS considers it misleading to confuse the effects of tillage practice vs. non-tillage practice with the effects of an application of glyphosate without proper negative control. In a laboratory study it could be shown that reproductive capacity of the collembolan species *Folsomia candida* was not influenced by the application of glyphosate containing plant protection product when applied at relevant environmental concentrations (Santos et al., 2010).

Arthropods in their natural environment can be exposed directly to pesticides after the application due to residues on food or due to contact with contaminated surfaces (such as plants, soil, surrounding substrate).
Risk analysis is currently based on so called beneficial arthropods which are important in the biological control of agronomic pests, typically through predation or parasitism including beetles, mites, wasp and spider. Tests are performed on glass plates or on extended laboratory tests with a 2 dimensional exposure on leaf substrates testing the formulated product for the determination of the median lethal dose (LD_{50}) and/or median effect on reproduction. Thereby test species were selected more for practical reasons because of their utility in agricultural production and feasibility in experimental setups than on the basis of their ecological relevance. At the same time effects on various developmental stages of arthropods, physiology, and behavior or prey consumption are not given consideration in traditional risk assessment. Bueno et al., (2008) could show that glyphosate containing products can be harmfull towards egg stages of *Trichogramma*, whereas at other parasitoid stages the same product was harmless. Sublethal effects of glyphosate were assessed in the laboratory on prey consumption, web building, fecundity, fertility and developmental time of progeny of a web weaver spider (*Alpaida veniliae*) in Argentina (Benamu et al., 2010) and on wolf spiders in north America (Evans et al., 2010). The authors concluded that the exposure to glyphosate containing products affects the behavior of the animals and their capacity to grow and persist in agroecosystems. In contrast, short term exposures (2h and one-day residues) of spiders and carabid beetles, respectively *Pardosa agricola* and *Poecilus cupreus*, did not affect mating or avoidance of the arthorpods, but (only) slightly slower movement (Michalkova et al., 2009).
These effects together with the indirect effects of herbicide treatment on the vegetation of their habitat receive less attention, even though they might have implications for the success of survival and reproduction.

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Schier, A. (2006): Field study on the occurrence of ground beetles and spiders in genetically modified, herbicide tolerant corn in conventional and conservation tillage systems. Journal of Plant Diseases and Protection. Special Edition XX:101-113

B.9.5.5 Summary of toxicity data on arthropods other than bees

The effects of the exposure of *Aphidius rhopalosiphi* to rates of 16, 12, 8, 6 and 4 L MON 52276/ha (nominally 5760, 4320, 2880, 2160, and 1440 g a.s./ha) were determined under extended laboratory test conditions. The 48h LR_{50} was higher than 16 L product/ha (nominally 5760 g a.s./ha). MON 52276 affected the reproductive performance of surviving wasps up to and including a treatment rate of 16000 mL product/ha, but leading to an increase in reproduction.
The effects of the exposure of *Typhlodromus pyri* to rates equivalent to 16, 12, 8, 6 and 3 L MON 52276/ha (nominally 5760, 4320, 2880, 2160, and 1080 g a.s/ha) were determined under extended laboratory conditions. The 7-day LR_{50} (median lethal rate) was found to be higher than 16 L/ha (nominally 5760 g a.s./ha), the maximum rate tested. MON 52276 had no adverse effects on the reproductive performance of surviving mites up to and including a treatment rate of 8 L formulation /ha (nominally 2880 g a.s./ha).
An extended laboratory study was conducted with MON 52276 on the reproduction capacity of the staphylinid beetle *Aleochara bilineata*. The beetles were exposed to rates up to 12.0 L MON 52276/ha applied on a soil substrate. No effects on mortality and reproduction capacity of *Aleochara bilineata* were observed. Accordingly, the resulting LR/ER_{50} was determined to be >12.0 L MON 52276/ha, equivalent to 4.32 kg a.s./ha.

B.9.5.6 Risk assessment

B.9.5.6.1 Tier 1 assessment using hazard quotients

In-field exposure
Non-target arthropods living in the crop can be exposed to residues from glyphosate-containing plant protection products by direct contact either as a result of overspray or through contact with residues on plants and soil or in food items. As an indirect effect foliar dwelling arthropods living on these weeds will loose their habitat if all vegetation in the target area is controlled.
The in-field exposure, given as predicted environmental rates, PER, for non-target arthropods resulting from the intended uses of glyphosate-containing plant protection products is calculated according to published agreement after ESCORT 2 workshop (Candolfi et al. 2011[2]) using the following equation:

$$PER_{in-field} = \text{Application rate (g a.s./ha)} \times \text{MAF}$$

where:

MAF = generic multiple application factor used to take into account the potential build-up of applied substances between applications. This factor integrates number of applications, application interval and degradation kinetics of the active substance.

Since glyphosate-containing plant protection products will be applied two times a season at a maximum application rate of 2x2160 g a.s./ ha, i.e. 4.32 kg a.s./ha, this worst case application scheme was identified and chosen for the risk assessment covering the risk assessment for the

[1] Candolfi, M.P.; Barrett, K.L.; Campbell, P.; Forster, R.; Grandy, N.; Huet, M.C.; Lewis. G.; Oomen, P.A.; Schmuck, R.; Vogt, H. (2001): Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. ESCORT2 Workshop European Standard Characteristics of Non-Target Arthropod Regulatory Testing. Wageningen, The Netherlands, 46 pp.

other applications with lower maximum annual application rate applying the following MAF values:
MAF_{foliar} =1.3; 10 days half-life and interval of 21 days (a ratio of 1:2)
MAF_{soil}= 1.5; $DT_{50\ Soil}$ 21 days and interval of 21 days (a ratio of 1:1)

The maximum predicted environmental rate (PER) occurring in the field after application of glyphosate-containing plant protection products at the maximum application rate is presented in the following table.

Table B.9.5-7: In-field predicted environmental rates (PER) for MON 52276

Scenario	Application rate (g a.s./ha)	in-field PER foliar (g a.s./ha)	in-field PER soil (g a.s./ha)
All crops	2x 2160 g a.s./ha, i.e. 4320 g a.s./ha	3672 g a.s./ha	4104 g a.s./ha

The maximum in-field exposure (predicted environmental rate [PER]) to foliar-dwelling and soil-dwelling organisms are two times 2.16 kg a.s./ha, corresponding to a maximum application rate 4.32 kg/ha glyphosate in any 12 month period across use categories (equivalent to 12 L MON 52276/ha).

The potential risk for non-target arthropods exposed in-field to glyphosate-containing plant protection products was assessed by comparing the environmental rate (PER in-field) to the lowest lethal rate (LR_{50}) estimated in toxicity tests with non-target arthropods. According to the Escort 2 concept, extended laboratory testing is formally triggered when the Tier-1 Hazard Quotient (HQ) trigger of 2 is breached.

$$In\ field\ HQ = \frac{In\text{-}\ field\ PER\ (g/ha)}{LR_{50}\ (g/ha)}$$

Table B.9.5-8: Acceptability criteria for higher tier data and HQ values

Test substance	Species	Test type	Endpoint L(E)R50 ml Prod./ha (g a.s./ha)	HQ In-field foliar	HQ In-field soil
MON 52276	*A. rhopalosiphi*	3D	LR_{50} > 16000 ml MON 52276/ha (5760 g a.s./ha)	< 0.6	-
	T. pyri	2D	ER_{50} ≥12000 ml MON 52276/ha (4320 g a.s./ha)	≤ 0.9	-
	A.bilineata	soil	ER_{50} >12000 ml MON 52276/ha (4320 g a.s./ha)	-	< 0.95

With regard to the endpoints from the extended laboratory tests on lethal and sublethal effects and the predicted rates of glyphosate in-field, the calculated HQ values show no risk for non-target arthropods following intended use of the lead formulation MON 52276 according to the label.

Off-field exposure

Exposure of non-target arthropods living in non-target off-field areas to MON 52276 will mainly be due to spray drift from field applications. Off-field predicted environmental rates (PER-values) were calculated from in-field PERs in conjunction with drift values published by the BBA (2000[2]) as shown in the following equation:

$$Off-field\ PER = \frac{Maximum\ in-field\ PER\ x \left(drift\ percentile / 100 \right)}{vegetation\ distribution\ factor\ (vdf)}$$

where:

vdf = vegetation distribution factor used in combination with test results derived from 2-dimensional exposure set-ups

To account for interception and dilution by three-dimensional vegetation in off-crop areas, a vegetation distribution or dilution factor (vdf, see above) is incorporated into the equation when calculating off-field exposure in conjunction with toxicity endpoints derived from two-dimensional studies (e.g. glass plate or leaf discs). For the results of the study with *T. pyri* exposed to MON 52276 a vegetation distribution factor has to be considered (study conducted in 2D environment).

A dilution factor of 10 is recommended by the Guidance Document, but has been questioned. The risk assessment procedure here considers a dilution factor of 5 more appropriated. For an endpoint resulting from 3-dimensional studies, i.e. where spray treatment is applied onto whole plants, the dilution factor is not used.

Since glyphosate-containing plant protection products will be applied two times a season at a maximum application rate of 2 x 2160 g a.s./ ha, i.e. 4.32 kg a.s./ha and an application interval indicated in the GAP is a minimum of 21 days the MAF values were calculated on the basis of a 10-day half-life and interval of 21 days (a ratio of 1:2).

MAF_{foliar} =1.3; 10 days half-life and interval of 21 days (a ratio of 1:2)
MAF_{soil}= 1.5; $DT_{50\ Soil}$ 21 days and interval of 21 days (a ratio of 1:1)

The drift rate at 1 m distance is ~~2.77~~ 2.38% of the double application rate (82th percentile drift). The drift factor (% drift/100) is therefore 2.38/100 = 0.0238. As for this herbicide a double ground-directed application is given, the field crop drift values are used for the indications “all crops”.

In the case of the intended use on mature crops before harvest, it must be pointed out, that the desiccation is not considered by the current assessment based on FOCUS (2001). For the particular situation of the siccation scenario, drift rates based on FOCUS (2001) might not mirrow the height of the plants and finaly might underestimate exposure. However, as recommended by the PPR panel (EFSA Journal 2014;12(7):3800) the current assessment based on FOCUS (2001) is followed, because new drift curves are currently not harmonised.
It is acknowledged that new drift values will be implemented in the nearest future. For sake of harmonisation, it was concluded that the present assessment should be based on the current agreed FOCUS values.

[2] BBA (Biologische Bundesanstalt für Land- und Forstwirtschaft) (2000): Abtrifteckwerte für Flächen- und Raumkulturen sowie für den gewerblichen Gemüse-, Zierpflanzen- und Beerenobstanbau. Bundesanzeiger 100, 26. Mai 2000, Köln, pp. 9879.

Nevertheless, the following uncertainties must be taken into account by MS and the possible underestimation of exposure might be considered:

The height of the sprayed crop has an influence on the drift pattern of PPP to the non-target habitats (e.g. Stallinga, H., J.M.G.P. Michielsen & J.C. van de Zande, 1999. Effect van gewashoogte op de drift bij een bespuiting in een graangewas. Instituut voor Milieu- en Agritechniek, IMAG-DLO Nota P99-71, Wageningen. 1999. 23pp.). The spray driftability is probably increasing the higher the equipment is distant from the ground level and the higher the canopy level. In general, this issue can be reflected by higher drift deposition values.
A study on standard spray drift deposition curves (van de Zande, J.C., D. Rautmann, H.J. Holterman & J.F.M. Huijsmans (2013): Joined spray drift curves for boom sprayers in The Netherlands and Germany. Draft Report, Plant Research International, Wageningen UR, Business Unit Agrosystems, Report 526, 72pp.) for a reference situation with a bare soil /short crop situation (crop height lower than 20 cm) and a cropped situation reports that separate spray drift curves can be described for the bare soil vs. cropped situation. The difference between the two situations vary –but can be roughly determined to be at least factor 2 higher in the cropped situation than on bare soil. In the case for crop canopy > 50 cm or special crop heights in vineyards, orchard and hops basic drift deposition values have been modified as well. Using the basic drift deposition values for field crops would probably underestimate risk to non target arthropods, when application in cereals is performed in BBCH 89 before harvest. Therefore, RMS proposed to mirror the height of the crop and the potential higher drift deposition in order to reduce risk to non-target arthropods in the off-crop habitat (The exposure scenario "ground crops x 2" was proposed).

~~New harmonised values at the EU level are under discussion and In order to respect a precautionary principle in the risk assessment for non-target arthropods the Ganzelmeier tables have been extended to take into account high crops like e.g. sunflowers by using a modified deposition factor (2.77% *2).~~

The resulting PERoff-field values are shown in the table below.

Table B.9.5-9: Off-field predicted environmental rates (PER) resulting from the intended uses of MON 52276

Study type	Max. rate (g a.s./ha)	MAF	in-field PER foliar (g a.s./ha)	Drift rate (% appl. rate)	vdf	Off-field PER (g a.s./ha)
~~2-D~~	~~2x 2160 g a.s./ha, i.e. 4320 g a.s./ha~~	~~1.3~~	~~2808 g a.s./ha~~	~~2.77 %~~	~~5~~	~~15.56~~
~~3-D~~	~~2x 2160 g a.s./ha, i.e. 4320 g a.s./ha~~	~~1.3~~	~~2808 g a.s./ha~~	~~2.77 %~~	~~1~~	~~77.78~~
~~soil~~	~~2x 2160 g a.s./ha, i.e. 4320 g a.s./ha~~	~~1.5~~	~~3240 g a.s./ha~~	~~2.77 %~~	~~1~~	~~89.75~~
2-D	2x 2160 g a.s./ha, i.e. 4320 g a.s./ha	1.3	2808 g a.s./ha	2.38 %	5	13.37
3-D	2x 2160 g a.s./ha, i.e. 4320 g a.s./ha	1.3	2808 g a.s./ha	2.38 %	1	66.83

soil	2x 2160 g a.s./ha, i.e. 4320 g a.s./ha	1.5	3240 g a.s./ha	2.38 %	1	74.52
~~2-D~~	~~2160 g a.s./ha~~	~~1~~	~~2160 g a.s./ha~~	~~5.54 %~~	~~5~~	~~23.93~~
~~3-D~~	~~2160 g a.s./ha~~	~~1~~	~~2160 g a.s./ha~~	~~5.54 %~~	~~1~~	~~119.66~~
2-D	2160 g a.s./ha	1	2160 g a.s./ha	2.77 %	5	299.16
3-D	2160 g a.s./ha	1	2160 g a.s./ha	2.77 %	1	59.83

Reduction of the amount of drift reaching the off-field areas can be achieved by implementing an in-field buffer strip of a given width. The resulting drift values (according also to spray-drift predictions of Ganzelmeier & Rautmann (2000)[3]) are given in the table below.
The calculation of HQ values for the active substance glyphosate according to Escort 2 is documented in the following table for off-field exposure due to spray drift. According to Regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2, the results of the assessment indicate an acceptable risk for non-target arthropods, if the calculated HQ values do not breach the trigger ≤ 2 describing the potential risk resulting from an exposure of non-target arthropods.

Table B.9.5-10: Maximum off-field predicted environmental rates (PER) of MON 52276 at increasing distances from the sprayed areas following intended uses. See text for details

Maximum Intended in-field rate (g a.s./ha)	**Species**	**Test type**	**L(E)R$_{50}$ L Prod./ha (g a.s./ha)**	PEROff-field at 1m (all crops: 2.38% drift) (cereals: 2.77% drift) ~~(2.77x 2 drift)~~ **(g a.s./ha)**	**HQ at 1m**	PEROff-field at 5m (all crops: 0.47% drift) ((cereals 0.57% drift) ~~(0.57 x 2 drift)~~ **(g a.s./ha)**	**HQ at 5m**	PEROff-field at 10m (all crops: 0.24% drift) (cereals 0.29% drift) ~~(0.29 x 2 drift)~~ **(g a.s./ha)**	**HQ at 10m**
~~2x max. 2160 g a.s./ha, i.e. max. 4320 g a.s./ha All crops / all seeded or trans-planted crops~~	~~*A. rhopalo-siphi*~~	~~3D~~	~~LR$_{50}$ > 16 (5760)~~	~~77.78~~	~~<0.1~~	~~16.01~~	~~<0.1~~	~~8.14~~	~~<0.1~~
	~~*T. pyri*~~	~~2D~~	~~ER$_{50}$ ≥12 (4320)~~	~~15.56~~	~~<0.1~~	~~3.20~~	~~<0.1~~	~~1.63~~	~~<0.1~~
	~~*A.bilineata*~~	~~soil~~	~~ER$_{50}$ >12 (4320)~~	~~89.75~~	~~<0.1~~	~~18.47~~	~~<0.1~~	~~12.53~~	~~<0.1~~
2x max. 2160 g a.s./ha, i.e. max. 4320 g a.s./ha All crops / all seeded or trans-planted crops	*A. rhopalo-siphi*	3D	LR$_{50}$ > 16 (5760)	66.83	<0.1	13.19	<0.1	6.7	<0.1
	T. pyri	2D	ER$_{50}$ ≥12 (4320)	13.37	<0.1	2.78	<0.1	1.35	<0.1
	A.bilineata	soil	ER$_{50}$ >12 (4320)	74.52	<0.1	15.23	<0.1	7.78	<0.1
max. 2160 g a.s./ha	~~*A. rhopalo-*~~	~~3D~~	~~LR$_{50}$ > 16 (5760)~~	~~119.66~~	~~<0.1~~	~~24.62~~	~~<0.1~~	~~12.53~~	~~<0.1~~

[3] Ganzelmeier H., Rautmann D. (2000) Drift, drift-reducing sprayers and sprayer testing. Pesticide Application, Aspects of Applied Biology 57

Maximum Intended in-field rate (g a.s./ha)	**Species**	**Test type**	**L(E)R$_{50}$ L Prod./ha** (g a.s./ha)	PERoff-field at 1m (all crops: 2.38% drift) (cereals: 2.77% drift) ~~(2.77x 2 drift)~~ (g a.s./ha)	**HQ at 1m**	PERoff-field at 5m (all crops: 0.47% drift) ((cereals 0.57% drift) ~~(0.57 x 2 drift)~~ (g a.s./ha)	**HQ at 5m**	PERoff-field at 10m (all crops: 0.24% drift) (cereals 0.29% drift) ~~(0.29 x 2 drift)~~ (g a.s./ha)	**HQ at 10m**
"Cereals and Oilseeds (pre-harvest)"	~~*siphi*~~								
	~~*T. pyri*~~	~~2D~~	~~ER$_{50}$ ≥12 (4320)~~	~~23.93~~	~~<0.1~~	~~4.92~~	~~<0.1~~	~~2.50~~	~~<0.1~~
	~~*A.bilineata*~~	~~soil~~	~~ER$_{50}$ >12 (4320)~~	~~23.93~~	~~<0.1~~	~~4.92~~	~~<0.1~~	~~2.50~~	~~<0.1~~
	A. rhopalo-siphi	*3D*	LR50 > 16 (5760)	59.83	<0.1	16.01	<0.1	8.14	<0.1
	T. pyri	*2D*	ER50 ≥12 (4320)	15.56	<0.1	3.20	<0.1	1.63	<0.1
	A.bilineata	*soil*	ER50 >12 (4320)	89.75	<0.1	18.47	<0.1	12.53	<0.1

For the off-field scenario, all calculated HQ values remain below the acceptability criterion.

B.9.5.6.2 Toxicity/exposure ratio (TER) approach (off-field exposure)

As an alternative to the Escort 2 approach, a risk assessment based on the TER approach is presented. In view of the RMS, this concept should be preferred for the off-field assessment to be in line with other areas of the environmental risk assessment. In principle, the same parameters are utilised as in the Escort 2 concept. The interspecies variability in sensitivity is no longer expressed in form of a 'correction factor', but is used as the TER acceptability criterion (assessment factor).
Deviating from the Escort 2 proposals, a vdf of 5 instead of 10 for extrapolation from 2D-testing to 3D-structures in reality is normally applied, based on the evaluation of experimental data.

$$\mathrm{TER} = \frac{\mathrm{E/LR}_{50}}{\mathrm{AR} \times \mathrm{MAF} \times \frac{\mathrm{drift}}{\mathrm{vdf}}}$$

where:
E/LR$_{50}$ toxicity in relevant test (lethal or sub-lethal endpoint, whichever lowest)
AR application rate
MAF multiple application factor
drift percent of drift depending on distance
vdf vegetation distribution factor

Table B.9.5-11: Non-target arthropods exposed to glyphosate: off-field risk assessment according to the TER concept

Maximum Intended in-field rate (g a.s./ha)	Species	L(E)R$_{50}$ L Prod./ha (g a.s./ha)	MAF	AR x MAF (g a.s./ha)	Drift	vdf	TER	Trigger
2x 2160 g a.s./ha, i.e. 4320 g a.s./ha	*A. rhopalosiphi*	LR$_{50}$ > 16 (5760)	1.7	3672	~~1 m: 2.77 %~~ 1 m: 2.38 %	5	330	5
	T. pyri	ER$_{50}$ ≥12 (4320)	1.7	3672	~~1 m: 2.77 %~~ 1 m: 2.38 %	1*	50	5
	A. bilineata	ER$_{50}$ >12 (4320)	1.9	4104	~~1 m: 2.77 %~~ 1 m: 2.38 %	1*	44	5

* not applicable, toxicity test was performed in a 3D environment

For the intended uses of MON 52276 according to the proposed use pattern with a serial application with 2 times 2160 g a.s./ha, the calculated TER values do achieve the acceptability criteria TER ≥ 5 and indicate an acceptable off-field risk to non-target arthropods.

B.9.5.6.3 Indirect effects via trophic interactions

Apart from potential direct (i.e. toxicological) effects, non-target arthropods can be affected indirectly by the use of herbicides. These effects trace back to the intended effect of the herbicides – eliminating competing plants – and the interruption of the food web going along with it. In fact, there is strong evidence that the use of herbicides (including glyphosate) considerably contributes to the ongoing loss of biodiversity in farmland invertebrate and vertebrate non-target species. For invertebrates, theses relationships are not yet as well-described as for vertebrates, especially farmland birds. Hence, the RMS limits the discussion on options for risk assessment of indirect effects and potential mitigation measures to chapter B.9.1.7.7.

B.9.6 Effects on earthworms (Annex IIA 8.4; Annex IIIA 10.6.1)

In the first EU peer reviewed evaluation of glyphosate in 2001, it was concluded that the acute risk to earthworms is low (Klenner and Bathelt, 1995, Wüthrich, 1990 and Handley, 1995). The lowest LC$_{50}$ >480 mg/ kg dry soil that was originally employed in the assessment of the risk for earthworms resulting from an exposure to glyphosate originated from the study of Hänisch (1991; ARW 96-00094) who used the active substance glyphosate in formulation. For the assessment of sublethal effects, the agreed endpoint originated from the study by Heyward and Mallett (2000; CEMS-1173), in which no effects were recorded at the highest concentration tested. For studies for which the former evaluation was still valid and did not need to be revised, please refer to the glyphosate monograph of 2001 and its addenda.

Table B.9.6-1: Toxicity of glyphosate technical to earthworms. Tests submitted for the first EU evaluation of glyphosate in 2001

Species	Test substance	Test design	EC$_{50}$ (mg a.s./kg dry weight soil)	Reference/GLP

Species	Test substance	Test design	EC_{50} (mg a.s./kg dry weight soil)	Reference/GLP
Eisenia fetida	Glyphosate acid in formulation (360 g a.s./l)	14 d acute	**>480**	Hänisch, 1991; ARW 96-00094 ICS 29428
Eisenia fetida	Glyphosate acid	14 d acute	>1000	ARW 96-00099 Wüthrich, 1990
Eisenia fetida	Glyphosate acid	14 d acute	>1000	ARW 96-00095 Handley et al., 1995
Eisenia fetida	Glyphosate IPA salt	14 d acute	>1000	IMW-92-0024-01 Van der Hoeven *et al.*, 1992 ARW 96-00091
Eisenia fetida	Glyphosate IPA salt	14 d acute	>5000	80-91-2078-06-91 Thun, 1995 ARW 96-00096
Eisenia fetida	Glyphosate IPA salt	56 d	**NOEC > 28.79** mg glyphosate IPA salt/kg dw soil; equivalent to **NOEC > 21.3** mg a.s. /kg dw soil **NOEC > 28.12** mg AMPA/kg dw soil	Heyward, J.C., Mallett, M.J., 2000; CEMR-1173

Values in bold: confirmed EU endpoints (SANCO/6511/VI/99-final, or EU Review Monograph)

Table B.9.6-2: Toxicity of glyphosate technical to earthworms. Tests submitted for the present renewal of approval of glyphosate

Species	Substance	System	Results (mg a.s./kg dry weight soil)	Reference
Eisenia fetida	Glyphosate acid	14 d acute	LC_{50} > 5770	Mallett, M.J., 2002 CEMR-1875; BVL no 2311027
Eisenia fetida andrei	MON 52276	14 d acute	LC_{50} > 1250 mg/kg dry soil equivalent to LC50 > 388 mg a.s./kg dry soil	Hoxter, K.A.; Smith, G.J., 1992; 139-306 BVL no 2317598
Eisenia andrei	AMPA	14 d acute	LC_{50} > 1000 mg AMPA/kg dry	Moser, T., Römbke, J., 2000; F13RA BVL no 2311029
Eisenia fetida andrei	AMPA	56 d	NOEC > 19.7 mg/kg dry soil	Noack, M., 2002; 011120FB BVL no 2311035
Eisenia fetida	AMPA	56 d	NOEC = 131.90 mg/kg dry soil (recalculated)	Servajean, E., 2003; 01-64-077-ES BVL no 2311037
Eisenia fetida andrei	MON0139 (63.81% w/w Glyphosate IPA salt)	56 d	NOEC > 1000 mg MON0139/kg dry soil equivalent to NOEC > 473 mg a.s. /kg dry soil.	Friedrich, S., 2009; 09 10 48 056 S BVL no 2311032
Eisenia fetida	Glyphosate IPA salt	56 d	NOEC > 28.79 mg glyphosate IPA salt/kg dw soil equivalent to NOEC > 21.3 mg a.s. /kg dw soil NOEC > 28.12 mg AMPA/kg dry soil	EU agreed endpoints (SANCO/6511/VI/99-final) Heyward, J.C., Mallett, M.J., 2000; CEMR-1173

Newly submitted studies on the toxicity of glyphosate for earthworms are summarised below.

B.9.6.1 Acute toxicity

KIIA 8.9.1/01

Author:	Mallett, M.J.
Title:	Sinon Glyphosate Technical: The Acute Toxicity to the Earthworm *Eisenia foetida*
Date:	29.11.2002
Doc ID:	2311027 /CEMR-1875
Guidelines:	OECD No. 207 (1984)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	20020727
Purity:	97.1 % w/w
2. positive control:	Reference substance: 2-chloracetamide

Species:	Earthworm (*Eisenia fetida*)
Age:	Definitive test: adults, ca. 7 – 8 months old
Weight:	336 - 407 mg (test initiation)
Source:	not stated
Food:	None
Acclimation period:	no data
Temperature:	18.6 – 20.5 °C (treatments), 19.0 – 20.5 °C, measured on day 0, 7 and 14
Photoperiod:	24 h light (mean:696lux, range: 581 – 787 lux), measured on day 0, 7, 14
Soil pH:	3.71 – 4.40 (treatments); 5.50 – 5.54 (control)
Soil moisture content:	30 - 35 %
Methods:	Since in a range finding test no mortality was observed at 10000 mg a.s./kg dry soil, the main test was conducted with only two test concentrations, 5770 and 10300 mg glyphosate acid/kg dry soil (equivalent to 5600 and 10000 mg a.s./kg dry soil) and a negative control was performed in parallel. The test item was mixed into the artificial soil substrate (10% Sphagnum-peat; 20% kaolin clay, 70% industrial quartz sand, pH adjusted to 5.5 – 6.5 with calcium carbonate). The moisture content was adjusted to about 35% using deionised water. Four replicate test containers (1000 mL glass beakers covered with polyethylene adhesive film and perforated with holes) during the definite test, were prepared for each treatment group. 10 adult earthworms per replicate were exposed for 14 days. The negative control was treated with deionised water only. As a toxic reference, earthworms were exposed to 2-chloracetamide/kg dry soil. Dead earthworms were counted after 7 and 14 days.Abnormal effects were noted after 7 and 14 days.All surviving earthworms per replicate were weighed as a group and average individual weights were calculated prior to test initiation and at day 14 after application. NOEC and LOEC were calculated using a non-parametric procedure (Steel's Many-one Rank Test, one-tailed) with the computer program Toxstat v 3.4.Biomass was compared to the control using a two-tailed t-test.

Results

The validity criteria according to OECD 207 are fulfilled –as no mortality was observed in the control group (required: < 10%).

The 14-day LC_{50} of the reference item 2-chloroacetamide/kg dry soil was 47.9 mg/ kg dry soil (95% CL: 43.8 – 51.9). This LC_{50} compares well with published data and is within the limit of 20 – 80 mg specified in ISO 11268-1 (1993).

Mortality was observed at both test concentrations. At 5600 mg a.s./kg dry soil 2.5% mortality and at 10000 mg a.s./kg dry soil 80%. No mortality was observed in the negative control.The average life weights of the earthworms decreased significantly at 5600 mg a.s./kg dry soil. At 5600 mg ae./kg dry soil no abnormalities of behaviour or appearance were noted.

Table B.9.6-3: Effects of glyphosate acid on earthworms

Test item (mg a.s./kg dry soil)	Mean weight change (%)	Mortality after 14 d (%)
Control	-8.5	0
5600	-14.2*	2.5
10000	-22.1	80*

* Significantly different when compared to the control, (α = 0.05)

At concentrations of 10000 mg a.s./kg dry soil at day 7 and 14, surviving worms were recorded as lethargic and of emaciated appearance. Two worms showed convulsive movements on day 7.

RMS Conclusions

The 14-day LC_{50} for earthworms (*Eisenia fetida*) exposed to glyphosate acid in an artificial substrate was determined to be >5770 – 10300 mg a.s./kg dry soil (5660 – 10000 mg a.s./kg dry soil. The corresponding NOEC was determined to be < 5770 mg a.s./kg dry soil (< 5600 mg a.s./kg dry soil) due to effects on biomass change.
The study is considered to be valid and acceptable.

KIIA 8.9.1/02

Author:	Moser, T., Römbke, J.
Title:	AMPA: Acute toxicity of AMPA technical material to the earthworm *Eisenia andrei* in an artificial soil test
Date:	13.09.2000
Doc ID:	2311029 /F13RA
Guidelines:	OECD 207
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	AMPA (aminomethyl phosphonic acid)
Lot/Batch #:	0693/07866
Purity:	100%
2. Vehicle and/or positive control:	Chloroacetamide
Species:	Earthworm (*Eisenia andrei*)
Age:	synchronized adults with clitellum, approx.. 5 months
Weight:	444.4 ± 69.5 mg
Source:	In house bred
Food:	none
Acclimation period:	At least 24 h
Temperature:	20-22 °C
Photoperiod:	24 h light (400 – 800 lux)
pH:	5.7 – 6.4 (start); 5.7 – 6.2 (end)
Water content:	54.9 – 60.5% (start); 52.5 – 60.0% (end)
Methods:	Earthworms were exposed in an artificial soil substrate (10 % sphagnum peat, air dried, finely ground; 20 % kaolin clay, approximately 69 % industrial quartz sand and 0.3 – 1 % g calcium carbonate). Four replicate test chambers were maintained in each treatment and for the controls, with 10 worms in each test chamber. Nominal test concentrations of 100 and 1000 mg AMPA/kg dry soil were thoroughly mixed into the soil substrate. The water content was adjusted to 50 ±10 % dry weight using deionised water. Negative control soil was treated with deionised water only. In a separate study, earthworms were exposed to the toxic reference substance chloroacetamide.

	Temperature and light intensity were monitored continuously. Water content and pH were determined at the beginning and the end of the test. The earthworms were exposed to the test item for 2 weeks and counted and weighed per replicate at the beginning and after 7 and 14 days of exposure. Behavioural changes and morphological alterations were recorded after 7 and 14 days. For biomass deviation, a one-way ANOVA followed by Dunnett's test was conducted (α = 0.05).

Results

The mortality in the control treatments did not exceed 10 %. The validity criteria according to guideline OECD 207 are therefore fulfilled.

The LC_{50} for the reference test item was determined to be 24.0 mg a.s./kg dry soil.

Table B.9.6-4: Effects of AMPA on survival and growth of *Eisenia andrei*

	Control	AMPA (mg/kg dry soil)	
		100	1000
Mortality (day 14) (%)	0	0	0
Biomass (day 14) (mg fresh weight)1)	346.8	333.6	289.8
Weight change (day 14) (%)1)	-21.3	-25.3	-35.1

No mortality was observed in any test item treatment concentrations or the control. After 14 days of exposure, mean percent biomass deviation was -21.3 % in the control group, -25.3% for the 100 mg AMPA/kg dry soil and -35.1% for the 1000 mg AMPA/kg dry soil treatments. The ANOVA and Dunnett-test showed a significant difference in biomass between control and the highest test item concentration (α = 0.05). No treatment related effects on behaviour or morphology of earthworms were observed.

RMS Conclusions

At the highest test concentration, no effects on mortality, behaviour or appearance of *Eisenia Andrei* were observed after 14 days. Therefore, the LC_{50} of AMPA was determined to be > 1000 mg AMPA/kg dry substrate. The NOEC value for biomass change in earthworms was determined to be 100 mg AMPA/kg dry substrate.

The study is considered to be valid and acceptable.

KIIIA 10.6.2/03

Author:	Hoxter, K.A.; Smith, G.J.
Title:	MON 52276: An acute study with the earthworm in an artificial soil substrate.
Date:	18.09.1992
Doc ID:	2317598/139-306
Guidelines:	OECD 207
GLP:	YES
Validity:	YES

Materials and Methods

Test item::	MON 52276 (Roundup)
Lot/Batch #:	LLN-9105-3135F

Purity:	30.95 %
2. Vehicle and/or positive control:	None
Species:	Earthworm (*Eisenia fetida andrei*)
Age:	Adults, with clitellum
Weight:	320 - 410 mg (test initiation)
Source:	Vittar & Associates, Mobile, Alabama, USA
Food:	None
Acclimation period:	Earthworms were acclimatised to the artificial soil for a period of around 24 h.
Temperature:	23 ± 1 °C
Photoperiod:	24 h light
Relative humidity:	20 ± 11%
Soil pH:	5.73 (test initiation); 5.22 – 5.59 (test termination)
Soil moisture content:	19 % (test initiation); 14.70 – 16.75 (test termination)
Methods:	The test was conducted with five test concentrations: 162, 270, 450, 750 and 1250 mg test item/kg dry soil and a negative control. The test item was suspended into deionised water and the suspension was mixed into the artificial soil substrate (10 % sphagnum peat; 20 % kaolin clay, 70 % quartz sand and 0.2 % calcium carbonate). The moisture content was adjusted to 19 % (35 % of the water holding capacity) using deionised water. Four replicate test containers (quart size glass jars containing approximately 750 g soil, wet weight) were prepared for each treatment group. 10 adult earthworms per replicate (a total of 40 worms) were exposed for 14 days. The negative control was treated with deionised water only. Temperature and relative humidity in the study room were recorded twice a day during the test period. Soil water content and pH were determined at the beginning and the end of the test. The replicates were examined for live and dead earthworms after 7 and 14 days. Effects on behaviour and appearance were noted after 7 and 14 days, when mortality was determined. All surviving earthworms per replicate were weighed as a group and average individual weights were calculated prior to test initiation and at day 14 after application. A statistical evaluation was not conducted due to a lack of observed mortality in this test. Descriptive statistics were used to determine toxicity endpoints.

Results

The validity criteria according to guideline OECD 207 are fulfilled as no mortalities were observed in the control group.

Table B.9.6-5: Mortality and observations of the earthworm (*Eisenia fetida andrei*) exposed to MON 52276

Nominal MON 52276 (mg/kg dry soil)	**Mortality Day 14 (%)**	**Observations[1]**		**Day 14 mean bodyweight (% of Day 0 value)**
		Day 7	Day 14	
0	0	40 AN	40 AN	+ 10.1
162	0	40 AN	40 AN	+ 3.7
270	0	40 AN	40 AN	+ 1.5
450	0	36 AN/4 SS	40 AN	- 4.0
750	0	24 AN/16 SS	39 AN/ 1 SS	+ 1.4
1250	0	28 AN/12 SS	34 AN/ 6 SA	+ 0.2

[1]AN – appeared normal; SS – shortened and stiff; SA – shortened & abnormally coloured.

No mortality was observed in any of the treatment groups and in the negative control. Mean body weight of earthworms was reduced in the treatment groups compared to control, in which 10% weight increase was observed. All worms at the 162 and 270 mg/kg dry soil treatment levels were normal in appearance and behaviour at each observation interval. At the 450 mg/kg dry soil concentration, four worms were noted as shortened and stiff at day 7, but all worms in this group appeared normal at day 14. In the 750 mg/kg dry soil group, sixteen worms were found to be shortened and stiff on day 7, with only one worm from this group remaining shortened and stiff by day 14. In the 1250 mg/kg dry soil group, twelve worms were noted as shortened and stiff on day 7, with six worms found to be shortened and abnormally coloured by day 14.

RMS Conclusions

The 14-day LC_{50} for earthworms (*Eisenia fetida andrei*) exposed to the test item MON 52276 in an artificial soil substrate was determined to be > 1250 mg/kg dry soil (>388 mg a.s./kg dry soil), the highest dose tested. The corresponding NOEC was determined to be 270 mg/kg dry soil (83.7 mg a.s./kg dry soil), based on abnormalities noted at higher dose levels.
The study is considered to be valid and acceptable.

B.9.6.2 Sublethal effects

KIIA 8.9.2/01

Author:	Friedrich, S.
Title:	MON 0139 - Sublethal toxicity to the earthworm *Eisenia fetida*
Date:	30.11.2009
Doc ID:	2311032 /09 10 48 056 S
Guidelines:	OECD 222 (2004)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	MON 0139 (glyphosate isopropylamine salt)
Lot/Batch #:	A8B60170S0
Purity:	63.81 % w/w glyphosate isopropylamine salt (analysed) 47.28 % w/w glyphosate acid equivalent (analysed)
2. Vehicle and/or positive control:	None
Species:	Earthworm (*Eisenia fetida andrei*)
Age:	Adults, approx. 3 months old with clitellum
Weight:	304 – 472 mg
Source:	In-house rearing
Food:	Air-dried and finely ground horse manure
Acclimation period:	Approx. 24 hours in the artificial substrate
Temperature:	18.6 – 21.8 ºC
Photoperiod:	16 h light / 8 h dark (600 Lux)

Soil pH:	6.1 – 6.2 (test start); 6.0 – 6.1 (test termination)
Soil moisture content:	35.1 – 35.2 % (test start); 34.6 – 34.8 % (test termination)
Methods:	A sublethal test was conducted with five nominal test concentrations of MON 0139, encompassing 30, 50, 100, 500 and 1000 mg test item/kg dry soil, equivalent to an analysed content of 14.1, 23.6, 47.3, 236, and 473 mg glyphosate acid equivalent/kg dry soil, respectively. In addition, a control group was exposed to soil mixed with deionised water only. The test concentrations were prepared by dispersing an exactly weighed amount of the test item in deionised water (stock solutions) and thereafter diluted to obtain different test concentrations, which were thoroughly mixed with the artificial soil, achieving desired test doses with a final nominal water content of 40-60 % of WHC. The artificial soil substrate was composed of 10 % Sphagnum-peat; 20 % kaolin clay, 69.5 % industrial quartz sand and 0.5 % calcium carbonate. Four replicate test containers (test item) and 8 replicate test containers (control) with 810 g soil wet weight (corresponding to 600 g dry weight) and 5 cm soil depth were prepared for each treatment group. 10 adult earthworms per replicate (a total of 40 worms) were exposed for 56 days. At test initiation, individual fresh weight and behavioural responses of earthworms were recorded. Behavioural and pathological symptoms including feeding activity were observed on a weekly basis. Four weeks after test initiation, number of surviving adult earthworms and fresh weight of surviving adult earthworms per replicate were recorded. At test termination (8 weeks after test initiation), number of surviving juveniles per replicate, were observed. The behavioural and pathological symptoms, including morphological alterations were observed 4 and 8 weeks after test initiation. Water content and pH measurements were performed at test initiation and at test termination. The temperature was continuously recoded throughout the test. Fisher's Exact Binomial Test and Dunnett's t-test were used for mean comparison. For statistical evaluation of the biomass change, mean fresh weight of surviving worms was used.

Results

The validity criteria according to guideline OECD 222 are fulfilled as each replicate (containing 10 adults) has produced ≥ 30 juveniles by the end of the test in the control and the coefficient of variation of reproduction was ≤ 30 % in the control. Also, the adult mortality over the initial 4 weeks of the test was ≤ 10 % in the control.

Table B.9.6-6: Lethal and sublethal effects of MON 0139 (glyphosate isopropylamine salt) on earthworms

MON 0139 (mg test item/kg dry soil)		Control	30	50	100	500	1000
Mortality of adult worms after 4 weeks (%)		0	0	2.5	2.5	0	0
Mean biomass change (%)		40.7	46.7	39.8	41.8	37.5	36.3
Juveniles after 8 weeks (mean No.)		79.0	78.5	83.8	71.8	80.3	74.3
CV (%)		18.7	19.1	15.0	34.1	28.7	22.1
Change of reproduction compared to control (%)		-	0.6	-6.0	9.2	-1.6	6.0
EC_{50}	Test item (MON 0139)	> 1000 mg/kg dry soil					
	glyphosate isopropylamine salt	> 638.1/kg dry soil					
NOEC	Test item (MON 0139)	1000 mg/kg dry soil					
	glyphosate isopropylamine salt	638.1/kg dry soil					

The test item MON0139 (glyphosate isopropylamine salt) caused no mortality at concentrations of 30, 500 and 1000 mg MON 0139/kg dry soil. No mortality occurred in the control group. At 50 and 100 mg MON 0139/kg dry soil, 2.5 % mortality was observed. No effects on behaviour (including feeding activity) of the worms were observed during the test.

The test item caused no statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) when compared to the control.

RMS Conclusions

The study is considered to be valid and acceptable.
The relevant NOEC for earthworms determined in this study is 1000 mg MON 0139 /kg dry soil, equivalent to 473 mg glyphosate acid equivalent/kg dry soil.
The EC_{50} of MON 0139 for earthworm was determined to be > 1000 mg test item/kg dry soil, equivalent to > 473 mg glyphosate acid equivalent/kg dry soil.

KIIA 8.9.2/02

Author:	Heyward, J.C., Mallett, M.J.
Title:	A laboratory investigation of the effects of Glyphosate and its breakdown product AMPA on reproduction in the earthworm *Eisenia fetida*
Date:	27.03.2000
Doc ID:	2311034 /CEMR-1173
Guidelines:	OECD 222
GLP:	YES
Validity:	YES

Materials and Methods

Test item 1:	Isopropylamine (IPA) salt of glyphosate
Lot/Batch #:	A9C 281
Purity:	62 % (45.9 % glyphosate acid equivalent)
Test item 2:	AMPA (aminomethylphosphonic acid)
Lot/Batch #:	PIT-8912-1385-A
Purity:	99.1%
Vehicle and/or positive control:	Positive control: Benlate® (50 % w/w benomyl) Reference item (in a separate study): 2-chloroacetamide
Species:	Earthworm (*Eisenia fetida*)
Age:	Adults, 7-10 month old
Weight:	386 - 477 mg (test initiation)
Source:	In-house culture based on a stock of worms obtained from Blades Biological, UK
Food:	Cattle manure
Acclimation period:	Earthworms were acclimatised to the artificial soil for a period of 29 days at 16 – 22.5 °C.
Temperature:	18 – 22 °C
Photoperiod:	16 h light: 8 h dark
Soil pH:	6.38 – 6.60 (test initiation); 6.83 - 6.96 (test termination)
Soil temperature:	18.4 – 19.6 °C
Soil moisture content:	37.9 % (60 % of the water holding capacity) (test initiation); 29.6 – 31.1 % (test termination)
Methods:	The test was conducted with two test concentrations of glyphosate IPA salt (5.76 and 28.79 mg/kg dry soil, equivalent to 4.27 and 21.31 mg glyphosate acid equivalent/kg

	dry soil), two test concentrations of AMPA (5.62 and 28.12 mg/kg dry soil), a negative control, and three concentrations of a positive control (Benlate®).The test item was suspended into/dissolved in deionised water and the suspension/solution was mixed with the water used for adjusting the soil moisture to 60 % of the water holding capacity. Afterwards, the solution was mixed into the artificial soil substrate (10 % peat; 20 % clay, 70 % silica sand and calcium carbonate to obtain a pH of 5.5-6.5). 1 g cow manure/ 100 g dry soil was added as feed. Four replicate test containers with 600 g dry soil were prepared for each treatment group. 10 adult earthworms per replicate (a total of 40 worms) were exposed for 56 days. Earthworms were fed with manure on day 1, 14, 21 and 28. Soil moisture was adjusted once a week by adding deionised water. A negative control was treated with deionised water only. As positive control, earthworms were exposed to three concentrations of Benlate® (2.66, 5.93 and 13.28 mg/kg).Temperature and light intensity were recorded daily during the test period. pH and soil temperature were determined at the beginning and the end of the test in one of the replicate vessels at each concentration. Soil moisture content was determined at day 0, 1, 7, 14, 21, 23, 28, 35, 42 and 56. Furthermore, toxicity of 2-chloroacetamide to *Eisenia fetida* was tested in a separate 14 d reference study. The replicates were examined for live and dead adult worms after 28 days at which time all adult worms were removed and the soil was replaced in the vessels. After a further 28 days (56 days total), the contents of the beakers were examined for juvenile worms and cocoons. All surviving earthworms per replicate were weighed as a group and average individual weights were calculated prior to test initiation and at day 28 after application. Mean percent changes in weights of live worms at 28 days and mean juvenile production per surviving adult worm at day 56 were tested for significant ($\alpha = 0.05$) inhibition compared to the controls using the Dunnett's Test (one tailed comparison) in the computer program TOXSTAT Release 3.0. The same test, but with a two-tailed comparison, was employed to test for significant differences between mean numbers of un-hatched cocoons because the test substances may have inhibited cocoon production or/and cocoon viability (cocoons may have been produced but unable to hatch). Each set of data was tested for normality before carrying out the parametric multiple comparison procedure using the Chi-square test and the Shapiro Wilks test, the data were also tested for homogeneity of variance using both the Hartley and the Bartlett's tests provided in the program TOXSTAT Release 3.0.

Results

The validity criteria according to guideline OECD 222 are fulfilled –as each replicate (containing 10 adults) has produced ≥ 30 juveniles by the end of the test in the control and the coefficient of variation of reproduction was ≤ 30 % in the control. Also, the adult mortality over the initial 4 weeks of the test was ≤ 10 % in the control.

The adult worms exposed to the reference item at concentrations of 5.93 and 13.28 mg Benlate®/kg dry soil showed a significantly reduced growth when compared to untreated controls at day 28. A significant reduction in juvenile production compared to untreated controls was seen for 2.66, 5.93 and 13.28 mg Benlate®/kg dry soil. At 5.93 mg Benlate®/kg dry soil, a significantly increased number of unhatched cocoons was observed when compared to the untreated control.

No significant difference in body weight change compared to untreated controls was noted for adult worms at any concentration or test item treatment.

No abnormal behaviour when compared to untreated controls was observed for adult worms at any concentration or test item treatment.

One adult worm died during the test at the lowest concentration of glyphosate IPA salt (5.76 mg/kg dry soil). This was not considered to be dose-related since no mortalities were observed at higher concentrations.

Table B.9.6-7: Effects of glyphosate IPA salt, AMPA and the positive control Benlate® on *Eisenia fetida*

Treatment	Adult worms		Juvenile production (at day 56)		Unhatched cocoons per surviving worm
	Mortality of adult worms (at day 28) (%)	Mean weight change (at day 28) (%)	Juveniles per surviving worm (mean No.)	Coefficient of variation (%)	(mean No.)
Control	0	+ 22	31.0	10	0.1
Benlate® (2.66 mg/kg)	0	+ 23	26.0 *	15	0.1
Benlate® (5.93 mg/kg)	0	+ 12 *	7.8 *	23	2.2 *
Benlate® (13.28 mg/kg)	0	- 24 *	0.0 *	0	0.7
Glyphosate[1] (5.76 mg IPA salt/kg)	2.5	+ 14	26.2	25	0.3(N)
Glyphosate[1] (28.79 mg IPA salt/kg)	0	+ 20	28.5	12	0.3(N)
AMPA (5.62 mg/kg)	0	+ 24	26.0	3	0.3(N)
AMPA (28.12 mg/kg)	0	+ 24	29.4	16	0.4(N)

* statistically (α = 0.05) different from controls.

[1] Glyphosate was tested as the IPA salt

N The numbers of unhatched cocoons present at the end of the test in the glyphosate and AMPA treatments were slightly higher than the controls but statistical analysis proved that this was probably due to random chance alone and was probably not due to the presence of glyphosate or AMPA

No significant differences were observed between mean juvenile production for untreated control worms and worms exposed to glyphosate IPA at any concentration tested. Similarly, for worms exposed to AMPA, no significant difference from the untreated controls was seen in terms of juvenile production. No significant differences were observed between number of unhatched cocoons present at day 56 in untreated controls and both concentrations of glyphosate IPA salt. Similarly, for AMPA, no significant difference from the controls was observed in terms of numbers of unhatched cocoons.

The 14 day LC_{50} was determined at 39.4 mg/kg dry soil (95 % confidence limits; 36.0 - 43.1 mg/kg dry soil).

RMS Conclusions

The study is considered to be valid and acceptable.

The NOEC for earthworms in this study is determined to be1000 mg MON 0139 /kg dry soil, equivalent to 473 mg glyphosate acid equivalent/kg dry soil.

Glyphosate IPA salt and the metabolite aminomethylphosphonic acid (AMPA) had no significant effect on growth or reproduction of *Eisenia fetida* after 56 days of exposure at concentrations up to 28.79 mg glyphosate IPA salt/kg dry soil (21.3 1 mg glyphosate acid equivalent/kg dry soil) and 28.12 mg AMPA/kg dry soil.

Therefore, the NOEC was determined to be 28.79 mg glyphosate IPA salt/kg dry soil (21.3 1 mg glyphosate acid equivalent/kg dry soil) and 28.12 mg AMPA/kg dry soil.

KIIA 8.9.2/03

Author:	Noack, M.
Title:	AMPA - Earthworm (*Eisenia fetida*), effects on reproduction
Date:	05.06.2002
Doc ID:	2311035 /011120FB
Guidelines:	DIN ISO 11268-2: 1998E
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	AMPA (aminomethyl phosphonic acid)
Lot/Batch #:	FA005563
Purity:	99 %
2. Positive control:	Derosal flüssig (31.5 % carbendazim)
Species:	Earthworm (*Eisenia fetida*)
Age:	synchronized adults with clitellum, 4 months
Weight:	300 – 600 mg
Source:	Biologische Bundesanstalt (BBA), Braunschweig, Germany
Food:	Dried litter of stinging nettle and porridge oats
Acclimation period:	2 days in artificial soil under test conditions
Temperature:	20 ± 2°C
Photoperiod:	16 h light/ 8 hours dark (413 - 546 lux)
pH:	5.45 – 6.30
Water content:	46.11 – 51.53%
Methods:	Earthworms were exposed in an artificial soil substrate (OECD 207, 10 % sphagnum peat, air dried, finely ground; 20 % kaolin clay, approximately 69 % industrial quartz sand and approx. 0.43 % calcium carbonate).Four replicate test chambers were maintained in each treatment, with 10 worms in each test chamber. Nominal test concentrations of 0.79, 3.94 and 19.7 mg AMPA/kg dry soil were thoroughly mixed into the soil substrate. The water content was adjusted to about 50 % of maximum water holding capacity (WHC) using demineralised water. Negative control soil was treated with deionised water only. As a toxic reference, earthworms were exposed in a separate study to Derosal flüssig (31.5% carbendazim) at concentrations of 1.26, 2.52 and 5.04 mg a.s./kg dry soil. The adult earthworms were exposed to the test item for 4 weeks; the adult worms were counted, removed and weighed per replicate. The remaining soil was returned to the reproductive test for additional 4 weeks. Thereafter, juveniles were counted. Temperature and light intensity were monitored continuously. Water content and pH were determined at the beginning and the end of the test. The adult earthworms were exposed to the test item for 4 weeks, after which the artificial soil was emptied onto a tray and the adult worms were counted, removed and weighed per replicate after they were washed under tap water and dried on filter paper. Missing worms and the earthworms, which failed to respond to gentle stimulation, were considered to be dead. The number of damaged earthworms (e.g. lack of movement, rigidity, etc.) was assessed at day 28 after application. Individual weight of the earthworms was recorded at day 28 after application. Reproduction was recorded8 weeks after the test initiation as mean number of juveniles

	per test container and replicate. As data for body weight changes and the reproduction were normally distributed and homogeneous, the Dunnett's test was used (multiple comparison, two-sided for weight and one sided smaller for reproduction, α = 0.05). NOEC and EC-values for reproduction were determined by regression analysis in an appropriate dose-response function.

Results

Each control replicate containing 10 adults produced ≥ 30 juveniles and adult mortality in the control treatments after four weeks did not exceed 10%. The coefficient of variation for reproduction in control groups was slightly higher than 30% at the end of the test. As one-way ANOVA showed that replicates were normally distributed and homogeneously with regard to variance, the validity criteria according to guideline OECD 222 are therefore still considered fulfilled.

Table B.9.6-8: Effects of AMPA on survival, growth and reproduction of *Eisenia fetida*

	Control	**AMPA (mg/kg dry soil)**		
		0.79	3.94	19.7
Mortality (day 28) (%)	0	0	0	0
Weight change (day 28) (%)[1]	-	+10.71	+1.79	+7.14
No of juveniles (day 56)	60±23	64±23	61±5	68±10
Reproduction (%) of control (56 days)[1)]	-	+7	+2	+13

No pathological symptoms or chances in behaviour of the adult earthworms were noted in any of the test item treatments and the control. During test period, body weights of earthworms in treated and control groups slightly increased or remained at starting level. No mortality was observed in any of the treatment groups and in the control. Different test item concentrations had no effects on the number of offspring. There was no statistically significant difference between the treated groups and the control.

The $EC_{50}$and NOEC for reproduction in the reference test item were determined to be 2.9 and 1.26 mg a.s./kg dry substrate, respectively.

RMS Conclusions

The study is considered to be acceptable.

The coefficient of variation for reproduction in control groups was slightly higher than 30% at the end of the test. Shapiro-Wilk´s Test on Normal Distribution (Number of residues = 16; Shapiro-Wilk´s W = 0.965; p(W) = 0.760) showed that treatment data do not significantly deviate from normal distribution. Based on the pre-selected significance level of 0.05, the Levene test indicates variance homogeneity. The validity criteria according to guideline OECD 222 are therefore still considered fulfilled. The 56-day no-observed-effect-concentration (NOEC) of AMPA for mortality, growth and reproduction of the earthworm *Eisenia fetida* was found to be 19.7 mg AMPA/kg dry soil, which was the highest concentration tested.

KIIA 8.9.2/04

Author:	Servajean, E.
Title:	Laboratory determination of the side-effects of aminomethyl phosphonic acid (AMPA) on the reproductive performance of earthworms (*Eisenia fetida*) using artificial soil substrate
Date:	20.01.2003
Doc ID:	2311037 /01-64-077-ES
Guidelines:	OECD 222
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	AMPA (aminomethyl phosphonic acid)
Lot/Batch #:	A0164351
Purity:	99.7%
Control:	Carbendazim (99.6%)
Species:	Earthworm (*Eisenia fetida*)
Age:	synchronized adults with clitellum, >2 months
Weight:	300 – 600 mg
Source:	In house bred
Food:	5 g of cattle manure with 6 mL of water per week
Acclimation period:	Not stated
Temperature:	19.0 to 21.5°C
Photoperiod:	12 h light / 12 hours dark (416 - 595 lux)
pH:	5.7 – 6.0 (start), 6.3 – 6.9 (end)
Water content:	43.9 – 46.2%
Methods:	Earthworms were exposed to AMPA in an artificial soil substrate (OECD 207, 10% sphagnum peat, air dried, finely ground; 20% kaolin clay, approximately 68% industrial quartz sand and approx. 1.8 g calcium carbonate). Four replicate test chambers were maintained in each treatment and eight for the controls, with 10 worms in each test chamber. Nominal test concentrations 58.6, 87.8, 131.9, 198.1, 297.1, 445.5, 668.5 and 1002.5 mg AMPA/kg dry soil were thoroughly mixed into the soil substrate. The water content was adjusted to about 50% of maximum water holding capacity (WHC) using demineralised water. Negative control soil was treated with deionised water only. As a toxic reference, earthworms were exposed to carbendazim at concentrations of 1.0, 2.2 and 5.0 mg a.s./kg dry soil. The adult earthworms were exposed to the test item for 4 weeks; the adult worms were counted, removed and weighed per replicate. The remaining soil was returned to the reproductive test for additional 4 weeks. Thereafter, juveniles were counted. Temperature and light intensity were monitored continuously. Water content and pH were determined at the beginning and the end of the test. The adult earthworms were exposed to the test item for 4 weeks, after which the adult worms were counted, removed and weighed per replicate after they were washed under tap water and dried. Missing worms and the earthworms, which failed to respond to gentle stimulation, were considered to be dead. Individual weight of the earthworms was recorded at day 28 after application and calculated as total weight reported to the number of survivals. Reproduction was recorded 8 weeks after the test initiation as mean number of

	juveniles per test container and replicate. For biomass deviation and production of juveniles, F-variance analysis was conducted by the applicant (α = 0.01). Statistic re-evaluation (Williams Multiple Sequential t-test Procedure) was performed by the RMS via ToXRatPro Version 2.10.

Results

The NOEC for reproduction in the reference test item was determined to be 2.2 mg a.s./kg dry soil, respectively.

Each control replicate containing 10 adults produced ≥ 30 juveniles and adult mortality in the control treatments after four weeks did not exceed 10 %. The coefficient of variation for reproduction in control groups 10.3 % at the end of the test. Therefore, all validity criteria according to guideline OECD 222 are fulfilled.

Statistic reevaluation was performed by the RMS (ToXRatPro, Version 2.10). Percent biomass deviation at the end of the exposure period of the adults were re-analyzed. Treatments were compared by the T-test procedure after Williams. Significance was Alpha = 0.05. A NOEC of 131.90 is suggested for the parameter biomass and number of juveniles.

Table B.9.6-9: Biomass change (%) after 28d of exposure of adult earthoworms to AMPA

	AMPA (mg/kg dry soil)								
No.	control	58.6	87.8	131.9	198.1	297.1	445.5	668.5	1002.5
1	90.5	87.5	88.9	83.4	86.1	91.9	78.3	64.4	67.2
2	90.9	90.5	90.2	88.6	83.7	85.3	75.2	72.1	65.6
3	94	91.7	90.9	87.3	78.9	82.6	80.5	70.8	63.4
4	89.2	86.2	90.1	94.5	84	92.9	76.7	63.4	66.6
Replicates	4	4	4	4	4	4	4	4	4
Mean	91.2	89.0	90.0	88.5	83.2*	88.2*	77.7*	67.7*	65.7*
Std.Dev	2.0	2.6	0.8	4.6	3.0	5.0	2.3	4.4	1.7
CV%	2.2	2.9	0.9	5.2	3.7	5.7	2.9	6.5	2.5

*statistically significant different from the control

Table B.9.6-10: Number of earthworm juvenils after 56 days exposure to AMPA

	AMPA (mg/kg dry soil)								
No.	control	58.6	87.8	131.9	198.1	297.1	445.5	668.5	1002.5
1	116	104	104	124	119	88	64	45	18
2	119	122	121	106	94	109	71	39	9
3	135	128	121	119	107	90	64	38	24
4	113	105	104	91	116	88	68	42	14
Replicates	8	4	4	4	4	4	4	4	4
Mean	120.6	114.8	112.5	110.0	109.0*	93.8*	66.8*	41.0*	16.3*
Std.Dev	12.4	12.1	9.8	14.8	11.2	10.2	3.4	3.2	6.3
CV%	10.z3	10.5	8.7	13.4	10.3	10.9	5.1	7.7	39.0

Mean percent biomass deviation in the earthwors was -9.5 % in the control group, ranging between -4.0 and -12.9 %. Loss of earthworm biomass was similar in the AMPA treated groups for treatment concentrations of up to 131.90 1 mg AMPA/kg dry soil (Williams Multiple Sequential t-test Procedure performed by RMS). At concentration of 198.1 mg/kg dry soil, treated animals lost significantly more biomass compared to the control group. A significant reduction in offspring number was observed in treatment concentrations higher

than 131.90 mg AMPA/kg dry soil. One earthworm died in one of the 668.5 mg AMPA/kg dry soil treatment and in the 2.2 and 5.0 mg Carbendazim/kg dry soil test units.

RMS Conclusions

The authors suggested the 56-day NOEC for AMPA regarding mortality, body weight and the reproduction rate of *Eisenia fetida* to be 198.1 mg AMPA/kg dry soil, set on earthworm reproduction. Statistic re-evaluation was performed by the RMS. A NOEC of 131.90 mg/kg is suggested on the basis of effects on earthworm biomass. Also the 56-day NOEC for earthworm juveniles production for AMPA was recalculated to 131.90 mg/kg.
The 56-day no-observed-effect-concentration (NOEC) of AMPA for mortality, body weight and the reproduction rate of *Eisenia fetida* was 131.90 mg AMPA/kg dry soil.

B.9.6.3 Field study

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B.9.6.4 Literature Review

Among soil organisms, eathworms are standard organisms in the risk assessment as they have an important role in the formation and maintenance of fertile soils. Besides laboratory studies submitted for the application for the renewal of approval of the active substance glyphosate following international guidelines, additional 21 references have been submitted dealing with soil organsims in general. Within these references, 5 studies (Casabe et al., 2007; Correia et al., 2012; Kaneda et al., 2009; Verrel et al., 2004 and Yasmin et al., 2003) focusing on earthworms have been considered to represent supporting information for risk assessment.

In the risk assessment for acute effects on soil organisms, behaviour is not included as a sensitive endpoint. However, these responses might also have negative consequences, e.g. – when worms move to the surface of contaminated soil- exposure to predators or to detrimental light. It could be shown that the activity of worms was influenced by the exposure to environmentally relevant concentration of commercial formulation of glyphosate (Verrel and Buskirk, 2004). The worms emerged onto the surface within 2 h after exposure. Nevertheless, after 48 h animals were found to be buried in the soil again. Authors concluded that acute exposure to the glyphosate containing plant protection product may compromise the survival of earthworms even though its direct toxicity appears low (Verrel & Buskirk, 2004).
Nevertheless it seems important to assess not only of the active ingredients, but also of the different formulations (Piola et al. 2013). Especially for aquatic organsims it was also demonstrated that commercial formulations can be more toxic than the active substance itself because of the adjuvants present in the formulations.

Effects on reproduction were examined by Casabé et al. (2007), Kaneda et al. (2009) and Yasmin et al. (2006) using commercial formulations with the recommended application rates. It is concluded that the observed responses will not impact the population of earthworm in nature. Santos et al. (2012) could also confirm that glyphosate application to agricultural fields did not seem to affect either earthworms or collembolans in the recommended field dose. Consistently Zhou (2012 and 2013) confirmed that glyphosate has very low toxicity to the earthworms.
However, it can not be excluded that with repeated appplications of glyphosate containing plant protection products during the season or year by year will have negative effects on the

biotic soil community. It is considered that herbicide application did not directly affect the mortality or reproduction but instead the biological activity of the animals.

In a reproduction test with *Eisenia fetida*, which was conducted with the active substance glyphosate itself (Correia et al., 2012), earthworms were kept in treated soil and were classified as alive after the evaluation period, but showed significant reduction in mean weight at all test concentrations. Moreover morphological abnormalities like elevating the body, coiling, and curling were observed in all specimens exposed to the highest concentrations of glyphosate (1000 mg/kg). Further behavioural abnormalities were described in terms of reduced casting production (Kaneda et al., 2009), reduced cocoon viability, a reduction in the feeding activity (Casabé et al., 2007) or reduced body weight (Yasmin et al., 2006). However, the test rates were similar or above the one tested in the offically submitted studies, so that the outcome of the risk assessment for earthworm did not change.

B.9.6.4.1.1.1.1 References

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Zhou, C. F., Wang, Y. J., Yu, Y. C., Sun, R. J., Zhu, X. D., Zhang, H. L., & Zhou, D. M. (2012). Does glyphosate impact on Cu uptake by, and toxicity to, the earthworm Eisenia fetida?. Ecotoxicology, 21(8), 2297-2305.

Chui-Fan Zhou , Yu-Jun Wang , Cheng-Cheng Li , Rui-Juan Sun , Yuan-Chun Yu , Dong-Mei Zhou (2013) Subacute toxicity of copper and glyphosate and their interaction to earthworm (Eisenia fetida). Environmental Pollution 180 (2013) 71-77

B.9.6.5 Summary of toxicity data on earthworms

In the first EU evaluation of the active substance glyphosate, the assessment of the acute risk for earthworms was based on the lowest LC_{50} > 480 mg/ kg dry soil, originated from the study of Hänisch (1991; ARW 96-00094) which used the active substance glyphosate in formulation (FSG 03090h Taifun forte). The study was a limit test, and no effect was observed at the highest concentration tested. Therefore, for evaluation of acute risk of glyphosate acid on *Eisenia fetida*, we propose to use the endpoint LC_{50} >5600 mg a.s./kg dry soil from the newly submitted test by Mallett (2002, BVL no 2311027).

For AMPA, a new acute toxicity study was conducted by Moser et al. (2000, BVL no 2311029). At the highest test concentration, no effects on mortality, behaviour or appearance of *Eisenia andrei* were observed after 14 days. Therefore, the LC_{50} of AMPA was determined to be > 1000 mg AMPA/kg dry substrate.

The endpoint for chronic toxicity of glyphosate to earthworms of the previous EU evaluation of glyphosate in 2001 (SANCO/6511/VI/99-final) was based on the study by Heyward & Mallett (2000) conducted with two test concentrations of glyphosate IPA salt (5.76 and 28.79 mg/kg dry soil, equivalent to 4.27 and 21.31 mg glyphosate acid equivalent/kg dry soil). In both concentrations, no significant differences were observed in comparison to the untreated control in relation to body weight, behavior or reproduction. The test was considered valid according to the OECD Test Guideline 222 and the endpoints were used for risk assessment of glyphosate (see table above).

A new chronic study was submitted by the applicant (Friedrich, 2009, BVL no 2311032). This study was also performed with glyphosate-isopropylamine-salt (30, 50, 100, 500 and 1000 mg MON 0139/kg dry soil, corresponding to 14.1, 23.6, 47.3, 236, and 473 mg a.s./ kg dry soil). In this study, no effects on behavior and body weight were observed. Treatments with 50 and 100 mg MON0139/kg dry soil showed a mortality rate of 2.5 %, that was not assessed as being statistically significant. Therefore the proposed endpoint is a NOEC = 1000 mg MON 0139/kg dry soil, corresponding to 473 mg of glyphosate acid. The proposed endpoint will be used for risk assessment.

AMPA was classified as a major metabolite occurring in relevant amounts (1 x ≥ 10 % of Application Rate in soil. For AMPA, a new study by Noack et al.(2002, BVL no 2311035) concerning the long-term toxicity towards earthworms was performed. For this study a re-evaluation of the deriven endpoints was performed by the RMS. A NOEC of 131.9 mg/kg was recalculated for biomass deviation. The 56-day no-observed-effect-concentration (NOEC) of AMPA was 131.9 mg/kg regarding earthworm reproduction (number of juvenils).

B.9.6.6 Risk assessment

MON 52276 is the leading formulation in the re-registration dossier of glyphosate. A study with MON 52276 was conducted leading to an LC_{50}>1250 mg/kg soil dry soil (IPA-salt) corresponding to >388 mg a.s/kg dry soil.

Exposure

According to the GAP, glyphosate containing plant protection products are intended to be applied at maximaum application rate of 2 x 2160 g a.s./ha. However, it is stated that the maximum application rate is supposed to be 4.32 kg/ha glyphosate in any 12 month period

across use categories. The resulting maximum PECsoil values for the active substance glyphosate and the major soil degradation products are presented in the table below.
Calculations considered the maximum application rate and a minimum of 0 % foliar interception for applications. PEC values for the soil metabolites were calculated considering the maximum percentage of their formation observed in either the aerobic degradation studies and correcting for molecular weight.

RMS recalculated the predicted environmental concentrations in soil (PECSoil) of glyphosate and its major soil metabolite aminomethylphosphonic acid (AMPA) using the program ESCAPE 2.0. PECSoil calculations are based on the recommendations of the FOCUS workgroup on degradation kinetics. A soil bulk density of 1.5 g/cm3, a soil depth of 5 cm and a tillage depth of 20 cm (arable crop)/5 cm (permanent crops) were assumed. The PECSoil calculations were performed with ESCAPE 2.0 based on the input parameters for glyphosate and its metabolite AMPA as presented in Section 8.

Due to the slow degradation of glyphosate and its metabolite AMPA in soil (DT_{90} > 365 d, field data) the accumulation potential of both substances needs to be considered. Therefore, an accumulated soil concentration ($PEC_{soil,accu}$) is used for risk assessment which comprises background concentration in soil ($PEC_{soil,bkgd}$) considering a tillage depth of 20 cm (arable crop) or 5 cm (permanent crops) and the maximum annual soil concentration $PEC_{soil,act}$ for a soil depth of 5 cm.

Table B.9.6-11: Results of PEC_{soil} calculation (soil bulk density 1.5 g/cm^{-3}, soil depth 5 cm) after maximal application of glyphosate to all crops

plant protection product:			Mon 52276				
use:			all crops – worst case approach				
Number of applications/intervall			1				
application rate:			4320 g ai/ha				
crop interception:			0 %				
active substance	**application**	**soil relevant application rate (g/ha)**	**PECact (mg/kg)**	**PECtwa 21 d (mg/kg)**	**tillage depth (cm)**	**PECbkgd (mg/kg)**	**PECaccu = PECact + PECbkgd* (mg/kg)**
glyphosate	1 x every year annual crops	4320	5.76	4.6290	20	0.2140	5.974
	1 x every year permanent crops	4320	28.8	23.14	5	0.8562	**6.6162**
AMPA	1 x every year annual crops	1527	2.0360	2.0128	20	1.0359	3.0719
	1 x every year permanent crops	1527	2.0360	2.0128	5	4.1437	**6.1797**

* a tillage depth of 20 cm was considered for calculating the background concentration

Toxicity to Exposure Ratio

The potential acute and long-term risk for earthworms resulting from an exposure to glyphosate, glyphosate IPA-salt as well as the major soil degradation products of glyphosate

was assessed by comparing the maximum PECsoil with the 14-day LC_{50} or the NOEC value to generate TER_A and TER_{LT} respectively.

The resulting TER values are shown in table below:

Table B.9.6-12: Ecotoxicological endpoints, PECsoil values and Toxicity to Exposure ratios to assess the risk for earthworms

Test substance	Timescale	Endpoint (mg /kg dry soil)	PECaccu = PECact + PECbkgd* (mg/kg)	TER	TER trigger
Glyphosate	acute	LC_{50} =5600 mg /kg dry soil	6.6162	846	10
AMPA	acute	LC_{50} >1000 mg /kg dry soil	6.1797	59	10
	long-term	NOEC =131.90 mg /kg dry soil	6.1797	21	5
MON 52276 (IPA-salt)	acute	LC_{50} >1250 mg/kg dry soil (388 mg a.e/kg dry soil)	6.6162	59	10
	long-term	-			5
MON 0139 (IPA-salt)	acute	-			10
	long-term	NOEC > 1000 mg MON 0139/kg dry soil (473 mg a.e./kg dry soil)	6.6162	72	5

* a tillage depth of 20 cm was considered for calculating the background concentration

The results of the assessment indicate an acceptable risk for earthworms due to the intended use according to the label.

B.9.7 Effects on other soil non-target mesofauna (Annex IIIA 10.6.2)

New studies have been conducted exposing *Hypoaspis aculeifer* and *Folsomia candida* to glyphosate IPA salt and AMPA, respectively. Details of the studies are given below and the toxicity endpoints are summarised.

KIIA8.9.2/05

Author:	Schulz, L.
Title:	MON 0139 - Effects on the reproduction of the predatory mite *Hypoaspis aculeifer*
Date:	18.12.2009
Doc ID:	2311040 /09 10 48 058 S
Guidelines:	OECD 226 (2008)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	MON 0139 (glyphosate isopropylamine salt)

Lot/Batch #:	A8B60170S0
Purity:	Nominal: 62 % w/w glyphosate isopropylamine salt(corresponding to 45.9 % w/w glyphosate acid equivalent) Analysed: 63.81 ± 0.29 % w/w glyphosate isopropylamine salt (corresponding to 47.28 ± 0.21 % w/w glyphosate acid equivalent)
Reference item	Perfekthion (Dimethoate, EC 400, 422.4 g/L analysed)
Species:	*Hypoaspis aculeifer* (Canestrini)
Age:	Adult mites
Source:	In-house culture from Katz Biotech AG, 15837 Baruth, Germany
Diet/Food:	*Tyrophagus putrescentiae* (Schrank) were fed every 2 days, before and during the test
Temperature:	19.7 – 21.9 °C
Composition of artificial soil	5 % sphagnum peat, 20 % kaolin clay, 0.3 % calcium carbonate, 74.7 % quartz sand, Deionised water
Soil water content:	Test start: 18.79 – 20.21 % (47.52 – 51.11 % of WHC) Test end: 18.65 – 20.11 % (47.17 – 50.87 % of WHC)
pH	Test start: 5.9 – 6.2, Test end: 5.3 – 5.4
Photoperiod:	16 hours light / 8 hours darkness
Light intensity:	588 lux
Methods:	MON 0139 was evaluated for mortality and reproductive reduction with *Hypoaspis aculeifer* at four application rates, equivalent to 50, 100, 500 and 1000 mg MON 0139/kg dry soil (= 23.64, 47.28, 236.40 and 472.80 mg glyphosate acid equivalent/kg dry soil). In addition a blank control with deionised water and a toxic reference (Perfekthion, 422.4 g/L dimethoate) were tested. Each test item concentration was tested with 40 mites (10/ test vessel), while the control group consisted of 8 replicates. For each test item concentration and for the control group 2 test vessels without mites were provided for measurement purposes. The mites were put in glass bottles with screw tops of 100 mL containing 20 g (dry weight) artificial soil with the requested test item concentrations and closed, but opened every second day for food supply and aeration. Two weeks after introducing the test organisms the parental and juvenile were counted. Water content and pH were determined at test start and end. Adult and juvenile mites were counted at test end. The statistical analysis was performed with the software ToxRat Professional 2,10. Fisher's Exact Binomial Test with Bonferroni Correction and Dunnett's test (α = 0.05) were used to compare the control with independent test item groups. Abbott's formula was used to correct for control mortality.

Results

All validity criteria according to OECD 226 were fulfilled, as adult mortality in the control treatments did not exceed 20%, the mean number of juveniles per replicates was > 50 at test end and the coefficient of variation of the number of juveniles per replicate was not higher than 30 % at test end.

After treatment with the reference item Perfekthion (Dimethoate, EC 400, 422.4 g/L analysed) at concentrations of 4.1, 5.12, 6.40, 8.00 and 10.00 mg a.s./ kg dry soil an EC_{50} (reproduction) of 4.9 mg test item/ kg dry soil was calculated. The EC50 value for the reduction of reproduction was within the range of 3-7 mg a.s./kg soil dry weight specified in OECD 226 (2008), the EC_{50} therefore showed that the test system was sensitive.

The test item MON 0139 caused no statistically significant mortality (Fishers' Exact Binominal Test, α = 0.05) of the adult *Hypoaspis aculeifer* at the end of the 14-day exposure period. In the control group, a parental mortality of 8.8 % was observed. The corrected mortality in the treatment groups was not higher than 4.1 %. Also no significant decrease in

reproduction of the mites was observed (Dunnett-t-test, α = 0.05). The results are summarized in the table below:

Table B.9.7-1: Mortality and reproduction of *Hypoaspis aculeifer* after exposure to MON 0139 in a 14d laboratory study

Test rate (mg/kg soil dry weight)	glyphosate acid equivalent (mg a.e./kg soil dry weight)	Mortality of parent mites after 14d (%)	Corrected mortality [1] (%)	Juveniles after 14 days (No.)	Reduction of reproduction vs. control (%)	Coefficient of variation (%)
Control	Control	8.8	-	190.5	-	8.9
50	23.64	10	1.4	176.8	7.2	12.3
100	47.28	12.5	4.1	173.5	8.9	12.4
500	236.40	10	1.4	182.3	4.3	11.7
1000	472.80	7.5	-1.4	207.8	-9.1	6.3

[1] calculated with Abbott 1925

RMS Conclusions

In a 14-day *Hypoaspis aculeifer* reproduction study, the EC_{50} for reproduction could not be calculated. It can be concluded that the EC_{50} for *Hypoaspis aculeifer* is higher than 1000 mg MON 0139/kg soil dry weight (472.80 mg glyphosate a.s./kg soil dry weight), the highest concentration tested.

The NOEC for reproduction of *Hypoaspis aculeifer* was determined to be 1000 mg MON 0139/kg soil dry weight (472.80 mg glyphosate a.s./kg soil dry weight), the highest concentration tested.

The study is considered to be valid and acceptable.

KIIA8.9.2/06

Author:	Friedrich, S.
Title:	MON 0139 - Effects on the reproduction of the collembolans *Folsomia candida*
Date:	02.02.2010
Doc ID:	2311042 /09 10 48 057 S
Guidelines:	ISO 11267 (1999)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	MON 0139 (Glyphosate isopropylamine salt)
Lot/Batch #:	A8B60170S0
Purity:	Nominal: 62 % w/w glyphosate IPA- salt (corresponding to 45.9 % w/w glyphosate acid equivalent) Analysed: 63.81 ± 0.29 % w/w glyphosate IPA- salt (corresponding to 47.28 ± 0.21 % w/w glyphosate acid equivalent)
Reference item:	Betosip (Phenmedipham EC 157 g/L)
Species:	*Folsomia candida* (Willem)

Age:	Juvenile springtails (10 – 12 d old)
Source:	In-house culture originally obtained from Biologische Bundesanstalt (BBA), Berlin, Germany
Diet/Food:	Approximately 2 mg granulated dry yeast at test start and after 14 days
Temperature:	20.4 – 21.1 °C
Composition of artificial soil	10 % sphagnum peat, 20 % kaolin clay, 0.5 % calcium carbonate, 69.5 % quartz sand Deionised water
Soil water content:	Test start: 34.9 – 35.2 % (54.4 – 54.9 % of WHC) Test end: 34.5 – 34.7 % (53.8 – 54.1% of WHC)
pH:	Test start: 6.01 – 6.08, Test end: 5.79 – 5.91
Photoperiod:	16 hours light / 8 hours darkness
Light intensity:	580 lux
Methods:	MON 0139 was evaluated for mortality and reproductive reduction with *Folsomia candida* at 5 application rates of 32, 50, 100, 500 and 1000 µLMON 0139/kg dry soil (19, 29, 59, 294 and 587 mg glyphosate acid equivalent/kg dry soil). In addition a blank control with deionised water and a toxic reference (Betosip) were conducted. Each test item concentration and the control were tested with 50 springtails (10/ test vessel). For each test item concentration and for the control group 2 test vessels without springtails were provided for measurement purposes. The springtails were put in a glass container (~ 150 mL) containing 30g (wet weight) artificial soil with the requested test item concentrations and covered with a glass lid for 28 d. Four weeks after introducing the test organisms the surviving adults and juveniles were counted. Water content and pH were determined at test start and end. Adults and juvenile springtails were counted at test end. The statistical analysis was performed with the software ToxRat Professional 2.10. Fisher's Exact Binomial Test with Bonferroni Correction and Welch t-test were used to compare the control with the independent test item groups for significance of parental mortality and reproductive reduction, respectively. Abbott's formula was used to correct for control mortality.

Results

All validity criteria according to OECD 232 were fulfilled, since the mean adult mortality did not exceed 20%, the mean number of juveniles per vessel was ≥ 100 and the coefficient of variation of juveniles was less than 30 %.

Treatment with the reference item Betosip (Phenmedipham EC 157 g/L) at concentrations of 50, 100, 200 and 400 mg test item/ kg dry soil resulted in significant effects on reproduction and the determined EC_{50} was 181.0 mg Betosip/kg dry soil. The EC_{50} value for the reduction of reproduction was within the range of 100-200 mg product/kg soil dry weight specified in ISO 11267 (1999), the EC_{50} therefore showed that the test system was sensitive.

No statistically significant effect (Fisher`s Exact Binomial Test, $p > 0.05$) on parental mortality or the number of offspring (Welch-t-test, $\alpha = 0.05$) was found for any concentration tested.

Table B.9.7-2: Mortality and reproduction of *Folsomia candida* exposed to MON 0139 in a 28d laboratory study

Test rate (mg /kg soil dry weight)	Test rate glyphosate acid equivalent (mg a.e./kg soil dry weight)	Mortality of parental collembolans after 4 weeks (%)	Corrected mortality [1] (%)	Mean number of juveniles after 4 weeks (No.)	Reduction of reproduction vs. control (%)	Coefficient of variation (%)
Control	Control	4	-	397.2	-	14.2
32	19	6	2	355.6	10	14.3
50	29	6	2	384.6	3	38.4
100	59	2	-2	344.4	13	10.8
500	294	0	-4	446.4	-12	20.0
1000	587	8	4	358.8	10	12.1

[1] calculated following Abbott 1925

RMS Conclusions

The NOEC was determined to be 1000 µL MON 0139/kg soil d.w. (587 mg glyphosate a.s./kg soil d.w.). The EC_{50} could not be calculated, but it can be concluded that the EC_{50} is higher than 1000 µL MON 0139/kg soil d.w. (587 mg glyphosate a.s./kg soil d.w.), this being the highest concentration tested. The study is considered to be valid and acceptable.

KIIA8.9.2/07

Author:	Schulz, L.
Title:	AMPA - Effects on the Reproduction of the Predatory Mite *Hypoaspis aculeifer*
Date:	07.09.2010
Doc ID:	2311044 /10 10 48 053 S
Guidelines:	OECD 226
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	AMPA (Aminomethylphosphonic acid)
Lot/Batch #:	GLP-0908-19984-A
Purity:	98.7 %
2. Reference item:	Dimethoate EC 400 (414.8 g/L analysed)
Species:	*Hypoaspis aculeifer* (Canestrini)
Age:	Adult mites
Source:	In-house culture originally obtained from Katz Biotech AG, 15837 Baruth, Germany
Diet/Food:	*Tyrophagus putrescentiae* (Schrank) were fed every 2 days, before and during the test
Temperature:	19.7 – 21.8 °C
Composition of artificial soil	5 % sphagnum peat, 20 % kaolin clay, 0.3 % calcium carbonate, 74.7 % quartz sand, Deionised water

Soil water content:	Test start: 17.40- 18.07 % (47.81 – 49.64 % of WHC) Test end: 17.10 – 17.55 % (46.98 – 48.22% of WHC)
pH	Test start: 5.8 – 6.1, Test end: 5.4 – 6.3
Photoperiod:	16 hours light / 8 hours darkness
Light intensity:	472 lux
Methods:	AMPA was evaluated with five application rates, equivalent to 40, 80, 160, 240 and 320 mg test item/kg dry soil. A blank control with deionised water and a toxic reference (Dimethoate EC 400) were conducted. Each concentration was tested with 40 mites (10/ test vessel), while the control group consisted of 8 replicates. For each concentration and for the control group 2 test vessels without mites were provided for measurement purposes. The mites were put in glass bottles with screw tops of 100 mL containing 20 g (dry weight) artificial soil with the test item concentrations and closed, but opened every second day for food supply and aeration. Two weeks after introducing the test organisms the parental and juvenile were counted. Water content and pH were determined at test start and end. Adults and juvenile mites were counted at test end. Fisher's Exact Binominal test with Bonferroni Correction for significance of parental mortality. Dunnett-t-test (α = 0.05) for significance of reproductive reduction. Statistical program: ToxRat Professional 2.10 (2009).

Results

All validity criteria according to OECD 226 were fulfilled, as adult mortality did not exceed 20 %, the mean number of juveniles per replicate was > 50 at test end and the coefficient of variation of the number of juveniles per replicate was not higher than 30% at test end.

After treatment with the reference item Dimethoate EC 400 at concentrations of 4.1, 5.12, 6.40, 8.00 and 10.00 mg a.s./kg dry soil an EC_{50} (reproduction) of 6.6 mg test item/ kg dry soil was concluded.

The test item AMPA caused no statistically significant mortality (Fishers's Exact Binominal Test, p > 0.05) of the adult *Hypoaspis aculeifer* at the end of the 14-day exposure period. In the control group no parental mortality was observed. The corrected mortality in the test item treatment groups was not higher than 5.0 %.

Also no significant decrease in reproduction was observed (Dunnett-t-test, α = 0.05). The results are summarised in the table below:

Table B.9.7-3: Mortality and reproductive reduction of *Hypoaspis aculeifer* after application of AMPA in a 14 d laboratory study

Test rate (mg /kg dry soil)	Mortality of parent mites after 14days (%)	Corrected mortality [1)] (%)	Mean number of juveniles after 14 days (No.)	Reduction of reproduction vs. control (%)	Coefficient of variation (%)
Control	0.0	-	220.6	-	13.3
40	5.0	5.0	228.0	-3.3	20.7
80	2.5	2.5	236.3	-7.1	7.7
160	2.5	2.5	209.3	5.2	6.0
240	0.0	0.0	237.3	-7.5	9.9
320	2.5	2.5	227.5	-3.1	20.7

[1] calculated with Abbott 1925

RMS Conclusions

In a 14-day *Hypoaspis aculeifer* reproduction study, the EC_{50} for reproduction could not be

calculated, but it can be concluded that the EC_{50} is higher than 320 mg AMPA/kg soil dry weight, the highest concentration tested. The NOEC for reproduction of mites exposed to AMPA was determined to be 320 mg AMPA/kg soil dry weight, the highest concentration tested.

The study is considered to be valid and acceptable.

KIIA8.9.2/08

Author:	Friedrich, S.
Title:	AMPA - Effects on the Reproduction of the collembolans *Folsomia candida*
Date:	07.09.2010
Doc ID:	2311046 /10 10 48 045 S
Guidelines:	OECD 232 (2009)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	AMPA (Aminomethylphosphonic acid)
Lot/Batch #:	GLP-0908-19984-A
Purity:	98.7 %
Reference item:	Boric acid (100%)
Species:	*Folsomia candida* (Willem)
Age:	Juvenile springtails (9 – 12 d old)
Source:	In-house culture originally obtained from Biologische Bundesanstalt (BBA), Berlin, Germany
Diet/Food:	Approximately 2 mg granulated dry yeast at test start and after 14 days
Temperature:	20.4 – 22.0 °C
Composition of artificial soil	5 % sphagnum peat, 20 % kaolin clay, 0.3 % calcium carbonate, 74.6 % quartz sand, Deionised water
Soil water content:	Test start: 24.9 – 25.1 % (57.8 – 58.2 % of WHC) Test end: 24.3 – 25.0 % (56.4 – 58.0 % of WHC)
Photoperiod:	16 hours light / 8 hours darkness
Light intensity:	750 lux
Methods:	AMPA was evaluated for mortality and reproductive reduction with *Folsomia candida* at five application rates, equivalent to 30, 54, 97.2, 175 and 315 mg test item/kg dry soil. In addition a blank control with deionised water and a toxic reference (100% boric acid) were conducted. Each test item concentration was tested with 40 springtails (10/ test vessel), while the control group consisted of 8 replicates. For concentration and for the control group 2 test vessels without springtails were provided for measurement purposes. The springtails were put in a glass container (~ 150 mL) containing 30g (wet weight) artificial soil with the requested test item concentrations and covered with a glass lid for 28 d. Four weeks after introducing the test organisms the parental and juvenile were counted. Water content and pH were determined at test start and end. Adults and juvenile springtails were counted at test end as well as physiological or pathological symptoms. Fisher's Exact Binominal test with Bonferroni Correction for significance of parental mortality Dunnett-t-test (α = 0.05) for significance of reproductive reduction Statistical program: ToxRat Professional 2.10 (1999).

Results

All validity criteria according to OECD 232 were fulfilled, since the mean adult mortality in control treatment did not exceed 20 %, the mean number of juveniles per vessel was ≥ 100 and the coefficient of variation of juveniles was less than 30 %.

Treatment with the reference item boric acid at concentrations of 44, 67, 97.2, 150 and 225 mg test item/ kg dry soil resulted in an EC_{50} of 108.6 mg test item/kg dry soil.

No statistically significant effects on parental mortality (Fisher's Exact Binominal Test, α = 0.05) or the number of offspring (Dunnett-t-test, α = 0.05) was found.

Table B.9.7-4: Mortality and reproduction of *Folsomia candida* after exposure to AMPA in a 28 d laboratory study

Test rate (mg AMPA/kg dry soil)	Mortality of parentl collembolans after 4 weeks (%)	Corrected mortality [1)] (%)	Mean number of juveniles after 4 weeks (No.)	Reduction of reproduction vs. control (%)	Coefficient of variation (%)
Control	6.3	-	931	-	15.1
30	5.0	-1	925	1	11.6
54	7.5	1	934	0	5.2
97.2	2.5	-4	946	-2	11.8
175	7.5	1	973	-4	20.1
315	2.5	-4	939	-1	21.3

RMS Conclusions

In a 28 d laboratory test to determine the effects of AMPA on the collembolan, *Folsomia candida*, the NOEC for reproduction was determined to be 315 mg AMPA/kg dry soil. The 28-d LC_{50} and EC_{50} could not be calculated, but it can be concluded that LC_{50} and EC_{50} are higher than 315 mg AMPA/ kg dry soil, the highest concentration tested.

The study is considered to be valid and acceptable.

B.9.7.1 Summary of toxicity data on other soil non-target macro-organisms

New studies have been conducted exposing *Hypoaspis aculeifer* and *Folsomia candida* to glyphosate IPA salt and AMPA, respectively. The toxicity endpoints are summarised.

Table B.9.7-5: Toxicity of glyphosate IPA-salt and its metabolite AMPA to soil mites and springtails

Species	Substance	Design	Endpoints	Reference
Hypoaspis aculeifer	Glyphosate IPA-salt	14 d chronic	EC_{50} > 1000 mg/kg dry soil > 472.8 mg a.e./kg dry soil NOEC=1000 mg/kg 472.8 mg a.e./kg	Schulz, 2009; 09 10 48 058 S BVL no 2311040
Folsomia candida	Glyphosate IPA-salt	28 d chronic	EC_{50} >1000 mg/kg > 587 mg a.e./kg dry soil NOEC= 1000 mg/kg 587 mg a.e./kg	Friedrich, 2010; 09 10 48 057 S BVL no 2311042
Hypoaspis aculeifer	AMPA	14 d	EC_{50} > 320 mg/kg dry soil	Schulz, 2010;

Species	Substance	Design	Endpoints	Reference
		chronic	NOEC=320 mg/kg dry soil	10 10 48 053 S BVL no 2311044
Folsomia candida	AMPA	28 d chronic	EC_{50} >315 mg/kg NOEC= 315 mg/kg	Friedrich, 2010; 10 10 48 054 S BVL no 2311046

B.9.7.2 Risk assessment of non-target soil mesofauna

Table B.9.7-6: Risk assessment of non-targen soil mesofauna exposed to glyphosate at intended uses

Test substance	Species	NOEC (mg/kg dry soil)	Maximum $PEC_{soil, plateau}$ (mg/kg dry soil)	TER_{LT}
Glyphosate IPA salt	*Hypoaspis aculeifer*	472.8 mg a.e./kg	6.6162	71
	Folsomia candida	587 mg a.e./kg	6.6162	89
AMPA	*Hypoaspis aculeifer*	320	6.1797	52
	Folsomia candida	315	6.1797	51

The TER values calculated using worst-case PEC_{soil} values for glyphosate acid and its metabolite AMPA exceeded the relevant triggers, indicating that the risk to soil macro organisms other than earthworms following intended uses of glyphosate is acceptable.

B.9.8 Effects on soil non-target micro-organisms (Annex IIA 8.5; Annex IIIA 10.7)

In the first EU peer review evaluation of glyphosate in 2001, four test were carried out with formulations and the active substance. It was stated that the inteded uses of glyphosate caused no negative effects on microbial activities in soils. For details on studies for which the initial evaluation was still valid, please refer to the initial monograph and its addenda.

New studies on nitrogen and carbon mineralisation have been carried out with glyphosate technical (Franco Perina, 2000, BVL no 2311047), MON 52276 (Hutcheson, 2012, BVL no 2316013) and the metabolite AMPA (Schulz, 2010, BVL no 2311050).

B.9.8.1 Laboratory testing

KIIA 8.10.1/01

Author:	Franco Perina, V.C.
Title:	Side-Effects of Glifosate Técnico Nufarm on Soil Microflora Carbon and Nitrogen Cycles
Date:	05.01.2000 Side-Effects of Glifosate Técnico Nufarm on Soil
Doc ID:	2311047 /RF-D1. 113/99
Guidelines:	OECD 216/ OECD 217
GLP:	Yes
Validity:	OECD 217: Yes

	OECD 216 No: For soil nitrogen cycle (OECD 216) test the variation between replicate control samples was more than ± 15%.

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	037-919-113
Purity:	95 % a.s (nominal), 95.49% a.s. (measured)
2. Vehicle/control:	None
Soil	LE (Typic Hapludox) and LR (Rhodic Hapludox)
Source:	Not stated
Water content	Not stated
Water holding capacity	Not stated
pH:	5.5 (LR), 7.0 (LE)
Total Org. C:	Not stated
Clay (< 0.002 mm):	39 % (LR), 24% (LE)
Silt (0.002 mm - 0.063 mm):	10 % (LR), 9 % (LE)
Sand (0.063 – 2.00 mm):	51 % (LR), 67 % (LE)
Temperature:	19 – 22 °C
pH:	5.53 – 6.27 (LR); 6.34 – 6.84 (LE)
Water content:	40- 60 % of WHC
Photoperiod:	24 hour dark
Methods:	The test substance was applied at 2.4 and 4.8 kg a.s./ha using three replicates per concentration. Negative controls with or without organic matter amendment were tested. 150 g soil samples were amended with organic matter at a rate of 0.5% dry soil equivalent for all treatments, except for control without organic matter amendment. Soils were incubated at 19 to 22°C in dark in covered glass flasks. Soil samples were removed 0, 14 and 28 days after treatment and analysed for soil dry mass, pH, nitrite, nitrate, nitrogen ammonical and short term respiration test. Nitrogen cycle: Ammonium-N analysis: 10 g of soil was placed in 250 mL wide-mouth bottle, to which 100 mL of 2M KCl was added. 1 mL of the filtered aliquot containing between 0.5 and 12 µg of NH_4+-N was placed into 25 mL volumetric flasks. 1 mL EDTA, 2 mL phenol nitroprussid and 4 mL hypochlorite buffer were successively added. The concentration of NH_4+-N was determined photometrically at 636 nm. Nitrate-N: 10 g of soil was placed in a 500 mL Erlenmeyer flask, then 0.5 g of $CaSO_4$ and 250 mL distilled water were added. For the analysis of nitrate-N, an aliquot of 25 mL of the extract was pipetted into 10 mL round bottom flask and 0.05g of $CaCO_3$ was added. Subsequently, 2 ml of phenoldisulfonic acid (25 g phenol in 150 mL of concentrated NH_4OH) was added. After 10 min, 20 ml of distilled water was added. The nitrate-N concentration was determined using a Hach Model DR 2010 absorbance spectrophotometer at 410 nm. Nitirite-N: an aliquot of 25 mL of the extract was pipetted into a 25 mL cell. The visual absorbance of each sample was determined at 507 nm using a Hach Model DR 2010 absorbance spectrophotometer. Carbon cycle: 2 g of soil samples were placed in 50 mL Erlenmeyer flasks, adding 0.5 mL of 2 µmol/mL of glucose- ^{14}C. In order to absorb CO_2 evolved from glucose degradation by soil microorganisms, a small glass flask (1 mL) was hung from the rubber cap, containing 0.2 mL of NaOH. After one hour of incubation in dark conditions, the glucose degradation was then stopped. The NaOH and filter paper strips were transferred into scintillation vials. The radioactivity was assessed in a Liquid

	Scintillation Analyser Packard model Tri-carb 1900, during 5 min/sample. Results were evaluated using Duncan's Multiple range Test at α = 0.01

Results

No adverse effects of glyphosate acid on soil carbon cycle were observed for both concentrations 28 days after application. For soil nitrogen cycle (OECD 216) test the variation between replicate control samples was more than ± 15 %.

Table B.9.8-1: Effects of glyphosate acid on soil nitrogen cycle

		Glyphosate acid (kg a.s./ha)				
		Control	**2.4**		**4.8**	
		(mg N/kg dry soil)	**(mg N/kg dry soil)**	**Dev. [a]**	**(mg N/kg dry soil)**	**Dev. [a]**
Soil: LR (Rhodic Hapludox)						
Day 0	Ammonium	22.66	21.61	-4.6	24.31	+7.3
	Nitrite	0.30	0.29	-3.3	0.40	+33.3*
	Nitrate	22.51	22.54	+0.1	23.11	+2.7
Day 14	Ammonium	27.34	34.92	+27.7*	37.50	+37.2*
	Nitrite	0.29	0.21	-27.6*	0.23	-20.7
	Nitrate	30.02	36.47	+21.5*	44.10	+46.9*
Day 28	Ammonium	13.13	11.32	-13.8	9.38	-28.6*
	Nitrite	0.26	0.24	-7.7	0.24	-7.7
	Nitrate	18.39	24.16	+31.4*	34.61	+88.2*
Soil: LE (Typic Hapludox)						
Day 0	Ammonium	30.01	27.87	-7.1	34.72	+15.7
	Nitrite	0.32	0.27	-15.6	0.27	-15.6
	Nitrate	22.58	22.74	+0.7	23.34	+3.4
Day 14	Ammonium	26.19	22.60	-13.7	24.50	-6.5
	Nitrite	0.26	0.29	+11.5	0.27	+3.8
	Nitrate	21.78	39.26	+80.3*	41.01	+88.3*
Day 28	Ammonium	16.82	18.71	+11.2	18.72	+11.3
	Nitrite	0.40	0.24	-40.0*	0.26	-35.0*
	Nitrate	18.39	31.67	+72.2*	25.77	+40.1*

[a] - = Deviation from control
* = Significant deviation from control according to OECD Guideline 216 and 217
- = inhibition, + = stimulation

Table B.9.8-2: Effects of glyphosate acid on soil carbon cycle

	Glyphosate acid (kg a.s./ha)				
	Control	**2.4**		**4.8**	
	Soil respiration [b]	**Soil respiration [b]**	**Dev. [a]**	**Soil respiration [b]**	**Dev. [a]**
Soil: LR (Rhodic Hapludox)					
Day 0	9.00	8.33	-7.4	9.06	+0.7
Day 14	16.06	16.19	+0.8	16.76	+4.4
Day 28	15.13	14.63	-3.3	16.53	+9.3
Soil: LE (Typic Hapludox)					
Day 0	12.80	13.00	+1.6	11.56	-9.7
Day 14	16.69	20.16	+20.8	17.56	+5.2
Day 28	16.43	18.06	+9.9	17.26	+5.1

[b] = Activity of soil microorganism in mmoles metabolized glucose/g soil/h
- = inhibition, + = stimulation

RMS Conclusions

The test item glyphosate acid caused no adverse effects on soil carbon cycle at test concentration of 2.4 and 4.8 kg a.s/ha, 28 days after treatment.
The nitrogen cycle test is considered not valid according to OECD guideline 216. According to the OECD 216 guidance document, the test concentrations recommended are the maximum predicted environmental concentration (PEC) and five times that concentration. Sufficient data was submitted to evaluated the risk of the representative formulation MON 52276.

Nevertheless, for the evaluation of the active substance glyphosate acid further data will have to be generated to assess the risk for non target micro-organisms. This is necessary as the active substance glyphosate acid is considered persistent in soil (the maximum DT_{50} value of unnormalised field dissipation studies of 116.4 days exceeds the trigger value of 60 days for soil).

KIIA 8.10.1/02

Author:	Schulz, L.
Title:	AMPA - Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests)
Date:	07.10.2010
Doc ID:	2311050 /10 10 48 010 C/N
Guidelines:	OECD 216 (2000) OECD 217 (2000)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	AMPA (Aminomethylphosphonic acid)
Lot/Batch #:	GLP-0908-19984-A
Purity:	98.7 %
2. Positive control:	Reference item: Dinoterb
Soil	Loamy sand soil “Wassergut Canitz” (agricultural soil)
Source:	Field “Schag 34/3” in the municipality of Canitz, Saxony, Germany.
Water content:	11.30% (g water/100 g dry soil)
Water holding capacity	36.56 % (g water/100 g dry soil)
pH:	6.3
Total Org. C:	1.43 %
Clay (< 0.002 mm):	9.1 %
Silt (0.063 mm > 0.002 mm):	38.2 %
Sand (≥ 0.063 – 2.00 mm):	52.7 %
Temperature:	19.7 – 21.8 °C

pH:	5.9 – 6.3
Water content:	41.46 – 44.71 % of WHC (nitrogen transformation test) 41.84 – 45.09 % of WHC (carbon transformation test)
Photoperiod:	24 hours darkness
Methods:	AMPA was applied at concentration rates encompassing 40, 80, 160, 320 and 640 mg test item/kg dry soil. In addition, a negative control (non-treated soil) was tested. Three replicate soil samples were prepared for each treatment rate and the control for the carbon transformation and nitrogen transformation tests. Soil C-transformation: For each replicate a sub-sample of 1000 g dry soil was mixed with deionised water. Water was added to the soil to achieve a water content of approximately 45% WHC. Water content was adjusted weekly to the required range of 40-50 % of WHC. The prepared soil was transferred to steel test vessels (4 L) under 19.7 – 21.8°C. C- transformation was determined for 12 hours on sampling days 0 (3 hours after application), 7, 14, 28, 42 and 56 days after application On each sampling occasion, 100 g samples of soil (dry soil) were taken, mixed with glucose by means of a hand-stirrer and placed into glass reaction flasks (500 mL). Then, glass vessels containing 18 mL of 1 M NaOH solution were placed in the reaction flasks and connected with a respirometer (BSB digi SELUTEC). Cumulative O_2-production (corresponding to the O_2 consumption by microorganisms) was determined over a 12-hour. Soil N- transformation: Sub-samples of 200 g dry soil were weighed into each test vessel (500 mL wide mouth glass flask). Lucerne meal (5 g/kg dry soil) was added to provide 1.0 g Lucerne meal per 200 g dry soil. One additional soil sample (without Lucerne meal) was used for determination of initial NH_4-N- and NO_3-N-content. The initial NH_4-N and NO_3-N content was 0.01 mg and 1.48 mg/100 g dry soil, respectively. The prepared soil was incubated in wide-mouth glass flasks (500 mL) at 19.7 – 21.8 °C. Soil samples (10 g dry soil per replicate) were sampled after 3 hours, 7, 14, 28, 42 and 56 days after application and NH_4-N, NO_3-N and NO_2-N contents were determined. Soil was extracted by adding 50 mL 1 M KCl solution to the equivalent of 10 g dry soil. Mineralized nitrogen was quantified using an Autoanalyzer II. Two-sided Students t-test for homogenous variances at $\alpha = 0.05$. For C- transformation, a two-sided Welch t-test for homogenous variance was additionally performed.

Results

28 days after application, no adverse effects on nitrogen and carbon transformation were observed up to and including a test concentration of 160 mg AMPA/kg dry soil. After the prolongation of the test to 56 days for the test concentrations 320 and 640 mg AMPA/kg dry soil, the measured variations of nitrogen and carbon transformations of > 25 % could be observed till the end of the study (56 days). This can be most likely attributed to the high phosphorus/nutrient content in AMPA.

In a different test, 28 days after application the toxic standard dinoterb caused effects of +37.6 %, +51.4 % and +27.1 % on nitrogen transformation and -30.5 %, -34.5 % and -28.8 % on carbon transformation at concentrations of 6.80, 16.0 and 27.0 mg dinoterb/kg dry respectively, and thus demonstrates the sensitivity of the test system.

All validity criteria according to OECD 216 and 217 were fulfilled, as the variation between replicate control samples was less than ± 15 %.

Table B.9.8-3: Effects of AMPA on soil nitrogen transformation:

	AMPA (mg test item/kg dry soil)										
	Control	**40**		**80**		**160**		**320**		**640**	
	NO_3-N	**NO_3-N**	**Dev. [a]**	**NO_3-N**	**Dev. [a]**	**NO_3-N**	**Dev. [a]**	**NO_3-N**	**Dev. [a]**	**NO_3-N**	**Dev. [a]**
Day 0	15.7	15.5	-1.1	15.7	2	15.4	-1.9	14.9*	-4.9	14.6*	-6.6
Day 7	23.1	23.6	2.5	27.3*	18.5	25.8	+11.7	30.5*	+32.2	33.5*	+45.2
Day 14	32.2	34.6	7.5	37.4*	16.3	35.1*	+9.2	42.9*	+33.3	43.9*	+36.5
Day 28	42.2	46.8*	10.7	47.7*	13	51.0*	+20.8	57.4*	+35.8	65.0*	+53.8
Day 42	55.4	-	-	-	-	-	-	72.1*	+30.2	78.1*	+41.1
Day 56	61.9	-	-	-	-	-	-	78.4*	+26.7	88.6*	+43.1

[a] - = Deviation from the control based on NO_3-nitrogen production
* = Significantly different from control (two-sided Student- t test for homogenous variances at $\alpha = 0.05$)
- = inhibition, + = stimulation

Table B.9.8-4: Effects of AMPA on soil carbon transformation

	AMPA (mg test item/kg dry soil)										
	Control	**40**		**80**		**160**		**320**		**640**	
	O_2[a]	**O_2[a]**	**Dev. [b]**	**O_2[a]**	**Dev. [b]**	**O_2[a]**	**Dev. [b]**	**O_2[a]**	**Dev. [b]**	**O_2[a]**	**Dev. [b]**
Day 0	12.0	11.9	-0.8	11.4*	-5.3	11.1*	-8.0	10.8*	-10.4	10.1*	-16.2
Day 7	11.9	11.0*	-7.1	10.3*	-13.2	9.9*	-16.9	9.5*	-20.2	8.3*	-29.7
Day 14	11.7	10.9*	-7.0	10.6*	-9.2	9.9*	-15.4	9.1*	-22.6	8.0*	-31.3
Day 28	10.9	10.0*	-7.9	9.5*	-12.9	8.9*	-18.5	8.1*	-25.7	7.0*	-35.3
Day 42	10.7	-	-	-	-	-	-	7.9*	-26.6	6.8*	-37.0
Day 56	10.1	-	-	-	-	-	-	7.4*	-26.1	6.2*	-38.8

[a] - = Oxygen consumption
[b] - = Deviation from the control based on NO_3-nitrogen production
* = Significantly different from control (two-sided Student- t test or two-sided Welch-t-test, respectively for homogenous variances at $\alpha = 0.05$)
- = inhibition, + = stimulation

RMS Conclusions

The test item AMPA caused no adverse effects on soil nitrogen transformation and on soil carbon transformation (< 25 % deviation from control) up and including a test concentration of 160 mg AMPA/kg dry soil at the end of 28-day incubation period.
The study is considered to be valid and acceptable.

KIIIA 10.7/03

Author:	Hutcheson, K.
Title:	MON 52276: Effect on soil microbial activity, Carbon and Nitrogen transformations
Date:	26.01.2012
Doc ID:	2316013 /CEMR-5259
Guidelines:	OECD 216/OECD 217
GLP:	Yes
Validity:	Yes

Materials and Methods

Test item:	MON 52276
Lot/Batch #:	A9K0106104
Purity/Density:	358.8 g/L glyphosate acid equivalent 30.68% w/w// 1.17 g/mL
Soil	LUFA standard soil type 2.3 (sandy loam soil)
Source:	LUFA Speyer, Germany, batch no. F2.34011
Water content:	35 ± 5 %
Water hold. capacity	35.6 %w/w
pH:	7.5
Total Org. C:	0.94 %
Microbial biomass	1.91 % of TOC
Clay (< 0.002 mm):	8.7 ± 1.7 %
Silt (0.002 mm-0.05 mm):	27.6 ± 3.8 %
Sand (0.05–2.00 mm):	63.7 ± 4.4 %
Temperature:	20 ± 2 °C
pH:	7.5 – 7.9
Water content:	38.9 % of WHC
Methods:	MON 52276 was applied at two test item treatment concentrations, 18.8 and 94 mg MON 57726/kg dry soil, equivalent to 12 and 60 L MON 52276/ha, using three replicates per treatment. A negative control (deionised water) was tested. Moisture content was maintained at 40 % (±5 %) of the MWHC and at each time point the weight of the moisture control vessel was determined as a guide to test vessel water loss. For soil nitrogen transformation, replicates were amended with Lucerne meal (0.5 %) as a nitrogen source (C- content: 41.67 % w/w, N-content: 2.54 % w/w giving a C/N ratio of 16.4:1). After 7, 14, 28 and 43 days, 50 g soil sample (based on dry weight) was removed for determination of NH_4^+, NO_2^- and NO_3. Soil extracts were prepared by extracting soil samples with 250 ml 2 M KCl and shaking for two hours and then centrifuged for 15 minutes. The supernatant was analysed using a Bran + Luebbe Autoanalyzer AA3 system. For soil microflora respiration, a 8.0 mg glucose amendment/g dry soil was used. Soil was taken from one replicate from each treatment from the carbon test for pH (water) determination at the start and end of the study, soil was taken from one replicate from each treatment from the carbon test for moisture and dry matter content determination at the end of the study. After 7, 14, 28 and 43 days, 100g soil sample (based on dry weight) was removed from each replicate for the determination of glucose-induced respiration rates in soil respiration chamber. Carbon dioxide evolution was measured using an infrared carbon dioxide analyser and automatic column switching unit (GHU) (ADC 2250 and GHU). The total CO_2 evolution over a period of at least 12 hours was calculated for each replicate.Statistical calculations are based on ANOVA followed by Dunnett's two-tailed test ($\alpha = 0.05$).

Results

Deviations from OECD 216 and 217 have been observe in terms of an increase in temperature for 4 hours during study (minimum 17.93 °C).

Statistical analysis showed there was a significant difference ($\alpha = 0.05$, Dunnett's two-tailed test) between the treatment rate of 94 mg MON 52276/kg dry soil and the control treatment for soil carbon.

As the difference in respiration rates between the treatment rates of MON 52276 (18.8 and 94 mg MON 52276/kg dry soil) and control is less than 25% at Day 28, the test item can be evaluated as having no long-term influence on carbon transformation in soils.
Statistical analysis showed there was a significant difference (α = 0.05, Dunnett's two-tailed test) between the treatment rate of 94 mg MON 52276/kg dry soil and the control treatment for nitrate production from Day 14 to 28.
As the difference in nitrate production between the treatment rates of MON 52276 (18.8 and 94 mg MON 52276/kg dry soil) and control is less than 25 % at Day 28, the test item can be evaluated as having no long term influence on nitrogen transformation in soils.

Table B.9.8-5: Effects of MON 52276 on soil nitrogen transformation in sandy loam : soil

		Nitrogen concentration (mg N/kg soil)		% deviation from control	
	Control	**18.8 mg/kg** (1×)	**94 mg/kg** (5×)	**18.8 mg/kg**	**94 mg/kg**
Nitrate transformation rates					
Day 0-7	-3.20	-3.48	-3.59	+8.84	+12.24
Day 7-14	+3.69	+4.69	+6.04	+27.14	+63.72
Day 14-28	+3.54	+3.69	+3.81	+4.31	+7.85
Nitrate (NO_{3-})					
Day 0	22.4	24.4	25.1	+8.93	+12.05
Day 7	0.0	0.0	0.0	-	-
Day 14	25.8	32.8	42.3	+27.13	+63.95
Day 28	75.3	84.5	95.7	+12.22	+27.09
Ammonium (NH_4^+)					
Day 0	10.3	10.7	11.2	+3.88	+8.74
Day 7	3.0	2.9	2.8	-3.33	-6.67
Day 14	1.6	1.6	1.6	+0.00	+0.00
Day 28	1.1	1.1	1.0	+0.00	-9.09

Table B.9.8-6: Effects of MON 52276 on soil microflora respiration

	CO_2 (mg CO_2/kg soil/h)			% deviation from control	
	Control	**18.8 mg/kg** (1×)	**94 mg/kg** (5×)	**18.8 mg/kg**	**94 mg/kg**
Day 0	16.08	16.16	17.24	+0.47	+7.19
Day 7	15.42	16.64	18.73	+7.97	+21.52
Day 14	15.42	16.93	18.77	+9.78	+21.71
Day 28	16.49	17.15	18.90	+3.96	+14.57

RMS Conclusions
MON 52276, when applied at 18.8 and 94 mg MON 57726/kg dry soil (5.76 and 28.8 mg a.s./kg dry soil), equivalent to 12 and 60 L MON 52276/ha, respectively, is not expected to cause significant long-term effects on soil microflora respiration and soil nitrogen transformation processes.

The study is considered to be valid and acceptable.

KIIA 8.10.1/04

During the revision of the active substance glyphosate for the renewal of Annex I inclusion (AIR2), the Rapporteur Member State (RMS) Germany requested the glyphosate task force (GTF) to submit a new N-transformation study following the OECD guideline 116, with the

active substance glyphosate acid. The following study was made available upon request by the European Food Safety Authority (EFSA)

Author:	Hutcheson, K.
Title:	Glyphosate technical (MON 77973): Effect on Soil Microbial Nitrogen Transformations
Date:	2014
Doc ID:	2716673/ Report No: CEMR-6237
Guidelines:	OECD 216 (2000)
GLP:	Yes
Validity:	Yes

Materials and Methods

Test item:	MON 77973
Lot/Batch #:	GLP-0807-19475-T
Purity/Density:	96.59% Glyphosate Acid
Soil	LUFA standard soil type 2.3 (sandy loam soil)
Source:	LUFA Speyer, Germany, batch no. Sp2.33113
Water content:	35 ± 5%
Water hold. capacity	36.2 %w/w
pH:	6.5
Total Org. C:	0.67%
Microbial biomass	4.35% of TOC
Clay (< 0.002 mm):	5.9 ± 2.5%
Silt (0.002 mm-0.05 mm):	33.9 ± 0.0%
Sand (0.05–2.00 mm):	60.3 ± 2.5%
Temperature:	20 ± 2 °C
pH:	6.0-6.6 (range between Day 0 and Day 28)
Water content:	42 % of WHC
Methods:	MON 77973 was applied at two test item treatment concentrations, 6.62 and 33.1 mg a.e./kg dry weight soil, equivalent to using three replicates per treatment.. In addition, a negative control (deionised water) was tested. Moisture content of the soil was maintained at 40% (□ 5%) of the MWHC and at each time point the weight of the moisture control vessel was determined as a guide to test vessel water loss. For soil nitrogen transformation, each replicate was amended with Lucerne meal (0.5%) as a nitrogen source. The Lucerne had a carbon content of 41.67% w/w and a nitrogen content of 2.54% w/w giving a C/N ratio of 16.4:1. Soil was taken from one replicate from each treatment for pH (water) determination at the start and end of the study, soil was taken from one replicate from each treatment for moisture and dry matter content determination at the end of the study. Soil nitrogen transformation: As soon as possible after dosing and after 7, 14, and 28 days, 50 g soil sample (based on dry weight) was removed for determination of NH4+, NO2- and NO3. Soil extracts were prepared by extracting soil samples with 250 ml 2 M KCl and shaking for two hours and then centrifuged for 15 minutes. The supernatant was analysed using a Bran + Luebbe Autoanalyzer AA3 system. Shapiro-Wilks and Bartletts Test followed by Dunnett's two-tailed test (α = 0.05).

Results

All validity criteria according to OECD 216 were fulfilled, as the variation between replicate control samples was less than ± 15%.

Statistical analysis showed there was no significant difference (α = 0.05, Dunnett's two-tailed test) between the treatment rates of 6.62 and 33.1 mg a.e./kg dry soil and the control treatment for nitrate production from Day 14 to 28.
As the difference in nitrate production between the treatment rates of MON 77973 (6.62 and 33.1 mg a.e./kg dry soil) and control is less than 25% at Day 28, the test item can be evaluated as having no long term influence on nitrogen transformation in soils at concentrations ≤ 33.1 mg a.e./kg dry soil.

Table B. 9.8-7 Effects of MON 77973 on soil nitrogen transformation in sandy loam soil

		Nitrogen concentration [mg N/kg soil]		**% deviation from control**	
	Control	**6.62 mg a.e/kg (1×)**	**33.1 mg a.e./kg (5×)**	**6.62 mg a.e/kg (1×)**	**33.1 mg a.e./kg (5×)**
Nitrate transformation rates					
Day 0-7	-3.47	-3.51	-3.56	+1.26	+2.52
Day 7-14	+1.04	+1.34	+1.39	+29.47	+33.68
Day 14-28	+4.10	+4.09	+4.18	-0.13	+2.13
Nitrate (NO_{3-})					
Day 0	24.3	24.6	24.9	+1.23	+2.47
Day 7	0.0	0.0	0.0	0.00	0.00
Day 14	7.3	9.4	9.7	+28.77	+32.88
Day 28	64.6	66.7	68.3	+3.25	+5.73
Ammonium (NH_4^+)					
Day 0	7.0	7.0	6.6	+0.00	-5.71
Day 7	2.4	2.4	2.4	+0.00	+0.00
Day 14	1.8	1.7	1.7	-5.56	-5.56
Day 28	0.8	0.8	0.8	0.00	0.00

- = inhibition, + = stimulation
Note: Some values are calculated using un-rounded values.

RMS Conclusions

MON 77973, when applied at 6.62 and 33.1 mg a.e./kg dry soil, is not expected to cause significant long-term effects on the soil nitrogen transformation process at concentrations ≤ 33.1 mg a.e./kg dry soil.

B.9.8.2 Literature Review

For the group of soil non-target micro-organisms, a database of 99 publications was collected by the notifier and selected 21 publications. The submitted publications were also evaluated by zRMS and have been assigned according to an UBA screening. Most of the studies submitted by the notifier dealt with the rhizobia of glyphosate-resistant crops and were therefore not assignable for risk assessment in the European Union. However, after screening 28 studies were recognised as informative with low weight (category UBA3), 18 publications have been considered as supportive information (UBA2) and only one publication from Cycon & Kaczynska (2004) has been classified as UBA1 (critical data, high weight of evidence in risk assessment). In this study, performed according to the OECD guidelines 216 and 217, the authors applied glyphosate at the field rate of 4.5 mg/ kg of soil (PEC) as well as at a 5-fold higher concentration (22.5 mg/ kg of soil). After 1, 7, 14 and 28 days of incubation, soil respiration rates (SIR – Substrate Induced Respiration) and the amounts of nitrate did not significantly differ from control soil.

Soil microorganisms play a very important role in soil fertility by assuming key ecological functions like matter decomposition and nutrient cycling. Therefore, information about how agricultural practices and especially pesticides significantly affect soil microorganisms is highly required in risk assessment. However, soil microbial diversity is extremely difficult to measure because of its high complexity (Tiedje *et al*. 1999). In practice, the risk assessment of soil non-target micro-organisms is hence often restricted to the measurement of the impact of pesticides on soil functional diversity (i.e. carbon and nitrogen mineralization rates, respiration rate, enzyme activities) or bacterial and fungal biomass.

In the case of glyphosate, only few studies failed to detect significant effect on soil functional diversity after application of the herbicide (e.g. Liphadzi, *et al.* 2005). Zabaloy, *et al.* (2008) reported that "the addition of glyphosate at a dose 10 times higher than the normal field application rates caused minor changes to soil microbial activity, bacterial density and functional richness". In rare cases, inhibitory effects have also been reported. In a land set-aside in the western part of Prague (Czech Republic), Ruzkova *et al.* (2011) found that repeated application of Roundup desiccation caused a significant increase of microbial biomass (+69 %), but also strongly decreased the immobilization of nitrates by the plants (nitrate-nitrogen ratio +86%) as well as the arylsulfatase activity (–28 %).

In some studies, differences in microbial parameters are more a function of time and site quality than pesticides doses. For example, Gomez et al. (2009) detected significant differences in microbial respiration over the time but not between doses of applied glyphosate. In Hart et al. (2009), seasonality was a significant determinant of denitrifier and fungal abundance. Parallelly, Busse et al. (2001) found that variation in microbial community size, activity and metabolic diversity depended more of time of year and land-use that herbicide treatment.

Nevertheless, glyphosate is an organophosphonate herbicide that can be easily used as a source of P, C or N by either by gram-positive or gram-negative bacteria (van Eerd et al., 2003). Therefore, in most studies, the application of glyphosate at expected or higher field concentration rates is correlated with an immediate and significant increase in soil respiration (Accinelli et al., 2002), microbial biomass (Lupwayi et al., 2004), C- and N-mineralizations (Lancaster et al., 2006; Haney et al., 2002a, 2002b). This stimulation of soil principal functional parameters is assumed to be linked to a rapid use of glyphosate as source of nutrients (Mijangos et al., 2009) usually correlated with a metabolisation of the pesticide. Araujo et al.

(2003) demonstrated in two Brazilian soils a rapid biodegradation of glyphosate by soil microorganisms with the formation the metabolite AMPA, resulting in short- and long-term positive effect of the herbicide on the soil microbial activity (increase of 10–15 % in the CO2 evolved and a 9–19 % increase in FDA hydrolyses in the presence of glyphosate).

This potential use of glyphosate as a source of P, C or N by soil non-target micro-organisms is likely to induce a shift in their community structures. Ratcliff et al. (2006) detected a community shift from fungal dominance to equal ratio with an enrichment of opportunistic copiotrophic bacteria. Community shifts from bacterial to fungal dominance were also recorded (Araujo et al., 2003). Lupwayi et al. (2004) observed herbicide-induced shifts in microbial composition even when diversity indices among treatments did not differ. This study points out the importance to assess both microbial diversity and composition when looking at the effects of pesticides on non-target micro-organisms. In microcosm experiments performed with sediment microbes, Widenfalk et al. (2008) focused their monitoring on various levels of microbial community organization. Community-level endpoints like bacterial activity, fungal and total microbial biomass were not affected by pesticide exposure, whereas endpoints recorded at the "sub-community level" (e.g. Phospholipid Fatty-acid Analysis, 16S rRNA genotyping, T-RFLP) demonstrated significant shifts in bacterial community composition even at environmentally relevant concentrations. The same authors concluded that "Any shifts in community structure will, however, only have consequences on ecosystem function if the tolerant microorganisms cannot compensate for biogeochemical functions normally carried out by inhibited or eliminated microbial groups". Such community shifts coupled with a loss of function are clearly illustrated in Lancaster et al. (2006). The authors looked at how the combinations of pesticides may affect soil microbial activity differently than pesticides applied alone. They found that after 30 days, soils treated with glyphosate alone (applied as Roundup WeatherMAX, Monsanto Co., St. Louis, MO) exhibited greater microbial biomass, cumulative C and N mineralization than all other treatments. However, the addition of "Roundup WeatherMax" reduced C mineralization in soils treated with the pesticides fluometuron, aldicarb, or mefenoxam + pentachloronitrobenzene formulations. The authors concluded that glyphosatebased herbicides might alter the soil microbial response to other pesticides.
Therefore, like stated in Lupwayi et al. (2004), community shifts might have long-term effects on soil biological processes and the relevance of microbial diversity and composition is of importance when assessing the ecosystem soil.

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B.9.8.3 Risk assessment

The risk assessment follows the EU Guidance Document on Terrestrial Ecotoxicology (SANCO 10329/2002).

Table B.9.8-8: Effects of glyphosate acid, its metabolite and the product MON 52276 on soil micro-organisms

Species	Substance	System	Results	Reference
Microflora	Glyphosate acid	Carbon-mineralisation 28-day study	No significant effects (>25%) on carbon mineralisation by day 28 at 2.4 and 4.8 kg/ha; NOEC= 4.8 kg/ha (6.4 mg/kg dry soil) Results for nitrogen mineralisation are invalid	Franco Perina, 2000, BVL no 2311047 /RF-D1. 113/99
Microflora	Glyphosate acid	Nitrogen-mineralisation 28-day study	No significant effects (> 25 %) on N transformation by day 28 at concentrations of up to 33.1 mg a.e./kg dry soil	Hutcheson, K. 2014 2716673/ Report No: CEMR-6237 OECD 216
Microflora	Glyphosate acid	Nitrogen- and Carbon-mineralisation 28 day study	No effects up to 18 kg as/ha (28.8 mg glyphosate acid/kg dry soi)l	EU Review Report glyphosate; 6511/VI/99 final, 2002
Microflora	AMPA	Nitrogen- and Carbon-mineralisation 28/56-day study	No significant effects (> 25 %) on C &N transformation by day 28 at concentrations of up to 160 mg/kg dry soil NOEC= 120 kg/ha (160 mg/kg dry soil)	Schulz, 2010 BVL no 2311050 /10 10 48 010 C/N
Microflora	MON 52276	Nitrogen- and Carbon-mineralisation 28-day study	No significant effects (> 25 %) on dehydrogenase activity and nitrogen mineralisation by day 28 at 18.8 and 94 mg MON 57726/kg dry soil (12 and 60 L MON 52276/ha) in loamy sand NOEC=12 and 60 L MON 52276/ha , corresponding to 5.7 and 29.1 mg acid equivalents kg dry soil	Hutcheson, 2011 BVL no 2316013 /CEMR-5259

The influence of the metabolite AMPA and the representative formulation MON 52276 on carbon- and nitrogen conversion rate is < 25 % (Trigger of SETAC-guideline) in comparision with untreated soil. For the active ingredient glyphosate effects on the nitrogen cycle test could not be assessed due to an invalid study according to OECD guideline 216.

MON 52276 is the representative formulation in the current EU review of glyphosate. MON 52276 caused no effects >25 % on soil microflora respiration and soil nitrogen transformation processes.

Maximum PECsoil values were provided in Section 8. As demonstrated by the comparison of PECsoil values and no-effect concentrations, no potential for adverse effects was identified for glyphosate, the representative formulation MON 52276 and the metabolite AMPA.

Table B.9.8-9: Assessment of the risk for soil micro-organisms exposed to glyphosate acid, its metabolite and the product MON 52276

Substance	Indication	Intended use	Application rate interception (g/ha) (%)	PECact (mg/kg)	PECaccu (mg/kg)*	Concent-ration effects (mg/kg)	MoS
AMPA	worst case	1 x every year annual crops	1527	2.0360	3.0719	160	52
		1 x every year permanent crops	1527	2.0360	6.1797		26
glyphosate	worst case	all crops annual	1 x 4320 (0%)	5.7600	5.974	6.4	1.1
		all crops, permanent	1 x 4320 (0%)	5.7600	6.6162		1.0
	001	all crops,annual	2 x 2160 (0%)	4.7514	4.9572		1.3
		all crops, permanent	2 x 2160 (0%)	4.7514	5.5746		1.1
	002	all crop, annual	1 x 1080 (0%)	1.4400	1.4935		4.3
		all crop, permanent	1 x 1080 (0%)	1.4400	1.6538		3.9
	003, 004	cereals	1 x 2160 (90%)	0.2880	0.2987		21.4
	005	oil seed rape	1 x 2160 (80%)	0.5760	0.5974		10.7
	006	orchard crop, vines, citrus&tree nuts	3 x 2880 (0%) 33% of area treated	2.5490	3.0648		2.1
	007	orchard crop, vines, citrus&tree nuts	3 x 2880 (0%) 50% of area treated	3.8235	4.5973		1.4
MON 52276	worst case	all crops annual	1 x 4320 (0%)	5.7600	5.974	29.1	4.9
		all crops, permanent	1 x 4320 (0%)	5.7600	6.6162		4.4
	001	all crops,annual	2 x 2160 (0%)	4.7514	4.9572		5.9
		all crops, permanent	2 x 2160 (0%)	4.7514	5.5746		5.2
	002	all crop, annual	1 x 1080 (0%)	1.4400	1.4935		19.5
		all crop, permanent	1 x 1080 (0%)	1.4400	1.6538		17.6
	003, 004	cereals	1 x 2160 (90%)	0.2880	0.2987		97.4
	005	oil seed rape	1 x 2160 (80%)	0.5760	0.5974		48.7
	006	orchard crop, vines, citrus&tree nuts	3 x 2880 (0%) 33% of area treated	2.5490	3.0648		9.5
	007	orchard crop, vines, citrus&tree nuts	3 x 2880 (0%) 50% of area treated	3.8235	4.5973		6.3

Based on laboratory testing with MON 52276, the Annex VI trigger value of > 25 % effects after 28 days was not exceeded at concentrations of 1× and 5× the maximum recommended

annual use rate for 4.32 kg a.s./ha. Therefore, the use of MON 52276 according to the proposed use pattern can be considered not to result in any unacceptable adverse effects for soil micro-organisms.

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (Annex IIA 8.6)

B.9.9.1 Effects on plants

In the first EU peer review evaluation of glyphosate in 2001, no risk assessment for terrestrial plant was performed, but studies on seed germination and seedling emergence and one study on vegetative vigour were evaluated. For studies for which no amendment of the initial evaluation was required, please refer to the initial monograph and its addenda.

In a non-GLP study, Bohn (1987, ARW 97-00101) exposed seven dicotyledonous and four monocotyledonous weed species to 5.7 and 11.2 kg a.s./ha and observed an inhibition of 17% for Ipomoea sp. (morning glory) and *Polygonum pensylvanicum* (Pennsylvania smartweed), whereas for most species no inhibition was observed. A test on vegetative vigor by Chetram and Lucash (1994, BVL no 2311054) exposed 10 different crops to glyphosate at application rates at up to 5.1 kg a.s./ha according to US EPA guidelines. All plant species showed differences compared to control in plant height and plant dry weight. The lowest (worst case) 21 day ER50 values of glyphosate acid were observed for tomato plants and were calculated to be 0.1457 kg a.s./ha for dry weight. This endpoint was used for risk assessment of non-target plants so far. Additionally, a study investigating effects of spray drift on native British plant species at several distances downwind was cited (Marrs et al. 1989; ARW 97-00100). Most sensitive species were *Digitalis purpurea*, *Centaurea nigra*, *Prunella vulgaris* and *Lychnis flos-cuculi*. The authors concluded that, when spraying with ground sprayers, buffer zones around nature reserves should be 5-10 m.

New studies on seedling emergence of terrestrial non-target plants have been carried out with glyphosate acid (Everett, C.J et al., 1996, BVL no 2311057), studies on effects of glyphosate acid (wettable powder formulation) in vegetative vigour tests (Harnish, W.N., 1994, BVL no 2311051; Everett, C.J et al., 1996, BVL no 2311055) and the lead formulation MON 52276 (Collings L. et al., 2005 BVL no 2311060).

A summary of the endpoints of the most sensitive species is presented in the following table whilst full details of these studies are provided below.

Table B.9.9-1: Effects of glyphosate and of plant protection products containing glyphosate on non-target plants

Species	Substance	System	ER_{50} g a.s./ha	Reference
Cyperus rotundus *Avena sativa,* *Triticum aestivum,* *Zea mays* *Allium cepa* *Beta vulgaris* *Lactuca sativa* *Brassica napus* *Cucumis sativa* *Glycine max* *Abelmoschus esculentus* *Rheum rhoponticum*	Glyphosate acid (formulated product, WP)	28 d Seedling emergence	> 4480 (seedling emergence, seedling dry weight) (not valid)	Everett *et al.*, 1996, RJ2008B BVL no 2311057
Solanum lycopersicum *Glycine max* *Lactuca sativa* *Raphanus sativus* *Cucmis sativus* *Brassica oleracea* *Avena sativa* *Lolium perenne* *Zea mays* *Allium cepa*	Glyphosate acid	21 d vegetative vigour	**146 (dry weight)**	Chetram, Lucash, 1994; MSL-13320 BVL no 2311054
Raphanus sativa *Glycine max* *Cucumis sativus* *Helianthus annus* *Lycopersicon esculentum* *Daucus carota* *Zea mays* *Triticum aestivum* *Avena sativa* *Allium cepa*	Glyphosate acid	21 d vegetative vigour	248.8 (survival) (not valid)	Harnish, 1994 BVL no 2311051
Cyperus rotundus *Avena sativa* *Triticum etsivum* *Zea mays* *Beta vulgarisLactuca sativaRaphanus sativus* ***Brassica napus*** *Cucumis sativus* *Glycine max* *Abelmoschus esculentus*	Glyphosate acid (wettable powder WP formulation)	28 d vegetative vigour	149 (visual damage) 150 (dry weight)	Everett *et al.*, 1996; RJ2009B BVL no 2311055
Beta vulgaris *Raphanus rapistrum* *Lepidium sativum* *Pisum sativum* *Lolium perenne* *Triticum aestivum*	MON 52276	22 d vegetative vigour	Not considered to be valid	Collings, L., Blake, N., 2005; CEA.104 ! BX-0928 BVL no 2311060
Zea mays *Avena sativa* *Allium cepa* *Triticum aestivum* *Cucumis sativus* *Brassica napus* *Raphanus sativus* *Glycine max* *Helianthus annuus* *Lycopersicon esulentum*	MON 52276	21 d vegetative vigour	**28.4 g a.e./ha. Valid with uncertainties**	Bergfield, A., 2014, ABC Study No. 80477 BVL no 2716933

B.9.9.1.1 Seedling emergence

KIIA 8.12./01

Author:	Everett, C.J., Fleming,T.M., Cole, J.F.H.
Title:	Glyphosate acid: A Tier 2 greenhouse study to assess the effects on seedling emergence of terrestrial non-target plants
Date:	19.09.1996
Doc ID:	2311057 /RJ2008B
Guidelines:	OECD/ EPA Guidelines, Subdivision J, Series 123-1 (a)
GLP:	Yes
Validity:	The validity of the present study according to OECD guideline 208 is not given, as no data on visible phytotoxic effects and the mean survival of emerged seedlings for the duration of the study in control group were reported.

Materials and Methods

Test item::	Glyphosate acid (formulated product)
Description:	Wettable powder (WP) formulation WF 2380
Lot/Batch #:	F12
Purity:	50 % a.s (nominal), 48.3 % a.s. (measured)
Vehicle and/or positive control:	None
Species:	7 Dicotyledons: (sugar beet [5], lettuce [6], oilseed rape [1], cucumber [4], soybean [7], okra [4] and rhubarb [4]) 5 Monocotyledons: (purple nutsedge [1], oat [2], winter wheat [1], maize [3], and onion [4])
Source:	1. Zeneca Agrochemicals, Jealott's Hill Research Station; 2. Cargill plc, Blandford Forum, Dorset DT11 7EG; 3. Zeneca seeds, Slater, Iowa, USA; 4. E W King, Kelvedon, Colchester, Essex, CO5 9PG; 5. Maribo UK, Potter Handworth, Lincoln Lincolnshire 6. J W Moles & Son, Stanway, Colchester, Essex, CO3 5PD 7. Zeneca ag, products, Wester Research, Centre, Richmond, California, USA.
Temperature:	Test 1: 10-42 °C: Oats, winter wheat, sugar beet, lettuce, radish, oilseed rape, onion and rhubarb (cool); 9 – 45.5 °C: purple nutsedge, maize, cucumber, soybean and okra (warm) Test 2: 12.0 – 33.5 °C (cool); 13.0 – 39.5 °C (warm);
Relative humidity:	Test 1: 6 – 90 % (cool), 3 – 100 % (warm) Test 2: 12 – 94 % (cool), 4 – 94 % (warm)
Photoperiod:	16 hours light / 8 hours dark Test 1: 96 – 6483 klux (cool), 71 – 669 klux (warm) Test 2: 68 – 394 klux (cool); 56 – 524 klux (warm);
Soil pH:	6.4 (test 1); 6.5 (test 2)
Soil organic matter content:	1.0 (test 1), 0.7 (test 2)
Methods:	Ten seeds per replicate per species were sown into disposable plastic seed trays (11 cm x 15 cm x 7 cm, with base holes) containing equal-sized compartments and filled to top (7 cm depth) with SC compost (0.7 – 1.0% organic matter). For each of the twelve species, three replicate trays were prepared for each treatment rate and two controls. After one day, seed trays were treated with 6 nominal test concentrations, 0.140, 0.280, 0.560, 1.12, 2.24 and 4.48 kg a.s./ha. In addition, two negative control groups (untreated test trays) were tested. Each test concentration was applied using a hydraulic track sprayer fitted with a single, even brass 8002 EVB Teejet. The trays were left to

	dry for 3 hours before being transferred to a glasshouse and watered. Because of a high control mortality for okra, soybean and lettuce during the first test (test 1) caused by extreme temperature conditions, a second test (test2) was carried out with these species. Seedling emergence was assessed three times a week by recording the number of seeds emerged per replicate. Plant development was observed 14 and 27 days after application (DAA) in test 1 and 14 and 28 DAA in test 2. Each replicate was assigned a single overall score at each assessment. Non-emergence was included in the overall rating as a percentage of decrease compared to controls. Observation of phytotoxic symptoms was also recorded. Control replicates were given a zero score and treated replicate were assessed relative to the control. The plants were harvested at 28 DAA by cutting the stem at soil level. Dry weight was assessed as g/ replicate for each species by drying in an oven at approximately 70 °C starting at the day of harvest until a constant weight was observed. Data were analysed using a t-test (α = 0.05), after an arcsine transformation of the data (for emergence only). No discernible dose response was detected and therefore the estimation of ERx values was not considered for both emergence and dry weight.

Results

The validity of the present study according to OECD guideline 208 is questionable, as no data on visible phytotoxic effects and the mean survival of emerged seedlings for the duration of the study in control group were reported. Generally these validity criteria ensure that optimal conditions can lead to treatment related effects.

Table B.9.9-2: Mean percent damage assessment of glyphosate acid (formulated product) treatment on seedling emergence after 27/28 days

Crop	**Rate (kg a.s./ha)**						
	Control	**0.140**	**0.280**	**0.560**	**1.12**	**2.24**	**4.48**
Purple nutsedge	-	5.0	3.3	1.7	11.7	6.7	1.7
Oat	-	5.0	8.3	10.0	0	1.7	3.3
Winter wheat	-	10.0	0	0	0	1.7	0
Maize	-	1.7	0	0	5.0	8.3	0
Onion	-	0	0	0	8.3	3.3	8.3
Sugar beet	-	3.3	3.3	3.3	3.3	0	10.0
Lettuce	-	1.7	13.3	13.3	13.3	5.0	3.3
Oilseed rape	-	13.3	13.3	10.0	10.0	0	11.7
Cucumber	-	3.3	0	6.7	0	6.7	1.7
Soybean	-	3.3	3.3	1.7	6.7	3.3	3.3
Okra	-	5.0	16.7	6.7	3.3	10.0	6.7
Rhubarb	-	5.0	5.0	0	3.3	6.7	15.0

Visual differences from the controls were evident for all species, this being generally attributable to stem decrease. However, there was no consistent dose response pattern and therefore effects were not considered be treatment-related. Mortality was observed at treated lettuce and okra plants. This mortality was not considered to be treatment-related as it generally occurred after full emergence.

With regard to the observed symtomology, it should also be noted that the test conditions recommended in the OECD 208 could not be maintained in the greenhouse (temperature

deviations reaching from 9 °C to 45.5 °C, relative humidity 3-100% and light intensitiy up to 669klux).
There was no significant dose-related reduction of mean dry weight. For purple nutsedge, three dose concentrations resulted in significant differences from the controls (0.280, 0.560 and 2.24 kg a.s./ha). The highest, third highest and lowest rates were however not significantly different from the controls and therefore no endpoint could be determined.

For all other species NOER were greater than the maximum rate (> 4.48 kg a.s/ha). Analysis of all species produced no dose repsonse and therfore EC values could not be determined.

RMS Conclusions
The ER_{50} of glyphosate acid was determined by the authors of the study to be > 4.48 kg a.s/ha respectively for both seedling emergence and seedling dry weight of oat, winter wheat, maize, onion, sugar beet, lettuce, oilseed rape, cucumber, soybean, okra and rhubarb.
RMS deems the study not to be valid. The validity of the present study according to OECD guideline 208 is not given, as no data on visible phytotoxic effects and the mean survival of emerged seedlings for the duration of the study in control group were reported. In control plants, stem elongation was decreased, probably due to high light intensity. With regard to the observed symtomology, it should be noted that the test conditions recommended in the OECD 208 could not be maintained in the greenhouse (temperature deviations reaching from 9 °C to 45.5 °C, relative humidity 3-100% and light intensitiy up to 669klux). Generally, the validity criteria concerning integrity of control plants ensure that optimal conditions ensure the detection of possible treatment related effects.

B.9.9.1.2 Vegetative vigour

KIIA 8.12./02

Author:	Harnish, W.N.
Title:	LX1146-02 (Glyphosate techn.) Tier II Non-target plant hazard evaluation - Terrestrial vegetative vigor
Date:	22.07.1994
Doc ID:	2311051 /236 GLY
Guidelines:	OECD/ EPA Guidelines, Subdivision J, Series 123-1 (b)
GLP:	Yes
Validity:	The validity criteria according to the OECD 227 (the seedling emergence is at least 70 %) can not be examined, as no data on seedling emergence in control group were reported.

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	206-JAK-119-1
Purity:	98.5 % (technical)
Control:	None
Species:	6 Dicotyledons: (carrot[1], cucumber[1], radish[1], soybean[3], sunflower[2], tomato[1]) 4 Monocotyledons: (field corn[2], oat [2], onion[1], wheat[2])

Source:	1. Burpee Seed, Warminster, PA. 2. Farmers supply, Co., Valdosta, GA 3. Pineland Plantation, Newton; GA
Temperature:	Approx. 12.2 °C – 37.8 °C
Relative humidity:	70 % - 94 %
Photoperiod:	10 h light / 14 h dark, approx. 5443– 42531 Lux
Soil pH:	5.5 - 5.6
Soil organic matter content:	0.94 - 1.5 %
Methods:	Seedlings were grown in plastic pots (approx.15 cm round) containing approximately 1 kg of pasteurised sandy soil. Small seeds (carrot, onion, radish and tomato) were planted at a depth of 0.5 to 1 cm and large seeds (field corn, wheat, oat, cucumber, sunflower and soybean) were planted at a depth of 1 to 1.5 cm. Soybean seeds were inoculated with commercial *Rhizobium japonicum*. Four replicate pots for each treatment were prepared for each species tested. At least 7 days prior to application, seedlings were grown to 1-3 true leaves and then thinned to five plants per replicate and their height recorded. The plants were treated with 5 nominal concentrations (adjusted to test item purity), encompassing 0.0056, 0.0112, 0.0235, 0.0471 and 0.0930 kg glyphosate acid/ha. One negative control group (treated with deionized water) was tested. Application was performed using a single nozzle hand-held CO2 pressurized sprayer, starting with the water control. Plants were not watered during the first 24-hour period to avoid wetting the plants foliage and dislodging spray residue. Because of poor rate response in most crops, a test continuation was initiated at five additional concentration rates, encompassing 0.0930, 0.1861, 0.3721, 0.5582 and 0.7442 kg glyphosate acid/ha. Seedling survival and plant height were recorded 7 days before treatment (DBT), on the day of treatment, 14 days after treatment (DAT) and 21 DAT. For dry weight measurements, plants were harvested 21 DAT and dried for a minimum of 24 h at approximately 100°C. Plant survival observations were recorded 7 DAT (6 DAT for the continuation test), 14 DAT (13 DAT for the continuation test) and 21 DAT. Phytotoxicity was evaluated 7, 14 and 21 DAT for initial test and 6, 13 and 21 DAT for the continuation test. Data were analysed using two-way ANOVA and an LSD test was performed as post-hoc. The actual ERx values were estimated by regression analysis using Lotus 1,2,3 Software.

Results

Plants were significantly affected by glyphosate treatments in all species tested. Among the monocotyledonous species, oat was most tolerant to glyphosate while all other species exhibited approximately the same level of sensitivity to glyphosate. Among the dicotyledonous species, sunflower and radish were the most sensitive species, whilst tomato, carrot and soybean exhibited lower sensitivity to glyphosate. For phytotoxicity, onion was the most tolerant monocot while other monocots tested showed approximately the same level of sensitivity to glyphosate.

Visual phytotoxicity was generally expressed within 13 days after the treatment and did not substantially increase by 21 days. Onion exhibited tip burn (necrosis at the leaf tip and margins) at 0.7442 kg a.s./ha, but no visual phytotoxicity at any of the lower rates. Oat exhibited visual phytotoxicity at a rate of 0.3721 kg a.s./ha, whereas wheat and field corn showed signs of visual phytotoxicity at rates as lower as 0.1861 kg a.s./ha. Glyphosate caused multiple shoots to develop at the soil line; higher application rates caused necrosis at the leaf tips.

For dicots, visual phytotoxicity occurred within 13 DAT and did no increase significantly by 21 days. When comparing the 21-day data, carrot was the most tolerant dicot with a NOER of 0.3721 kg glyphosate acid/ha and exhibited no phytotoxicity at rates below 0.5582 kg a.s./ha.

The injury observed was chlorosis and stunting for carrot. With the exception of soybean (NOER = 0.1861 kg glyphosate acid/ha), the NOER for dicots was 0.0930 kg glyphosate acid/ha. For radish and sunflower, mortality was observed at the two highest rates tested and significant treatment effects were also noted in plant height and dry weight.

Table B.9.9-3: ER_{50} and NOER values for glyphosate acid on survival, dry weight and plant height

Crop	Endpoint (kg glyphosate acid/ha)					
	Survival		Dry weight		Plant height	
	ER_{50}	NOER	ER_{50}	NOER	ER_{50}	NOER
Onion	> 0.7442	0.7442	n.d.	0.0930	> 0.7442	0.0930
Field corn	> 0.7442	0.7442	0.6400	0.0930	> 0.7442	0.0930
Oat	> 0.7442	0.7442	>0.7442	0.7442	>0.7442	0.7442
Wheat	> 0.7442	0.7442	0.6478	0.0930	>0.7442	0.0930
Soybean	> 0.7442	0.7442	0.6758	0.1861	0.6590	0.1861
Radish	0.2488	0.0930	0.2623	0.0930	0.6904	0.0930
Cucumber	> 0.7442	0.7442	> 0.7442	0.7442	> 0.7442	0.1861
Sunflower	**0.3508**	0.1861	**0.2959**	0.0930	**0.2993**	0.0930
Tomato	> 0.7442	0.7442	0.5335	0.1861	> 0.7442	0.0930
Carrot	> 0.7442	0.7442	0.6512	0.3721	> 0.7442	0.1861

RMS Conclusions

The study is not acceptable. The validity criteria according to the OECD 227 could not be examined, as no data on seedling emergence in control group were reported. The lowest 21 day ER_{50} values of glyphosate were determined for sunflower and were calculated to be 0.3508, 0.2959 and 0.2993 kg glyphosate acid/ha for survival, dry weight and plant height. The lowest 21-day NOER value was observed for plant height and visual phytotoxicity and determined to be 0.0930 kg glyphosate acid/ha.

KIIA 8.12./03

Author:	Chetram, R.S., Lucash, K.J.
Title:	Tier 2 Vegetative vigor nontarget phytotoxicity study using Glyphosate
Date:	4.01.1994
Doc ID:	2311054 /MSL-13320
Guidelines:	OECD
GLP:	YES
Validity:	YES

Materials and Methods

Test item::	Glyphosate acid (N-phosphonomethylglycine)
Lot/Batch #:	RUD-9302-4778-T (technical); RUD-9203-3961-A (analytical standard)
Purity:	96.6 % (technical); 99.8% (analytical standard)
Control:	Deionized water containing a non-ionic surfactant

Species:	6 Dicotyledons: (soybean[1], lettuce[2], cabbage[3], cucumber[4] radish[3] tomato[3]) 4 Monocotyledons: (oat[5], Ryegrass[6], corn [3],onion[3])
Source:	1. Azlin Seed Co.; 2. Germain's Seed Co.; 3. Burpee Seed Co.; 4. Carolina Seed Co. 5. Northrup King, 6. Omni Seed Co.
Temperature:	19 °C – 44 °C(base test); 17 °C – 40 °C (test continuation)
Relative humidity:	40 % - 90 %(base test); 37 % - 90 (test continuation)
Photoperiod:	Approx. 14 h light/ 10 h dark at. 38212– 45639 Lux (base test) Approx. 13 h light/ 11 h dark 24541– 19052 Lux (test continuation)
Soil pH:	7.9
Organic matter content:	1.1 %
Methods:	Seedlings were grown in plastic pots (approx.10 cm x 10 cm x 7.6 cm) filled with soil/perlite mixture. Soybean, cucumber, oat and corn were planted at 2.5 cm while the remaining six crops were planted at a depth of 1.3 cm in four replicates. Seedlings were grown to 1-3 true leaves and thinned to five plants of uniform height per pot. Glyphosate was diluted in water containing two drops of Triton surfactant per 250 mL of water (to represent a realistic spray concentration of surfactant) and applied once with an overhead flat-fan nozzle at approximately 378 L/ha (40 gallons/acre).The plants were treated with seven nominal concentrations, (0.0785, 0.1569, 0.3138, 0.6276, 1.2329, 2.5778 and 5.0436 kg a.s./ha). One negative control group (treated with deionized water) was tested. All applications of glyphosate were performed indoors with a spray booth equipped with a single Teejet 8001-E nozzle and a compressed air cylinder. After treatment plants were kept in a greenhouse at 17 – 44 °C and 37 – 90 % relative humidity under natural light supplemented with artificial lighting for 18 h per day. During the first 48 hours after treatment, pots were hand watered to prevent the test item from being washed off. As a no-observable effect concentration level was not reached for radish and tomato, a test continuation was initiated for both species using six nominal concentrations, encompassing 0.0049, 0.0099, 0.0202, 0.0392 and 0.0785 kg a.s./ha. Plant height was recorded prior to treatment and 21 days after treatment. Phytotoxicity ratings were recorded 7, 14, and 21 days after treatment. 21 days after treatment, surviving plants were cut at soil level and dry weight was recorded. Prior to application, samples (10 mL) of each test solution were collected and analysed immediately by HPLC method to verify the concentrations of the test item in the test solutions. Analysis of variance, followed by a one-tailed Dunnett's multiple comparison test were used for data analysis. The ERx values were determined using regression analysis (TableCurve™ Curve Fitting Software).

Results

Visual phytotoxicity, plant height and plant dry weight of all crops were significant affected by glyphosate treatments. Except for soybean and onion, a significant effect on mortality was observed for all species exposed to glyphosate.

HPLC analysis showed an average recovery of glyphosate ranging from 100 % to 107 % and 105 % to 110 % of the nominal test concentrations for the first test and the test extension, respectively. Ecotoxicological endpoints were evaluated using nominal concentrations of the test item. The validity criteria according to the OECD 227 were fulfilled.

RMS Conclusions

The lowest 21 day ER_{50} values of glyphosate acid were observed for tomato plants and were calculated to be 0.1457 kg a.s./ha for plant dry weight. This endpoint has been determined to be relevant for risk assessment so far.

The validity criteria according to the OECD 227 are fulfilled. The lowest 21 day ER_{50} values of glyphosate acid were observed for tomato plants and were calculated to be 0.5156, 0.3362 and 0.1457 kg a.s./ha for survival, plant height and dry weight, respectively. The lowest 21-

day NOER values were determined to be 0.0785 kg a.s./ha (tomato and radish), 0.3138 kg a.s./ha (tomato and radish), 0.0392 kg a.s./ha (tomato and radish), and 0.0392 kg a.s./ha (tomato) respectively for visual phytotoxicity, survival, dry weight and plant height.

Table B.9.9-4: ER_{50} and NOER values for non target plants exposed to glyphosate acid. Effects on plant survival, dry weight and plant height

Crop	Endpoint (kg a.s./ha) at Day 21					
	Survival		Plant height		Plant dry weight	
	NOER	ER_{50}	NOER	ER50	NOER	ER_{50}
Ryegrass	1.232	4.592	0.627	2.352	0.627	1.344
Corn	0.627	1.680	0.627	0.918	0.627	0.750
Onion	5.040	> 5.040	0.627	> 5.040	0.627	1.792
Oat	2.576	> 5.040	0.627	1.344	0.157	0.874
Soybean	5.040	> 5.040	0.627	1.568	0.314	0.974
Lettuce	1.232	2.800	0.627	1.344	0.314	0.762
Cucumber	2.576	4.032	0.314	1.456	0.314	0.896
Cabbage	1.232	4.592	0.627	1.456	0.157	0.739
Radish	0.314	0.918	0.078	0.358	0.039	0.246
Tomato	0.314	0.515	0.039	0.336	0.039	**0.146**

KIIA 8.12./04

Author:	Everett, C.J., Fleming,T.M., Cole, J.F.H.
Title:	Glyphosate acid: A Tier 2 greenhouse study to the effects on vegetative vigour of terrestrial n-target plants.
Date:	16.09.1996
Doc ID:	2311055 /RJ2009B
Guidelines:	OECD/ EPA Guidelines, Subdivision J, Series 123-1 (a)
GLP:	Yes
Validity:	The validity of the present study according to OECD guideline 227 is questionable, as no data on seedling emergence and plant survival in control group were reported. Nevertheless, data from damage assessment show that plant number in replicates were not reduced and not impaired. Therfore the study is considered to be acceptable.

Materials and Methods

Test item::	Glyphosate acid (formulated product)
Description:	Wettable powder (WP) formulation
Lot/Batch #:	F12
Purity:	50 % a.s (nominal), 48.3 % a.s. (measured)
Positive control:	None

Species:	7 Dicotyledons: (sugarbeet[4], lettuce[5], radish[6], oilseed rape[1], cucumber[6], soybean[7], okra[6]) 4 Monocotyledons: (purple nutsedge[1], oat[2], winter wheat[1],, maize[3])
Source:	1. Zeneca Agrochemicals, Jealott's Hill Research Station; 2. Cargill plc, Blandford Forum, Dorset DT11 7EG; 3. Zeneca seeds, Slater, Iowa, USA; 4. Maribo UK, Potter Handworth, Lincoln Lincolnshire; 5. J W Moles & Son, Stanway, Colchester, Essex, CO3 5PD; 6. E W King, Kelvedon, Colchester, Essex, CO5 9PG: 7. Zeneca AG, products, Wester Research, Centre, Richmond, California, USA.
Temperature:	Test 1: 10-42 °C: Oats, winter wheat, sugar beet, lettuce, radish, and oilseed rape (cool) 9 – 45.5 °C: purple nutsedge, maize, cucumber, soybean and okra (warm) Test 2: 8.0 – 27.0 °C: radish, and oilseed rape (cool)
Relative humidity:	Test 1: 6 – 86 % (cool), 3 – 100% (warm); Test 2: 24 – 80% (cool)
Photoperiod:	16 hours light / 8 hours dark Test 1: 80 – 651 klux (cool), 77 – 606 klux (warm) Test 2: 67 – 199 klux (cool)
Soil pH:	5.4 – 6.4 (test 1); 6.7 (test 2)
Soil organic matter content:	1.0 - 1.1 % (test 1), 1.0 % (test 2)
Methods:	Seedlings were grown (in 10 cm- diameter plastic pots containing 9 cm depth of compost) to the 1-3 true leaf stage from untreated seed in SC compost (1.0 - 1.1% organic matter, pH 5.4 – 6.7) in a glasshouse. Applications of the formulated product were made using a hydraulic track sprayer fitted with a single even brass 8002 EVB Teejet. The plants were treated with six concentrations, 0.00615, 0.0184, 0.0553, 0.166, 0.498 and 1.49 kg a.s./ha. Two negative control groups (untreated plants) were tested. Each test concentration was applied in three replicates containing five plants. After spraying, plants were left to dry for at least an hour before being transferred to a glasshouse and watered. Due to a localised aphid infestation of radish and oilseed rape during the test (test 1), a second test (test2) was carried out. Plant damage and visual phytotoxicity was observed 6, 13, 21 and 27 days after application (DAA) in test 1 and 7, 14, and 28 DAA in test 2. Each plant was observed individually and compared to a representative healthy control plant of the same replicate and species. Assessments were carried out using a percentage damage scale. For plant dry weight, plants were harvested at 28 DAA by cutting the stem at soil level. For each replicate, all plants were pooled together. Dry weight was assessed as g/replicate for each species by drying from the day of harvest until a constant weight was observed. Dose response relationship for dry weight was estimated by non-linear logistic regression. The ERx values were calculated as reduction from the observed control mean and Fieller's Theorem was used calculate their respective 95% confidence limits.

Results

All species except purple nutsedge were severely affected (60 – 100%) at the two highest treatment rates. Purple nutsedge was affected at the highest rate only. Predominant visual phytotoxicity symptoms were overall stunt, leaf necrosis, leaf chlorosis and senescence.

The dry weight of all species except purple nutsedge was significantly affected ($\alpha = 0.05$) at the two highest test rates. Purple nutsedge was affected only at the highest test item treatment rate. Weight of winter wheat, lettuce, oilseed rape and okra was also significantly reduced at the third highest test item treatment rate (0.166 kg a.s./ha) and for soybean at the test item treatment rate of 0.0553 kg a.s./ha. Dry weight of winter oats was significantly reduced at all rates tested, except at 0.0553 kg a.s./ha.

Table B.9.9-5: ER_{25} and ER_{50} values for plants exposed to glyphosate acid. Data on visual phytotoxicity and plant dry weight

Crop	Endpoint (kg a.s./ha)			
	Damage assessment		Plant dry weight	
	ER_{25}	ER_{50}	ER_{25}	ER_{50}
Purple nutsedge (*Cyperus rotundus*)	0.891	1.293	0.851	1.253
Oat (*Avena sativa*)	0.274	0.415	0.281	0.376
Winter wheat (*Triticum aestivum*)	0.270	0.390	0.178	0.242
Maize (*Zea mays*)	0.268	0.386	0.397	0.423
Sugar beet (*Beta vulgaris*)	0.254	0.416	0.199	0.377
Lettuce (*Lactuca sativa*)	0.307	0.466	0.254	0.402
Radish (*Raphanus sativus*)	0.228	0.417	0.488	1.078
Oilseed rape (*Brassica napus*)	0.083	**0.149**	0.106	0.150
Cucumber (*Cucumis sativa*)	0.138	0.254	0.205	0.359
Soybean (*Glycine max)*	0.190	0.415	0.174	0.358
Okra (*Abelmoschus esculentus*)	0.256	0.507	0.205	0.346

RMS Conclusions

The validity of the present study according to OECD guideline 227 is questionable, as no data on seedling emergence and plant survival in control group were reported. Nevertheless, data from damage assessment show that plant number in replicates were not reduced and not impaired. Therefore the study is considered to be acceptable. Moreover the study represents the sole data source for endpoints, which are determined at 28d after treatment. The worst case ER_{50} values of glyphosate were observed for oilseed rape and were calculated to be 0.149 kg glyphosate acid/ha and 0.150 kg glyphosate acid/ha, respectively for visual damage assessment and plant dry weight.

KIIA 8.12./05

Author:	Collings, L., Blake, N.
Title:	Evaluation of the toxicity of Glyphosate and Paraquat to terrestrial non-target plants
Date:	19.09.2005
Doc ID:	2311060 /CEA.104 ! BX-0928
Guidelines:	OECD 208B (draft, 2000)
GLP:	NO
Validity:	NO

Materials and Methods

Test item::	MON 52276 (360 g a.s./L EC)
Lot/Batch #:	not available
Purity:	not stated
Positive control:	None

Species:	4 Dicotyledons: (*Beta vulgaris*, *Raphanus rapistrum*, *Lepidium sativum*, *Pisum sativum*) 2 Monocotyledons: (*Lolium perenne*, *Triticum aestivum*)
Source:	Herbiseed (Berkshire, U.K.)
Temperature:	18 °C/12 °C day/night
Relative humidity:	not specified, substrates were watered daily
Photoperiod:	14 h light/ 10 h dark
Soil pH, Soil organic matter content	No data
Methods:	Seedlings were grown in pots filled with sterilised Kettering loam and Derby Quartz (mixture loam and grit: 5:1). For sugar beet, rape, garden cress and pea, 6 seeds and for ryegrass and winter wheat, 12 seeds were planted per pot at a depth of 1 cm. Once a day or as required to ensure the soil surface remains moist, pots were watered with an automatic overhead boom pre-spray. Following seedling emergence, plants were thinned by removing half of the seedlings. Each treatment/crop combination was replicated four times. Prior to treatment, seedlings were grown to at least 2-4 true leaves. MON 52276 was applied indoors with a Mardrive pot sprayer at 225 L/ha at a height of 50 cm and at a pressure of 2.2 bar. The plants were treated with seven nominal concentrations, encompassing 0.00004, 0.0004, 0.004, 0.04, 0.4, 2.0 and 4.0 L prod/ha. One negative control group was tested. After treatment plants were kept in a greenhouse at 12 – 18°C h per day. Phytotoxicity ratings, according to a nine point scoring system were recorded for the first 4 days and at approximately 7, 15 and 22 days after treatment. All plots were harvested 22 days after treatment to determine fresh shoot weight. The weight of plants in one pot was combined. Data for the No Observed Effect Rates (NOER) were analysed using one-way ANOVA and Dunnett's t-test was performed as post-hoc. The highest concentration not significantly different from the control was identified as the NOER. ER_{50} values were calculated in a separate report (Levine, S.L. & Orr, T.B., 2011, MSL0024009 IIIA 17.1/01). EC_{50} values and 95% confidence intervals were calculated from mean fresh shoot weights for each glyphosate treatment obtained 20 to 22 days after post-emergent application, using a standard 3-parameter logistic model with the software package GraphPad Prism version 5.04 (GraphPad Software, Inc.).

Results

Phytotoxicity ratings were recorded after 22 days after treatment The highest concentration not significantly different from the control was identified as the NOER.

Among dicotyledonous species, Garden cress (*Lepidum sativum*) was the most sensitive species from day 2 after application of MON 52276 until the end of the study (NOER = 0.04 L/ha equivalent to 14.4 g a.s./ha). Sugar beet (*Beta vulgaris*) and rape (*Raphanus rapistrum*) responded quickly to and exhibited significant effects on vegetative vigour one day after application at an application rate of of 720 g a.s./ha (equivalent to 2.0 L/ha, 50 % field application rate). NOER values of 14.4 g a.s./ha (equivalent to 0.04 L prod/ha, 10 % field appliacation rate) after 22 days was 0.04 L prod/ha.

Among monocotyledonous species, winter wheat was more sensitve to MON 52276 than perennial ryegrass (NOER = 0.04 L/ha, equivalent to 14.4 g a.s./ha). The NOEC for fresh shoot weight was the same for all species tested (NOER= 0.4 L/ha, equivalent to 144 g a.s./ha).

Table B.9.9-6: NOER of MON 52276 to monocotyledonous and dicotyledonous plants based on phytotoxicity and fresh shoot weight

Crop	Phytotoxicity (L/ha)							Fresh shoot weight (L/ha)
	1 day	2 days	3 days	4 days	7 days	15 days	22 days	
Sugar beet	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Rape	0.4	2.0	0.4	0.4	0.4	0.4	0.4	0.4
Garden Cress	n.d.	0.04	0.04	0.04	0.04	0.04	0.04	0.4
Pea	n.d.	n.d.	0.4	0.4	0.4	0.4	0.4	0.4
Perennial ryegrass	n.d.	0.4	0.04	0.4	0.4	0.4	0.4	0.4
Winter wheat	n.d.	0.4	0.4	0.4	0.4	0.4	0.04*	0.4
n.d.: No significant differences between any treatment and the control * 0-00004 L prod/ha also differed significantly from the control on this one sampling occasion								

RMS Conclusions

In this study, no mean plant survival data for the control are available. Treated plants were evaluated over a period of 22 days following treatment. Endpoints were determined for biomass and visual effects but only NOER are presented. After 22 days, the lowest NOER values of 14.4 g a.s./ha (equivalent to 0.04 L prod/ha) for MON 52276 were observed for garden cress and winter wheat.

Due to the lack of raw data including standard deviation values, no evaluation of possible concentration–response relationships was possible. No ER_{50} could be recalculated. The study is not considered to be valid and acceptable.

No additional study showing the toxicity of the lead formulation MON 52276 was provided. Since it can not be excluded that the formulants in the product enhance toxicity, the lack of further data evaluating the representative formulation MON 52276 has consequences for the risk assessment. Please refer to section B.9.9.1.5.

A valid study assessing effects on non-target plants is required for the plant protection product, as the risk cannot be reliably predicted on the basis of the active substance data generated. The test shall provide the ER_{50} values of the plant protection product to non-target plants.

KIIIA 10.8/01

During the revision of the active substance glyphosate for the renewal of Annex I inclusion (AIR2), the Rapporteur Member State (RMS) Germany requested the glyphosate task force (GTF) to submit a new vegetative vigor study following the OECD guideline 227, with the representative formulation MON 52276 and to include the most sensitive species in previously performed studies tomato [*Lycopersicum esculentum*] (Chetram and Lucash, 1994). A study assessing effects on non-target plants is required for the plant protection product, as the risk could not be reliably predicted on the basis of the active substance data.

The formulation study was made available upon request by the European Food Safety Authority (EFSA) (Bergfield, 2014, BVL no 2716933, GLP, not published).

Author:	Bergfield, A.
Title:	MON 52276: Effects on the Vegetative Vigor of Non-Target Terrestrial Plants (Tier II)

Date:	17 October 2013
Doc ID:	2716933 /80477
Guidelines:	OECD Guideline 227
GLP:	YES
Validity:	YES with major uncertainties

Materials and Methods

Test item::	MON 52276, a formulated product containing glyphosate as the active ingredient
Lot/Batch #:	Lot number: GLP-1308-22862-F; ABC reference number MM-8685-00001
Purity:	Purity: 30.45 wt% glyphosate acid
Positive control:	None
Species:	6 Dicotyledons: (*Cucumis sativus, Brassica napus, Raphanus sativus, Glycine max, Helianthus annus, Lycopersicon esculentum*) 4 Monocotyledons: (*Zea mays, Avena sativa, Allium cepa, Triticum aestivum*)
Source:	*Zea mays* / N48-G6F2; Syngenta SeedCare *Avena sativa* / Ogle; Ohio FoundationSeeds *Allium cepa* /Yellow Granex; Park Seed Co. *Triticum aestivum* / Triple IV; L.A. Hearne Company *Cucumis sativus* /Straight Eight; NE Seed *Brassica napus* / Dwarf Essex; Johnny's Selected Seeds *Raphanus sativus* / Crimson Giant; Sustainable Seed Company *Glycine max* / Williams 82; Missouri Foundation Seeds *Helianthus annuus* / 3433 NS/DM; Syngenta Seeds, Inc *Lycopersicon esulentum* / Beefsteak; NE Seed
Temperature:	28 °C/17°C max/ min 294/21.4°C max/ min (Cucumber, Oilseed Rape, Radish, and Tomato)
Relative humidity:	32-92 RH % 27-73 RH % (Cucumber, Oilseed Rape, Radish, and Tomato)
Photoperiod:	16 h light/ 8 h dark
Soil pH, Soil organic matter content	pH5.9; % org mateer: 2.5; This soil was categorized as sandy loam based on the USDA textural class
Methods:	The test was replicated 4 times. At initiation, each pot contained five plants per pot, except for cucumber which contained three plants per pot. Pots were planted with an exaggerated no. of seeds to allow for thinning to a uniform stand prior to initiation. The plants were grown for approx. 2-5 weeks - the plants had 2-4 leaves upon test initiation. The test substance were dosed by spraying the control or appropriate test substance spray solution onto the foliage of the seedlings and surface of the soil within the test pots using an overhead track sprayer (De Vries Manufacturing). The single nozzle sprayer was equipped with a TeeJet 4001 E nozzle and operated at 40 psi. Natural sunlight supp.with 600 watt high pressure sodium grow lights set on a 16/8h photoperiod provided the lighting during the treatment exposures. The test pots were positioned within the greenhouses in a complete randomized block design. The testing for corn, oat, onion, wheat, soybean, and sunflower was performed in Greenhouse 7; testing for cucumber, oilseed rape, radish, and tomato was performed in Greenhouse 8. Temperature and relative humidity were recorded continuously in using electronic data loggers. Light was measured using an OWL-1 light logging system. Observations of survival (numbers of live plants present and cumulative mortality) and phytotoxicity ratings (i.e., visual injury assessments) were performed on a weekly basis for all species. If there was any visible normal coloration of the seedling, that plant was reported as alive. Data collected included percent survival, visual injury (i.e., phytotoxicity), shoot length, and replicate shoot fresh weight at termination (21days). The data were analyzed using analysis of variance to compare treatments to the control to determine the no effect concentration (NOEC). Regression analysis was used to estimate the effective

	rate to elicit a 25 and 50% detrimental effect (ER25, and ER50) for length and weight data. The NOEC, ER25, and ER50 rates (g a.e./hectare) for the effects of MON 52276 on plant survival, shoot height, and shoot weight from the vegetative vigor test are summarized in the following table. All Statistical computations were performed using SAS Version 9.3 software.

Results

The average temperature and relative humidity was 24.7°C and 52%,(Greenhouse 7 corn, oat, onion, wheat, soybean, and sunflower) and was 25.7°C and 45%, (Greenhouse 8 cucumber, oilseed rape, radish, and tomato), respectively.
During the test period, the average daily photosynthetically active radiation (PAR) was 170 µEs-1 m-2 (daily accumulated PAR was 10 E/m2) with an average 16 hours light for Greenhouse 7 and was 173 µEs-1 m-2 (daily accumulated PAR was 10 E/m2) with an average 16 h light for Greenhouse 8, respectively.
Testing was conducted at seven rates of up to 1.280 g a.e./hectare. Phytotoxicity ratings were recorded after 21 days after treatment.
Plants were affected by glyphosate treatments in all species tested. After 21 days, the lowest NOER values <20 g a.e./ha for MON 52276 was observed for cucumber.
Among the dicotyledonous species, tomato was the most sensitive species concerning the endpoint shoot weight with an ER_{50} value of 30 g a.e./ha, whereas cucumber exhibited the lowest endpoint for shoot length with an ER_{50} value of 28.4 g a.e./ha. Among the monocotyledonous species, wheat was the most sensitive species concerning the endpoint shoot weight with an ER_{50} value of 38.2 g a.e./ha.

Table B.9.9-7: NOEC, ER_{25}, and ER_{50} (g a.e./hectare) for the effects of MON 52276 on plant survival, shoot height, and shoot weight from the vegetative vigor test

Species	% Survival [a]			Shoot Length [a]			Shoot Weight [a]		
	NOEC	ER25	ER50	NOEC	ER25	ER50	NOEC	ER25	ER50
Corn	320	522	854	40	55.8	207	40	87.1	131
Oat	160	204	252	80	112	204	80	91.7	120
Onion	160	536	916	20	99.7	389	80	103	163
Wheat	40	70.2	85.9	20	32.5	120	20	29.1	38.2
Cucumber	40	NC	**76.7**	< 20	18.3	**28.4**	>20	28.4	39.2
Oilseed Rape	320	NC	632	160	202	689	80	96.0	153
Radish	160	305	431	40	130	1144	40	55.3	94.9
Soybean	80	134	179	20	33.0	75.3	20	34.7	52.9
Sunflower	40	92.2	117	20	21.4	50.9	< 20	21.9	31.1
Tomato	80	92.4	108	20	22.9	46.7	< 20	19.5	**30.0**

[a] Concentrations are expressed as g a.e./Ha.
NC = Not calculated

RMS Conclusions

Despite the assumption that the study can be considered to be valid as criteria according to OECD 227 are fullfilled (the seedling emergence is at least 70%; and in the controls the plants

do not exhibit visible phytotoxic effects) major uncertainties remain concerning the acceptability of the study:

OECD 227 states that tests should be performed with light intensity of 350±50 E/m2/s. The guideline recommends additional lighting to become necessary if intensity decreases below 200 E/m2/s, wavelength 400-700 nm except for certain species which light requirements need less light intensity. Nevertheless, the study presented was conducted with an average daily photosynthetically active radiation (PAR) of 170 µEs-1m-2 and 173 µEs-1m-2 (daily accumulated PAR was 10E/m2) with an average 16 hours light, for greenhouse 7 and 8, respectively.

As light is an pivotal environmental factor that might be expected to affect a plastid-localized pathway, RMS questions the reliability of the endpoints from the study with half the recommended ight intensity. Glyphosate inhibits the shikimic acid pathway located in plastids by binding to EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), an enzyme of the aromatic amino acid biosynthetic pathway. The inhibition of the enzyme prevents the plant from synthesizing the essential aromatic amino acids needed for protein biosynthesis and causes plant death due to the disturbance of the carbonmetabolism required to produce aromatic amino acids and their derivatives in plants (Amrhein et al., 1980; Herrmann, 1995b; Bromilow and Chamberlain, 2000). It is not clear, if a reduced light intensity during plant growth might decrease carbon flow through the shikimic acid pathway. Light might correspond to need for secondary aromatic metabolite as it stimulates plant growth and also the demand for e.g. lignin precursors (Weaver and Herrmann, 1997). Moreover reduced light might have induced shade avoidance responses, which include increases in plant height, as well as a reduction in biomass and leaf numbers. Consequently, major uncertainties exist in terms of a reliable exposure of test plants and concerning the full potential of glyphosate action to affect a down regluated plastid localized pathway under the used light conditions.

Related to this topic it was shown that shoot lenght can be considerd as an enpoint with limited reliability (Sharkhuu et al., 2014). The authors demonstrated that the growth inhibitory potential towards shoot length varied according to the time of day the spray was applied[…] Growth of shoots was arrested completely when plants were sprayed during the first half of the day, whereas glyphosate spraying in late afternoon caused only a partial inhibition of shoot length. In particular when glyphosate was applied ante meridiem it was lethal, wheras plants survived the application occurred later.

Uncertaintiy is even increased by the fact that the recommeded light intensity was reduced. Therefore, RMS can not exclude the possibility that sensitivity of the test species is underestimated under the proposed environmental condidtions and with the choice of the endpoint shoot length.

Nevertheless, this study displays the only dataset provided for the representative formulation MON 52276 and therefore, includes information about the relevance of the formulants. In general toxicity studies with the commercial product are more appropriate than studies with the active ingredient only for the assessment of the effects on non-target plants.

KIIA 8.16./01

In the following study the relative post-emergence phytotoxicity between glyphosate and the metabolite aminomethylphosphonic acid (AMPA) was compared considering both crop and annual weed species.

Author:	Levine, S.L.; Orr, T.B.
Title:	Comparative Post-Emergence Phytotoxicity of AMPA and Glyphosate to Crop and Annual Weed Species
Date:	10 April 2012
Doc ID:	2312026 / MSL0024009
Guidelines:	no
GLP:	NO
Validity:	nd

Materials and Methods

Test item::	Glyphosate isopropylamine (IPA) salt (N-phosphonomethylglycine) AMPA (Aminomethylphosphonic acid)
Lot/Batch #:	-
Purity:	
Positive control:	Deionized water containing 0.4% emulsifier-L (cyclo-L)
Species:	9 Dicotyledons: (cocklebur, hemp sesbania, lambsquarters, morning glory, smartweed, soybean, sugar beet, velvetleaf, wild buckwheat) 8 Monocotyledons: (barnyard grass, corn, crabgrass, green foxtail, proso millet, rice, sorghum, wheat)
Source:	Obtained from a supply of viable seeds kept in suitable storage facilities by Monsanto to maintain viability
Methods:	At planting, containers were packed with a sufficient quantity of sterilized silt loam soil to provide a uniform medium for plant growth. Seeds were planted in sufficient quantity to give between 5 and approximately 30 specimens per replicate depending on the species planted and covered with a half-inch layer of soil. After planting, plants were moved to the greenhouse with supplementary lighting to support good plant growth and placed on a bench containing capillary matting. Sufficient tap water was provided to promote germination and provide good plant growth. Nominal test concentrations for foliar applications were prepared from a 1% stock solution for glyphosate acid equivalent and AMPA and applied as needed to achieve the desired rate of application to young plants (approximately two weeks after planting) using a spray bottle fitted with a DeVilbiss No. 152 atomizer nozzle. Low rates required further dilution of the 1% stock solution to 0.1% and 0.01% stock solutions to ensure accuracy in pipetting. To complete the formulation prior to application 0.4% of emulsifier-L (cyclo-L) was added to each spray bottle and then water was added in sufficient volume to provide a spray volume of 200 gallons/A. The spray solutions were uniformly applied to the foliage of the plants. The plants were inspected approximately twice per week by a trained observer and observations were recorded. Phytotoxicity was recorded as visual percent injury (chlorosis) relative to the untreated control and evaluated two weeks after test initiation. Phytotoxicity was recorded as visual percent injury (chlorosis) relative to the untreated control and evaluated two weeks after test initiation. The percent injury observations were used as the phytotoxicity endpoint to calculate EC50 values in this analysis. For this linear and quantitative scale a value of 0 = 0% chlorosis, 10 = 10% chlorosis and so on. Statistical calculations: EC50 values were calculated using a 3-parameter logistic model with the software package GraphPad Prism version 5.04 (GraphPad Software, Inc.). The maximum asymptote was constrained in the logistic model to 100% to re-

	flect the maximum potential response based on percent injury observations.

Results

EC_{50} values for glyphosate acid and AMPA were compared on a molar basis to assess relative toxicity. EC_{50} molar ratios were calculated as EC_{50} AMPA/ EC_{50} glyphosate acid and ranged from 3.4 for hemp sesbania to 86.8 for common lambsquarters. In all cases, the ratios were greater than two, indicating that AMPA has less than 50% of the herbicidal activity of glyphosate.

Table B.9.9-8: EC50 values for AMPA and Glyphosate to Crop and Annual Weed Species based on units of moles/ha

Species Common name	Species Scientific Name	Glyphosate Acid EC50 (Moles/ha)	AMPA EC50 (Moles/ha)	EC50 Molar Ratio 1
BARNYARD GRASS	*Echinochloa crus-galli*	4.202	103.972	24.745
COCKLEBUR	*Xanthium strumarium*	4.123	25.963	6.297
CORN	*Zea mays*	1.710	42.346	24.762
CRABGRASS	*Digitaria ischaemum*	2.320	70.661	30.458
GREEN FOXTAIL	*Setaria veridis*	2.146	42.790	19.937
HEMP SESBANIA	*Sesbania exaltata*	5.933	20.159	3.398
LAMBSQUARTERS	*Chenopodium album*	2.303	199.869	86.773
MORNING GLORY	*Ipomoea sp.*	6.913	128.603	18.602
PROSO MILLET	*Panicum miliaceum*	1.949	43.668	22.401
RICE	*Oryza sativa*	5.537	87.650	15.831
SMARTWEED	*Polygonum pensylvanicum*	4.882	37.682	7.718
SORGHUM	*Sorghum bicolor*	3.310	97.663	29.504
SOYBEAN	*Glycine max*	5.618	92.677	16.496
SUGAR BEET	*Beta vulgaris*	3.291	39.237	11.923
VELVETLEAF	*Abutilon theophrasti*	5.204	142.432	27.370
WHEAT	*Triticum aestivum*	4.703	131.732	28.007
WILD BUCKWHEAT	*Polygonum convolvulus*	3.287	25.821	7.856
AVERAGE		3.9667	78.407	22.475

EC_{50} ratio calculated as EC_{50} AMPA/ EC_{50} glyphosate. Results were calculated with full precision mode in Excel and may differ slightly from hand calculated values.

RMS Conclusions

EC_{50} molar ratios were calculated as EC_{50} AMPA/ EC_{50} glyphosate acid and ranged from 3.4 for hemp sesbania to 86.8 for common lambsquarters. In all cases, the ratios were greater than two, indicating that AMPA has less than 50% of the herbicidal activity of glyphosate.

B.9.9.1.3 Literature Review

For the group of terrestrial non-target plants (NTP), a comprehensive database of 87 peer-reviewed papers was collected by the notifier. The notifier considered one publication from

Boutin et al. (2010), measuring the variability in phytotoxicity testing using crop and wild plant species, to be rated in category "Klimisch2" (Klimisch, 1997) and annotated with minimal remarks. The remaining papers were considered not acceptable for risk assessment.

The submitted publications were also evaluated by RMS and have been assigned according to an UBA screening. From this screening, 27 studies were recognized as supporting information (category UBA2). Most of the cited studies were performed with formulated products rather than with the active ingredient alone. Surfactants or additives may be contained in significant amounts in plant protection products. The function of these compounds is supposed to enhance the herbicidal activity of the active ingredient by e.g. improving the dispersal and retention on the leaf surface or the uptake of glyphosate. Considering that herbicide sensitivity among crops species or within the same crop can be extensive and that, depending on the species included in testing, conclusions regarding the phytotoxicity of any given herbicide may differ (White and Boutin 2007), it is essential for current regulatory risk assessment to take into account toxicity data on the possible synergistic effects of the products in the assessed formulation in order to avoid underestimation of the toxicity of glyphosate containing products.

The use of herbicides to control weeds in target areas may affect non-target terrestrial plants (NTP) also in off- field situation. Potentially at risk are -besides NTP-, non-target arthropods or birds and mammals that are dependent on these plants for food and shelter. The objective of the risk assessment towards NTP, especially for herbicides, is to ensure that they will not be harmed by unintended exposure due to drifting into the off- field area outside the intended spray zones. Under optimal spraying conditions and appropriate application techniques, total spray drift (the portion of herbicide achieving off-field area) was considered to range from (2.77% to 29.2% of the volume applied) depending on the crop to be sprayed (Ganzelmeier & Rautmann, 2000).

Several publications were evaluated that simulate glyphosate drift with different test organisms (Deeds et al., 2006; Ellis et al., 2003; Felix et al., 2011; Gove et al., 2007; Koger et al., 2005; Nandula et al., 2007; Pfleeger et al., 2011). At tested rates corresponding to predicted environmental exposure, the authors detected visual injuries to test plants depending on test concentration, time of treatment, crop variety and experimental approach. Gove et al. (2007) even recommend the adoption of a buffer zones of at least 5 m to protect woodland species from the impacts of agrichemicals. Pfleeger et al. (2011) conducted toxicity test in greenhouse and under natural conditions and found that visual injury is not to be necessarily the most sensitive endpoint, but that reproductive endpoints in many cases were more sensitive that vegetative ones. Therefore, the proposes that more restrictive regulations (safety factors) should be imposed to account for the variability in sensitivity observed between greenhouse- and field-grown plants (Pfleeger et al. 2011). The study of Boutin et al. (2007) supports the inclusion of an uncertainty factor in risk assessments to account for the intrinsic variability in plant sensitivity to herbicides. It could be shown in Boutin et al.(2010), that crops and wild plant species responded quite variably when they were tested in different seasons as well as when tested under different environmental conditions. These findings are in line with uncertainties of phytotoxicity testing described by Olzyk et al. (2004), who addresses current trends in general risk assessment of plants in US.
Furthermore to concerns about the effectiveness of singlespecies greenhouse tests variability in sensitivity or the appropraite endpoint parameter for the protection of nontarget plants, the importance of sublethal effects of repeated drift events or exposure to mixtures of herbicides to plants is unknown.

More limitations to current phytotoxicty testing were described, taking into account that herbicides can influence plant communities in terms of species composition and diversity. Greenhouse microcosms were more sensitive than single-species tests and changes in community structure were observed in herbicide-treated microcosms that would not be predicted from single-species testing (Dalton and Boutin, 2010). The authors of this study concluded that even though single-species tests are useful as they can demonstrate clear dose–response patterns independently from other factors influencing growth, these test are unable to predict changes in community structure that may have long-term consequences. Pfleeger et al. (2014) determine effects of multiple applications of herbicides on small constructed plant communities and provided evidence that levels of glyphosate can affect native vegetation and that there were differences in species sensitivity. RMS agrees that it is important that the system-wide effects of the increasing impacts on ecosystems should be continously evaluated to for a better protection of plant communities. Therefore, traditional ERA might be complemented taking into account a regional agroecosystem level - with exposure estimations considering the extensive usage of herbicides in varios indications and large area (human factor in the maintance of agricultural management).

Additionally, it was shown that foliar applied glyphosate to target plants is released into the rhizosphere and might negatively affect non-target plants, disease problems and nutritional status (Neumann et al., 2006; Eker et al., 2006). It was reviewed by Johal and Huber, 2009 that glyphosate can affects disease development, including increasing soil pathogen populations or immobilizing micronutrients involved in disease resistance (Johal and Huber, 2009).Much of the concern regarding glyphosate is related to its effects within the rhizosphere (Kremer and Means 2009, Druille et al., 2013ab, Fernandedez et al. 2009). Nevertheless it also suggest that interactions between soil and cropping practices shape the influence of glyphosate use on rhizosphere microorganisms, and not glyphosate alone. Again it is important to state that even small effect of glyphosate itself on functionally important components of the agroecosystem can have a impact when bearing in mind the extensive usage of glyphosate in varios indications and large area. It is therefore recommend to maintain environmental monitoring in soil to survey these effects.

The decrease of certain plant species in agriculture landscape might associate with impacts on birds and mammals, as well as arthropods by influencing food resources or plant cover to reproduce or to hide from predators (Norris and Kogan 2005). Ecological side effects might even be stronger in diverse and species rich forest ecosystems. McMullin et al.(2012) showed that glyphosate reduced the abundance, respectively in 40 % and 56 % of the boreal forest lichen species. This study shows on the one hand that sensitivity to herbicide applications is relevant to forest managers, as for example lichen vegetation provides food, habitat for invertebrates and on the other hand highlights the limitations of current phytotoxicity testing by neglecting indirect effects or limiting species testing, which shall represent plant species in a whole ecosystem. In addition the extensive studies with the active substance glyphosate in the field of plant physiology provides a detailed information on photosynthesis, assimilation and translocation, as well as oxidative stress and shows that glyphosate has several secondary or indirect effects on plant physiology which may also explain its herbicidal effects (Gomez 2014).

The intensive agricultural management also causes indirect effects due to changes in agricultural management and can reduce the diversity of weed flora, with subsequent impacts on other non-target taxa (Marshall et al. 2003). RMS agrees that is necessary to strike a

balance between adequate weed control, and the requirement to support biological diversity. Moreover, RMS also favours modifications to crop management in selected areas of fields, such as conservation headlands and supports the extensiveness of uncropped strips to provide sufficient resources for biodiversity of non target (plant) organisms.

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B.9.9.1.4 Summary of results on non-target plants

The potential effects of glyphosate acid on seedling emergence could not suffuciently demonstrated, as the study by Everett, et al. (1996, BVL no 2311057) is not considered as valid by the RMS. The validity of this study according to OECD guideline 208 is not given, as no data on visible phytotoxic effects and the mean survival of emerged seedlings for the duration of the study in control group were reported. In control plants, stem elongation was decreased, pobably due to high light intensity. With regard to the observed symtomology, it should be noted that the test conditions recommended in the OECD 208 could not be maintained in the greenhouse (temperature deviations reaching from 9 °C to 45.5 °C, relative humidity 3-100 % and light intensitiy up to 669 klux). Generally, the validity criteria concerning integrity of control plants ensure that optimal conditions might lead to treatment related effects and optimal growth and test conditions.

Two new studies on vegetative vigour non-target terrestrial plants of glyphosate acid have been evaluated (Harnish, 1994, BVL no 2311051 and Everett, 1996, BVL no 2311055). In the study of Harnish (1994, BVL no 2311051) endpoints were determiend at day 21. The lowest 21 day ER_{50} values of glyphosate in the study was calculated for radish to be 0.2488, 0.2623 and 0.6904 kg glyphosate acid/ha for survival, dry weight and plant height). The study of Everett (1996, BVL no 2311055) represents the sole datasource for the derivation of endpoints, which are determined at 28d after treatment. The lowest ER_{50} values in the study was calculated for oilseed rape to be 0.149 kg glyphosate acid/ha and 0.150 kg glyphosate acid/ha, respectively for visual damage assessment and plant dry weight. The test on vegetative vigor by Chetram and Lucash (1994, BVL no 2311054) observed the lowest 21 day ER_{50} values for tomato plants and the endpoint was calculated to be 0.146 kg a.s./ha for dry weight. This endpoint was used so far for risk assessment in the national and EU plant protection product authorization process.

During the revision of the active substance glyphosate for the renewal of Annex I inclusion (AIR2), the Rapporteur Member State (RMS) Germany requested the glyphosate task force (GTF) to submit a new vegetative vigor study following the OECD guideline 227, with the representative formulation MON 52276 and to include the most sensitive species in

previously performed studies tomato [Lycopersicum esculentum] (Chetram and Lucash, 1994). Major uncertainties remain concerning the acceptability oft the study. RMS can not exclude the possibility that sensitivity of test species is underestimated under the environmental conditions and with the choice of the endpoint shoot length. Nevertheless, this study displays the only dataset provided for the representative formulation and therefore includes information about the relevance of the formulants. In general toxicity studies with the commercial product are more appropriate than studies with the active ingredient only for the assessment of the effects on non-target plants.

Conclusion:
RMS highlights the importance of toxicological assessment towards non target plants not only of the single active substance, but also of the different formulations during product authorization

B.9.9.1.5 Risk assessment for non-target plants

The risk assessment for terrestrial non-target plants refers to the off-field area being exposed due to spray drift. The risk assessment was conducted according to the recommendations of EU Guidance Document on Terrestrial Ecotoxicology (SANCO 10329/2002). The effects of glyphosate on the seedling emergence and vegetative vigour of monocotyledonous and dicotyledonous crop species were assessed in laboratory studies in order to predict lethal effects due to spray drift.

In general, phytotoxicity studies are conducted under GLP conditions and using approved methods like the OECD 227 guideline in order to keep consistent in evaluation and across different herbicides. The OECD 227 guideline states further that plants are evaluated through 21 - 28 days from treatment. The most sensitive species was tomato in the vegetative vigor test with the active ingredient glyphosate from the study of Chetram and Lucash (1994, BVL no 2311054) This species was not evaluated in the study of Everett (1996, BVL no 2311055). A comparison of all available endpoints of tested species in both 21d-studies (by Harnish, W.N., 1994, BVL no 2311051 and Chetram and Lucash, 1994, BVL no 2311054) with the endpoints of the same species in the 28 d-studies revealed an increase of toxicity endpoints of a factor of about 1.8. Consequently, it must be assumed that testing the most sensitive species tomato for a period of 28 days would similarly lower the observed endpoint. Using the former EU endpoint $ER_{50,\ 21day}$ for tomato plants (0.146 kg a.s./ha for dry weight) as well as the lowest 28 d endpoint of the sensitive species oilseed rape (ER_{50}= 0.149 kg a.s. /ha) might not sufficiently display the toxicity endpoint.

Therefore the risk might not be reliably predicted and the assessement based on the endpoint for the active ingredient glyphosate acid can be considerd on a preliminary basis.
Final risk assessment should then be performed on the level of product authorisation with reliable data-sets providing the ER_{50} values of the plant protection product to non-target plants.

Herbicides are formulated to increase their efficacy by the addition of e.g surfactants, solvents and antifoam compounds. These formulated agents are frequently even more toxic than glyphosate alone, especially for non –target organisms, especially aquatic organisms.
Since it can not be excluded that the formulants enhance toxicity, the lack of toxicity data of the inert ingredients in the formulation and the commercial product will create uncertainty about potential harmful effects of formulated products towards non-target plants. Therefore,

toxicity studies with the commercial product are more appropriate than studies with the active ingredient only for the assessment of the effects on non-target plants. It is evident from the available PPP authorization data that the glyphosate-containing formulated products typically are about a factor of 3 more toxic than the active ingredient itself (confidential data not shown). In order to respect a precautionary principle in the preliminary risk assesment for non-target plants, the acceptability criteria was therefore modified when assessing glyphosate-containing formulated products (TER ≥ 15 instead of TER ≥5). This approach was retaining all proposed use patterns including the proposed application rates, but takes into consideration an adequate bufferstrips to protect non–target plants off- field area due to spray drift.

During the revision of the active substance glyphosate for the renewal of Annex I inclusion (AIR2), the Rapporteur Member State (RMS) Germany requested the glyphosate task force (GTF) to submit a new vegetative vigor study following the OECD guideline 227, with the representative formulation MON52276. RMS sees uncertainties concerning the acceptability oft the study, because recommendations of the OECD guideline 227 were neglected and therefore the toxicity of the representative formulation MON52276 might be understimated due to unfauvorable environmental condidtions. These concerns could not be finalised due to a lack of further studies according to OECD227. Still the current assessment does not permit the conclusion that differently formulated plant protection products containing the active substance will not have any harmful effect on non-target plants.

Nevertheless, this study displays the only dataset provided for the representative formulation and includes information about the relevance of exclusive representative formulants. In general toxicity studies with the commercial product are more appropriate than studies with the active ingredient only for the assessment of the effects on non-target plants. RMS proposes that concerned Member States shall request the submission of additional studies according to OECD227 with the commmercial products particular during product authorziation, in order to evaluate specific sensitive endpoints corresponding to the product to be authorzied and to determine adequate bufferstrips for the protection of non–target plants in off- field areas.

For the risk assessment the following assumptions concerning spray drift reaching off-crop was taken into consideration:

Around the treated area in orchards non target plants will be exposed to glyphosate acid in plant protection products mainly via spray drift. The amount of spray drift reaching off-crop habitats is calculated using the 90th percentile estimates derived by the BBA (2000) from the spray-drift predictions of Ganzelmeier & Rautmann (2000). PERdrift values at 1, 5, and 10 meters were calculated based on the maximum application rate and the driftable portion of application rate for soil directed application:

Single application : 2.77 % at 1 m from treated field edges, 0.57 % at 5 m and 0.29 % at 10 m

Double application : 2.38 % at 1 m from treated field edges, 0.47 % at 5 m and 0.24% at 10 m

~~In the case of the intended use on mature crops before harvest, the drift rate to be considered should mirror the height of the plants. The spray driftability is increased the higher the equipment is distant from the ground level and the higher the canopy level. In general, this issue is reflected by higher drift deposition values. In the case for crop canopy >50cm or special crop heights in vineyards, orchard and hops. Using the basic drift deposition values for~~

~~field crops would therefore underestimate risk to non target plants, when application in cereals is performed in BBCH 89 before harvest. Therefore, we proposed to mirror the height of the crop and the potential higher drift deposition in order to reduce risk to non-target plants in the off-crop habitat.~~
~~New harmonised values at the EU level are under discussion. In order to respect a precautionary principle in the risk assessment for non-target plants the Ganzelmeier tables have been extended to take into account high crops like e.g. sunflowers by using a modified deposition factor (2.77% *2).~~
~~The exposure scenario "ground crops x 2" was chosen. For late application times in cereals and oilseeds indicated as crop maturity, the following driftable portion were assumed:~~

~~Late application: 2.77 % x 2 at 1 m from treated field edges, 0.57 % x 2 at 5 m and 0.29 % x 2 at 10m were assumed.~~

In the case of the intended use on mature crops before harvest, it must be pointed out, that the desiccation scenario is not considered by the current assessment based on FOCUS (2001). For the particular situation of the siccation scenario, drift rates based on FOCUS (2001) might not mirrow the height of the plants and finaly might underestimate exposure. Therfore, the following uncertainties must be taken into account by MS and the possible underestimation of exposure might be considered:

The height of the sprayed crop has an influence on the drift pattern of a plant protection product to the non-target habitats (e.g. Stallinga, H., J.M.G.P. Michielsen & J.C. van de Zande, 1999. Effect van gewashoogte op de drift bij een bespuiting in een graangewas. Instituut voor Milieu- en Agritechniek, IMAG-DLO Nota P99-71, Wageningen. 1999). The spray driftability is probably increasing the higher the equipment is distant from the ground level and the higher the canopy level. In general, this issue can be reflected by higher drift deposition values.
A study on standard spray drift deposition curves (van de Zande, J.C., D. Rautmann, H.J. Holterman & J.F.M. Huijsmans (2013): Joined spray drift curves for boom sprayers in The Netherlands and Germany. Draft Report, Plant Research International, Wageningen UR, Business Unit Agrosystems, Report 526, 72pp.) for a reference situation with a bare soil /short crop situation (crop height lower than 20 cm) and a cropped situation reports that separate spray drift curves can be described for the bare soil vs. cropped situation. The difference between the two situations vary –but can be roughly determined to be at least factor 2 higher in the cropped situation than on bare soil. In the case for crop canopy >50 cm or special crop heights in vineyards, orchard and hops basic drift deposition values have been modified as well. Using the basic drift deposition values for field crops would probably underestimate risk to non target plants, when application in cereals is performed in BBCH 89 before harvest. Therefore, RMS proposed to mirror the height of the crop and the potential higher drift deposition in order to reduce risk to non-target plants in the off-crop habitat (The exposure scenario "ground crops x 2" was proposed).

However, as recommended by the PPR panel (EFSA Journal 2014;12(7):3800) the current assessment based on FOCUS (2001) is followed, because new drift curves are currently not harmonised. It is acknowledged that new drift values will be implemented in the nearest future. For sake of harmonisation, it was concluded that the present assessment should be based on the current agreed FOCUS values.

Multiple application factor were considered to take into account the potential build-up of applied substances between applications according to Candolfi et al. 2001 ESCORT2 workshop. RMS proposed to take into account this artificial MAF of 1.7 for two applications for the following reason: Plants, in contrast to e.g. arthropods, are sessile and will be affected by each spray drift event. In conclusion plant will be affected at each single application event. The development of approaches to address the impact from multiple exposure events via appropriate extrapolation factors is appreciated.

The Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target terrestrial plants (EFSA Journal 2014;12(7):3800) proposes an effect-based method for assessing the risk from multiple applications. It is concluded that the toxicological Independent Action (IA) concept can also be applied for estimating cumulative effect levels due to subsequent exposure to the same compound. For a series of n equal drift values, the single effect levels are equal and are combined into a cumulative effect according to the IA formula described in Appendix E4.2. Taking into account the dose-response curve of study Bergfield (2013, BVL no 2716933) the calculation can be based on ER_{25} (18.3 g a.s./ha) and ER_{50} (28.4 g a.s./ha) and results the same risk mitigation measures as proposed by RMS for the application 2 x 2160 g a.s./ha.

PER off-field= Maximum in-field PER (including MAF) x % drift

PER off-field (double application)= Application rate x 1.7 x 2.38 % drift (1 m), 0.47 % drift (5 m), 0.24 % drift (10 m),

~~PER off-field (crop maturity, plant height >1 m)= Application rate x 5.54 % x drift (1 m), 1.14 % drift (5 m), 0.58 % drift (10 m)~~

PER off-field (crop maturity, plant height >1 m) = Application rate x 2.77 % x drift (1 m), 0.57 % drift (5 m), 0.29 % drift (10 m)

PER off-field (orchard, actual application rate 50 %)= Application rate x 2.77 % drift (1 m), 0.57 % drift (5 m), 0.29 % drift (10 m) x 0.5

PER off-field (orchard, actual application rate 50 %, triple application)= Application rate x 2.3x 2.01 % drift (1 m), 0.41 % drift (5 m), 0.20 % drift (10 m) x 0.5

The resulting maximum off-field predicted environmental rates (PER off-field) are summarized in the following table.

Table B.9.9-9: TER values for terrestrial non-target plants exposed to glyphosate acid are given in the following table

Scenario	ER_{50} (g a.s./ha)	PERin-field (g a.s./ha)	Distance (m)	PERoff-field (g a.s./ha)	TER	TER with 50 % drift reduction	TER with 75 % drift reduction	TER with 90 % drift reduction
All crops (all seeded and transplanted crops)	28.4	2 x 2160	1	87.4	**0.3**	**0.6**	**1.3**	**3.2**
			5	17.3	**1.6**	**3.3**	6.6	16.4
			10	9.9	**2.9**	5.7	11.5	28.7
		2 x 1440	1	58.3	**0.5**	**1.0**	**1.9**	**4.9**
			5	11.5	**2.5**	**4.9**	9.9	25
			10	5.9	**4.8**	9.6	19	48
		1 x 1440	1	39.9	**0.7**	**1.4**	**2.8**	7.1
			5	8.21	**3.5**	6.9	14	35
			10	3.5	8.1	16	33	81
		1 x 1080	1	29.9	**0.9**	**1.9**	**3.8**	9.5
			5	6.2	**4.6**	9.2	18	46
			10	3.1	9.2	18	37	92
Orchard crops, vines including citrus & tree nuts		1 x 2880	1	79.8 x 0.5 *	**0.7**	**1.4**	**2.8**	7.1
			5	16.4 x 0.5*	**3.5**	6.9	14	35
			10	8.4 x 0.5*	6.7	14	27	68
		1 x 2160	1	59.8 x 0.5*	**0.9**	**1.9**	**3.8**	9.5
			5	12.3 x 0.5*	**4.6**	9.2	19	46
			10	5.2 x 0.5*	10.9	21,8	43,7	109,2
		3 x 1440	1	66.6 x 0.5*	**0.9**	**1.7**	**3.4**	8.6
			5	13.6 x 0.5*	**4.2**	8.4	17	42
			10	6.6 x 0.5*	8.6	17	34	86
~~Cereals, Oilseeds (pre-harvest)~~		~~1 x 2160~~	~~1~~	~~119.7~~	~~**0.2**~~	~~**0.5**~~	~~**0.9**~~	~~**2.4**~~
			~~5~~	~~24.6~~	~~**1.2**~~	~~**2.3**~~	~~**4.6**~~	~~12~~
			~~10~~	~~12.5~~	~~**2.3**~~	~~**4.5**~~	~~9.1~~	~~23~~
		~~1 x 1440~~	~~1~~	~~79.8~~	~~**0.4**~~	~~**0.7**~~	~~**1.4**~~	~~**3.6**~~
			~~5~~	~~16.4~~	~~**1.7**~~	~~**3.5**~~	~~6.9~~	~~17~~
			~~10~~	~~8.4~~	~~**3.4**~~	~~6.8~~	~~14~~	~~34~~
		~~1 x 1080~~	~~1~~	~~59.8~~	~~**0.5**~~	~~**0,9**~~	~~**1.9**~~	~~**4,7**~~
			~~5~~	~~12.3~~	~~**2.3**~~	~~**4,6**~~	~~9,2~~	~~23.1~~
			~~10~~	~~6.3~~	~~**4.5**~~	~~9,0~~	~~18.0~~	~~45.1~~
		~~1x 720~~	~~1~~	~~39.9~~	~~**0.71**~~	~~**1.4**~~	~~**2.8**~~	~~7.1~~
			~~5~~	~~8.2~~	~~**3.5**~~	~~6.9~~	~~14~~	~~35~~

			~~10~~	~~4.2~~	~~6.8~~	~~14~~	~~27~~	~~68~~
Cereals, Oilseeds (pre-harvest)		1 x 2160	1	59.83	**0.5**	**0.9**	**1.9**	**4.7**
			5	12.31	**2.3**	**4.6**	9.2	23.1
			10	6.32	**4.5**	9.0	18	45
		1 x 1440	1	39.9	**0.71**	**1.4**	**2.8**	7.1
			5	8.2	**3.5**	6.9	14	35
			10	4.2	6.8	14	27	68
		1 x 1080	1	29.92	**0.9**	**1.9**	**3.8**	9.5
			5	6.16	**4.6**	9.2	18	46
			10	3.16	9.0	18.0	36.0	89.9
		1x 720	1	19.94	**1.4**	**2.8**	5.7	14
			5	4.10	6.9	13	27	69
			10	2.11	13	27	54	134
TER in bold are below the relevant trigger of 5.								

***** Because applications are made round base of trunk and to the intra-rows, (inner strips between two trees within a row), application rates per ha are expressed per 'unit of treated surface area' the actual application rate per ha orchard or vineyard will only be 50 % of the reported rate.

Based on the predicted rates of glyphosate in off-field areas, the TER values describing the risk for non-target plants following exposure to glyphosate indicate acceptable risks providing that the following risk mitigation measures are taken into account:

Mitigation measure for plants exposed to glyphosate acid: Buffer strips

Tabelle 9.9-10: Mitigation measure for plants exposed to glyphosate acid: Buffer strips

Intended uses	**Application rate (g a.s./ha)**	**Buffer strip (m) without drift reduction**	**Buffer strip (m) with x % drift reduction**
Orchard crops, vine including citrus & tree nuts*	1 x 2880	10 m	None with 90%
	1 x 2160	10 m	None with 90%
	3 x 1440	10 m	None with 90%
All crops (all seeded and transplanted crops)	2 × 2160	trigger not reached	5m-75%
	2 × 1440	trigger not reached	5m-75%
	1 × 1440	10m	None with 90%
	1 × 1080	10m	None with 90%
~~Cereals, Oilseeds (pre-harvest)~~	~~1 x 2160~~	~~trigger not reached~~	~~5m-90%~~
	~~1 × 1440~~	~~trigger not reached~~	~~5m-75%~~
	~~1 × 1080~~	~~trigger not reached~~	~~5m-75%~~
	~~1x 720 g~~	~~10 m~~	~~1m-90%~~
Cereals, Oilseeds (pre-harvest)	1 x 2160	trigger not reached	5m-75%
	1 × 1440	10m	None with 90%
	1 × 1080	10m	None with 90%
	1x 720 g	5m	None with 75%

***** Because applications are made round base of trunk and to the intra-rows , (inner strips between two trees within a row), application rates per ha are expressed per 'unit of treated surface area' the actual application rate per ha orchard or vineyard will only be 50 % of the reported rate.

For the intended use 'orchard crops, vine including citrus & tree nuts' the exposure towards the off-field area was reduced to 50% of the reported rate of the actual application rate per ha, when applications are made round base of trunk and to the intra-rows or inner strips between two trees within a row. With an application rate at 1 x 2880 g a.s./ha, 1 x 2160 g a.s./ha, 3 x 1440 g a.s./ha (applic. rate /treated area), corresponding to an application rate of 50% based on cropped area, the acceptability criteria are not met. The risk for non-target plants arising from this intended use are considered acceptable only, when including the risk mitigation measure in terms of a 10m buffer strip. If 90 % drift reduction nozzles and a buffer strip of 1 m are used, acceptable risk is achieved. Especially in these cases it appears necessary to protect off-field areas by a fixed distances to protect non target plants and biodiversity. Plant protection products must not be applied to the limit of the in-field areas in the case of strip-application but only inner strips between two trees should be treated. Only a inner strip application combined with the risk mitigation measures buffestrip and drift reducing nozzles can protect the function of the off-field area.

For the intended use 'all crops' for all seeded and transplanted crops with an application rate at 2 x 2160 g a.s./ha, 2 x 1440 g a.s./ha the acceptability criteria are not met. The risk for non-target plants arising from this intended use are considered acceptable including risk mitigation measures including drift reduction and buffer strips. If 75 % drift reduction nozzles and a buffer strip of 5 m are used, acceptable risk is achieved. For the intended uses with an application rate at 1 x 1440 g a.s./ha and 1 x 1080 g a.s./ha risk can be mitigated with 10 m buffer strips.

For the intended use 'pre-harvest' in cereals and oilseeds acceptability criteria are not met without any risk mitigation measures.
For the cereals scenario with an application rate of max. 1 x 2160 g a.s./ha a buffer of 5 m including 75% drift reduction nozzles should be applied in order to protect non target plants when using drift rates based on FOCUS (2001). For further information concerning the uncertainties and possible underestimation of risk in this particular scenario please refer to the introduction of this chapter. If 90 % drift reduction nozzles are used the application rate 1 x 1440 g a.s./ha and 1 x 1080 g a.s./ha pose an acceptable risk The risk for non-target plants arising from this intended use are considered acceptable without drift reduction only for the application of max. 1 x 720 g a.s/ha and a buffer of 5 m.

A late herbicide application before harvest time poses additional ecological risks for surface water, ground water and for non-target species. We refer to Directive 2009/128/EC of the European Parliament and of the Council that establishes a framework for Community actions to achieve a more sustainable use of pesticides (Plant Protection Framework Directive). Directive 2009/128/EC commit Member States to adopt national action plans (NAP) for sustainable use of plant protection products in Article 4. The aims are to further reduce the risks and impacts to human health and the natural environment associated with the use of pesticides and to limit the application of pesticides to the necessary degree. Member States might consider the appropriateness of additional herbicide uses, especially when addressing sustainable use of plant protection products according to Directive 2009/128/EC.

The protection aims according to Regulation EC 1107/2009 – the absence of unacceptable effects on biodiverstity and the ecosystem – do not explicitly differentiate between in- and off-field habitats. Considering the flora species as an integral part of the biodiversity of

non-target species and the strong evidence that the use of herbicides including glyphosate contributes to the ongoing loss of biodiversity in farmland invertebrate and vertebrate (especially farmland bird) species additional risk mitigation measures might be regarded at member state level depending on the agricultural and landscape conditions.

~~Table B.9.9-7: Preliminary TER values for terrestrial non-target plants exposed to glyphosate acid are given in the following table~~

~~Scenario~~	~~ER_{50}~~ ~~(g a.s./ha)~~	~~PERin-field~~ ~~(g a.s./ha)~~	~~Distance~~ ~~(m)~~	~~PERoff-field~~ ~~(g a.s./ha)~~	~~TER~~	~~TER with 90 % drift reduction~~
~~All crops (all seeded and transplanted crops)~~	~~146~~	~~2 x 2160~~	~~1~~	~~87.4~~	~~**2**~~	~~17~~
			~~5~~	~~17.3~~	~~**9**~~	~~-~~
			~~10~~	~~9.9~~	~~15~~	~~-~~
		~~2 x 1440~~	~~1~~	~~58.3~~	~~**3**~~	~~25~~
			~~5~~	~~11.5~~	~~**13**~~	~~-~~
			~~10~~	~~5.9~~	~~25~~	~~-~~
		~~1 x 1440~~	~~1~~	~~39.9~~	~~**4**~~	~~37~~
			~~5~~	~~8.21~~	~~18~~	~~-~~
			~~10~~	~~3.5~~	~~41~~	~~-~~
		~~1 x 1080~~	~~1~~	~~29.9~~	~~**5**~~	~~49~~
			~~5~~	~~6.2~~	~~24~~	~~-~~
			~~10~~	~~3.1~~	~~47~~	~~-~~
~~Orchard crops, vines including citrus & tree nuts~~		~~1 x 2880~~	~~1~~	~~79.8 x 0.5 *~~	~~**4**~~	~~37~~
			~~5~~	~~16.4 x 0.5~~	~~18~~	~~-~~
			~~10~~	~~8.4 x 0.5~~	~~41~~	~~-~~
		~~1 x 2160~~	~~1~~	~~59.8 x 0.5~~	~~**5**~~	~~49~~
			~~5~~	~~12.3 x 0.5~~	~~24~~	~~-~~
			~~10~~	~~5.2 x 0.5~~	~~47~~	~~-~~
		~~3 x 1440~~	~~1~~	~~66.6 x 0.5~~	~~**4**~~	~~44~~
			~~5~~	~~13.6 x 0.5~~	~~22~~	~~-~~
			~~10~~	~~6.6 x 0.5~~	~~44~~	~~-~~
~~Cereals, Oilseeds (pre-harvest)~~		~~1 x 2160~~	~~1~~	~~119.7~~	~~**1**~~	~~**12**~~
			~~5~~	~~24.6~~	~~**6**~~	~~60~~
			~~10~~	~~12.5~~	~~**12**~~	~~-~~
		~~1 x 1440~~	~~1~~	~~79.8~~	~~**2**~~	~~18~~
			~~5~~	~~16.4~~	~~**9**~~	~~-~~
			~~10~~	~~8.4~~	~~17~~	~~-~~
		~~1 x 1080~~	~~1~~	~~59.8~~	~~**2**~~	~~24~~
			~~5~~	~~12.3~~	~~**12**~~	~~-~~
			~~10~~	~~6.3~~	~~23~~	~~-~~
		~~1x 720~~	~~1~~	~~39.9~~	~~**4**~~	~~37~~
			~~5~~	~~8.2~~	~~18~~	~~-~~
			~~10~~	~~4.2~~	~~35~~	~~-~~
~~**TER in bold are below the modified relevant trigger of 15**~~						

~~* Because applications are made round base of trunk and to the intra-rows , (inner strips between two trees within a row), application rates per ha are expressed per 'unit of treated surface area' the actual application rate per ha orchard or vineyard will only be 50 % of the reported rate.~~

~~Based on the predicted rates of glyphosate in off-field areas, the TER values describing the risk for non-target plants following exposure to glyphosate indicate acceptable risks providing that the following risk mitigation measures are taken into account:~~

~~**Table B.9.9-8: Mitigation measure for plants exposed to glyphosate acid: Buffer strips**~~

~~**Intended uses**~~	~~**Application rate (g a.s./ha)**~~	~~**Buffer strip (m) without drift reduction**~~	~~**Buffer strip (m) with 90% drift reduction**~~
~~Orchard crops, vine including citrus & tree nuts*~~	~~1 x 2880~~	~~5 m~~	~~-~~
	~~1 x 2160~~	~~5 m~~	~~-~~
	~~3 x 1440~~	~~5 m~~	~~-~~
~~All crops (all seeded and transplanted crops)~~	~~2 × 2160~~	~~10 m~~	~~-~~
	~~2 × 1440~~	~~10 m~~	~~-~~
	~~1 × 1440~~	~~5 m~~	~~-~~
	~~1 × 1080~~	~~5 m~~	~~-~~
~~Cereals, Oilseeds (pre-harvest)~~	~~1 x 2160~~	~~trigger not reached~~	~~5m~~
	~~1 × 1440~~	~~10 m~~	~~-~~
	~~1 × 1080~~	~~10 m~~	~~-~~
	~~1x 720 g~~	~~5 m~~	~~-~~

~~Only for the intended use 'pre-harvest' in cereals and oilseeds with one application rate at 1 x 2160 g a.s./ha, the acceptability criteria is not met. The risk for non-target plants arising from this intended use is considered unacceptable without drift reduction. If 90 % drift reduction nozzles are used majority of these scenarios pass the risk assessment at 1m. For the cereals scenario with an application rate of max. 1 x 2160 g a.s/ha we would propose both the use of 90 % drift reduction nozzles and a buffer of 5 m.~~

Desiccation of crops

A herbicide used as desiccant accelerates the drying process of crops like cereals, peas, beans (for animal forage), oilseed rape, and linseed in order to obtain an even crop maturation (e.g. Dickeduisberg et al. 2012). Pre-harvest application of herbicides should also control late weed infestation in the field. However, target of a late herbicide application near crop maturation is to schedule the crop harvest date. While the direct weed control -e.g in lodged grain- is a plant protection measure to prevent crop loss and follows the principles of good agricultural practice, the treatment of crops to facilitate and time crop harvest date is considered by Cook et al. (2010) as a economically motivated agronomic measure.

An additional application of a non-selective herbicides poses ecological risks, like the entry into surface waters or ground waters (see Volume 3, B.8, chapter 6.3; Monitoring data), consumption of resources (chemicals, fuel, water) and encourages the development of resistant species. Potentially at risk are- besides non-target-plants, non-target arthropods or birds and mammals, which are dependent on these plants for food and shelter. Desiccation as agronomic measure leads to additional risks to non-target organisms in the off-field and the in-field spray zones. Additional costs for the chemicals, grain losses through lanes at the crossing with equipment, reduced crop marketability (potential pesticide contamination of food, seeds and brewer's grain excluded) should also be considered together with environmental risks.

An important objective of the environmental risk assessment for herbicides is to ensure that non-target organisms in the off-crop will not be harmed by unintended drift exposure. In the

case of the intended use on mature crops before harvest, the drift rate to be considered should mirror the height of the plants. In consequence, high drift percentiles should be considered for the late application times indicated as crop maturity. Depending on the application rates (e.g >720 g glyphosate/ha), risk mitigation measures like buffer-zones should be implemented in order to protect non-target organisms in non-target areas. In-field buffer zones are to be implemented to mitigate the risks.
We refer to Directive 2009/128/EC of the European Parliament and of the Council that establishes a framework for Community actions to achieve a more sustainable use of pesticides (Plant Protection Framework Directive). Directive 2009/128/EC commits Member States to adopt national action plans (NAP) for sustainable use of plant protection products in Article 4. The aims are to further reduce the risks and impacts to human health and the natural environment associated with the use of pesticides and to limit the application of pesticides to the necessary degree. Member States might consider the appropriateness of additional herbicide uses when addressing sustainable use of plant protection products according to Directive 2009/128/EC.

References

Cook, S., Wynn, S., Clarke, J. 2010: Glyphosate- a necessary herbicide. How valuable is glyphosate to UK agriculture and environment? Outlooks on pest management, December 2010, 280-283.

Dickeduisberg, M., Steinmann, H., Theuvsen, L. (2012): A survey on the use of glyphosate in German arable farming. Julius-Kühn-Archiv 434, 2012

B.9.9.1.6 Indirect effects via trophic interactions

Following the current version of the EU Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2 final), the RMS confines the quantitative risk assessment for terrestrial plants to the off-field area in this report. However, it should be noted that the protection aims according to Regulation EC 1107/2009 – the absence of unacceptable effects on biodiversity and the ecosystem – do not explicitly differentiate between in- and off-field habitats. Here, it is relevant that in the course of the recent revision of the PPP data requirements (Annex to Commission Regulation (EC) No 283/2013), the former phrase “non-target plants are plants outside the cropped area” in the non-target higher plants chapter has been deleted. Apart from the fact that flora species are an integral part of the biodiversity of non-target species, there is strong evidence that the use of herbicides including glyphosate contributes to the ongoing loss of biodiversity in farmland invertebrate and vertebrate species, especially birds. In chapter B.9.1.7.7 we discuss the options for risk assessment of indirect effects on birds and mammals via trophic interactions and potential mitigation measures which might be decided on at member state level.

B.9.9.2 Effects on amphibians

B.9.9.2.1 Literature Review

Since the 2001 EU glyphosate evaluation, a number of acute and chronic amphibian toxicity studies with glyphosate and commercial glyphosate-based formulations have been published. For this review of the literature, acute studies are considered to be 96 hours or less. However, also so called chronic studies did mostly evaluate lethality, apart from few investigations of

the effect of glyphosate formulations on weight and/or performance at metamorphosis. Only few studies assessed the toxicity of glyhosate formulation on terrestrial stages of amphibians.

Most of the acute and chronic amphibian study results were generated in laboratory toxicity tests. However, some of the studies were performed in the field using *in situ* enclosures or field mesocosms, in order to assess impacts under representative natural conditions, and chemical and biological monitoring studies conducted under conditions directly relevant to product use. Studies were evaluated based on criteria of reliability and relevance/adequacy for risk assessment.

A significant amount of research exists on the toxicity of glyphosate and several glyphosate-based formulations for amphibians.

Acute studies with glyphosate acid and glyphosate IPA for sensitive Gosner stage 25 tadpoles show medial lethal values comparable to values obtained with fish in regulatory studies and in the literature. The LC_{50} values for amphibian exposed to glyphosate and its salts range from >17.9 to >466 mg a.s./L (see table below).

Table B.9.9-11: Effect values reported in peer reviewed literature for amphibians: glyphosate acid and salts of glyphosate

Species	Substance	Study duration (h)	LC_{50} (mg a.s./L)	Reference
B.10 Amphibians				
Crinia insignifera tadpoles	Glyphosate acid	96	103.2	Bidwell and Gorrie 1995 glyphnosubm_023
Crinia insignifera adult	Glyphosate acid	96	75.0	Bidwell and Gorrie 1995 glyphnosubm_023
Litoria moorei tadpoles	Glyphosate acid	48	81.2	Mann and Bidwell 1999 glyphnosubm_024
Litoria moorei tadpoles	Glyphosate acid	48	121.0	Mann and Bidwell 1999 glyphnosubm_024
Crinia insignifera adult	Glyphosate acid	48	83.6	Mann and Bidwell 1999 glyphnosubm_024
Rana clamitans	Glyphosate IPA	96	>17.91	Howe et al., 2004 glyphecotox_025
Lymnodynastes dorsalis tadpoles	Glyphosate IPA	48	>400.0	Mann and Bidwell 1999 glyphnosubm_024
Litoria moorei tadpoles	Glyphosate IPA	48	>343.0	Mann and Bidwell 1999 glyphnosubm_024
Crinia insignifera tadpoles	Glyphosate IPA	48	>466.0	Mann and Bidwell 1999 glyphnosubm_024
Heleioporus eyrei tadpoles	Glyphosate IPA	48	>373.0	Mann and Bidwell 1999 glyphnosubm_024

The effects of different glyphosate-based formulations on amphibian survival have been evaluated on almost 30 species of amphibians (e.g. Howe et al., 2004; Cauble and Wagner, 2005; Dinehart et al., 2010; Edginton et al., 2004; Jayawardena et al., 2011; Jones et al., 2010 and 2011; Relyea 2012).

The medial lethal concentration for amphibian exposed to formulation of glyphosate containing specific surfactant classes are far lower than for glyphosate acid or its salts (see Table B.9.9-2). The surfactants displaying a high toxicity in glyphosate-based formulations usually belong to the classes of polyoxyethoxylated alkylamines (POEA; e.g. ethoxylated tallow- and cocoamines), or are e.g. fatty nitrogen derivate etheramine. For the implications resulting from these obervations, please see chapter B.9.11.

Table B.9.9-92: Effect values reported in peer reviewed literature for amphibians: glyphosate formulations and surfactants; GLY: glyphosate; POEA: polyoxyethoxylated alkylamine; w: with; w/o: without

Species	Substance	Study duration (hours or days)	LC_{50} (mg a.e./L)	Reference
Amphibians				
Rana pipiens; Gosner 25	Roundup Original GLY w POEA	96 h	2.9	Howe et al., 2004 glyphecotox_025
Rana sylvatica, Gosner 25		96 h	5.1	
Bufo americanus; Gosner 25		24 h	4.2	
		48 h	<4.0	
Rana clamitans Gosner 25		48 h	2.0	
Rana clamitans Gosner 25	POEA	48 h	2.2	Howe et al., 2004 glyphecotox_025
Rana clamitans Gosner 25	Roundup Bioactive ® GLY w/o POEA	48 h	> 17.9	Howe et al., 2004 glyphecotox_025
Rana clamitans Gosner 25	Touchdown ® GLY w/o POEA	48 h	> 17.9	Howe et al., 2004 glyphecotox_025
Rana clamitans Gosner 25	Glyfos BIO ® GLY w/o POEA	48 h	> 17.9	Howe et al., 2004 glyphecotox_025
Rana cascadae tadpole	Roundup Original? GLY w POEA	48 h	3.2	Cauble and Wagner, 2005 glyphecotox_049
Spea bombifrons Gosner 29	Roundup WeatherMAX® GLY w/o POEA; w surfactants of POEA similar toxicity	48 h	1.9	Dinehart et al., 2010 glyphecotox_064
Spea multiplicata Gosner 29		48 h	2.1	
Xenopus laevis Gosner 25	Vision® GLY w POEA	96 h	0.9	Edginton et al., 2004 glyphecotox_066 [a)]
Bufo americanus Gosner 25		96 h	1.7	
Rana clamitans Gosner 25		96 h	1.4	
Rana pipiens Gosner 25		96 h	1.1	
Scinax nascius Gosner 25	Glyfos® GLY w POEA?	96 h	2.6 b) 1.3	Lajmanovich et al., 2003 glyphecotox_078
Rhinella arenarum Gosner 25	Roundup UltraMAX® GLY w/o POEA; w surfactants of POEA similar toxicity	48 h	2.4	Lajmanovich et al. 2011 glyphecotox_080
Rana sylvatica Gosner 25	Roundup Original MAX®	21 d	2.9	Relyea, 2012 glyphecotox_088

Species	**Substance**	**Study duration (hours or days)**	**LC_{50} (mg a.e./L)**	**Reference**
Rana pipiens Gosner 25	GLY w/o POEA w surfactants of POEA similar toxicity	21 d	2.9	
Bufo americanus Gosner 25		21 d	2.5	
Rana sylvatica Gosner 26	Roundup Original MAX®, GLY w/o POEA; w surfactants of POEA similar toxicity	18 d	2.1	Jones et al., 2010 glyphecotox_075 c)
Bufo americanus Gosner 26		18 d	2.3	
Rana catesbeiana Gosner 25	Roundup Original MAX®, GLY w/o POEA; w surfactants of POEA similar toxicity	23 d	2.2	Jones et al., 2011 glyphecotox_076 d)
Rana clamitans Gosner 25		23 d	2.6	
Hyla versicolor Gosner 25		23 d	2.0	

a) Values reported for test series with pH 7.5; lower toxicity at pH 6.0
b) Value refer to mg formulation/L
c) Values reported for early application day 0; lower toxicity with split applications
d) Values reported for low animal density (single species); higher toxicity if kept at higher densities

Comparable to the findings regarding glyphosate as salt or acid, also the range in LC_{50} values reported for tadpoles when exposed to formulations of glyphosate is comparable to the range of LC_{50} values reported for fish. A first mechanistic explanation proposed why fish and tadpoles have very similar acute sensitivities to the surfactants that are added to glyphosate-based formulations relates to the toxic mode of action of surfactants. Increasing the permeability of cell membranes, addition of surfactants results in loss of osmotic or ionic stability at the gill. Consequently, the mode of action of surfactants to aquatic organisms could explain why the range of sensitivities for amphibians and fish in acute tests are similar when exposed to comparable glyphosate-based formulations. It should be noted here that only few data assessing the effect of glyphosate and glyphosate-based products on terrestrial stages of amphibians. Studies with other products (Brühl et al. 2013; Belden et al. 2010) have shown that terrestrial stage of amphibians do experience environmental concentration far higher than a medial lethal rate (LR_{50}) at authorized field uses. Therefore, if the acute risk for the aquatic stages of amphibian seem to be covered by a proper assessment of the risk to fish, this is not the case for the terrestrial stages. The risk assessment for bird and mammals has long been taken also as protective for amphibian in terrestrial environments. Since it has been shown by Brühl et al. (2013) that juvenile amphibian exposed to other products die at authorized field rates –and for some products even at 1/10 of field rates- the conclusions of the risk assessment for birds and mammals for a specific product do not cover necessarily the risk of exposed terrestrial amphibian stages.

Further studies evaluated were performed in the field in controlled enclosures (Thompson et al., 2004; Edge et al., 2011; Wojtaszek et al., 2004) employing specific formulations for forest applications with overspray scenarios for surface water ponds. These studies were not performed with dose response design and report amphibian survival rates and other parameters at given concentrations supposed to be environmentally relevant. Since exact exposure scenario was not always quantifiable and mean lethal concentrations mostly not reached in the chosen study design, these results are not directly utilizable for the ecotoxicological assessment of glyphosate formulations for amphibians.

Regarding chronic toxicity enpoints that do not relate merely to long-term effects on amphibian survival rate, Cauble and Wagner (2005) studied the effects of glyphosate formulations on larval methamorphosis. In glyphosate treatments (1 mg a.e./L), there are

indications of earlier metamorphosis and smaller size of *Rana cascadae* when compared to the control.

Also Howe et al. (2004) monitored in lab studies several chronic enpoints (e.g. forelimb emergence, tail damage and maximum tail height, snout-vent-length of metamorphs; gonadal histology to determine sex ratios). Significant tail damages and reduced tail lengths were recorded in treatments with the Roundup Original ® formulation and in treatments with the surfactant POEA. No effects on chronic endpoints were determined when the amphibian were exposed to glyphosate alone. POEA containing formulations showed displaced sex ratios towards intersex individuals. Again, this was not observed in treatment with glyphosate technical. However, results were not always strictly dose-dependent.

The studies by Cauble and Wagner (2005) and Howe et al. (2004) have been criticized by the Notifier as regards to experimental and/or reporting deficiencies. Not all critical points are shared by RMS. RMS believes that the findings pointing at chronic toxicity of surfactants in glyphosate-based formulations are not exaustively resolved by a critique of the study set up. Even if the cited studies suffer from experimental difficulties, the results indicate effects of ethoxylated surfactants on amphibian metamorphosis. The implications of these findings for the potential registration of glyphosate-based formulation with surfactants of significant toxicity are discussed in chapter B.9.11. The lead formulation for the assessment of glyphosate as active substance does not contain surfactants of overt toxicity.

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B.9.10 Effects on biological methods of sewage treatment (Annex IIA 8.7)

KIIA 8.15./01

Author:	Magor S.E., Daniel, M., Shillabeer, N.
Title:	Glyphosate technical: Determination of toxicity to *Pseudomonas putida*
Date:	19.06.2000
Doc ID:	2311061 /BL6889/B
Guidelines:	Water quality - *Pseudomonas putida* growth inhibition test (*Pseudomonas* cell multiplication inhibition test) International Standard ISO 10712
GLP:	YES
Validity:	NO

Materials and Results

Test item:	Glyphosate acid
Lot/Batch #:	P30
Purity:	97.6 %
Vehicle and/or positive control:	3,5-dichlorophenol (97 %), Growth medium
Species:	*Pseudomonas putida*, strain NCIMB9494
Source of organisms:	National Collections of Industrial and Marine Bacteria Ltd., Aberdeen, UK
Temperature:	27.0±0.5 °C
Methods:	The effects of glyphosate acid on *Pseudomonas putida* growth inhibition were evaluated in a 16-hour static toxicity test. The test concentrations of 1.0, 3.2, 10, 32, and 100 mg a.s./L in test medium were prepared in duplicate and sterile conditions in conical flasks. These test solutions were prepared by adding the appropriate amount of a 500 mg/a.s./L stock solution (0.125 g glyphosate acid in 250 mL deionised water) directly into the flasks. Flasks containing 1.0, 3.2, 10, 32, and 100 mg a.s./L (single replicates) of the reference toxic substance (3,5-dichlorophenol) and three control flasks were also prepared. Four mL growth medium, 1 mL inoculum and deionised water were added to obtain a final volume of 50 mL test solution. After shaking at 27.0±0.5°C (in an in incubator) at 150 rpm for 16±1 hours the optical density of the contents of each flask were measured at 600 nm with an Uvikon 930 spectrophoto-meter.

Results

The validity criteria according to ISO 10712 were not fulfilled, as the control inoculum has not multiplied by a factor of at least 60 within the test period. Test inoculum optical density was mesaured at beginng to be 0.532. The EC_{50} of the reference substance 3,5-dichlorophenol was between 10 mg/L and 30 mg/L.

Table B.9.10-1: The effects of glyphosate acid on *Pseudomonas putida*

Nominal concentration (mg a.s./L)	Mean optical density	Mean inhibition (%)
Control	0.859	-0
1.0	0.836	3
3.2	0.838	2
10	0.842	2
32	0.868	0
100	0.878	0
3,5-DCP 1.0	0.839	2
3,5-DCP 3.2	0.857	0
3,5-DCP 10	0.851	1
3,5-DCP 32	0.055	94
3,5-DCP 100	0.047	95

Conclusions

The 16-h IC_{50} for *Pseudomonas putida* exposed to glyphosate acid was >100 mg/L based on nominal concentration. The NOEC after 16 h was 100 mg test item/L. The study is not considered to be valid and acceptable.

KIIA 8.15./02

Author:	Wüthrich, V.
Title:	Assessment of the acute toxicity of glyphosate technical on aerobic waste-water bacteria
Date:	7.10.1990
Doc ID:	2311064 / 277830
Guidelines:	OECD 209 (1984)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	229-Jak-5-1
Purity:	98.9 %
Positive control:	3,5-dichlorophenol (97 %)
Source of activated sludge	Domestic waste-water treatment plant (ARA, Sissach, Switzerland
Temperature:	22 °C
pH:	7.5-7.7
Methods:	The effects of glyphosate acid on activated sludge were determined in a 3-hour exposure laboratory study. Activated sludge from a domestic waste-water treatment plant was exposed to the test item at concentrations of 3.2, 10, 32, 50, and 100 mg a.s./L, 2 untreated controls and a toxic reference (3,5-dichlorophenol at concentrations of 1.0, 3.2, 10, 32, 50, and 100 mg a.s./L). A stock solution of 500 mg a.s./L was prepared by dissolving glyphosate acid in bi-distilled water. The sludge was sieved, centrifuged and the solid material re-suspended in tap water and again centrifuged. This procedure was repeated a further 2 times. An aliquot of the final sludge suspension was made up with Soerensen buffer to 1 litre. To that mixture, 50 mL OECD recommended synthetic sewage feed were added. Glass flasks were filled with appropriate aliquots of stock solutions, water and activated sludge up to 500 mL final volume and aerated with an air flow of about 0.2 L/minute. After 180 minutes of aeration at 22 °C, samples were taken from the test flasks for oxygen measurement over a period of up to 10 minutes. The inhibitory effect of the test item is expressed as oxygen consumption per minute. Respiration rate (oxygen consumption expressed as mg O_2 per litre per hour) was expressed as percent inhibition relative to the control.

Results

The test is regarded to be valid since the variance between the control samples was 12.7 %. Furthermore, it was shown that the inhibitory effects of of 3,5-DCP was in the range expected and specified in the pertaining guideline OECD 209. The EC_{50} for the toxic reference 3,5-DCP was found to be 8.6 mg/L.

The table shows that the respiration rate of the sludge was not inhibited (-7.8%) at the highest concentration of glyphosate acid of 100 mg a.s./L. The influence of glyphosate acid on oxygen consumption of activate sludge is presented below.

Table B.9.10-2: Influence of glyphosate acid on oxygen consumption of activate sludge

Nominal concentration (mg a.s./L)	Oxygen consumption (mg O_2 per litre per hour)	(Mean mg O_2 per litre per hour and (deviation))	Inhibition (%)
Control	1.02	1.085 (12.7%)	-
Control	1.15		-
3.2	1.16	-	-6.9
10	1.09	-	-0.5
32	1.15	-	-6.0
50	1.09	-	-0.5
100	1.17	-	-7.8
3,5-DCP 1.0	1.11	-	-2.3*
3,5-DCP 3.2	1.07	-	1.4*
3,5-DCP 10	0.38	-	65.0*
3,5-DCP 32	0.07	-	93.5*
3,5-DCP 50	0.05	-	95.4*

RMS Conclusions

The EC_{50} for waste-water micro-organisms exposed to glyphosate acid was determined to be >100 mg/L.

B.9.10.1 Summary of results

The effects on sewage treatment were considered as not relevant, as the assessment of the acute toxicity of glyphosate technical on aerobic waste-water bacteria did not show a significant influence on the oxygen consumption of activate sludge.

Table B.9.10-3: Effects of glyphosate on biological methods of sewage treatment

Test substance Test type	Test organism	Endpoint (mg/L)	Reference
Glyphosate acid	Activated sludge (domestic)	3 h EC_{50} >100	Wüthrich, 1990; 277830 BVL no 2311064

B.9.11 Surface active substances in glyphosate-based formulations

B.9.11.1 Literature Review

In glyphosate-based formulations – as in almost all plant protection products (PPP) –, a varying amount of co-formulants are added to improve the handling and efficacy of the product. A great amount of the co-formulants might consist of water to which substances with antifoaming or surface active properties are added.
Surfactants do have a mode of action that attack membranes, so to permit the active substance to enter cells and reach the target. A class of non-ionic surfactants, the so called alkylamine ethoxylates (ANEO), exert an (eco)toxicological effect that can be detected in glyphosate-based formulations. Polyethoxylated alkylamine (POEA) are non-ionic surfactants belonging to the alkylamine ethoxylates.

The lead formulation for the assessment of glyphosate as active substance for PPP in the European Union does not contain alkylamine ethoxylates as surfactant. Nevertheless, since several glyphosate-based products are formulated with alkylamine ethoxylates, RMS considers it adequate to provide general background informations to other Member States in the European Union to facilitate the assessment of the risk arising from glyphosate-based PPP other than the lead formulation.
The toxicity of glyphosate-based products is greatly enhanced if the active substance is formulated with alklyamine ethoxylates (e.g. Figure B.9.11–1).

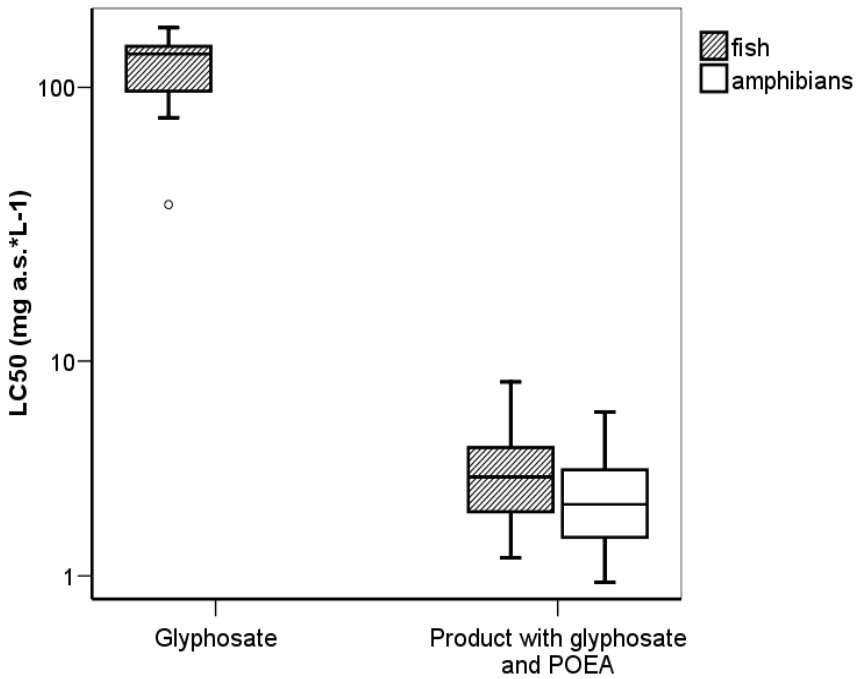

Figure B.9.11-1: LC_{50} values determined for fish and amphibians exposed to glyphosate or to glyphosate-based products containing polyethoxylated alkylamines. Data submitted for authorization of different products. Box gives median and 50%, whiskers 75% values

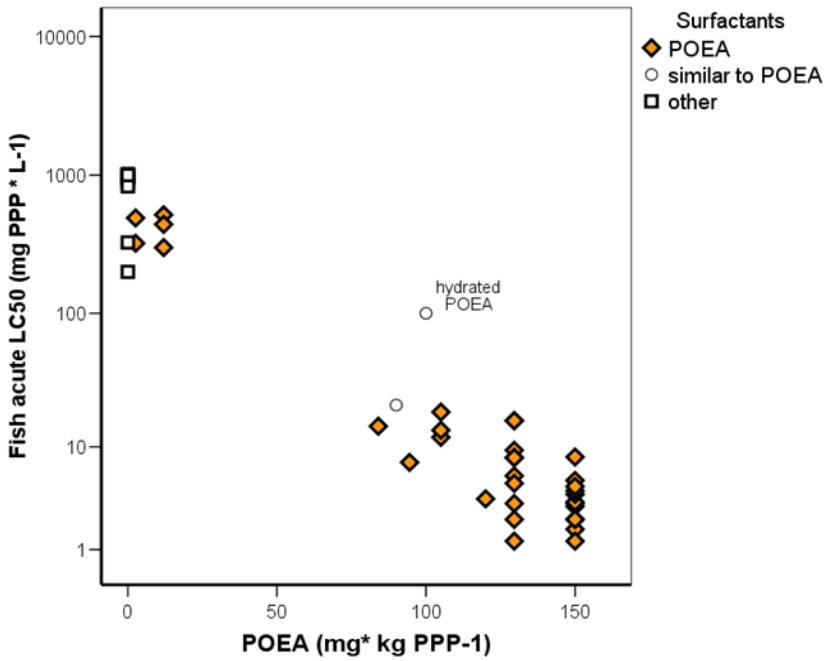

Figure B.9.11-2: LC_{50} for fish exposed to several glyphosate-based PPPs with different surfactants as a function of product surfactant content. POEA: polyethoxylated alkylamines; similar to POEA: other alkylamine ethoxylates. Other: other surfactant classes

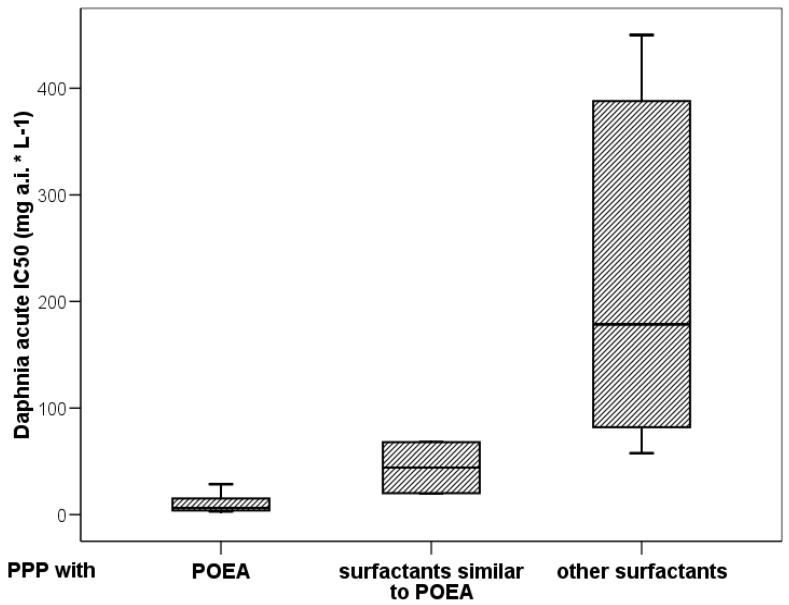

Figure B.9.11-3: LC_{50} for Daphnia exposed to several glyphosate-based PPPs: with different surfactants. POEA: polyethoxylated alkylamines; similar to POEA: other alkylamine ethoxylates. Other: other classes. Data submitted for authorization of different products. Box: median and 50 %, whiskers 75 % values

As can be seen in Figure B.9.11–1, the acute toxicity of PPP with glyphosate and POEA for fish and amphibians (stage Gosner 25, see Gosner 1960) is comparable, as was discussed in the respective chapter.
The clearly higher toxicity of some PPP with glyphosate as active substances can be predicted from the surfactant 'class' in the formulation and can be depicted as relative to the content of the surfactant and not of the amount of active substance in the product (Figure B.9.11–2).
Similar data showing enhanced toxicity of glyphsate-based PPP when formulated with POEA are available for Daphnia (Figure B.9.11–3) and algae (data not shown).

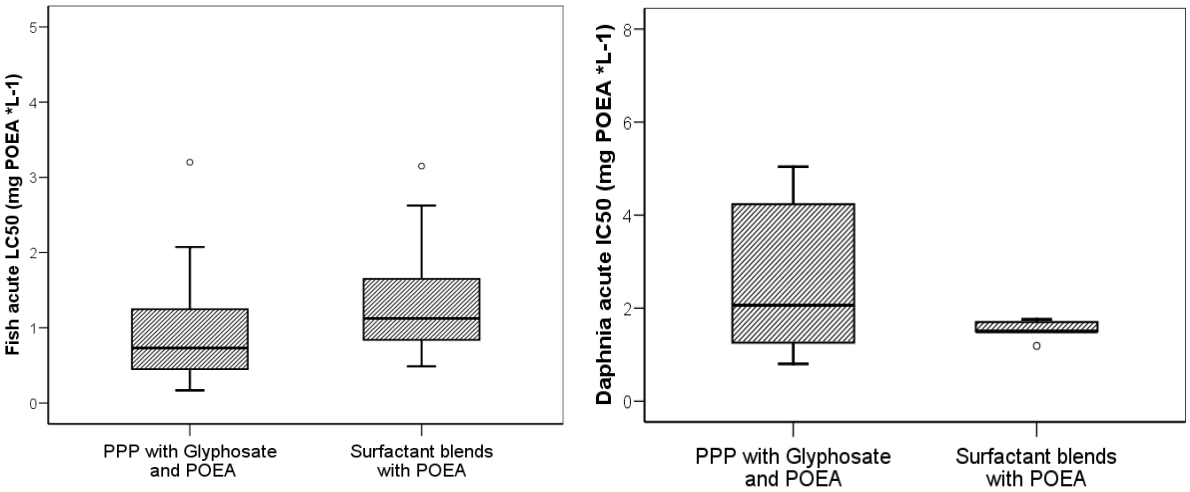

Figure B.9.11-4: LC_{50} data for fish and daphnids exposed either to glyphosate-based products containing alkylamine ethoxylates or to the surfactants alone. Data submitted for authorization of different products. Box gives median and 50 %, whiskers 75 % values

During authorisation processes for different glyphosate-based products, several toxicity data were generated with fish, daphnid and algae exposed to the surfactant blend alone (Figure B.9.11–4). All data show that the toxicity of the surfactant does drive the toxicity of the product, in part because glyphosate itself is only moderately acutely toxic to the tested organisms.

Summarising the data available, the acute risk for non-target organisms exposed to glyphosate-based products containing alkylamine ethoxylates can be assessed in the opinion of RMS based on the product tests submitted with the registration dossiers.
Hovewer, several studies published and peer reviewed that were submitted with this dossier and allocated to different themes (e.g. fish, aquatic invertebrates, algae, amphibians) do rise concerns on the effects of glyphosate and glyphosate-based formulation on endpoints regarding genotoxicity, mutagenicity, development or reproduction of non target organisms (Paganelli et al., 2010; Romano et al., 2010 and 2012; Gasnier et al., 2009; Dallegrave et al., 2003 and 2007; Hokanson et al., 2007; Walsh et al., 2000, McDaniel et al., 2008).
For the evaluation of studies with glyphosate and glyphosate-based products in in-vitro and in-vivo experimental set-ups with the aim to detect possible endocrine, genotoxic, carcenogenetic effect please refer also to the specific chapters assessing human toxicity (Vol.

1, chapter 2.6.7.2, developmental toxicity and teratogenicity and respective Vol. 3 of this report).
RMS considers several of the observed effects to be mediated by the surfactants included in the formulatios. In different studies, the effects were clearly more pronounced in treatments where the tested products contained alkylamine exthoxylated surfactants (e.g. Benachour et al., 2007 and 2009); glyphosate acid treatment (when tested alone) did extremely seldom show any effects on biomarkers or higher endpoints (e.g. Quassinti, 2009) . In the paper of Mesnage et al. (2013), the direct actue toxic effects of co-formulants belonging to the alkylamine ethoxylates class has been identified and very well characterised.
In Vol. 3, chapter B.6.13 of this report, an evaluation of the potential chronic toxicity, carcinogenicity reproduction and developmental toxicity of POEA is performed.
All (eco)toxicological data available give strong evidence that the toxicity of glyphosate-base formulation with POEA arisises from the surfactant. Nevetheless, even if this evidence relieves for the time being the active substance glyphosate from the suspect of being potentially carcenogenetic, endocrine disruptive and mutagenic, it does not tell the same for the surfactant class of the alkylamine ethoxylates.
Walsh et al. (2000) reported that glyphosate based formulations, but not glyphosate alone, might affect the steroidogenesis pathway by inhibiting the progesterone production. Levine et al. (2007) determined that Roundup® branded formulation and a Roundup blank formulation without glyphosate decreased the hCG-stimulated increase in progesterone production. These findings indicate that the effect on progesterone is largely attributable to the surfactant, insofar as it decreases progesterone production upon mitochondrial membrane disruption.
Other finding (e.g. Dallegrave et al., 2007, Knapp, 2007 and 2008) give indication on reproductive toxicity of a commercial Roundup® formulation and the surfactant formulation MON 0818 (POEA) on reproduction. As stated in Vol. 3, chapter B.6.13.3 "(...) Nonetheless, the published findings suggest that offspring development was in fact a particularly sensitive target of Roundup and the POE-tallowamine. The findings in young male rats might indicate impairment of spermatogenesis (...)".
More indications exist that glyphosate-based products with ethoxylated alkylamines might interfere with the endocrine system of vertebrates (e.g. estrogen synthesis, aromatase activity Soso et al., 2007; Richard et al., 2005). Moreover, it is not clear how glyphosate-based PPP with alkylamine exthoxylated affect the process of amphibian methamorphosis (please refer to the respective chapter B.9.11).
Therefore, the authorization of glyphosate-based products with alkylamine exthoxylated surfactants might require the generation of further data. The requests should cover the clarification of the effect of POEA on endocrine enpoints (e.g by a fish screening assay) and thyroid mediated processes (e.g. by extended amphibian metamorphosis tests) with representative POEA.

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B 9.12 Endocrine disruption potential of the active ingredient towards wildlife

Under Regulation (EU) 1107/2009 “endocrine disrupting properties” has been introduced as a hazard based cut-off criterion to be considered when evaluating active substances of plant protection products. Regarding wildlife, regulation EU 1107/2009 requires: “An active substance… shall only be approved if,…, it is not considered to have endocrine disrupting properties that may cause adverse effects on non-target organisms…“ In contrast, agreed procedures are not finalised regarding how substances with endocrine disrupting properties are to be identified or how risk is assessed. In the case of the re-evaluation of the active substance glyphosate information from mammalian studies (please refer to Annex B.6.1) is available and an interpretation of ecotoxicological studies towards endocrine disrupting indications is possible. The OECD conceptual framework for testing and assessment of potential endocrine disrupters (OECD, 2012a) includes several guidelines to support the testing and assessment. Within this guidance it is proposed to use assays originally not specifically designed to detect endocrine disrupting chemicals. Therefore, the following studies were assessed for their potential to detect endocrine disrupting properties:

1) Fish sexual development test and Fish Reproduction Partial Lifecycle Test

A short term study with *Brachydanio rerio* (KIIA 8.2.3/01) was conducted. This study is only 7 days in duration. Although sensitively reacting with respect to mortality, the test has no relevance for detecting endocrine mechanism. A fish full life cycle test was performed using *Pimephales promelas* (fathead minnow) and is included in the 2001 EU Evaluation of glyphosate. The chronic endpoint for the 255 day fish full life-cycle study with the fathead minnow was 25.7 mg a.s./L. No effect on survival, growth or reproduction of adult fathead minnow or progeny were observed when exposed to concentrations of up to 25.7 mg a.e./L for up to 8 months (255 days).

Additional chronic fish data included confirms a low chronic toxicity of glyphosate to fish (KIIA 8.2.4/02). This 85-day (60 days post-hatch) study with rainbow trout (*Oncorhynchus mykiss*) exposed to glyphosate resulted in NOEC and LOEC values for percent normal fry at hatch, hatching success, fry survival, length and weight to be 9.63 and > 9.63 mg a.s./L (the highest concentration tested), respectively, based on mean measured concentrations. However, none of the relevant parameters - indicating endocrine activity - i.e. reproductive

success (fertilization rate, numbers of eggs, sex ratio or vitellogenin content etc.) were assessed.

2) Avian Reproduction Assay (KIIA 8.1.4/03 and KIIA 8.1.4/01)

It is proposed to consider whether there are any indications of endocrine disruption in the submitted chronic studies with birds, such as deviating male-female-ratio of chickens, organ weights, increased incidence of tumors and pathological observations.

The study reviewed in KIIA 8.1.4/03 provides no information about male-female-ratio of chickens. During the test period the hatchling weight and survival of the animals were not affected at any concentration. The reproductive parameters to be determined according to the OECD guideline were not statistically significant with two exceptions. A statistically significant reduction in egg weight occurred at the highest test dose of 1000 mg glyphosate technical/kg diet. Since hatchling weight and survival were not statistically significant, it can be assumed that the observed changes in egg weight do not represent a population relevant adverse effect. The NOEAL for bobwhite quail exposed to glyphosate acid in a one-generation reproduction study was determined to be 1000 mg glyphosate technical/kg feed, corresponding to 96.3 mg/kg/bw/d.

Another study (KIIA 8.1.4/01) was submitted, which however is not considered to be acceptable and valid. Nevertheless it provides gross pathological observations on sexual organs. At test termination, for female animals increased regressing and regressed ovaries were observed at 1000 and 2500 ppm. In male animals, at 1000 ppm elevated numbers of small tests were observed. This effect was not observed in the highest does of 2500 ppm treatment. These findings were considered by authors to be incidental to treatment.

3) Larval Amphibian Growth & Development Assay (not submitted)

4) Mollusc Partial Lifecycle Assays (not submitted)

5) Chironomid Toxicity Test (not submitted)

RMS Conclusions

Glyphosate was included on the US EPA Endocrine Disruptor Screening Program's (EDSP) first list of 67 compounds to Tier 1 Screening. The US EPA published the criteria for inclusion on List 1 was strictly based on exposure potential, not hazard, specifically stating in the Federal Register (2009, ASB2012-12041); Recently, the first publicly data available from the glyphosate Tier 1 assays under the US EPA Endocrine Disruptor Screening Program were reported (Saltmiras & Tobia 2012, ASB2012-12016) for the Hershberger and Uterotrophic assays. Glyphosate has not potential to interact with androgenic or estrogenic pathways under these GLP studies following the US EPA 890 Series Test Guidelines. For further information please refer to the comprehensive review of the available data on the endocrine disrupting properties of Vol.3 B 6.1. In addition, published studies on developmental toxicity, reproductive toxicity and an endocrine disrupting potential of glyphosate and glyphosate based formulations include in vitro studies, in vivo studies and epidemiological studies. The RMS concludes in Volume 3 that "on basis of this comprehensive and high quality data package the active substance glyphosate is not considered to be an endocrine disruptor or a teratogenic substance". Many studies are specifically discussed in a comprehensive glyphosate review by Williams et al. (2012, please refer to volume B 6.1).

Additionally to the studies concerning mammalian toxicity were evaluated in the literature review of Appendix 9. Several information is availbale in peer-reviewed literature (Walsh et al. 2000, Levine et al. 2007, Dallegrave et al., 2007, Knapp, 2007 and 2008). For detailed information please refer to chapter B 9.11 B (Surface active substances in glyphosate-based formulations). The effects observed were attributable not to the active substance itself, but to direct acute toxicity of the surfactant system.

Moreover, a few peer reviewed studies have been published on the endocrine disrupting potential of glyphosate related to wildlife. Most of these studies use glyphosate containing preparations instead of the pure active substance glyphosate. Xie et al. (2003) investigated the estrogenic potencies of four herbicides, non-ionic surfactants, and the mixture of herbicides with surfactants using an in vivo rainbow trout vitellogenin assay. In this study glyphosate did not induce elevated levels of vitellogenin in juvenile rainbow trout compared with control fish. This was in line with results by Le Mer, et al. (2012), who also could not observe induced production of vitellogenin or a change in sex ratio in early life stages of stickleback. RMS provided a review on the Surface active substances in glyphosate-based formulations with special emphasis on alkylamine ethoxylated compounds and specifically on polyethoxylated tallowamine (POEA). In conclusion it is stated that the authorization of glyphosate-based products with alkylamine exthoxylated surfactants might require the generation of further data. The requests should cover the clarification of the effect of POEA on endocrine endpoints (e.g by a fish screening assay) and thyroid mediated processes (e.g. by extended amphibian metamorphosis tests) with representative POEA.
McDaniel, et al. (2008) performed a field survey on the impact of an exposure to the sum of possible substances employed in agricultural management on amphibian biomarkers and development. The investigation of the effects of single substances was not part of the study and the impact of the sole active ingredient glyphosate could not be determined. Generally, it should be considered that studies which have no relevance for the risk assessment of glyphosate itself, might give evidence that mixtures of pesticides potentially have endocrine effects in amphibians inhabiting farm ponds and agricultural drains in intensive agriculture.

In Europe, more than 300 different glyphosate-containing herbicides from over 40 manufacturers are sold. Glyphosate is used predominating for stubble management, pre-sowing application, as well as for forestry management and use in non agricultural areas and desiccation (Dickeduisberg, 2012). Glyphosate based formulations are widely used and must therefore be considered the most significant herbicide with a widespread release to diverse ecosystems. Even though no endocrine disrupting properties have been detected so far, it is recommended that analytical-chemical monitoring of environmental concentrations of glyphosate and its degradation products in aquatic and terrestrial ecosystems should ongoing in order to support regulatory activities.

B.9.13 Calculation of the 21day time-weighted –average (twa) for glyphosate in grass foliage used in the mammalian risk assessment

The methodology used to calculate the TWA for glyphosate in grass foliage for the long-term mammalian risk assessment follows the procedure described in the Guidance Document on Terrestrial Ecotoxicology (1). With the approach outlined in the Guidance Document on Terrestrial Ecotoxicology, residues are assumed to follow the standard pattern of first order exponential decline. The decline of glyphosate residue in grass was characterised using data from 22 residue trials each of which had a Day 0 value. Of these 22 trials, 18 of the trials were

from 4 separate Monsanto reports (references 2 - 5) and 4 trials were from 2 separate Cheminova reports (references 6 and 7). MON 2139 and a comparable Cheminova formulation were used in these grass residue trials. The grass residue values from the Cheminova trials were taken directly from the Glyphosate Monograph (reference 8); the Cheminova reports themselves were not reviewed.
The dissipation of glyphosate in grass was estimated using the standard first-order dissipation model:

(1) $C_t = C_i \times e^{-kt}$

where k is the first order rate constant, Ci is the initial residue concentration, and Ct is the residue concentration at time t. Residue half life time (DT50) in days for these grass land trials was calculated with equation 2.

(2) $DT_{50} = \frac{-\ln 0.5}{k}$

In each Monsanto report, residual glyphosate in mg/kg dry matter in grass was normalised to 1 kg a.s./ha and these values were plotted against time in days. For the Monsanto residue trials, many of the later sampling intervals were taken after plant desiccation. Therefore, for the purpose of accurately characterising glyphosate dissipation kinetics in grass, the glyphosate residues in mg/kg normalised to 100% dry matter content were used to eliminate the effect of sample weight losses during desiccation (Table II-1). However, since the final sampling day in the Cheminova trials was on Day 5, when grass desiccation was negligible, correction for moisture content was not necessary (Table II-2).
The dissipation of glyphosate was modelled with equation 1 using nonlinear regression (9). For 20 of the 22 trials, the standard first-order dissipation model provided an adequate fit for glyphosate dissipation (R2 > 0.8). The standard first-order dissipation model inadequately fit one Monsanto trial and one Cheminova trial (coefficient of determination, R2 □ 0.600). For these two trials, the DT50 was estimated by identifying the first day when a measured value had greater than 50% dissipation. Since the DT50 was estimated in this fashion for these two trials, the glyphosate residues in Tables II-1 and II-2 are also expressed as a percentage of the initial concentration, which was set at 100% for Day 0 after treatment. The average DT50 for the 22 trials was 2.8 days.
The linear first-order rate constant corresponding to a DT50 of 2.8 days was calculated using equation 3:

(3) $k = \frac{-\ln 0.5}{DT_{50}}$

which results in a rate constant k of 0.2476 days-1.

The 21-day time-weighted average (TWA) was calculated using equation 4:

$$(4)\quad TWA = \frac{(1-e^{-kt})}{kt}$$

where k is the first order rate constant calculated using equation (3), and t is the window of time over which the TWA is calculated (i.e. 21 days). Using these parameters for k, and t, the 21-Day TWA is calculated to be 0.19 for the active substance glyphosate acid.

Glyphosate residues in grass following a single treatment of Roundup® (MON 2139, SL/360). Source: Monsanto Field Residue Studies:

Country, Year Trial, ID	**App. Rate (kg a.s./ ha)[1]**	**NRG 100% of DM [2]**	**% of Day 0 a.s. residue**	**DAT[3]**		***R*²**	***k* (days⁻¹)**	**DT₅₀ (days)**	**Glyphosate Monograph Reference; Monsanto Report No.**
Great Britain, 1981									
SU 8125	1.08	101	100	1 h		0.990	0.4106	1.7	RIP95-01242 MLL 30.080
		27	26.7	3					
		12	11.9	7					
SU 8125	2.88	67	100	1 h		0.997	0.3251	2.1	
		27	40.3	3					
		5	7.5	7					
SU 30117	1.08	247	100	1 h		0.997	0.9587	0.72	
		14	5.7	3					
		8	3.2	7					
		7	2.8	9					
		6	2.4	10					
		3	1.2	14					
SU 30117	2.88	130	100	1 h		0.976	0.7063	0.98	
		14	10.8	3					
		11	8.5	7					
		9	6.9	9					
		10	7.7	10					
		3	2.3	14					
SU 30119	1.08	193	100.0	1 h		0.809	0.1456	4.8	
		175	90.7	4					
		38	19.7	9					
		9	4.7	11					
SU 30119	2.88	161	100.0	1 h		0.901	0.1550	4.5	
		123	76.4	4					
		30	18.6	9					
		13	8.1	11					
France, 1981									
811	0.72	168	100.0	0		0.976	0.4576	1.5	RIP95-01245 MLL 30.082
		9	5.4	5					
		23	13.7	8					
		5	3.0	12					
811	1.08	134	100.0	0		0.950	0.3768	1.8	
		9	6.7	5					
		27	20.1	8					
		5	3.7	12					
The Netherlands, 1982									
NL 8207	1.44	682.0	100.0	0		0.998	0.4230	1.6	RIP95-01264

Country, Year Trial, ID	App. Rate (kg a.s./ ha)[1]	NRG 100% of DM [2]	% of Day 0 a.s. residue	DAT[3]		R^2	k (days^{-1})	DT_{50} (days)	Glyphosate Monograph Reference; Monsanto Report No.
		77.0	11.3	5					MLL 30.101
		31.7	4.6	10					
Denmark, 1981									
Villbach (GE)-1981-0181Vi	1.80	162.9	100	0		0.844	0.1415	4.9	RIP95-01273 MLL 30.132
		36.0	22.1	7					
		52.6	32.3	14					
Villbach (GE)-1981-0281Vi	1.80	496.3	100	0		0.994	0.1537	4.5	
		184.4	37.2	7					
		37.0	7.5	14					
Lettgunbrun n (GE)-1981-0981LE	1.80	437.9	100	0		0.961	0.2616	2.6	
		51.2	11.7	7					
		69.4	15.8	14					
Villbach (GE)-1981-0481Vi	1.80	190.7	100	0		0.937	0.1098	6.3	
		69.0	36.2	7					
		59.0	30.9	14					
Denmark, 1983									
Vogach (GE)-19B	1.44	158.9	100	0		0.995	0.9083	0.76	RIP95-01273 MLL 30.132
		9.9	6.2	3					
		8.3	5.2	7					
		3.3	2.1	10					
		4.4	2.8	14					
Untermehlha usen (GE)-1983	1.44	169.6	100	0		0.990	0.2852	2.4	
		16.4	9.7	7					
		16.2	9.6	10					
		13.0	7.7	14					
Schoneberg	1.44	257.2	100	0		*	*	10 [4]	
		155.8	60.6	3					
		144.6	56.2	7					
		123.9	48.2	10					
		151.0	58.7	14					
Utphe (GE)-1983	1.44	354.9	100	0		0.961	0.1718	4.0	
		78.7	22.2	7					
		62.7	17.7	14					
		39.0	11.0	21					
Meiling (GE)-1983	1.44	253.9	100	0		0.997	0.9014	0.77	
		16.6	6.5	3					
		6.0	2.4	7					
		6.3	2.5	10					
		8.3	3.3	14					

[1] a.s. = glyphosate acid.
[2] NRG 100% of DM = residual glyphosate mg/kg normalised to 1 kg a.s./ha and corrected to 100% dry matter content. Values taken directly from Monsanto reports.
[3] DAT = Days After Treatment.
[4] Estimated DT_{50} value based on time when approximately 50% dissipation was reached.
* Did not fit standard 1st order dissipation model.

Glyphosate residues in grass following a single treatment of CHE 3607 (SL/360). Source: Cheminova Field Residue Studies (cited in Glyphosate Monograph):

App. Rate (kg a.s. /ha) [1]	Residue (mg a.s./kg wet weight)	% of Day 0 a.s. Residue	DAT[2]	R^2	k (days^{-1})	DT_{50} (days)	Glyphosate Monograph Reference; Cheminova Report no.
Great Britain, 1992							
2.16	237.6	100.0	4 h	0.987	1.9629	0.35	RIP95-01308 IF-93/04572-01
	45	18.9	1				
	19.6	8.2	3				
	9.6	4.0	5				
1.08	87.6	100.0	4 h	0.937	2.0879	0.33	
	14.6	16.7	1				
	14.3	16.3	3				
	8.3	9.5	5				
2.16	252.3	100.0	4 h	0.951	0.4885	1.4	RIP95-01312 IF-93/13842-01
	131	51.9	1				
	72.1	28.6	3				
	36.8	14.6	5				
1.08	90.4	100.0	4 h	*	*	3 [3]	
	142.8	158.0	1				
	39.8	44.0	3				
	17.3	19.1	5				

[1] a.s. = glyphosate acid.
[2] DAT = Days After Treatment.
[3] Estimated DT_{50} value based on time when approximately 50% dissipation was reached.
* Did not fit standard 1st order dissipation model.

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B.9.14 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[4]
KIIA 8.1.1 (OECD)	[redacted]	1997	Glyphosate acid. Acute oral toxicity (LD50) to Bobwhite quail ISN 400/963858 SYN GLP: Y, published: N 2310906 /	N	SYD
KIIA 8.1.1 (OECD)	[redacted]	1996	Glyphosate: Acute oral toxicity to Japanese Quail 1413/4-1011 NUF GLP: Y, published: N 2310909 /	N	NUF
KIIA 8.1.1 (OECD)	[redacted]	1999	Avian single-dose acute oral toxicity test in Japanese Quail with the chemical product Glifosate Técnico Nufarm D.8.1 - 382/99 NUF GLP: N, published: N 2310910 /	N	NUF
KIIA 8.1.1 (OECD)	[redacted]	1996	Glyphosate: Acute oral toxicity to Mallard Duck 1413/5-1011 NUF GLP: Y, published: N 2310912 /	N	NUF
KIIA 8.1.1 (OECD)	[redacted]	2003	MON 78623: An acute oral toxicity study with the Northern Bobwhite WL-2002-151 MON GLP: N, published: N 2310915 /	Y	MOD
KIIA 8.1.4 (OECD)	[redacted]	1999	Glyphosate Acid: A reproduction study with the Northern Bobwhite (Colinus virginianus) 123-186 SYN GLP: Y, published: N 2310916 /	N	SYD

[4] Only notifier listed

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KIIA 8.1.4 (OECD)	[redacted]	1999	Glyphosate Acid: A reproduction Study with the Mallard (Anas platyrhynchos) 123-187 SYN GLP: Y, published: N 2310918 /	N	SYD
KIIA 8.1.4 (OECD)	[redacted]	1978	One-Generation Reproduction Study - Bobwhite Quail; Glyphosate Technical. 139-141 MON GLP: N, published: N 2310921 /	N	MOD
KIIA 8.1.4 (OECD)	[redacted]	1978	One-Generation Reproduction Study - Mallard Duck; Glyphosate technical. 139-143 MON GLP: N, published: N 2310923 /	N	MOD
KIIA 8.10.1 (OECD)	Franco Perina, V.C.	2000	Side-Effects of Glifosate Técnico Nufarm on soil microflora: Carbon and Nitrogen Cycles RF-D1. 113/99 NUF GLP: Y, published: N 2311047 /	N	NUF
KIIA 8.10.1 (OECD)	Schulz, L.	2010	AMPA - Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests) 10 10 48 010 C/N GTF GLP: Y, published: N 2311050 /	Y	EGT
KIIA 8.10.1 (OECD)	Hutcheson, K.	2014	Glyphosate technical (MON 77973): Effect on Soil Microbial Nitrogen Transformations CMS-6237 GLP: Y, published: N 2716673 /	Y	MOD
KIIA 8.12 (OECD)	Harnish, W.N.	1994	LX1146-02 (Glyphosate techn.) Tier II Non-target plant hazard evaluation - Terrestrial vegetative vigor 236 GLY CHE GLP: Y, published: N 2311051 /	N	CHE
KIIA 8.12 (OECD)	Chetram,R.S., Lucash, K.J.	1994	Tier 2 Vegetative vigor nontarget phytotoxicity study using Glyphosate MSL-13320 MON GLP: Y, published: N 2311054 /	N	MOD
KIIA 8.12 (OECD)	Everett, C.J., Fleming,T.M., Cole , J.F.H.	1996	Glyphosate acid: A Tier 2 greenhouse study to athe effects on vegetative vigour of terrestrial n-target plants. RJ2009B SYN GLP: Y, published: N 2311055 /	N	SYD

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KIIA 8.12 (OECD)	Everett, C.J., Fleming , T.M., Cole , J.F.H.	1996	Glyphosate acid: A Tier 2 greenhouse study to assess the effects on seedling emergence of terrestrial non-target plants RJ2008B SYN GLP: Y, published: N 2311057 /	N	SYD
KIIA 8.12 (OECD)	Collings, L., Blake, N.	2005	Evaluation of the toxicity of Glyphosate and Paraquat to terrestrial non-target plants CEA.104 ! BX-0928 MON GLP: N, published: N 2311060 /	Y	MOD
KIIA 8.15 (OECD)	Magor S.E., Daniel, M., Shillabeer, N.	2000	Glyphosate technical: Determination of toxicity to Pseudomonas putida BL6889/B SYN GLP: Y, published: N 2311061 /	N	SYD
KIIA 8.15 (OECD)	Wüthrich, V.	1990	Assessment of the acute toxicity of glyphosate technical on aerobic waste-water bacteria 277830 CHE GLP: Y, published: N 2311064 /	N	CHE
KIIA 8.16.1 (OECD)	Levine, S.L., Orr, T.B.	2012	Comparative post-emergence phytotoxicity of AMPA and Glyphosate to crop and annual weed species MSL0024009 MON GLP: N, published: N 2312026 /	Y	MOD
KIIA 8.16.2 (OECD)	Schneider, K., Staedtler, T.	2010	Residues of glyphosate in arthropods after spray application in an arable field - magnitude and time course of residue decline 10153 GTF GLP: Y, published: N 2312028 /	Y	EGT
KIIA 8.2.1 (OECD)	[redacted]	1995	Glyphosate acid: Acute toxicity to rainbow trout (Oncorhynchus mykiss) BL5552/B SYN GLP: Y, published: N 2310924 /	N	SYD
KIIA 8.2.1 (OECD)	[redacted]	1995	Glyphosate acid: Acute toxicity to Bluegill Sunfish (Lepomis macrochirus) BL5553/B SYN GLP: Y, published: N 2310926 /	N	SYD
KIIA 8.2.1 (OECD)	[redacted]	2000	Acute Toxicity of Glifosate Técnico Nurfarm to Zebrafish (Brachydanio rerio) RF-D61.47/99 NUF GLP: Y, published: N 2310928 /	Y	NUF

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[4]
KIIA 8.2.1 (OECD)	[redacted]	2006	Glyphosate Technical: Acute Toxicity to Common Carp (Cyprinus carpio) 2060/015 NUF GLP: Y, published: N 2310930 /	Y	NUF
KIIA 8.2.1 (OECD)	[redacted]	1998	96-Hour Acute Toxicity Study in Rainbow trout with (Aminomethyl)Phosphonic Acid (Static). 232469 AGC GLP: Y, published: N 2310933 /	N	AGC
KIIA 8.2.1 (OECD)	[redacted]	1993	AMPA: Acute toxicity to rainbow trout (Oncorhynchus mykiss) BL5070/B SYN GLP: Y, published: N 2310934 /	N	SYD
KIIA 8.2.1 (OECD)	[redacted]	2003	MON 78623: A 96-hour Static Acute Toxicity Test with the Rainbow Trout (Oncorhynchus mykiss) WL-2002-149 MON GLP: Y, published: N 2310937 /	Y	MOD
KIIA 8.2.3 (OECD)	[redacted]	2000	Chronic Toxicity of Glifosate Técnico Nufarm to Zebrafish larvae (Brachydanio rerio) RF-D62.16/99 NUF GLP: Y, published: N 2310938 /	Y	NUF
KIIA 8.2.4 (OECD)	[redacted]	2010	Glyphosate acid: Early life-stage toxicity test with rainbow trout (Oncorhynchus mykiss) under flow-through conditions 1005.029.321 CHE GLP: Y, published: N 2310941 /	Y	CHE
KIIA 8.2.4 (OECD)	[redacted]	2011	AMPA (Aminomethylphosphonic acid): An early life-stage toxicity test with the fathead minnow (Pimephales promelas) WL-2010-328 GTF GLP: Y, published: N 2310943 /	Y	EGT
KIIA 8.3.1.1 (OECD)	McAllister, W.A., Forbis, A.D.	1978	Acute Toxicity of Technical Glyphosate (AB-78-201) to Daphnia magna AB 78-201 MON GLP: N, published: N 2310945 /	N	MOD
KIIA 8.3.1.1 (OECD)	Wüthrich, V.	1990	48-Hour Acute toxicity of Glyphosate techn. to Daphnia magna (OECD-Immobilization Test) 272968 CHE GLP: Y, published: N 2310947 /	N	CHE

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[4]
KIIA 8.3.1.1 (OECD)	Franco Perina, V. C.	2000	Acute toxicity of glifosate tecnico Nufarm to Daphnia magna RF-D51.39/99 NUF GLP: Y, published: N 2310948 /	Y	NUF
KIIA 8.3.1.1 (OECD)	Morris, D.S., Kent S.J., Banner, A.J., Wallace, S.J.	1996	Glyphosate acid: Acute toxicity to Daphnia magna BL5551/B SYN GLP: Y, published: N 2310950 /	N	SYD
KIIA 8.3.1.1 (OECD)	Jahnke, M.	1994	Acute Toxicity in Daphnia magna; Test Article: 'Glyphosate isopropylamine salt' 83-91-0737-00-93 ADM GLP: Y, published: N 2310952 /	N	ADM
KIIA 8.3.1.1 (OECD)	Franco Perina, V. C.	2000	Acute toxicity of glifosato IPA tecnico Nufarm to Daphnia magna RF-D51.017/00 NUF GLP: Y, published: N 2310954 /	Y	NUF
KIIA 8.3.1.1 (OECD)	Palmer, S.J., Kendall, T.Z., Krueger, H.O.	2003	MON 78623: A 48-Hour Static Acute Toxicity Test with the Cladoceran (Daphnia magna) WL-2002-150 MON GLP: Y, published: N 2310957 /	Y	MOD
KIIA 8.3.1.1 (OECD)	Bogers, M.	1998	Acute Toxicity Study in Daphnia magna with (Aminomethyl)Phosphonic Acid (Static). 232471 AGC GLP: Y, published: N 2310959 /	N	AGC
KIIA 8.3.1.1 (OECD)	Palmer, S.J., Kendall, T.Z., Krueger, H.O.	2011	HMPA (Hydroxymethylphosphonic acid): A 48-hour static acute toxicity test with the cladoceran (Daphnia magna) WL-2010-329 GTF GLP: Y, published: N 2310961 /	Y	EGT
KIIA 8.3.2.1 (OECD)	Magor, S.E., Shillabeer, N.	1999	Glyphosate acid: Chronic toxicity to Daphnia magna BL6535/B SYN GLP: Y, published: N 2310962 /	N	SYD
KIIA 8.3.2.1 (OECD)	Minderhout, T., Kendall, T.Z., Krueger, H.O	2011	AMPA (Aminomethylphosphonic acid): A semi-static life cycle toxicity test with the Cladoceran (Daphnia magna) WL-2010-327 GTF GLP: Y, published: N 2310965 /	Y	EGT
KIIA 8.4 (OECD)	Dias Correa Tavares, C.M	2000	Acute toxicity of glifosate tecnico NUFARM to Selenastrum capricornutum RF-D2.44/99 NUF GLP: Y, published: N 2310966 /	N	NUF

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[4]
KIIA 8.4 (OECD)	Smyth, D.V., Kent, S.J., Morris, D.S., Morgan, D.J., Magor, S.E.	1995	Glyphosate acid: Toxicity to the green alga Selenastrum capricornutum BL5550/B SYN GLP: Y, published: N 2310968 /	N	SYD
KIIA 8.4 (OECD)	Smyth, D.V., Shillabeer, N., Morris, D.S., Wallace, S.J.	1996	Glyphosate acid: Toxicity to blue-green alga Anabaena flos-aquae BL5698/B SYN GLP: Y, published: N 2310970 /	N	SYD
KIIA 8.4 (OECD)	Smyth, D.V., Kent, S.J., Morris, D.S., Shearing, J.M., Shillabeer, N.	1996	Glyphosate acid: Toxicity to the marine alga Skeletonema costatum BL5684/B SYN GLP: Y, published: N 2310972 /	N	SYD
KIIA 8.4 (OECD)	Hughes, J. S.	1987	The toxicity of Glyphosate technical to Skeletonema costatum 1092-02-1100-3 MON GLP: N, published: N 2310975 /	N	MOD
KIIA 8.4 (OECD)	Smyth, D.V., Kent, S.J., Morris, D.S., Johnson, P.A., Shillabeer, N	1996	Glyphosate acid: Toxicity to freshwater diatom Navicula pelliculosa BL5673/B SYN GLP: Y, published: N 2310976 /	N	SYD
KIIA 8.4 (OECD)	Egeler, P., Baumann, J.	2002	A study on the Toxicity of Glyphosate isopropylamine salt 62.5% to Algae (Pseudokirchneriella subcapitata) A-99-02-04 ALS GLP: Y, published: N 2310979 /	Y	AAP
KIIA 8.4 (OECD)	Desjardins, D., Kendall, T. Z., Krueger, H.O.	2003	MON 78623: A 72-hour toxicity test with the freshwater alga (Selenastrum capricornutum) WL-2002-148 MON GLP: Y, published: N 2310983 /	Y	MOD
KIIA 8.4 (OECD)	Bogers, M.	1998	Fresh Water Algal Growth Inhibition Test with (Aminomethyl)Phosphonic Acid 232458 AGC GLP: Y, published: N 2310985 /	N	AGC
KIIA 8.4 (OECD)	Porch, J.R. Kendall, T.Z., Krueger, H.O.	2011	HMPA (hydroxymethylphosphonic acid): A 72-hour toxicity test with the freshwater alga (Pseudokirchneriella subcapitata) WL-2010-330 ! 139A-396A GTF GLP: Y, published: N 2310987 /	Y	EGT
KIIA 8.6 (OECD)	Smyth, D.V., Kent, S.J., Morris, D.S., Cornish, S.K., Shillabeer, N	1996	GLYPHOSATE ACID: Toxicity to duckweed (Lemna gibba) BL5662/B SYN GLP: Y, published: N 2310988 /	Y	SYD

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[4]
KIIA 8.6 (OECD)	Scheerbaum, D.	1999	Glyphosate 62% IPA-Salt, aquatic plant toxicity test using Lemna gibba 980909FH ADM GLP: Y, published: N 2310990 /	N	ADM
KIIA 8.6 (OECD)	Mallett, M.J., Turnbull, G.	2002	IPA Salt of Glyphosate: Effects on Lemna minor CEMR-1873 SIN GLP: Y, published: N 2310992 /	Y	SNN
KIIA 8.6 (OECD)	Wenzel, A	2012	Effect of MON77973 (Glyphosate acid) on the Growth of Myriophyllum aquaticum in the Presence of Sediment. Test with a subsequent Recovery Period CHE-015/4-80/A GTF GLP: Y, published: N 2310995 /	Y	EGT
KIIA 8.6 (OECD)	Wenzel, A.	2012	Effect of AMPA (Aminomethylphosphonic acid) on the Growth of Myriophyllum aquaticum in the Presence of Sediment, with a subsequent Recovery Period CHE-022/4-80/A GTF GLP: Y, published: N 2310997 /	Y	EGT
KIIA 8.6 (OECD)	Porch, J.R., Kendall, T.Z., Krueger, H.O.	2011	HMPA (hydroxymethylphosphonic acid): A 7-day static-renewal toxicity test with Duckweed (Lemna gibba G3) 139A-397 GTF GLP: Y, published: N 2310999 /	Y	EGT
KIIA 8.7.1 (OECD)	Kleiner, R.	1995	Testing Toxicity to Honeybee - Apis mellifera L. (laboratory) according to EPPO Guideline No 170. Glyphosate (tec.) 95 10 48 065 ADM GLP: Y, published: N 2311000 /	N	ADM
KIIA 8.7.1 (OECD)	Weyman, G. S.	1996	Glyphosate: Acute contact and oral toxicity to honeybees 1413/3-1018 NUF, CHE GLP: Y, published: N 2311002 /	N	NUF, CHE
KIIA 8.7.1 (OECD)	Thompson, H.M.	1998	Glyphosate Acid: Acute Contact and Oral Toxicity to Honey Bees (Apis mellifera) FN9700 SYN GLP: Y, published: N 2311004 /	N	SYD
KIIA 8.7.1 (OECD)	van der Steen, J.J.M.	1995	Honey Bees (Apis mellifera L.), oral toxicity study in the laboratory with Glyphosate 141907 AGC GLP: Y, published: N 2311007 /	N	AGC

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[4]
KIIA 8.7.1 (OECD)	Halsall, N.	2003	Laboratory bioassays to determine acute oral and contact toxicity of MON 78623 to the honeybee, Apis mellifera MON-02-10 ! MT-2002-108 MON GLP: Y, published: N 2311012 /	Y	MOD
KIIA 8.7.2 (OECD)	van der Steen, J.J.M.	1995	Honey Bees (Apis mellifera L.), contact toxicity study in the laboratory with Glyphosate 142335 AGC GLP: Y, published: N 2311009 /	N	AGC
KIIA 8.7.2 (OECD)	Franco Perina, V.C.	2000	Acute Contact Toxicity of GLIFOSATO IPA TECHNICO NUFARM to Honey Bees (Apis mellifera L.) RF-D4.017/00 NUF GLP: Y, published: N 2311010 /	N	NUF
KIIA 8.7.2 (OECD)	Kleiner, R.	1995	Testing Toxicity to Honeybee - Apis mellifera L. (laboratory) according to EPPO Guideline No 170. Glyphosate (tec.) 95 10 48 065 ADM GLP: Y, published: N 2311014 /	N	ADM
KIIA 8.7.2 (OECD)	Weyman, G.S.	1996	Glyphosate: Acute contact and oral toxicity to honeybees 1413/3-1018 NUF, CHE GLP: Y, published: N 2320519 /	N	NUF, CHE
KIIA 8.7.2 (OECD)	Thompson, H.M.	1998	Glyphosate Acid: Acute Contact and Oral Toxicity to Honey Bees (Apis mellifera) FN9700 SYN GLP: Y, published: N 2320520 /	N	SYD
KIIA 8.7.2 (OECD)	Halsall, N.	2003	Laboratory bioassays to determine acute oral and contact toxicity of MON 78623 to the honeybee, Apis mellifera MON-02-10 ! MT-2002-108 MON GLP: Y, published: N 2320521 /	Y	MOD
KIIA 8.7.3 (OECD)	Thompson, H.M.	2011	Glyphosate: Study to determine potential exposure of honeybee colonies to residues under semi-field conditions V7YH1002 GTF GLP: Y, published: N 2311016 /	Y	EGT
KIIA 8.7.4 (OECD)	Thompson, H.M.	2012	Glyphosate: Evaluating potential effects on honeybee brood (Apis mellifera) development V7YH1001 GTF GLP: Y, published: N 2311020 /	Y	EGT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[4]
KIIA 8.8.2.1 (OECD)	Stevens, J.	2011	A rate-response extended laboratory test to determine the effects of MON 52276 on the parasitic wasp, Aphidius rhopalosiphi (Hymenoptera, Braconidae) MON-09-2 ! MT-2009-405 MON GLP: Y, published: N 2311022 /	Y	MOD
KIIA 8.8.2.2 (OECD)	Fallowfield, L.	2010	An extended laboratory bioassay of the effects of MON 52276 on the predatory mite, Typhlodromus pyri (Acari: Phytoseiidae) MON-09-3 ! MT-2009-404 GTF GLP: Y, published: N 2311024 /	Y	EGT
KIIA 8.8.2.3 (OECD)	Spincer, D.	2010	An extended laboratory test to determine the effects of MON 52276 on the ground-active beetle, Aleochara bilineata (Coleoptera, Staphylinidae) MON-09-4 ! MT-2009-403 MON GLP: Y, published: N 2311026 /	Y	MOD
KIIA 8.9.1 (OECD)	Mallett, M.J.	2002	Sinon Glyphosate Technical: The Acute Toxicity to the Earthworm Eisenia foetida CEMR-1875 SIN GLP: Y, published: N 2311027 /	Y	SNN
KIIA 8.9.1 (OECD)	Moser, T., Römbke, J.	2000	AMPA: Acute toxicity of AMPA technical material to the earthworm Eisenia andrei in an artificial soil test F13RA SYN GLP: Y, published: N 2311029 /	Y	SYD
KIIA 8.9.2 (OECD)	Friedrich, S.	2009	MON0139 - Sublethal toxicity to the earthworm Eisenia fetida 09 10 48 056 S GTF GLP: Y, published: N 2311032 /	Y	EGT
KIIA 8.9.2 (OECD)	Heyward, J.C., Mallett, M.J.	2000	A laboratory investigation of the effects of Glyphosate and its breakdown product AMPA on reproduction in the earthworm Eisenia fetida CEMR-1173 MON GLP: Y, published: N 2311034 /	Y	MOD
KIIA 8.9.2 (OECD)	Noack, M.	2002	AMPA - Earthworm (Eisenia fetida), effects on eproduction 011120FB ADM GLP: Y, published: N 2311035 /	Y	ADM

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[4]
KIIA 8.9.2 (OECD)	Servajean, E.	2003	Laboratory determination of the side-effects of aminomethyl phosphonic acid (AMPA) on the reproductive performance of earthworms (Eisenia fetida) using artificial soil substrate 01-64-077-ES ALS GLP: Y, published: N 2311037 /	Y	AAP
KIIA 8.9.2 (OECD)	Schulz, L.	2009	MON0139 - Effects on the reproduction of the predatory mite Hypoaspis aculeifer 09 10 48 058 S GTF GLP: Y, published: N 2311040 /	Y	EGT
KIIA 8.9.2 (OECD)	Friedrich, S.	2010	MON0139 - Effects on the reproduction of the collembolans Folsomia candida 09 10 48 057 S GTF GLP: Y, published: N 2311042 /	Y	EGT
KIIA 8.9.2 (OECD)	Schulz, L.	2010	AMPA - Effects on the Reproduction of the Predatory Mite Hypoaspis aculeifer 10 10 48 053 S GTF GLP: Y, published: N 2311044 /	Y	EGT
KIIA 8.9.2 (OECD)	Friedrich, S.	2010	AMPA - Effects on the Reproduction of the collembolans Folsomia candida 10 10 48 045 S GTF GLP: Y, published: N 2311046 /	Y	EGT
KIIIA1 10.2.2.1 (OECD)	Lintott, D.R.	1992	MON 52276: Acute toxicity to rainbow trout, Oncorhynchus mykiss, under Flow- through test conditions TO-91-296 GLP: Y, published: N 2317591 /	Y	MON
KIIIA1 10.2.2.1 (OECD)	Lintott, D.R.	1992	MON 52276: Acute toxicity to common carp, Cyprinus carpio, under flow- through test conditions TO-91-298 GLP: Y, published: N 2317595 /	Y	MOD
KIIIA1 10.2.2.2 (OECD)	Lintott, D.R.	1992	MON 52276: Acute toxicity to the water flea, Daphnia magna, under flow- through test conditions TO-91-295 GLP: Y, published: N 2317596 /	Y	MOD
KIIIA1 10.2.2.3 (OECD)	Neven, B.	1992	Alga, Growth Inhibition Test. Effect of MON 52276 on the Growth of Selenastrum capricornutum LI-91-389 GLP: Y, published: N 2317597 /	Y	MOD

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[4]
KIIIA1 10.4.2 (OECD)	Baxter, I.	2001	Laboratory bioassays to determine acute oral and contact toxicity of MON 52276 to the honeybee, Apis mellifera MON-00-2 version 2 MON GLP: Y, published: N 2316011 /	N	EGT
KIIIA1 10.6.2 (OECD)	Hoxter, K. A., Smith, G. J.	1992	MON 52276: An acute toxicity study with the earthworm in an artificial soil substrate 139-306 ! WL-91-272 GLP: Y, published: N 2317598 /	Y	MOD
KIIIA1 10.7.1 (OECD)	Hutcheson, K.	2012	MON 52276: Effect on soil microbial activity, Carbon and Nitrogen transformations CEMR-5259 GTF GLP: Y, published: N 2316013 /	Y	EGT
KIIIA1 10.8.2.1 (OECD)	Dengler, D.	2002	Assessment of toxic effects of MON 52276 on aquatic plants using the duckweed Lemna gibba GA-2002-051 MON GLP: Y, published: N 2316015 /	Y	EGT
KIIIA1 10.8. (OECD)	Wenzel, A.	2012	Effect of MON52276 (Glyphosate formulation) on the growth of Myriophyllum aquaticum in the presence of sediment, with a subsequent recovery period CHE-016/4-80/A GTF GLP: Y, published: N 2316017 /	Y	EGT
KIIIA1 10.8(OECD)	Bergfield, A.	2014	MON 52276: Effects on the Vegetative Vigor of Non-Target Terrestrial Plants (Tier II) 80477 GLP: Y, published: N 2716933	Y	MOD

Codes of owner

AAP	Arysta LifeScience S.A.S
AGC	AgriChem B.V.
CHE	Cheminova A/S
EGT	European Glyphosate Task Force AIR 2
ADM	ADAMA AGAN Ltd
NUF	Nufarm GmbH & Co KG
MOD	Monsanto Europe S.A./N.V.
SNN	Sinon Corporation
SYD	Syngenta Agro GmbH

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Annex B.9 (Appendix)

Evaluation of peer-reviewed literature regarding ecotoxicity (KIIA 8.16)

RMS: Germany **Co-RMS: Slovakia**

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B.9 Evaluation of peer-reviewed literature regarding ecotoxicity of glyphosate

B.9.13 Purpose and matter of subject of the literature survey

Article 8.5 of the regulation 1107/2009/EC stipulates the addition of open, peer-reviewed scientific literature with a submission of a dossier approval of an active substance in the European Union. Literature that was published within the last 10 years before the submission should be included. For this reason, the notifier Glyphosate Task Force (GTF) provided the available peer-reviewed literature from the public domain that refers to effects of glyphosate, the glyphosate-salt, its relevant metabolites and the representative formulation MON52276 to the regulatory bodies. This survey reviews the literature provided under Section 6, Point 8 of the dossier. The relevance of peer-reviewed literature that has been submitted by the notifier for consideration in the environmental risk assessment of the active ingredient glyphosate was assessed. Moreover, also several Non-Governmental Organization (NGO) provided also peer-reviewed literature from the public domain that refers to effects of glyphosate and glyphosate-based products.
The pre-selection of the studies that were subjected by the notifier to in-deep reliability and relevance analysis after Klimisch et al. (1997) was reviewed. For this purpose, the criteria of Carr and Bleeke (2012) to rank the available literature were adjusted where appropriate. In RMS opinion, the strictly formal criteria employed by the notifier according to Klimisch et al. (1997) would possibly not cover all environmental impacts that have been described in publicly available literature in direct relationship to glyphosate use. In particular the following evaluation-steps were performed:

1. Revision of identified and submitted literature
2. Evaluation of the notifier's selection for detailed description with the cumulative bibliography
3. Supplement of relevant studies
4. Evaluation of the published literature regarding significance of results, the quality of statistical evaluation, plausibility of conclusions after EFSA 2011a (complemented by Klimisch et al. (1997) and Küster et al. (2009, 2010))
5. Assessment and supplementation of the evaluation of the notifier after Klimisch et al. 1997, as found in DocMIIA, section 6, point 8 (Anonymous 2012a, in the following 'DocM')
6. Summary of the results and evidence on outstanding publications regarding the ERA of glyphosate

B.9.13.1 Methodology of the literature search

B.9.13.2 Process of retrieving literature by the notifier

The notifier GTF conducted systematic literature research as stipulated by Article 8.5 of the regulation 1107/2009/EC for the period between the years 2001 and 2011. The notifier did not proceed exactly after EFSA (2011a).
For this purpose, five literature databases, namely 'Web of Science (SM)', 'Biosis Previews ®', CAB Abstracts ®', 'Medline ®' and 'Chemical Abstracts Plus' were queried for

Glyphosate- or its metabolites related peer-reviewed literature. The exact strategy has been described by Carr and Bleeke (2012) in detail. The results that were obtained by querying the databases for glyphosate-specific keywords were further filtered for the question of focusing on the fate and the characterization of unintended effects of glyphosate. In each of the years 2001 and 2011, between about 200 and 300 papers were identified, summing up to 2.770 citations in total (80% peer-reviewed). The area of ecotoxicology was covered by 483 peer reviewed publications that came with the submission of the dossier. Additional 180 publications were cited in the text, but t were not submitted (coded 'rel0_nosub_cit', see below), referring e.g. to an Earth Open Source publication on glyphosate effects (Antoniou 2011), mainly older than 10 years and therefore not necessarily included in the submission.

B.9.13.3 Procedures of sighting and classifying the submitted literature by zRMS

Steps of the procedure

- DocMIII, pp. 288-651, (glyphapplic_007) was scanned systematically, and all the references to the literature found were marked and labelled unambiguously in the pdf. Bookmarks with chapter headers and identity numbers for easy lookup were added to the pdf.
- Bookmarks for ease of navigation were also added to DocL (glyphapplic_016).
- Unique identity-numbers were assigned to each of the literature entries of DocM, DocL and the pdf-files of the submitted peer-reviewed publications. Notation is as follows:
    - glyphapplic = essential documents submitted by the notifier with the application for renewal of approval as substantial part
    - glyphnosubm = documents that were not submitted with the application but cited in the text of DocM as supplementary data
    - glyphecotox = literature submitted with the application
- The exact sources (journal/issue/pages) necessary to identify a publication, which were provided by DocL (glyphapplic_016) were added to the original reference list of the managing body BVL. This was necessary for all of the publications.
- The information on the relevance and reliability given by the notifier on available literature was added to the references (relevance, reliability, Klimisch rating).
- Citations were added to the references that were not submitted but cited in the text of DocM (glyphnosubm_xxx).
- Non-classified literature by the notifier (rel1_sub_nocit+norev) was assigned in a screening to the best-fit test area (e.g. fish, amphibians, soil microbes, see chapter 'References').
- Comprehensive analysis and classification of the open literature.

Completion and assignment of 'assessment-area' categories

It was desired to follow the notifier's categorisation in general. These are the 13 chapters of the survey at hand (sub-structure of Annex B.9). The literature was categorised as 'birds', 'fish', 'amphibians', 'aquatic invertebrates', 'aquatic plants including algae', 'bees', 'terrestrial non-target arthropods', 'soil microbial community', 'plant disease and plant

nutrient status', 'earthworms and soil macro-organisms', 'terrestrial non-target plants', 'POEA' and 'DART/ED'.
However, to characterise the main topics of a study it has been necessary to introduce some new classes of study topics, especially for the studies that were completely out of the scope of the ERA. The assignment has been done for 431 studies that were submitted but neither cited nor reviewed in DocM (290 were rated *rel1_sub_nocit+norev*).

- **Other**: not assignable to a category
- **Soil Organisms**: Collembolans, soil mites, additional to **'Earthworms'** category
- **General**: not assignable to a certain category, e.g. 'general consideration of herbicide use on terrestrial ecosystems'
- Reptiles
- Molluscs
- **Fate:** falsely assigned by the notifier to the 'ecotoxicology' category
- **Soil quality**: includes indicators of soil quality other than organism related
- Aquatic Microbes
- Monitoring
- Vertebrates/Mammals
- **NTP:** studies not assigned by the notifier, were classified 'NTP' while dealing with the effectiveness of herbicides to antagonize weeds. This was because the studies could give indication of the sensitivity range of weed species.
- Modelling
- Genotoxicity
- **Pathogens**: Refers to the section 'plant disease', which was dealt with by the notifier in the fate section of DocM (Anonymous 2012b).

B.9.13.4 Analysis of reliability and relevance of peer-reviewed literature

Notifier

The notifier explained how studies were evaluated introducing an (arbitrary) categorization of the publications (taken from the definitions of Carr and Bleeke 2012). The notifier described the strategy of filtering and classifying the available literature as reproduced below from DocM (Anonymous 2012a).
'The peer-reviewed publications were divided into the four key disciplines within the submission that address exposure and hazard (toxicology, ecotoxicology, residues and environmental fate). Some publications contained information relevant to more than one technical discipline. In some cases, the disciplines originally assigned during the search process were revised to match the disciplines within the submission (for example, publications on effects of glyphosate on soil microorganisms were classified as 'environmental fate' in the original literature search but were reclassified as 'ecotoxicology' for the submission). The peer-reviewed publications identified for inclusion during the literature search were reviewed within each discipline and classified into one of the categories listed below <u>by the notifier</u>.

- Category 0 publications
  These are publications in which glyphosate is only mentioned as an example substance

or is discussed/studied in a context that is not relevant or related to any of the regulatory sections or the exposure/hazard assessments within this submission; the publication is therefore outside of the scope of this submission.

- Category 1 publications
  These are publications that discuss glyphosate in a context relevant or related to the regulatory dossier sections and the conclusions fall within the conclusions of the exposure/hazard assessment. The publication is submitted with minimal or no comment or discussion.

- Category 2 publications
  These are publications that discuss glyphosate in a context relevant or related to the regulatory dossier sections and have conclusions that call into question the endpoints/conclusions in the exposure/hazard assessment. Additionally, Category 2 also includes publications with conclusions that support the risk/hazard assessment, and may be included in discussion of other relevant publications. For selected Category 2 publications, an OECD Tier-II type summary is provided in addition to a reliability assessment according to Klimisch et al 1997 5 (Klimisch rating); limited comments and critical remarks are provided, as appropriate.
- Category 3 publications
  These are publications that discuss glyphosate in a context relevant or related to (1) non-regulatory endpoints that need to be addressed as per new Regulation (EC) 1107/2009; (2) sensitive allegations that have emerged or could emerge in the media; or (3) the regulatory dossier sections and have conclusions that are in disagreement with endpoints/conclusions in the exposure/hazard assessment (although the experimental design seems relevant at first glance). An OECD Tier-II type summary was provided and a Klimisch rating assigned, and supplemented with critical review and discussion.
- Category ‘E’ publications
  These are peer-reviewed publications that were cited in the Earth Open Source document. This category includes publications that were already captured by the notifier literature search and are addressed within the appropriate discipline, as well as publications that were out of scope of the search (primarily as a result of being published prior to 2001). Publications already captured in the literature search are assigned a Category 1, 2 or 3 rating (as appropriate) in addition to a Category ‘E’ rating. An OECD Tier-II type summary has been prepared and a Klimisch rating assigned for each of the Category E publications. All Category ‘E’ publications are reviewed within the appropriate discipline, with most of the reviews provided within the toxicology dossier under Section IIA 5.10.’

For notifier relevance category (2 and) 3 studies, a formal and more or less comprehensive evaluation based on Klimisch et al. (1997) on the reliability of a study was conducted by the notifier. There are 4 categories: ‘reliable without restriction’, ‘reliable with restriction’, ‘not reliable’ and ‘not assignable’.
The relevance categories triggering the Klimisch evaluation are indicated by column ‘UBA Classification study according to notifier treatment’ in the comprehensive reference list (refer to chapter 7 and MS Excel sheet attached).

Rapporteur member state

Based on the general criteria of Klimisch et al. (1997), Küster et al. (2009) aimed to further develop and specify the demands on the reliability of peer-reviewed literature data, in particular for regulatory requirements on the ERA of pharmaceuticals. The categories of Küster et al. (2009) were taken over as follows:

- **Category I**
  Data are reliable without restriction according to the instructions in the EMEA guideline (EMEA 2008) and are therefore usable within the environmental risk assessment. This category includes data from the literature or reports,
  - > Which were carried out or generated according to internationally accepted test guidelines (e.g. OECD, ISO).
  - > In which the test parameters documented are based on a specific (e.g. national) testing guideline (e.g. DIN).
  - > In which all parameters described are closely related/comparable to a guideline method.
- **Category II**
  Data are reliable with restriction and are usable within the environmental risk assessment. This category includes data from the literature or reports
  - > In which the test parameters documented do not totally comply with the respective test guideline, but are sufficient to evaluate the data.
  - > In which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented, plausible and scientifically acceptable.
- **Category III**
  Data are not reliable and are not usable within the environmental risk assessment. This category includes data from the literature or reports,
  - > Where the documentation is not sufficient for an assessment.
  - > Which were carried out or generated using a non-accepted method or which are not well documented, plausible and scientifically acceptable?
  - > Which are only listed in short information (e.g. abstracts, summaries, data safety sheets) or non-peer-reviewed literature (e.g. diploma/master thesis).

The evaluation criteria set by Küster et al. (2009) were further refined by international groups of regulators, leading to the latest publicly available initiative on the harmonisation of evaluation criteria by Kase et al. (2012). The reliability and the relevance of literature underlie separate examination. Therefore, even not reliable data can have supporting character. Test data with high reliability and high relevance will have high weight in risk assessment. Test data with either high reliability or high relevance could function as supportive evidence in risk assessments: see Küster et al. (2012).

RMS opted for following evaluation criteria:

High reliability	**Supporting data in risk assessment: UBA2 (Klimisch 2 und 3)**	**Critical data: High weight in risk assessment: UBA1 (Klimisch 1)**
iability	**Low weight in risk assessment , data not assignable; UBA3 (Klimisch**	**Supporting data in risk assessment: UBA2 (Klimisch 2 und 3)**

Figure B.9.13-1: Assignment of literature data to categories of high and low reliability and relevance for ERA. Modified after Klimisch et al. (1997).

There are criteria to identify 'critical', 'supporting' data and 'low weight data' for the risk assessment.
Critical data are data of sensitive species, typically including endpoint for risk assessment (as long as they are reliable and relevant). Supporting data are not described as critical datasince they may suffer from deficiencies. All reliable and relevant data are used. These include studies using less sensitive species/endpoints, studies using non-standard statistical methods. This can help e.g. identifying sensitive taxa, results and conclusions can be combined for risk assessment and for derivation of uncertainty. All available toxicity data, both critical and supporting, are subject to assessment and are reported. Uncertainty levels in risk assessment are estimated by the use of critical and supporting data and extrapolation from all available data.

Finally, the information provided by the experimental studies that were evaluated has been summarized in tables and concluded for its use in ERA. The table below shows a blank table with dummy records that was developed for the particular use in this study.

Table B.9.13-1: Blank general evaluation table with dummy records used as a form to be filled in this survey

litID **Assessment area**	**Author**	**Year**	**Title**	**Source**
Reliability				
Purpose of the study, Description of endpoints				…
Test compound, application procedure, exposure period, protocol				…
Experimental approach, Statistical design, test environment				…
Test organisms				…
Biological effects				…
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				…
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				…
3 Is the ecotoxicological manifestation level appropriate for the assessment, e.g. gene induction vs. apical endpoints like growth or reproduction?				…
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				…

2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	…
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	…
Concluding weight of evidence/proposed action	**…**
Type of information (Critical, supporting, low weight)	**…**
Consideration/concluding score	**…**

B.9.13.5 Assessment of the open literature submitted

The literature on the ecotoxicological effects of glyphosate, the glyphosate salt, its metabolites and diverse commercial formulations was categorized according to 12 areas. The areas of risk assessment were covered for birds, fish, amphibians, aquatic invertebrates, aquatic plants including algae, terrestrial non-target arthropods, soil microbes, plant diseases and plant nutrient status, earthworms and soil macro-organisms, terrestrial non-target plants, the special case of polyethoxylated tallowamines as surfactive additives and endocrine disruption/reproductive toxicity.
For the whole area of ecotoxicological studies, the notifier submitted many studies that were rated category 1 or 2 and were therefore not considered for further analysis. Some studies were labelled category 3 (DocL) but not processed further in the text. Those studies were not categorised in one of the twelve areas of risk assessment and were for this reason not listed in the overview paragraph of each section. The following table gives an overview on the total dataset and the structure of the classification by the notifier.

Table B.9.13-2: Classification of glyphosate related open, peer-reviewed literature that is used by this evaluation according to its submission, citation and revision status. Frequencies of the submitted peer-reviewed data is given.

Category	Notifier relevance, submittance, citation, review status	No. of publications
Category 0: not submitted, but cited in DocM	rel0_nosub_cit	180
Category 0: not submitted and cited in DocM	rel0_nosub_nocit	2
Category 1: submitted and cited but not reviewed in DocM	rel1_sub_cit+norev	6
Category 1: submitted only, not used and reviewed in DocM	rel1_sub_nocit+norev	290
Category 2: Submitted and revied in DocM	rel2_sub_cit+rev	7
Category 2: submitted only, not used and reviewed in DocM	rel2_sub_nocit+norev	106
Category 3: not submitted, but used in DocM and fully evaluated	rel3_nosub_cit+rev	11
Category 3: Submitted and reviewed in DocM	rel3_sub_cit+rev	59
Category 3:	rel3_sub_nocit+norev	14

submitted only, not used and reviewed in DocM		
Official documents provided with the application, thus not object of revision	submit_notifier	19
Total number of studies submitted with the application		**694**

In December 2013, the draft re-assessment report (dRAR) for the active ingredient glyphosate was delivered to the European Food Safety Authority (EFSA). Therefore, the evaluation included publicly available litarature for the period of 2001 to 2011/20012.
EFSA made the summary dossiers publicly available and provided access report submitted by RMS for other Member States and the public in 2014. After the date of submission, during the adminstrative process and the public launching period of the evaluation a few more peer-reviewed relevant articles were newly published (mainly period of (2012-2014) and needed to be considered in a revised DAR. The evaluation of these publications will be presented in the revised RAR according to the above suggested evaluation scheme of (eco)toxicological data in the process of risk assessment. This is by differentiation between:

low weight in risk assessment	(low reliability plus low relevance),
supporting evidence in risk assessment	(high reliability plus low relevance or low reliability plus high relevance),
high weight in risk assessment	(high reliability plus high relevance).

The following sections summarise the results and discussion as found in the published literature. View of the RMS will be given in the final summariesing chapter after the results of the study are presented and discussed.

B.9.13.6 Assessment of the open literature from Non-Governmental Organization (NGO)

In total, over 100 studies on ecological risk assessment were cited and submitted by NGOs. All submitted publications were evaluated by RMS. Out of the submitted publications, over 60 studies were recognized as overlapping with publications submitted by the notifer. Relevant and reliable studies (UBA2) have been described in the above mentioned evaluation table and have been considered for the literature review process.

B.9.13.7 Effects on birds (KIIA 8.16)

All of the four submitted publications regarding the toxicity of glyphosate to birds have been subjected to detailed description and evaluation in the following.

Oliveira et al. (2007)

glyphecotox_001 Birds+DART/ED	**Oliveira, AG, Telles LF, Hess RA, Mahecha GAB,**	**2007**	**Effects of the herbicide Roundup on the epididymal region of drakes *Anas platyrhynchos*.**	**Reprod Toxicol 23 (2):182-91. DOI: 10.1016/j.reprotox.2006.11.004.**

	Oliveira CA			

Reliability	
Purpose of the study Description of endpoints	Test in vivo the hypothesis that glyphosate affects the steroidogenic acute regulatory protein (StAR) and aromatase activities responsible for androgenic and estrogenic hormones in birds
Test compound, application procedure, exposure period	Weights of testes and epididymal regions; morphometric, histochemical analyses (enzymatic activities), immunohistochemical analyses of ductules and ducts (androgen receptor expression) and hormone level analyses (testosterone, estradiol) were performed
Experimental approach, Statistics, test environment	Glyphosate as Roundup with 360 g glyphosate/L; 480 g/L isopropylamine salt, Presumably) daily application of 2 water diluted solutions of 5 and 100 mg/kg bw of Roundup via gavage for 15 days)
Test organisms	*Anas platyrhynchos*
Biological effects	Non-GLP
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	The mallard duck as a standard model species is considered appropriate for the respective research question.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Since the daily doses tested were below the relevant NOELs in standard tests (201 mg ai/kg bw/day) even though statistically significant, effects on the endocrine system and the reproductive tract of male individuals are considered relevant for the population indeed.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The endpoints reflect a broad range of possible deformations of the male reproductive system.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Glyphosate was tested in a Roundup formulation that was not further specified (e.g. surfactants used). The effects could not be assigned to the active ingredient glyphosate alone.
2 Do the tested concentrations relate to predicted environmental concentrations?	PEC_{sw} FOCUS step 1 = 0.101 mg ai/L
3 Have parameters influencing the endpoints been considered adequately?	Environmental parameters and the conditions of animal housing during the experimental period were not mentioned, but the study was conducted under the ethical principles of the researching University.
Concluding weight of evidence/proposed action	The unusual way of exposure for reproductive studies via gavage and not via dietary uptake is criticized by the notifier. For the mallard duck as an aquatic dabbling bird it is assumed acceptable as a worst case exposure scenario.
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Eason, T.H., Scanlon, P.F. (2007)

glyphecotox_358	**Eason, T.H., Scanlon, P.F.**	***2002***	**Effects of Atrazine and Glyphosate ingestion on body weight and nutritional well-being of *Coturnix quail***	**Zeitschrift Fur Jagdwissenschaft 48:281-285**

Reliability	
Purpose of the study Description of endpoints	The aim of this project was to test the effects on a quail species (*Coturnix coturnix japonica*) of ingesting foods treated with Glyphosate. Atrazine was tested in the same experimental design. Body weight, liver weight, body fat content
Test compound, application procedure, exposure period	The procedure of calculating the exposure concentrations is very fiddly and has not been resulted in definite body weight related values. In the summary, concentrations in the food items? Of 347, 1388, and 3470 ppm have been mentioned. The calculations were based on recommended field application rates, according to the supplier's labels, but have never been specified by the authors. The common name of the test compound 'glyphosate' was misspelled as 'glycophosphate' in the German and English summaries.
Experimental approach	12 control quails, 10 male quails used for each of the three treatments.
Test organisms	Adult male, Japanese quail, (*Coturnix japonica*)
Biological effects	No effects of glyphosate reported.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	-/-
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	-/-
3 Is the ecotoxicological manifestation level appropriate for the assessment?	-/-
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	-/-
2 Do the tested concentrations relate to predicted environmental concentrations?	-/-
3 Have parameters influencing the endpoints been considered adequately?	-/-
Concluding weight of evidence/proposed action	The poor quality of the paper suggests that the experimenters had no or minor experience with animal toxicological testing and should have never given the permission to kill in sum 72 vertebrate organisms. The study was not further considered relevant after checking the experimental design.
Type of information (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA3**

Stoleson et al. (2011)

glyphecotox_614	**Stoleson, S.H., Ristau, T.E., deCalesta, D.S., Horsley, S.B.**	**2011**	**Ten-year response of bird communities to an operational herbicide-shelterwood treatment in a northern hardwood forest**	**Forest Ecology and Management 262 (7):1205-1214. DOI: 10.1016/j.foreco.2011.06.017.**

Reliability	
Purpose of the study Description of endpoints	Long-term monitoring study of bird occurrence in a 'Shelterwood system' after seed cut and herbicide application (a silvicultural system in which overstory trees are removed in a series of cuts designed to achieve a new, even-aged stand under the shelter of remaining trees, http://en.wikipedia.org/wiki/Shelterwood_cutting). Fixed-radius point counts of birds at two points per plot: overall abundance, abundance of migratory guilds, nesting guilds, vegetation cover, avian community similarity

Test compound, application procedure, exposure period	Herbicide-treated plots were sprayed with a tank mix containing 364 ml glyphosate (Accord®) and 24 ml sulfometuron methyl (Oust ®) in 38 L water per ha
Experimental approach, Statistics, test environment	Repeated measures randomized split-plot experimental design, half of each of 10 plots was treated once with herbicide in August of 1994, remaining 5 plots as controls Time-series between 1992-1994 (pre-treatment period) and 1994-2004 (post-treatment period) **Statistics:** Generalized linear mixed models to model the effects of year, site, herbicide treatment and cutting sequence on vegetation and avian target variables. Site as a random effect, and year, herbicide treatment, and cutting sequence as fixed effects. Shannon Evenness scores were modelled using a Beta distribution and a logit link function, other diversity indices with a Gaussian distribution and identity link, vegetation cover modelled using a lognormal distribution and identity link, whereas bird abundances were modelled using a Poisson distribution and a log link function (Littell et al., 2006). maximum-likelihood (REML) method and the Kenward–Roger procedure to adjust the denominator degrees of freedom Post-hoc tests to identify years with significant differences between control and experimental treatments were conducted using Tukey–Kramer tests Multiple regression analyses to determine the effects of understory vegetation variables on the abundance of ground and shrub birds, and the effects of overall bird abundance and time since treatment on the similarity of avian communities pre- and post-treatment. We used analysis of similarities to test the null hypothesis that avian community structure did not differ significantly between herbicide and control plots.
Test organisms	Naturally occurring North American bird species, vegetation
Biological effects	Long-term monitoring study of bird occurrence in a 'Shelterwood system' after seed cut and herbicide application (a silvicultural system in which overstory trees are removed in a series of cuts designed to achieve a new, even-aged stand under the shelter of remaining trees, http://en.wikipedia.org/wiki/Shelterwood_cutting). Fixed-radius point counts of birds at two points per plot: overall abundance, abundance of migratory guilds, nesting guilds, vegetation cover, avian community similarity
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	The 'natural' composition of plant and bird species at all life stages has been analysed. This level of complexity is highly appropriate for ERA.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	-/-
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Endpoints refer to population and ecosystem level effects.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Since a tank mix has been tested consisting of a mixture of glyphosate and sulfometuron methyl, the effects could not be assigned to a single substance. The toxicity of the mixture used can be estimated assuming a 'concentration addition model', probably leading to glyphosate as the determining factor (*analysis not conducted within the scope of this survey*).
2 Do the tested concentrations relate to predicted environmental concentrations?	Yes, the tank mixture was applied at recommended application rates.
3 Have parameters influencing the endpoints been considered adequately?	The statistical design (GLM) included time, herbicide treatment, site, cutting sequence as explanatory variables in a model; so ecologically potential influencing factors in this design have been adequately considered.

Concluding weight of evidence/proposed action	The silvicultural practice of shelterwood systems and thus the experimental approach is quite specific for North American practices and not transferable to European agricultural practices. Nevertheless, the authors describe an impressive example of indirect effects of herbicides on 'ecosystem level' and the complexity of an assessment that is not covered by 'standard ERA' by far. The transferability is further restricted by the use of a tank mixture of two herbicides. The study is recommended to be considered as an example of indirect effects and profound statistical analysis of monitoring data.
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Sullivan, T.P., Sullivan, D.S. (2003)

glyphecotox_615	**Sullivan, T.P., Sullivan, D.S.**	**2003**	**Vegetation management and ecosystem disturbance: impact of glyphosate herbicide on plant and animal diversity in terrestrial systems**	**Environ. Rev. 11 (1):37-59. DOI: 10.1139/a03-005.**

Reliability	
Purpose of the study Description of endpoints	Comprehensive review on effects of Glyphosate on the biodiversity of NTP, NTA, birds and mammals under the general assumption of a relatively non-harmful environmental impact of the substance within vegetation management practices Species abundance, numbers (richness) and diversity indices (Shannon)
Test compound, application procedure, exposure period	Not possible to describe in detail, since the study is a literature compilation, not an original experimental work <u>Application rates</u>: Forest ecosystems: between 1.1 and 3.3 kg Glyphosate/ha once a year Agriculture/Wetland: variable dose-rates, nor further specified by the authors
Experimental approach, Statistics, test environment	Considered only: Measures within 'vegetation control' or 'vegetation management' programs for enhancing crop production (not the same as weed control in European countries) Peer-reviewed journal publications describing studies on vascular plants, small mammals, large mammalian herbivores, terrestrial invertebrates in forests or agricultural landscapes. Considered here: **findings on birds** Studies must provide numbers and composition of species (for richness estimation) in terrestrial ecosystems For birds, 7 studies have been analysed, the total number of replicate situations was 10 for statistical comparisons. Effects were given mainly in relative changes compared to the pre-treatment period and between control and treatment
Test organisms	Birds (songbirds and waterfowl)
Biological effects	Short-term (mainly first year after application) effects on species numbers (decline) and abundance (increase), dominance of most common species (increase) Over the whole study periods, most effects disappeared In total: Very small differences between controls and treatments over several years of the studies
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Communities of naturally occurring bird species in field monitoring studies have been assessed over 2-4 years, which could be ecologically highly relevant.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Since the methodology was not described in detail for each of the studies the statistical significance could not be judged. The studies were conducted on population level and could therefore considered relevant on this particular level of organisation
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Population changes over time is amongst the highest possible levels of manifestation
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The test substances were not uniform and not described in more detail than the mere mentioning of 'glyphosate' as the test substance.
2 Do the tested concentrations relate to predicted environmental concentrations?	Yes, because recommended field rates have been tested.
3 Have parameters influencing the endpoints been considered adequately?	-/-
Concluding weight of evidence/proposed action	The paper deals with the impact of the Anglo-Saxon practice of managing the vegetation for purposes of enhancing forest and other crop yields. This includes especially the control of roadside vegetation and intends the maintenance of ecological processes in terrestrial ecosystems. However, the review shows the transiency and indirectness of effects of Glyphosate treatments on the biodiversity of birds, most probably mediated by ephemeral changes of the (shrub) vegetation.
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Santillo et al. (1989)

BVL Nr. 2716490	**Santillo, D.J., Brown, P.W., Leslie Jr., D.M.**	**1989**	**Response of songbirds to glyphosate-induced habitat changes on clearcuts**	**J, WILDL_ MANAGE, 53(1):64-7 1**

Reliability	
Purpose of the study Description of endpoints	Examination of breeding bird populations and habitats on glyphosate (Roundup)-treated and untreated c1earcuts in northcentral Maine.
Test compound, application procedure, exposure period	All herbicide treatments we re g lyphosate applied by he licopter at a rate of 4.7 L active ingredient/4 2.1 L water/ha;
Experimental approach, Statistics, test environment	Two clearcu ts previously treated with herbicides and 4 untreated clea rc uts were selected to provide a 3-year range of posttreatment effects during our 2-year study; We censused breeding birds with the spotmap method (Williams 1936) and delineated territories according to standards of the International Bird Census Committee (1970).
Test organisms	Foliageg leaning insectivores (including warblers (Parulidae)) and ground-gleaning omnivores (including sparrows and darkeyed junco).
Biological effects	Treatment of clearcuts with glyphosate herbicide reduced the complexity of vegetation through 3 years posttreatment compared to untreated clearcuts. Total numbers of birds, common yellowthroats, sparrows and alder flycatchers were less abundant ($P < 0.05$) on treated clearcuts than on untreated clearcuts.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation
2 Do the tested concentrations relate to predicted environmental concentrations?	Yes, because recommended field rates have been tested.
3 Have parameters influencing the endpoints been considered adequately?	-/-
Concluding weight of evidence/proposed action	Authors state that leaving untreated palches of vegetation and staggering herbicide treatments on large clearcuts will maintain bird populations similar to those on untreated clearcuts. RMS emphasiszes that herbicides cause indirect effects and highlights the need for risk mitigation measures by the Member States, proposing compensation measures as a suitable tool.
Type of information (Critical, supporting, low weight)	**Critical**
Consideration/concluding score	**UBA1**

B.9.13 7.1 Summary of the relevant literature on birds

There was no critical data that must imperatively be included in an environmental risk assessment for the active substance glyphosate. It was not possible to distinguish between the effect of the technical glyphosate and the surface-active substances added to the commercial formulations by the experimental designs used. Two publications gave clues on indirect effects of glyphosate use on the biodiversity of birds on a regional scale in a long-term by subtle alterations of the vegetation structure. RMS emphasises that herbicides cause indirect effects and highlights the need for risk mitigation measures by the Member States, proposing compensation measures as a suitable tool.

B.9.13.8 Effects on other terrestrial vertebrates

Santillo et al. (1989)

BVL Nr. 2716491	**Santillo, D.J., Brown, P.W., Leslie Jr., D.M.**	**1989**	**Response of small mammals and habitat to glyphosate application on clearcuts**	**J, WILDL. MANAGE, 53(1):164-172**
Reliability				
Purpose of the study Description of endpoints	Authors examined effects of herbicide induced habitat changes on small mammals in northcentral Maine.			
Test compound, application procedure, exposure period	All herbicide treatments we re g lyphosate applied by he licopter at a rate of 4.7 L active ingredient/4 2.1 L water/ha;			
Experimental approach, Statistics, test environment	clearcuts previously treated with herbicides and 4 untreated clea rc uts were selected to provide a 3-year range of posttreatment effects during our 2-year study; Sampling of small mammals by removal trapping.			
Test organisms	Invertebrates as well as shrews, mouses and voles.			

Biological effects	Insectivorous comprised 72% os small mammal caputres and were less anundant for all 3 years post treatment. Herbivorous were less abundant and 2 years post treatment. Omnivorous were equally abundant on treated and untreated clearcuts.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation
2 Do the tested concentrations relate to predicted environmental concentrations?	Yes, because recommended field rates have been tested.
3 Have parameters influencing the endpoints been considered adequately?	-/-
Concluding weight of evidence/proposed action	RMS emphasiszes that herbicides cause indirect effects and highlights the need for risk mitigation measures by the Member States, proposing compensation measures as a suitable tool.
Type of information (Critical, supporting, low weight)	**Critical**
Consideration/concluding score	**UBA1**

B.9.13 8.1 Summary of the relevant literature on terrestrial vertebrates

Please refer to sections regarding amphibians and to the summary of the relevant literature on surface active substances in glyphosate-based formulations (Vol. 3; chapters B.9.9.2 and B.9.11).

B.9.13.9 Effects on aquatic organisms

The following sections summarise the results and discussion as found in the published literature. RMS assessment is given in the final summarising chapter after the results of the studies are presented and discussed.

B.9.13 9.1 Fish (KIIA 8.16)

Filizadeh, Y., Islami, H.R. (2011)

glyphecotox_007	**Filizadeh, Y., Islami, H.R.**	**2011**	**Toxicity determination of three sturgeon species exposed to glyphosate**	**Iranian Journal of Fisheries Sciences 10 (3):383-392**

Reliability	
Purpose of the study Description of endpoints	Determination of the acute toxicity (LC_{50}) of glyphosate formulation towards three juvenile sturgeon species; endpoints. mortality, swimming behaviour (not analysed).
Test compound, application procedure, exposure period	Roundup formulation (not further specified) with 41 weight % Glyphosate, content of POEA not specified; Non-GLP, protocol resembles acute fish toxicity testing after guideline OECD 203.
Experimental approach, Statistics, test environment	Dose-response study, 10 doses between 10 and 100 mg a.i./L, irregular spacing, non-geometric series; three treatment replicates with 8 fishes each; mortality was recorded after 6, 12, 24, 48, 96, 168 hours; static exposure in 100L replicate tanks Finney's Probit regression and 95% confidence limits for derivation of LC_{50}; comparison between species by One-way ANOVA and Tukey's post-hoc test, data tested for normality by Kolmogorov-Smirnov-test; Fish were fed daily.
Test organisms	*Huso huso, Acipenser stellatus, Acipenser persicus*
Biological effects	LC_{50} between 70-74 mg glyphosate/L after 6 h and 8-137 mg/L after 168 h of exposure; reference to acute studies is the value after 96h: between 20 and 26 mg a.i./L; differences in the sensitivities of the three species 96 and 168h after exposure (ANOVA); no mortality in control groups.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	The test species are considered of temperate to sub-tropical origin and generally as suitable for an indication of intrinsic sensitivity as the standard species in ERA. The juveniles could be considered as most sensitive stages.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	-/-
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Since mortality was assessed by the study, the biological level of assessment is appropriate for population level effects.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	This could not be judged because a detailed description of the test item, i.e. proportion of surfactant in the respective formulation is unknown.
2 Do the tested concentrations relate to predicted environmental concentrations?	The predicted environmental concentration of the application applied by the notifier was about 0.1 mg a.i./L and thus far below the concentrations tested here.
3 Have parameters influencing the endpoints been considered adequately?	The deviations in oxygen, ammonia and nitrite were rather high, but it was not indicated how the SD was calculated (all systems, only controls?). If differences in water parameters occurred treatment related, this could cause effects beyond the toxic effects of Glyphosate.

Concluding weight of evidence/proposed action	**The description of the study is deficient; however, the LC_{50}'s 96 hours after exposure (20-26 mg a.i./L) are below the acute studies provided by the notifier and are located rather near the chronic toxicity of a full life cycle test (25.7 mg/L). Because the content of POEA that is usually grossly determining the toxicity of Roundup formulations was not stated by the authors, the study could not be taken into account as additional or critical information on the ERA of the active substance glyphosate.**
Type of information (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA3**

Guilherme et al. (2010)

glyphecotox_008	**Guilherme, S., Gaivão, I., Santos, M.A., Pacheco, M.**	**2010**	**European eel (*Anguilla anguilla*) genotoxic and pro-oxidant responses following short-term exposure to Roundup® a glyphosate-based herbicide.**	**Archives of Environmental Contamination and Toxicology 62 (1):107-117. DOI: 10.1007/s00244-011-9686-7.**

Reliability	
Purpose of the study Description of endpoints	Description of genotoxicity and oxidative stress indicating endpoints in fish at environmentally relevant concentrations after short term exposure of 1 and 3 days Genotoxicity: Comet assay: strand breaks, alkali labile sites expressed in a Genetic Damage Index (GDI) ; ENA - erythrocytic nuclear abnormalities: irreparable lesions Oxidative stress by: catalase activity, glutathione-S-transferase, glutathione peroxidase and glutathione reductase) and non-enzymatic (total glutathione content) antioxidants, lipid peroxidation (LPO).
Test compound, application procedure, exposure period	Glyphosate as Roundup with isopropylammonium-salt 485 g/L (360 g/L = 30.8 % a.i.) and 16 % POEA as surfactant. Exposure for 1 and 3 days. Application procedure not described in detail. Non-GLP, but procedures were well described and referenced to other peer-reviewed protocols, sounds reliable.
Experimental approach, Statistics, test environment	Static exposure for 1 and 3 days of 6 fishes in each of six 20L aquaria; divided into 2 treatment replicates for control, 58 µg Roundup/L (equals 18 µg glyphosate/L) and 116 µg Roundup/L (36 µg glyphosate/L).
Test organisms	European eel *Anguilla anguilla*, average length 25 cm, average weight 32 g
Biological effects	Increasing DNA damage with increasing exposure time and glyphosate concentration was measured in the Comet assay; for ENA more pronounced effects after 3-days exposure; no oxidative stress was recorded.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	The European eel is a species that can be considered both to water and sediment phase of cold and warm temperate environments, and is thus most suitable to cover worst-case exposure scenarios.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	The true replication of the experiment was poor in fact. There were 6 pseudoreplicate fish that should be taken as averages for further statistical evaluation. It was not mentioned clearly but suspected that 1 aquarium equalled the true treatment replicates and the 6 replicate fish per treatment have been used for statistics as pseudo-replicates. The replication was not considered independent. Hence, it was not possible to judge the reliability of the data analyses.
3 Is the ecotoxicological manifestation level appropriate for the	The endpoints measured can be taken as early warning indicators of genotoxic and oxidative stress at the individual level but could not be used for the risk assessment for populations of eels and other temperate fishes in a real environment.

assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation containing POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	According to the paper at hand (max. PEC surface water 677 µg/L) and the RA of the notifier of 101 µg/L in a FOCUS step 1 scenario, the concentrations are quite realistic.
3 Have parameters influencing the endpoints been considered adequately?	No, environmental parameters were not measured explicitly during the experimental period.
Concluding weight of evidence/proposed action	The study is well conducted, except for the statistical evaluation. Regardless, the results can be taken as an indicator of genotoxic but not of oxidative stress effects of realistically low water concentrations in fish. Study describes physiological parameters, no mortality endpoints are stated. Micronuclei test (MN) and erythrocytic nuclear abnormalities (ENA) results did not significantly differ between studied treatments and control.Comet assay: significant effects indicating DNA damage in erythrocytes at concentrations of 58 and 116 µg/L. This paper reports physiological studies with a commercial formulation, which could not be taken into account as critical information for the assessment of the active substance glyphosate itself. Nevertheless it shows that commercial formulations of glyphosate might have elicit effects.
Type of information (Critical, supporting, low weight)	**supporting information**
Consideration/concluding score	**UBA2**

Hued, A.C. et al. (2012)

glyphecotox_009 Fish	**Hued, A.C., Oberhofer, S., de los Ángeles Bistoni, M.**	**2012**	**Exposure to a Commercial Glyphosate Formulation (Roundup) Alters Normal Gill and Liver Histology and Affects Male Sexual Activity of *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes)**	**Archives of Environmental Contamination and Toxicology 62 (1):107-117. DOI: 10.1007/s00244-011-9686-7.**

Reliability	
Purpose of the study Description of endpoints	Histological lesions of the neotropical, South-American fish species *Jenynsia multidentata* after acute and subchronic exposure to sublethal concentrations of Glyphosate. LC_{50} after 96h, male sexual activity after 7d and 28d of exposure, gill and liver histopathological analyses after termination of toxicity experiments; scores from 0-6 alterations to describe the degree of histological findings.
Test compound, application procedure, exposure period	Roundup Max granular (Monsanto, Argentina), containing 74.7 % Glyphosate and 25.3 % surfactants (presumably POEA). Application procedure is not described in detail. Static exposure for 96h, 7d and 28d Non-GLP, but procedures used are well documented and referenced in the literature cited.
Experimental approach, Statistics, test environment	Short-term testing: static test, nominal 5, 10, 20, 35, 60, and 100 mg Roundup/L for 96 h, duplicates of control and treatment groups, 4 male, 4 female fishes per duplicate. Subchronic testing: 0.5 mg Roundup/L of two groups of 9 individuals (5 male, 4

	female) for 7 and 28 days No clear indication if the duplicates of the treatments have been the replicates for statistics, or if the replicate fishes have been taken for the testing procedures and for the calculation of the importance indices.
Test organisms	*Jenynsia multidentata* (onesided livebearer) male and female fish standard lengths (means ± SDs) were 36.34 ± 4.16 and 43.71 ± 7.46 mm, respectively. The mean weight was 0.58 ± 0.21 g for male fish and 1.12 ± 0.5 g for female fish.
Biological effects	LC_{50} (96h)= 19.02 mg Roundup/L = 14.2 mg a.i./L; subchronic exposure caused significantly lower numbers of copulations per male, similarly for 7d and 28d exposure. Several dose-dependent pathological alterations of gill and liver histology in the acute tests, for the subchronic testing the effects were more pronounced in the 28d-exposure group; since the single histological endpoints did not show unambiguous results, the total histopathogical index showed significant dependent effects at 0.5 mg Roundup/L (equals 0.37 mg a.i./L)
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	The species has a special mode of reproduction, as being sexually dimorphic (male differentiate a gonopodium if needed) and livebearers. It could be seen as a well suited model for sexual behaviour of males as claimed by the authors, but the species is very unlikely a taxonomically and behaviourally representative of temperate European fish species. It is therefore considered as not relevant for ERA.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The histological endpoints ‘gill’ and ‘liver’ measured could serve as indicators of general individual vitality (stress level) of an organism and its reproduction fitness. It has been shown that the sexual system of males was affected. This could have severe effects on the stability of a fish population on the long term.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Although not specified precisely, the tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	Most of the concentrations tested in the acute testing exceeded environmentally concentrations by far. The concentration of 500 µg/L tested in the subchronic test resemble real measured concentrations of surface waters.
3 Have parameters influencing the endpoints been considered adequately?	The environmental parameters have been held constant, the light cycle was 12:12 hours light/dark. There was no measurement of e.g. water quality parameters that could cause additional stress concealing toxicant effects.
Concluding weight of evidence/proposed action	**There are some obscurities in the description of the statistics and the test substance. The study could be taken as a further source of information that realistic concentrations of Glyphosate in surface waters could have a pronounced long-term effect on the populations of fishes. It is not distinguishable if the effect on the endpoints was due to the active ingredient glyphosate or (more likely) to the surfactant that was contained at 15.3 % in the formulation.**
Type of information (Critical, supporting, low weight)	**supporting information**

Consideration/concluding score	**UBA2**

Kelly et al. (2010)

glyphecotox_010 Fish	**Kelly, D.W., Poulin, R., Tompkins, D.M., Townsend, C.R.**	**2010**	**Synergistic effects of glyphosate formulation and parasite infection on fish malformations and survival**	**Journal of Applied Ecology Volume: 47 Issue: 2 Pages: 498-504 Url: http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2664.2010.01791.x/pdf DOI: 10.1111/j.1365-2664.2010.01791.x ISSN: 1365-2664 (online)**

Reliability	
Purpose of the study Description of endpoints	Synergistic effects of multiple stressors, i.e. the combined effect of glyphosate and trematode parasites *Telogaster opisthorchis* on juvenile *Galaxias anomalus* freshwater fish (only the 1st of two independent experiments is evaluated here). Survival and spinal deformation of juvenile fish
Test compound, application procedure, exposure period	Glyphosate 360 (commercial formulation, Ravensdown, New Zealand), 360 mg a.i/L, 10-20 % POEA surfactant Infective trematodes *Telogaster opisthorchis* were provided via the intermediate host *Potamopyrgus antipodarum* snails. Non-GLP
Experimental approach, Statistics, test environment	The toxic potential of the herbicide glyphosate is assumed to enhance the disastrous effect of parasite infections: Experiment combined 4 treatments, 8-fold replicated in 32 aquaria, each equipped with 4 fish: 1) Controls: No parasite, no glyphosate, 2) parasite, no glyphosate, 3) No parasite, 0.36 mg glyphosate/L, 4) Parasite, 0.36 mg glyphosate/L Data tested on significance by first log10 or square root transforming and then applying ANOVA procedure followed by 'Fisher's protected least significant difference (FPLSD).
Test organisms	Juvenile *Galaxias anomalus* freshwater fish, *Telogaster opisthorchis* trematode infection mediated by infected host snails *Potamopyrgus antipodarum*.
Biological effects	No difference in survival for herbicide and parasite treatments alone, but for the combination treatment 4); spinal deformations were more frequent in parasitized fish treatment 2) and in the combination 4).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes, or rather there is no indication that juvenile stages of New Zealand freshwater fish should be less suitable for ERA than others are.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	The effects described in this study regarding the synergistic effect were clear.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Survival and spinal deformation are relevant endpoints.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The conclusion from this study is only valid for glyphosate formulations that contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	Yes, 0.36 mg a.i./L it could be seen as the ‚upper edge' of the distribution of possible concentrations in real surface waters.
3 Have parameters influencing the endpoints been considered adequately?	No, parameters of water quality that could cause further stress have not been regarded by the authors.
Concluding weight of evidence/proposed action	**The experiment shows a general mechanism of the ecotoxicological theory: many multiple stressors act additively on the endpoints observed; Study is of limited value for ERA because the factors are not considered separately and safety factors should cover uncertainties caused by synergisms or**

	enhanced toxicity.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Salbego et al. (2010)

glyphecotox_020 Fish	**Salbego, J., Pretto, A., Gioda, C.R., de Menezes, C.C., Lazzari, R., Radunz Neto, J., Baldisserotto, B., Loro, V.L.**	**2010**	**Herbicide formulation with glyphosate affects growth, acetylcholinesterase activity, and metabolic and hematological parameters in piava (Leporinus obtusidens)**	**Arch Environ Contam Toxicol 58 (3):740-5. DOI: 10.1007/s00244-009-9464-y.**

Reliability	
Purpose of the study Description of endpoints	Effects of long-term glyphosate exposure on growth, Acetylcholinesterase activity and various metabolic and hematological endpoints in the omnivorous fish *Leporinus obtusidens* (piava). Weight gain, condition factor (weight * length^{-3}), daily food consumption Hematocrit, haemoglobin, total erythrocyte counts, total leukocyte counts and blood protein from blood samples, liver and muscle glycogen, tissue protein, Acetylcholinesterase activity from homogenates of brain and muscles
Test compound, application procedure, exposure period	Glyphosate as the isopropylamine salt in the commercial formulation ‚Roundup' that contained 48% of the acid equivalent of the salt; content of POEA in the formulation not stated. Exposure time 90 days. Water renewal conditions: every 4 days water exchange, water conc. were followed for 8 days by chemical analysis of glyphosate and the main metabolite AMPA: remained nearly constant over the test period of 8 days to check for appropriate exposure, it is questionable if the measurements were realistic because of identical parent and metabolite concentrations.
Experimental approach, Statistics, test environment	2 replicate 250 L tanks for the control, 1 mg Roundup/L, 5 mg Roundup/L treatments; 30 fish per tank, 180 fish in sum. Weight and length measurements of 15 individuals per tank at days 30 and 60, 90 days after start of the experiment measurement of the remaining individuals. Blood samples of 8 ind. at day 90. No indication of how many ind. were sampled for brain and liver tissues Multiple Comparison by ANOVA followed by Tukey's post-hoc test, prior test for homogeneity of variances.
Test organisms	Omnivorous fish *Leporinus obtusidens* (Piva)
Biological effects	No mortality, no effects on condition factor and daily food consumption but significantly reduced, dose- and time dependent weight and length gains over the whole experimental period recorded. Significant effects have been observed at the lower concentration of 1 mg Roundup/L (equals 0.48 mg a.e. of the salt/L). Most metabolic and hematologic endpoints showed significantly reduced or enhanced parameters for both of the concentrations tested.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	This subtropical fish species is indigenous for few rivers in Brazil and thus could not be seen as a good representative for general ecotoxicological effect assessment.
2 Is the magnitude of effects of significance	Most endpoints showed significant effects around the NOEC of glyphosate for Zebrafish (43.2 mg a.i./L).

to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The authors propose to take enzymatic activities and the hematological parameters as indicators for exposure to glyphosate rather than as assessment endpoints. It remained unclear whether the endpoints measured could be taken as good indicators of the individual fitness of a fish, which would affect the population integrity in the end.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	There was some confusion and obscurities regarding the indication of the test substance as glyphosate or Roundup in the text.
2 Do the tested concentrations relate to predicted environmental concentrations?	Glyphosate concentrations exceed expectable surface water conc. by far (at least Factor 10) as reported by the paper.
3 Have parameters influencing the endpoints been considered adequately?	No
Concluding weight of evidence/proposed action	**Physiological endpoints measured do not contribute to an ERA and the species is not representative for common protection goals. There was no indication of the percentage content of tallow amine surfactants within the Roundup formulation tested.**
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Tierney et al. (2006)

glyphecotox_022 Fish	**Tierney, K.B., Ross, P.S., Jarrard, H.E., Delaney, K.R., Kennedy, C.J.**	**2006**	**Changes in juvenile coho salmon electro-olfactogram during and after short-term exposure to current-use pesticides**	**Environ Toxicol Chem 25 (10):2809-17**

Reliability	
Purpose of the study Description of endpoints	Effects of glyphosate on the olfactory sense of the coho salmon by recording the electro-olfactogram after exposure to an odorant Inhibition of the field potential of olfactory sensory neurons measured as the EOG peak amplitude size (in mV), determination of a median inhibitory concentration (IC50) The study tested six pesticides in a joint approach, thus the controls of each 3 compounds have been pooled for strengthen the statistical analysis.
Test compound, application procedure, exposure period	Glyphosate technical (purity 99 %) was directly added to the test aquaria, concentrations of 0.1, 1, 10, 100 mg a.i./L were chosen well below the LC50 of 22 mg a.i./L for the Coho salmon because the authors assume an enhanced effect of the usually added surfactants (e.g. POEA) Exposure for 30 minutes, post exposure 60 minutes.
Experimental approach, Statistics, test environment	Fish were fixed in a water flow-through system and exposed to the test compound and olfactorily stimulated by 2 second-pulses of L-serine as the odorant. Measurements at 2, 5, 10, 15, 20, 25, and 30 min during exposure; at 2, 5, 10, 15, 20, 30, 40, 50, and 60 min post-exposure. Pooled controls of 3 pesticides, N = 18; N = 6 for each of the treatments Differences between treatments: Two-way (time and treatment), repeated-measures analysis of variance followed by a Holm-Sidak post hoc test EOG response curves (% pre-exposure potential) were fitted to a three-

	parameter exponential decay model.
Test organisms	Coho salmons (Canada), *Oncorhynchus kisutch*
Biological effects	Significant drops of the EOG occurred at 1 mg a.i./L that is far below the LC50 for the Coho salmon. The IC50 was 10.9 mg/L (95% CI, 6.72–16.8 mg/L) 2 min after the exposure.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	The study was very thoroughly designed and conducted. Despite the fact that no standardised guideline was followed, the results appear to be reliable. There was a relatively high number of replicates and the findings are emphasised by analysing time series, which allows for an assessment of the persistence of an effect.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The authors state the main importance of the olfactory sense for the salmons survival. It is not discussed if the extent and duration of a reduction of the EOG may have ecological consequences to natural populations of salmons. The experimental assembly was very artificial and could cause stress whilst preventing behavioural responses of the fish (e.g. avoidance of exposure). The transferability into realistic scenarios seems to be difficult.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Glyphosate was tested as the technical substance, bearing in mind the enhanced toxicity of formulations containing surfactants such as POEA. The results are considered relevant for ERA
2 Do the tested concentrations relate to predicted environmental concentrations?	It relates more to the standard toxicity endpoints than to environmental concentrations.
3 Have parameters influencing the endpoints been considered adequately?	-/-
Concluding weight of evidence/proposed action	**Very interesting and well conducted study with a comprehensive description of the experimental design and statistics. The endpoints are considered ecologically relevant, but the validity and relevance are lowered by the artificial design.**
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2**

Soso et al. (2007)

glyphecotox_021 Fish	**Soso, A.B., Barcellos, L.J.G., Ranzani-Paiva, M.J., Kreutz, L.C., Quevedo, R.M., Anziliero, D., Lima, M., da Silva, L.B., Ritter. F., Bedin, A.C., Finco, J.A.**	**2007**	**Chronic exposure to sub-lethal concentration of a glyphosate-based herbicide alters hormone profiles and affects reproduction of female Jundia (Rhamdia quelen)**	**Environmental Toxicology and Pharmacology 23 (3):308-313. DOI 10.1016/j.etap.2006.11.008.**
Reliability				
Purpose of the study Description of endpoints	Description of the effect of a glyphosate based herbicide on hormones of female *Rhamdia quelen*. Gonado-somatic index (GSI) and liver-somatic index (LSI); Cortisol (F), 17 –beta-estradiol (E2) and testosterone (T) concentrations; swim-up fry production; liver			

	enzymes AST (aspartate aminotransferase), ALT (alanine aminotransferase)
Test compound, application procedure, exposure period	Roundup WG, 640 g a.i./kg powder weight, reapplied every 9th day of the experimental period, Renewal experiment, Water concentration: 3.6 mg a.i./L
Experimental approach, Statistics, test environment	8 females/treatment and sampling date were sampled prior to glyphosate application and at 1, 10, 20, 30 and 40 days following exposure. Student's t-test or ANOVA followed by Tukey's multiple range test
Test organisms	Adult Jundia, *Rhamdia quelen*, a South American catfish, 400-600 g body weight
Biological effects	No significant differences between control and treatment groups for both GSI and LSI at any of the sampling dates; slightly higher concentrations of cortisol at day 20 and 40, lower conc. of estradiol at day 40, no differences in testosterone levels, fertility parameters were only significantly lowered in the treatment group for the endpoint 'transferred swim-up fry'.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes, as reproduction parameters have been tested.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	The cortisol levels of the treatment group have been slightly higher from the start of the experiment. The authors argue that fish have been generally stressed by the experimental environment. This could lead to non-representative responses to additional stress events (such as toxic, chemical stressors). The very indiscernible or inconsistent effects might be of minor ecological meaning.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Since not only biochemical endpoints as indicators have been measured but also the realised reproduction rate as the number of viable fry has been measured, a comparison of different endpoints and thus an appropriate assessment is possible.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Except the indication that the glyphosate formulation consists of ‚water-dispersible granules' (WG), no further specification of the test substance was made
2 Do the tested concentrations relate to predicted environmental concentrations?	The single concentration used is far above expectable concentrations in the environment (roughly around 500 µg a.i./L).
3 Have parameters influencing the endpoints been considered adequately?	No
Concluding weight of evidence/proposed action	The design of the study as a limit-test makes the interpretation of the tendencies and results difficult. The study has shown slight effects at elevated concentrations. An extrapolation to lower doses would most probably not reveal significant effects, in the given statistical design. The POEA content of the formulation was not stated.
Type of information (Critical, supporting, low weight)	supporting
Consideration/concluding score	UBA2

De Menezes et al. (2011)

glyphecotox_338 Fish	**De Menezes, C.C., da Fonseca, M.B., Loro, V.L., Santi, A., Cattaneo, R., Clasen, B., Pretto, A.,**	**2011**	**Roundup Effects on Oxidative Stress Parameters and Recovery Pattern of**	**Archives of Environmental Contamination and Toxicology 60 (4):665-671. DOI:**

	Morsch, V.M.		***Rhamdia quelen***	**10.1007/s00244-010-9574-6.**

Reliability	
Purpose of the study Description of endpoints	The impact of the herbicide Glyphosate in the commercial formulation Roundup was tested on enzyme biomarkers in tissues of the juvenile silver catfish (*Rhamdia quelen*). Lipid peroxidation (LPO, thiobarbituric acid reactive species assay), Protein carbonylation both in liver, brain and muscle tissues; Oxidative stress by Catalase enzymatic activity, by Superoxide dismutase activity, by Glutathion S-transferase levels, by nonenzymatic antioxidants (ascorbic acid, nonprotein thiols).
Test compound, application procedure, exposure period	Glyphosate as 'Roundup 48%' (control, 0.45 mg a.i./L, 0.95 mg a.i/L), containing POEA as surfactant; After 4 days, 50% water renewal an new application of a.i. to maintain exposure levels (amount not specified, but measured concentrations prove the sufficient exposure).
Experimental approach, Statistics, test environment	Exposure for 8 days, recovery period in clean water for further 8 days 3 treatments, 2 replicate 250 L tanks, 8 fish per tank; Two-way ANOVA, followed by Tukey´s post-hoc test, N = 8 was taken as the replication in statistical tests
Test organisms	Juvenile *Rhamdia quelen* fish (mean 20 g weight, 11 cm length) from aquaculture
Biological effects	Oxidative stress markers, as Lipid peroxidation and protein carbonyl levels, were significantly affected in most tissues after the exposure period, but returned to the control level after the recovery period. Data for 5 antioxidant endpoints showed only very few deviations from control, this was during the exposure and the recovery period (e.g. GST decreased during exposure and increased after recovery periods).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes, since juveniles are often recognized as the most sensitive life-stages of fish species towards chemical stressors.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Overall, there was little indication of significant responses towards the stressor 'Roundup48'. The experimental period was relatively short-termed (acute to sub-chronic test design), and thus a population relevant effect could not be extrapolated from the results of this experiment.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Principally, biomarkers of stress could be taken as general indicators of toxic action of a test compound that could have an effect at higher levels of organisation, e.g. population level. The degree of uncertainty for the extrapolation to population level, which is relevant for ERA is unknown. The results could be taken as a hint that the substance is detoxified by the test organisms, which could lead to highly reactive Oxygen species.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The test substance was Roundup containing POEA. The surfactants are likely to cause a significant portion of the observed effects
2 Do the tested concentrations relate to predicted environmental concentrations?	The concentrations tested were far above expectable in the environment but at the suppliers' recommended concentrations in flooded rice cultures in tropical regions. The concentrations within the ERA in Europe are derived from non-rice cultures and thus are not transferable without further considerations to the tested scenario.
3 Have parameters influencing the endpoints been considered adequately?	None
Concluding weight of evidence/proposed action	There was little indication of adverse effects of the tested formulation on biomarker concentrations in different tissues of *Rhamdia quelen* at elevated concentrations

	compared to predicted environmental concentrations after spray application of Glyphosate. The findings support the classification of Glyphosate even in a formulation containing the potentially more toxic POEA as non to moderately toxic towards fish. Nevertheless, it cannot be distinguished between the effect of POEA and glyphosate.
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2**

Kreutz et al. (2011)

glyphecotox_434 Fish	**Kreutz, L.C., Barcellos, L.J.G., de Faria Valle, S., de Oliveira Silva, T., Anziliero, D., dos Santos, E.D., Pivato, M., Zanatta, R.**	**2011**	**Altered haematological and immunological parameters in silver catfish (*Rhamdia quelen*) following short term exposure to sublethal concentration of glyphosate**	**Fish Shellfish Immunol 29 (4):694-7. DOI: 10.1016/j.fsi.2010 .06.003.**

Reliability	
Purpose of the study Description of endpoints	The impact of sublethal doses of glyphosate was tested on haematological and immunological responses of Silver catfish fingerlings. Number of erythrocytes, lymphocytes, thrombocytes, total leukocytes, immature circulating cells, phagocytic index of coelomic cells, lysozyme, total peroxidase, bacteria agglutination, bactericidal activity, haemolytic activity, in serum.
Test compound, application procedure, exposure period	commercial available glyphosate (N-phosphonomethyl glycine, 360 mg/L) 10% of the LC_{50} after 96 hours of silver catfish was tested = 0.730 mg a.i./L, static exposure. Exposure period for haematological parameters was 96 hours, immunological endpoints were measured after 24 hours and 10 days.
Experimental approach, Statistics, test environment	7-10 fish per tank, triplicate tanks, but replication used for statistics was 7 (fish individuals per treatment).
Test organisms	Male and female fingerlings of silver catfish (*Rhamdia quelen)*, 18±8 g weight for immunological studies, juveniles of 80-100 g for haematological studies,
Biological effects	Total leukocytes, lymphocytes, thrombocytes and erythrocytes counts were significantly lower ($p < 0.01$), and the number of circulating immature cells were significantly higher ($p < 0.01$). Haematocrit, monocytes and neutrophil, as well as glucose and total plasma proteins were not different between the groups significant reduction ($p < 0.05$) of phagocytic index after 24h, no effect after 10 days No effect on bactericidal activity. Natural bacterial agglutination titer measured against formalin-inactivated pathogenic *A. hydrophila* was significantly lower ($p < 0.05$) in glyphosate exposed fingerlings, either at 24 h or 10 days. Serum lysozyme: lowered after 10 days, myeloperoxidase: lowered after 24 h, natural complement haemolytic activity: no effect.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	The threshold for considering observed effects as significant was set to 1% Type I-error probability, which points together with the numerous positive and negative significant deviations from the control level to a low variability of measurements and thus a high reliability of the statistics.
3 Is the	A common methodology of transferring concentrations of immunological relevant

ecotoxicological manifestation level appropriate for the assessment?	blood cells to higher levels of organisation and thus to draw conclusions for an environmental risk on population level is scarcely available. Assuming large interspecies differences in critical concentrations leading to an effect on an individual and the lack of a well-established reference system for *Rhamdia quelen*, there is no indication of the results of this study for an ERA.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, for glyphosate without surfactant addition.
2 Do the tested concentrations relate to predicted environmental concentrations?	In the of expectable concentrations (PEC surface water about 0.5 mg a.i./L
3 Have parameters influencing the endpoints been considered adequately?	No, but conditions have been held sufficiently constant amongst the experimental units.
Concluding weight of evidence/proposed action	**The study could be taken as supplementary information that environmentally relevant concentrations of glyphosate could induce subtle changes of the haematological status of fish. It could not be assessed if those changes have the potential to affect the health status of an individual and thus to cause effects relevant for a whole population.**
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2**

Kreutz et al. (2008)

glyphecotox_436 Fish	**Kreutz, L.C., Barcellos, L.J.G., Silva, T.O., Anzilierol, D., Martins, D., Lorenson, M., Marteninghe, A., da Silva, L.B.**	**2008**	**Acute toxicity test of agricultural pesticides on silver catfish (*Rhamdia quelen*) fingerlings**	**Fish Shellfish Immunol 30 (1):51-7. DOI: 10.1016/j.fsi.2010.09.012.**

Reliability	
Purpose of the study Description of endpoints	The mortality (LC_{50}) caused by different pesticides (amongst them the herbicide glyphosate) was determined for silver catfish (*Rhamdia quelen)* fingerlings Mortality after 96 h.
Test compound, application procedure, exposure period	Glyphosate as Roundup (N-phosphonomethylglycine), (360g L^{-1}) 2, 4, 8, 16, 32 mg a.i./L under static conditions
Experimental approach, Statistics, test environment	210 fingerlings uniformly distributed in 21 40-L plastic aquaria 5 concentrations, 3 replicates per treatment; 96 hours exposure period During acclimatisation period, 20 % water exchange per day, after treatmet exchange was stopped.
Test organisms	*Rhamdia quelen* (silver catfish) fingerlings, 60-day-old mixed-sex fingerlings weighing between 2 and 4 g
Biological effects	LC_{50} after 96 hours = 7.3 (6.5 – 8.2) mg a.i./L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	The 50% lethal dose was deduced from a ‘useful’ and usual statistical design and could thus be considered as relevant for the population of silver catfish as other acute mortality studies.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Yes, as mortality under laboratory conditions and acute exposure is commonly agreed.

3 Is the ecotoxicological manifestation level appropriate for the assessment?	The 50% lethal dose was deduced from a 'useful' and usual statistical design and could thus be considered as relevant for the population of silver catfish as other acute mortality studies.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, most probably the Roundup formulation contained POEA as surfactants, which could also explain the elevated toxicity of the test item compared to other studies with different fish species reported in the literature (cited herein).
2 Do the tested concentrations relate to predicted environmental concentrations?	Not relevant, since that was an acute dose-response test design to derive an LC50 from a Probit distribution.
3 Have parameters influencing the endpoints been considered adequately?	Conditions in the test containers have been maintained at non-harmful ranges.
Concluding weight of evidence/proposed action	**The LC_{50} for the exposure of *R. quelen* reported here is far below toxicities reported from other acute studies with fish under laboratory conditions. This is most probably due to the composition of the tested formulation of glyphosate (Roundup), as discussed by the authors as well. The study can be seen as additional evidence of enhanced toxicity caused by the POEA in glyphosate formulations. However, the study is not suited to trigger the aquatic risk assessment of glyphosate.**
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Folmar et al. (1979)

glyphnosubm_012	**Folmar, LC, Sanders HO, Julin AM**	**1979**	**Toxicity of the Herbicide Glyphosate and Several of Its Formulations to Fish and Aquatic Invertebrates.**	**Archives of Environmental Contamination and Toxicology 8: 269-278**

Reliability	
Purpose of the study Description of endpoints	Comparison of the acute toxicity of the technical grade glyphosate, the formulated herbicidal product 'Roundup' and the tallow amine surfactant. Mortality of rainbow trouts, fathead minnows, channel catfish, bluegills, determination of LC_{50} 24 and 96 hours after exposure. Additionally midges, scuds, daphnids were tested (also after 48h). The paper is mainly referred in the POEA section of the literature survey in DocM for the comparison between surfactant POEA and the active substance glyphosate. The year of publication was beyond the 10-years scope of the literature collection and thus discarded by the notifier. Here, the acute laboratory part of the study is reported only with no consideration of temperature effects and other aspects covered by this publication. Additional tests regarded avoidance behaviour, reproductive potential and stream drift of different organisms.
Test compound, application procedure, exposure period	Technical glyphosate (Isopropylamine salt, 480.42 g/L), Roundup with surfactant (360.32 g/L), surfactant Protocol: Methods recommended for static toxicity testing (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975) Static exposure of fish and amphibians for 24 and 96 hours
Experimental approach, Statistics, test environment	The exact number and spacing of concentrations tested in the acute studies were not reported in the publication. 10 fish per test concentration have been tested at 12°C (*O. mykiss*) and 22°C water

	temperature (other species). Methods of Litchfield and Wilcoxon to derive LC50's (and (EC50's for invertebrates)
Test organisms	Rainbow trout (*Oncorhynchus mykiss*, synonym *Salmo gairdneri*), Fathead minnow (*Pimephales promelas*), Channel catfish (*Ictalurus punctatus*), Bluegill (*Lepomis macrochirus*)
Biological effects	The paper reports acute LC_{50}-values for four fish species (in mg/L)

	Glyphosate acid		*Roundup*		*POEA*	
Species	24h	96h	24h	96h	24h	96h
Oncorhynchus mykiss	140.0	140.0	8.3	8.3	2.1	2.0
Pimephales promelas	97.0	97.0	2.4	2.3	1.4	1.0
Ictalurus punctatus	130.0	130.0	13.0	13.0	18.0	13.0
Lepomis macrochirus	150.0	140.0	6.4	5.0	3.0	3.0

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes, mainly standard test species have been tested.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	The endpoint mortality in acute studies with single species is of biological significance indeed and a widely accepted assessment aspect for the aquatic environment.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Yes, see above points 1 and 2
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The comparison between the POEA surfactant, technical grade glyphosate and the formulated product Roundup touches the key concern on the use of the herbicide glyphosate. It allows for factoring out the toxicity of the two components of a mixture surfactant and active ingredient.
2 Do the tested concentrations relate to predicted environmental concentrations?	The tested concentrations were not reported in the publication, which is a strong deficit and could be regarded the only reason to reject the revision of the results.
3 Have parameters influencing the endpoints been considered adequately?	PH and temperature were tested systematically. At suboptimal conditions of pH and temperatures the toxicity of the surfactant and Roundup in particular, was increased.
Concluding weight of evidence/proposed action	**The paper is considered one of the key publications on the enhancing effect of adding tallow amine surfactants to glyphosate-based herbicides and could not be ignored even in a recent risk assessment. Since the concentration series and the spacing factors were not described appropriately, the study has formally a low reliability. Nevertheless, it could be shown that most of the toxicity of the product was due to the POEA. Nowadays, products formulated by means of POEA are not expected to be neither notified nor registered in the future. The publication supports this practice.** Note: **the review in glyphnosubmit_540 falsely reports that here the glyphosate acid was tested, whereas the IPA salt was applied.**
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2**

Evrard et al. (2010)

glyphecotox_367 Fish	**Evrard, E., Marchand, J., Theron, M., Pichavant-Rafini, K., Durand, G., Quiniou, L., Laroche, J.**	**2010**	**Impacts of mixtures of herbicides on molecular and physiological responses of the European flounder *Platichthys flesus***	**Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 152 (3):321-331. DOI: 10.1016/j.cbpc.2010.05.009.**

Reliability	
Purpose of the study Description of endpoints	Effects of glyphosate (Roundup Ultra) and its first metabolite AMPA on the physiological response of the European flounder (*Platichthys flesus*) using genetic transcription patterns Real-time PCR assays were conducted on several candidate transcripts identified by the SSH assay (mRNA analysis) as indicators of liver injury. Nine gene transcripts in liver were analysed: betaine homocysteine methyltransferase (BHMT) transcript; apolipoprotein E1 transcript; chemotaxin (LECT2) transcript; α-2-macroglobulin transcript; anti thrombin III transcript; C1 inhibitor precursor (C1Inh) transcript; ubiquitin transcript; ATP synthase Fo subunit 6 transcript; cytochrome B transcript. Blood parameters and the physiological 'condition factor'
Test compound, application procedure, exposure period	Measured mean concentrations [nominal] over 62 days of exposure G-tank: Roundup Ultra, with unknown contents and identities of surfactants and the glyphosate salt *0.16 µg/L* [2 µg/L] plus AMPA *2.27 µg/L [2 µg/L]* GAMA2-tank Glyphosate 0.15 µg L [1.25 µg/L]+ AMPA 1.53 µg/L [1.25 µg/L]+ mecoprop 0.27 µg/L − [0.5 µg/L]+ acetochlor 0.36 µg/L [0.5 µg/L] + 2,4D 0.23 µg/L [0.5 µg/L]
Experimental approach, Statistics, test environment	3 replicate fish per treatment were taken from one of the three tanks (control, G, GAMA2), One sampling prior exposure, 3 sampling dates after exposure 15, 32, 62 days after treatment.
Test organisms	European flounder (*Platichthys flesus*)
Biological effects	Results reported here only for G-tank: BHMT, Apolipoprotein E1, Chemotaxin, macroglobulin and ATPase were highly significantly altered 62 days after exposure at 0.16 µg glyphosate/L. No impacts on physiological indices
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	The extrapolation from gene transcript alteration to populations of corresponding fish species or even all fish should be proven by experiments that address the question of population vitality at the same test concentrations which is not discussed by the authors.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	See point 2 above
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Two different difficulties with the test diminish the relevance of the test for an ERA: a complex mixture was tested and the composition regarding surfactants is unknown. The combination AMPA + glyphosate could be taken as representative for glyphosate because AMPA is the main metabolite and the measured concentration of glyphosate is assigned the relevant final concentration.
2 Do the tested concentrations relate to predicted environmental concentrations?	Conc. are far below highest expectable PECinis and thus of high environmental relevance.
3 Have parameters influencing the endpoints been considered adequately?	No particular test design to check for influencing parameters

Concluding weight of evidence/proposed action	**Main finding: Low concentrations of glyphosate are suited to alter the gene expression patterns of the liver of European flounders.** **There are many uncertainties in transferring the results of this study to populations of fish, which is the relevant organisational level for an environmental risk assessment.** **A simple and a complex mixture has been tested so far, that causes a limited use of the results.**
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2**

Cavalcante et al. (2008)

glyphecotox_316	**Cavalcante, D.G.S.M., Martinez, C.B.R., Sofia, S.H.**	**2008**	**Genotoxic effects of Roundup® (R) on the fish *Prochilodus lineatus***	**Mutation Research-Genetic Toxicology and Environmental Mutagenesis 655 (1-2):41-46. DOI 10.1016/j.mrgentox.2008.06.010.**

Reliability	
Purpose of the study Description of endpoints	The aim of this work was to evaluate the genotoxic effects of Roundup® in *P. lineatus* acutely exposed to the herbicide for different periods, using the comet assay, micronucleus test and the occurrence of erythrocytic nuclear abnormalities (ENAs).
Test compound, application procedure, exposure period	Roundup®® (360 g glyphosate/L or 41% of glyphosate, Monsanto Brazil ThisRoundup®® concentration Test concentration not directly stated. corresponds to 75% of the LC50 of this herbicide to *P. Lineatus*. The 96 h-LC50 of Roundup® was 13.69 mg/L.
Experimental approach, Statistics, test environment	Cell viability assay for erythrocytes and gill cells using the trypan blue exclusion method; Alkaline comet assaywas performed according to Singh et al. and Speit and Hartmann with some modifications as described by Vanzella et al. Two-tailed Student t test. Differences between means were considered significant when $p < 0.05$.
Test organisms	Juveniles of *Prochilodus lineatus* (Valenciennes, 1847), with 9.6±5.4 g and 9.7±1.81cm (mean±S.D., N= 50), were supplied by the Hatchery Station of Londrina State University.
Biological effects	In the micronucleustest micronucleus (MN) and erythrocytic nuclear abnormalities (ENA) were not significantly different from the respective negative controls. Comet assay showed significant effects towards DNA damage in erythrocytes.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	The test species are considered of temperate to sub-tropical origin. Indication of species variability for the standard in ERA.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Cell viability, nuclear abnormalities dependent on repair mechanisms
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Traditionally, survival, growth and reproduction of individuals are chosen as endpoints of the classic laboratory tests for ecotoxicity. No mortality assessed, low relevance for traditional ERA.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation, the tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to	Testing exceeded environmentally realistic concentrations.

predicted environmental concentrations?	
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	**Physiological study with the commercial formulation . No distinction between the activie substance and surfactants.**
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Langiano et al. (2008)

glyphecotox_452	**Langiano, V.dC., Martinez, C.B.R.**	**2008**	**Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus***	**Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 147 (2):222-231**

Reliability	
Purpose of the study Description of endpoints	Toxicity of Roundup® to *P. lineatus* and to evaluate the responses of this fish at biochemical, physiological and histological levels, after acute exposure to sub-lethal concentrations of the herbicide
Test compound, application procedure, exposure period	7.5 and 10 mg/L Roundup®
Experimental approach, Statistics, test environment	Parameters observed were mortality and histological alterations , physiology, Student's t-test, ANOVA.
Test organisms	Neotropical fish *Prochilodus lineatus*
Biological effects	Exposure to sub-lethal concentrations of Roundup® promoted an increase in plasma glucose, indicating a typical response to stress. The induction of liver catalase activity indicates the activation of antioxidant defenses, probably due to increased hydrogen peroxide generation. Roundup® exposure also induced a variety of liver histological alterations that might impair normal organ functioning. 96 h-LC50 of Roundup® was 13.69 mg/L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	The test species are considered of temperate to sub-tropical origin. Indication of species variability for the standard ERA.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Parameters observed: mortality and histological alterations
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Traditionally, survival, growth and reproduction of individu-als are chosen as endpoints of the classic laboratory tests for ecotoxicity.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation , the tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	Testing exceeded environmentally realistic concentrations.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	*Prochilodus lineatus* is more sensitive to Roundup® than rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar).* Physiological study with the commercial formulation . No distinction between the active substance and surfactants.

Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Ferreira et al. (2010)

glyphecotox_376	**Ferreira, D., da Motta, A.C., Kreutz, L.C., Toni, C., Loro, V.L., Barcellos, L.J.G.**	**2010**	**Assessment of oxidative stress in *Rhamdia quelen* exposed to agrichemicals**	**Chemosphere 79 (9):914-921. DOI: 10.1016/ j.chemosphere.2010.03.024.**

Reliability	
Purpose of the study Description of endpoints	Verification whether MP, Gly, and Teb are potential oxidative stress inducers in *R. quelen*, and whether their effects could provoke histopathological changes in the liver of this fish species.
Test compound, application procedure, exposure period	Commercial formulation containing the herbicide glyphosate 6.6% of the LC50–96h, as previously determined by Kreutz et al. (2008 (glyphosate based herbicide (1.21 mg/L of Roundup®™).)
Experimental approach, Statistics, test environment	Physiological study evaluating oxidative stress, enzymatic responses, ANOVA
Test organisms	*Rhamdia quelen*
Biological effects	Survival rate was not altered at that concentrations. Glyphosate containing product did not alter reactive substances in liver, but decrease in CAT activity, no visible histological changes.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	American species. Indication of species variability for the standard ERA.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	No visible histological changes.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	No visible histological changes. Traditionally, survival, growth and reproduction of individuals are chosen as endpoints for the classic laboratory tests for ecotoxicity.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formualtion not stated. No information about surfactants.
2 Do the tested concentrations relate to predicted environmental concentrations?	Based on modelling assumptions, the concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L: 0.185 mg /l probably realitsic worse case concentrations;
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	No visible histological changes, Indication for general fitness
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Haller et al. (2003)

glyphecotox_399	**Haller, W.T., Stocker, R.K.**	**2003**	**Toxicity of 19 adjuvants to juvenile Lepomis macrochirus (bluegill sunfish)**	**Environmental Toxicology and Chemistry 22 (3):615-619**

Reliability	
Purpose of the study Description of endpoints	Nineteen adjuvants, many used as surfactants for aquatic herbicide applications, were applied in static bioassay to bluegill sunfish (*Lepomis macrochirus*) for 96 h to determine median lethal concentrations (LC50).
Test compound, application procedure, exposure period	MON 0818
Experimental approach, Statistics, test environment	Surfactants are added to the tank mix as a percentage (v/v) of the total volume, in contrast to herbicide application rates.
Test organisms	bluegill sunfish
Biological effects	Ethoxylated tallow amine products were the most toxic, having LC50 values of 1.6 and 2.9 ppm. Seven alcohol/glycol-based surfactants had 96-h LC50 values of 4.0 to 11.6 ppm polysiloxane- or silicone-based surfactants had toxicities of 18.1 to 29.7 ppm, limonene-based products had LC50 values of 10.2 and 30.2 ppm.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*Lepomis macrochirus*
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	mortality
3 Is the ecotoxicological manifestation level appropriate for the assessment?	While toxicity of adjuvants has not been a focus of concern.for aquatic applications, the data reported here will give resource managers guidance into the acute toxicities of some of the commercially available adjuvants and assist in the development of invasive plant management programs with an acceptable margin of safety.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	POEA: MON 0818
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Monsanto's MON 0818 and Entry II are 68 to 73% and.35% ethoxylated tallow amine surfactants, respectively.The material safety.data sheet for MON 0818 lists 96-h toxicity to bluegill sunfish.at 1.3 ppm, similar to the 1.6-ppm LC50 obtained in this study.
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Zhidenko et al. (2007)

glyphecotox_670	**Zhidenko, A.A., Kovalenko, Y.M.**	**2007**	**The influence of Roundup® on the dynamics of histological changes in organs of carps**	**Hydrobiological Journal 43 (2):93-99**

Reliability	
Purpose of the study Description of endpoints	Identification of the dynamics of histological parameters in carp organism under the action of Roundup® (0.004 mg/dm3) and their possible influence on functional deviations in fish were the aim of this study.
Test compound, application procedure, exposure period	Roundup®
Experimental approach, Statistics, test environment	Histological observations
Test organisms	Two-year-old carps (*Cyprinus carpio L.)* weighing 200–300 g
Biological effects	Action of Roundup® at its 0.004 mg/dm3 contents in water environment leads to ambiguous alterations in organs of carp. The least deviations have taken place in the brain and gills, insignificant abnormalities were in the intestine and the greatest were in the muscles and liver; the latter organs are the most sensitive. Histologic changes in the liver of carp, which are connected with the granular and vacuolar-drop dystrophy, lead to the death of hepatocytes and to necrotic changes and, as a consequence, to the functional liver failure and to the formation of bilestones. The muscle fiber hypotrophy under the influence of Roundup® leads to destructive changes in skeletal muscles.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*Cyprinus carpio L*
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Histologic changes in the liver of carp, which are connected with the granular and vacuolar-drop dystrophy, lead to the death of hepatocytes and to necrotic changes and, as a consequence, to the functional liver failure.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Histological observations are an indication for general fitness. Survival, growth and reproduction of individuals are chosen as endpoints of the classic laboratory tests for ecotoxicity.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Although not specified precisely, the tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	Environmentally realistic concentrations have been used (0.004 mg/l)
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Action of Roundup® and environmentally realistic concentrations leads to alterations in organs of carp which might lead to functional changes in organ function.
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Ortiz-Ordoñez et al. (2003)

glyphecotox_532	**Ortiz-Ordoñez, E., Uría-Galicia, E., Ruiz-Picos, R.A., Duran, A.G.S., Trejo, Y.H., Sedeño-Díaz, J.E., López-López, E.**	**2011**	**Effect of Yerbimat Herbicide on Lipid Peroxidation, Catalase Activity, and Histological Damage in Gills and Liver of the Freshwater Fish *Goodea atripinnis***	**Archives of Environmental Contamination and Toxicology 61 (3):443-452. DOI: 10.1007/s00244-011-9648-0.**

Reliability	
Purpose of the study Description of endpoints	Determination of acute toxicity and evaluation of changes of biochemical parameters due to exposure to Yerbimat.
Test compound, application procedure, exposure period	In Mexico, one of the most widely used glyphosate-based herbicides is Yerbimat, which has agricultural as well as aquatic weed control applications.
Experimental approach, Statistics, test environment	– static bioassay at 96 h (LC50) – chronic exposure (75 days) – Probit Analysis v.1.5 software.
Test organisms	*Goodea atripinnis* (6.0 ± 0.5 cm standard length and 3.0 ± 0.5 g weight)
Biological effects	The 96-h LC50 value was 38.95 ± 0.33 mg/L. Yerbimat induced significant decreases in CAT activity in the gills of 9.88 and 53.3% at 1/10 of the LC50 and 1/5 of the LC50, respectively, Hypertrophy was evidenced by loss of the normal structure of the gills, and gill filaments were inflamed due to the abnormal size of the cells at 30–75 days of exposure, hepatic cells displayed increasing vacuolation, in which vacuoles increased in both number and size, and nuclei were displaced toward the cell periphery.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Mexican fish species
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Histological alterations in the gills and liver that might impair normal organ functioning
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Mortalitiy and biochemical alterations, Indication for general fitness.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation, probably containing relevant toxic surfanctants , probably POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	Based on modelling assumptions, the concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Biochemical damage, as evidenced by high LPX and CAT inhibition in gill tissue, was apparent following chronic Yerbimat exposure, indicative of damage due to oxidative stress, might lead to cellular damage and death.
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Cattaneo et al. (2011)

glyphecotox_315	**Cattaneo, R., Clasen, B., Loro, V.L., de Menezes, C.C., Pretto, A., Baldisserotto, B., Santi, A.L., de Avila, L.A.**	**2011**	**Toxicological Responses of *Cyprinus carpio* Exposed to a Commercial Formulation Containing Glyphosate**	**Bulletin of Environmental Contamination and Toxicology 87 (6):597-602. doi: 10.1007/s00128-011-0396-7.**

Reliability	
Purpose of the study Description of endpoints	The effects of commercial glyphosate.formulation on the activity of acetylcholinesterase (AChE) enzyme and oxidative stress were studied in *Cyprinus carpio*.
Test compound, application procedure, exposure period	Roundup® (648 g/L of isopropylamine salt of Glyphosate, 480 g/L of acid equivalent Ghyphosate and 594 g/L of inert ingredients), at concentrations of 0 (without herbicide), 0.5, 2.5, 5.0 and 10.0 mg/L.
Experimental approach, Statistics, test environment	Exposition for 96 h to 0, 0.5, 2.5, 5.0 and 10.0 mg/L and then equal recovery period in water without herbicide. Tissue samples (brain and muscle) were obtained, two-way ANOVA followed by Tukey–Kramer multiple range tests.
Test organisms	*Cyprinus carpio*
Biological effects	The activity of AChE was inhibited in the brain and in the muscle after exposure. However, after recovery period brain and muscle AChE activity increased.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	AChE in brain and muscle. The inhibition by glyphosate might lead to an accumulation of acetylcholine, causing the stimulation of the receptors.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Indication for general fitness
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial herbicide formulation containing the active ingredient glyphosate. It also contains the surfactant, POEA, which is known to be more toxic than glyphosate to fish.
2 Do the tested concentrations relate to predicted environmental concentrations?	Probably exceed worse case concentrations. Based on modelling assumptions, the concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Short-term exposure can affect their physiological conditions, nevertheless no discrimination between glyphosate and POEA possible.
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Modesto et al. (2010)

glyphecotox_511	**Modesto, K.A., Martinez, C.B.R.**	**2010**	**Effects of Roundup® Transorb on fish: Hematology, antioxidant defenses and acetylcholinesterase activity**	**Chemosphere 81 (6):781-787. DOI: 10.1016/j.chemosphere.2010.07.005**

Reliability	
Purpose of the study Description of endpoints	The objective of this work was to evaluate its effects on hematological and biochemical parameters of *P. lineatus*.
Test compound, application procedure, exposure period	Roundup® Transorb (RDT, 480 g glyphosate/ L) at two nominal concentrations of 1 and 5 mg /L was used.
Experimental approach, Statistics, test environment	Blood samples for hematological analysis, liver for antioxidants analysis, and brain and muscle for acetylcholinesterase (AChE) determination
Test organisms	Neotropical fish *Prochilodus lineatus.*
Biological effects	No fish mortality in any of the experimental groups. Hematologic alterations appeared only after 96 h exposure, when fish showed an increase in the hematocrit and in the number of both red and white blood cells, lipid peroxidation (LPO) returned to control levels after 24 and 96 h exposure to RDT.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Mortality and enzymatic parameters
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	The exposure to RDT for 96 h led to an inhibition.of AChE in brain and muscle but at rates which may not be considered a life-threatening situation.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Indication for general fitness.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Roundup® Transorb is a commercial formulation probably containing surfactants. Limited validity regarding effects of Glyphosate that does not contain the same surfactant.
2 Do the tested concentrations relate to predicted environmental concentrations?	Exceeding the predicted concentrations: Based on modelling assumptions, the concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Hematological parameters in fish can change in response towards chemical stressors; however, these alterations are non-specific to a wide range of substances. After 24 and 96 h the antioxidant defenses were apparently enough to combat ROS, preventing the occurrence of oxidative damage. The exposure to RDT for 96 h led to an inhibition of AChE in brain and muscle but at rates which may not be considered a life-threatening situation.
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Evrard et al. (2003)

glyphecotox_367	**Evrard, E., Marchand, J., Theron, M., Pichavant-Rafini, K., Durand, G., Quiniou, L., Laroche, J.**	**2010**	**Impacts of mixtures of herbicides on molecular and physiological responses of the European flounder *Platichthys flesus***	**Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 152 (3):321-331. DOI: 10.1016/j.cbpc.2010.05.009.**
Reliability				

Purpose of the study Description of endpoints	The effects of a simple mixture of a glyphosate-based formulation and AMPA and of a more complex mixture of herbicides (glyphosate/AMPA/ mecoprop/acetochlor/2,4D) were explored on the molecular and physiological responses of the European flounder *Platichthys flesus*.
Test compound, application procedure, exposure period	Roundup® Ultra solution contains the monoisopropylamine salt of N glyphosate and surfactants that were not identified in terms of chemical composition and concentration on the product label. The corresponding percentage of Roundup®solution was 0.0055% in the glyphosate/AMPA tank (G tank) nominal concentrations of 2 µg /L glyphosate (from Roundup® solution) and 2 µg/ L AMPA; this was known as the G tank.
Experimental approach, Statistics, test environment	Flounders were sampled after 0, 15, 32 and 62 days of exposure. Suppression subtractive hybridization, mRNA expression analysis, blood samples and physiological measurements, Principal Component Analysis.
Test organisms	Juvenile flounders *Platichthys flesus* (n = 300, length = 7–12 cm)
Biological effects	No significant difference was detected in the variation patterns of physiological parameters between contaminated and control fishes during the experiment; expression of three markers among the nine tested, namely BHMT, apolipoprotein E1 and chemotaxin, was altered by both types of pesticide mixture. these genes being implicated in stress response, but also in multiple biochemical pathways linked to the responses to abiotic and biotic factors of the experimental environment (light, salinity, social interaction, feeding…).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	European flounder *Platichthys flesus*
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Level of assessment is not appropriate for population level effects; LC_{50} stated.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Traditionally, survival, growth and reproduction of individuals are chosen as endpoints of the classic laboratory tests for eco-toxicity
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation Roundup® Ultra
2 Do the tested concentrations relate to predicted environmental concentrations?	Environmentally relevant concentrations of herbicides (4 µg/L)
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Significant alterations of liver gene expressions were detected for contaminated vs control fishes, particularly at the levels of methionine metabolism, lipid transport and metabolism, immunity and respiratory chain.
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Smith et al. (2003)

glyphecotox_602	**Smith, B.C., Curran, C.A., Brown, K.W., Cabarrus, J.L., Gown, J.B., McIntyre, J.K., Moreland, E.E., Wong, V.L., Grassley, J.M., Grue, C.E.**	**2004**	**Toxicity of four surfactants to juvenile rainbow trout: Implications for use over water**	**Bulletin of Environmental Contamination and Toxicology 72 (3):647-654. DOI 10.1007/s00128-004-0292-5.**
Reliability				

Purpose of the study Description of endpoints	Comparison of 4 surfactants using effect on survival and behaviour as endpoints
Test compound, application procedure, exposure period	R-11, Li700, HaSTEN, Agri DEX
Experimental approach, Statistics, test environment	96h static acute test(USEPA 1996)
Test organisms	*Oncorhynchus mykiss*
Biological effects	Erratic swimming, on-bottom gilling, inability to maintain horizontal orientation R11: LC50 96h = 6 ppm Li700: LC50 96h = 17 ppm HASTEN: LC50 96h = 74 ppm Agri DEX : LC50 96h = 271 ppm
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*Oncorhynchus mykiss*
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Surfactant toxicity has limited validity regarding effects of products with different surfactants. Nevertheless, it shows significance to evaluate on product level.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Surfactant toxicity was assessed.
2 Do the tested concentrations relate to predicted environmental concentrations?	No MW stated
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Surfactant apos environmental hazard, displaying non-specific narcosis .
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Kreutz et al. (2003)

glyphecotox_436	**Kreutz, L.C., Barcellos, L.J.G., Silva, T.O., Anzilierol, D., Martins, D., Lorenson, M., Marteninghe, A., da Silva, L.B.**	**2008**	**Acute toxicity test of agricultural pesticides on silver catfish (*Rhamdia quelen*) fingerlings**	**Fish Shellfish Immunol 30 (1):51-7. DOI: 10.1016/j.fsi.2010.09.012.**

Reliability	
Purpose of the study Description of endpoints	Investigation on the acute toxicity and the lethal concentration (LC50) of four herbicides, two fungicides and two insecticides to silver catfish fingerlings.
Test compound, application procedure, exposure period	Roundup® , 540-2160 g/ha
Experimental approach, Statistics, test environment	For the LC_{50} determinations, 210 fingerlings were uniformly distributed in 21 40-L plastic aquaria, keeping fish density below or equal to 1g /L. Each product was tested using 5 to 6 different concentrations, with 3 repetitions each.
Test organisms	*Rhamdia quelen*

Biological effects	96h-LC_{50} = 7.3 mg/L; 6.5–8.3; Lethargy, swimming at the water surface and erratic swimming (mainly vertical swimming) were the main behavioral changes observed.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*Rhamdia quelen*
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Mortality was observed and LC_{50} determined.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Roundup® Transorb is a commercial formulation probably containing surfactants. Limited validity regarding effects of Glyphosate that does not contain the same surfactant.
2 Do the tested concentrations relate to predicted environmental concentrations?	Recommended application rates were tested. Based on modelling assumptions, the concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	The 96-h LC50 determined for the glyphosate-based herbicide Roundup® (7.3 mg/L) was much lower than that for the active substance glyphosate itself.
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Xie et al. (2003)

glyphecotox_006	**Xie, L., Thrippleton, K., Irwin, M.A., Siemering, G.S., Mekebri, A., Crane, D., Berry, K., Schlenk, D.**	**2005**	**Evaluation of estrogenic activities of aquatic herbicides and surfactants using an rainbow trout vitellogenin assay**	**TOXICOLOGICAL SCIENCES 87(2), 391–398 (2005)**

Reliability	
Purpose of the study Description of endpoints	Investigation on the estrogenic potencies of four herbicides, nonionic surfactants, and the mixture of herbicides with surfactants using an in vivo rainbow trout vitellogenin assay.
Test compound, application procedure, exposure period	Glyphosate from Dow AgroSciences Indianapolis
Experimental approach, Statistics, test environment	Dose-response exposure. Plasma vitellogenin levels determination, Field evaluation.
Test organisms	Juvenile rainbow trout (standard length: 11.5 ± 2.2 cm)
Biological effects	Single Concentration Exposure: Pesticides triclopyr, glyphosate, and diquat dibromide did not induce elevated levels of vitellogenin in juvenile rainbow trout compared with control fish.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*yes*
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to predicted environmental concentrations?	Based on modelling assumptions, the concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Glyphosate did not induce elevated levels of vitellogenin in juvenile rainbow trout compared with control fish.
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Le Mer, C. et al. (2012)

glyphecotox_888 2716852	**Le Mer, C. et al.**	**2012**	**Effects of chronic exposures to the herbicides atrazine and glyphosate to larvae of the threespine stickleback (*Gasterosteus aculeatus*)**	**Ecotoxicology and Environmental Safety 89 (2013) 174 13181**

Reliability	
Purpose of the study Description of endpoints	The aim of this study was to determine the effect of exposures to the common herbicides atrazine and glyphosate to larval sticklebacks, using growth, survival and the reproductive biomar kers VTG, SPG and sexual differentiation as endpoints in larvae exposed to herbicides.
Test compound, application procedure, exposure period	Glyphosate (purity 96%), DHT (purity 99 %) and EE2 (purity 98 %)
Experimental approach, Statistics, test environment	Larval sticklebacks (24h old) were exposed for 42 d to four concentrations (0.1,1,10 and 100 mg/L) of either ATR or GLY, a sea water control, a carrier (acetone) control and positive controls for estrogenic (0.05 mg/L ethinylestradiol, EE2) and androgenic (3 mg/L dihydrotestosterone, DHT) effects. Authors investigated the responses of these endpoints in fish exposed to 17a-ethinylestradiol (EE2) and dihydrotestosterone (DHT) as positive controls for estrogenic and androgenic effects.
Test organisms	three-spinestickleback (*Gasterosteus aculeatus*)
Biological effects	There were no significant effects of ATR and GLY exposures on larval survival or growth. VTG was induced after the EE2 exposure, yet neither ATR nor GLY induced production of VTG and SPG.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Authors could not observe induced production of vitellogenin or a change in sex ratio in early life stages of stickleback.
Type of information (Critical, supporting, low weight)	**Supporting information**

Consideration/concluding score	**UBA2**

Fan, et al. (2013)

	Fan, J. Y., Geng, J. J., Ren, H. Q., Wang, X. R., & Han, C.	**2013**	**Herbicide Roundup® and its main constituents cause oxidative stress and inhibit acetylcholinesterase in liver of *Carassius auratus.***	**Journal of Environmental Science and Health, Part B, 48(10), 844-850.**
Reliability				
Purpose of the study Description of endpoints	In this study, crucian carp or *Carassius auratus*, was chosen as the testing aquatic organism exposed to Roundup R and its main constituents. The purpose of this paper is to compare the toxicity of Roundup R and its main constituents (G.I.S and POEA) to *C. auratus* under different exposure concentrations.			
Test compound, application procedure, exposure period	Roundup , containing 41% (w/v) G.I.S and 18% (w/v) POEA, was obtained from Monsanto (St. Louis, MO, USA). G.I.S (41% purity) was purchased from Sigma Chemical (St. Louis, MO, USA). POEA was purchased from Haian petrochemical complex (Jiangsu, China), 50% water was replaced daily by adding fresh Roundup R, G.I.S and POEA solution. Authors set the concentration of Roundup R (0.032 mg/L) as the middle concentration which approximates the actual concentration of glyphosate (< 0.021 mg/L) in the environment. Besides, 0.16 mg/L and 0.0064 mg/L concentrations of Roundup R were set at higher concentrations and at lower concentrations simultaneously.			
Experimental approach, Statistics, test environment	ROS analysis, superoxide dismutase (SOD) activity, degree of lipid peroxidation by determination of malondialdehyde (MDA), AChE activity, Statistical analysis, ANOVA			
Test organisms	crucian carp or *Carassius auratus*			
Biological effects	Hydroxyl radical could be induced by exposing Roundup (43%–111%), glyhosate (90%–124%) and POEA to 142%–157%. A decreased SOD activity was observed in fish exposed to glyphosate and POEA. The contents of MDA significantly increased when exposed to Roundup R at all concentrations, 0.16 mg/L G.I.S and 0.032 mg/L POEA. The exposure led to an inhibition of AChE in livers overall during the experimental periods. POEA was more toxic than Roundup R or glyphosate.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	nd			
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd			
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Biochemical parameters were analysed, no lethal or reproductive endpoint. Nevertheless, they might be sensitive biomarkers for toxicity testing.			
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?	Isopropylamine salt of glyphosate (G.I.S) and Roundup containing POEA; therefore limited validity for other products and the active substance glyphosate itself.			
2 Do the tested concentrations relate to predicted environmental concentrations?	Probably slightly exceeding worse case assumptions. Based on conservative modelling assumptions, the worst-case concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.			
3 Have parameters influencing the endpoints been considered adequately?	nd			
Concluding weight of evidence/proposed action	The present study showed that Roundup, glyphosate and POEA may cause changes in the metabolic and enzymatic parameters of fish.			

Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Nwani et a. (2013)

BVL Nr.: 2716842	**Nwani, C. D., Nagpure, N. S., Kumar, R., Kushwaha, B., Kumar, P., & Lakra, W. S.**	**2013**	**Induction of micronuclei and nuclear lesions in *Channa punctatus* following exposure to carbosulfan, glyphosate and atrazine.**	**Drug and chemical toxicology, (0), 1-8.**

Reliability	
Purpose of the study Description of endpoints	Investigation on the ability to induce micronucleus and morphological alterations in the peripheral blood cells of *C. punctatus*. The extent of genotoxic and cytotoxic damage induced by the pesticides and the feasibility of application of these endpoints in biological monitoring of environmental genotoxicants were further discussed.
Test compound, application procedure, exposure period	Roundup, manufactured by Monsanto India Ltd. (glyphosate, 41% soluble liquid (SL), CAS No.1071-83-6); glyphosate 8.1, 16.3, 24.4 mg/L
Experimental approach, Statistics, test environment	Semi-static system as standard methods (APHA, AWWA & WPCF, 2005) with change of solution on every alternate day to maintain the concentration of the pesticide; 96h exposure; Micronucleus assay and nuclear lesions: probit analysis
Test organisms	air-breathing fish *Channa punctatus*
Biological effects	The 96h-LC50-values were estimated by probit analysis as 0.27, 32.0 and 42.0 mg/L, respectively, for carbosulfan, glyphosate, and atrazine using semi-static bioassays. Glyphosate exposure induces at 96 h the frequency of MN significantly (2.5%) compared to control.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	no
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product. The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	Based on conservative modelling assumptions, the worst-case concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	The study, indicated that the MN and NL assays are sensitive tools for evaluating the mutagenic and genotoxic effects of carbosulfan, glyphosate and atrazine. Nevertheless study investigated the commercial products and therfore, emphasizes the importance to assess commercial products for their ability to induce genotoxicity. The induction of nuclear lesions was concentration and duration dependent with the record of highest frequency at 24.4 mgL at 96 h.

Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Richard, et al (2014)

BVL Nr.: 2716843	**Richard, S., Prévot-D'Alvise, N., Bunet, R., Simide, R., Couvray, S., Coupé, S., & Grillasca, J. P.**	**2014**	**Effect of a Glyphosate-Based Herbicide on Gene Expressions of the Cytokines Interleukin-1β and Interleukin-10 and of Heme Oxygenase-1 in European Sea Bass, *Dicentrarchus labrax* L.**	**Bulletin of environmental contamination and toxicology, 1-6.**

Reliability	
Purpose of the study Description of endpoints	The aim of this study was to evaluate the impact of Roundup Ultra 360 on the immune system of the European sea bass, which has a high commercial value both from fisheries and aquaculture in Mediterranean countries.
Test compound, application procedure, exposure period	Roundup Ultra 360 manufactured by Monsanto Europe S.A. (Belgium) was used in this work. This herbicide contains 360 g/L glyphosate (purity 41 %).
Experimental approach, Statistics, test environment	The expression levels of il-1b and il-10 cytokines genes in the spleen, gills and intestines in European sea bass exposed to Roundup were compared with unexposed control fish.
Test organisms	European Sea Bass, *Dicentrarchus labrax* L.
Biological effects	A group of fish was exposed to 647 mg/L of Roundup for 96 h. This treatment did not alter gene expression levels of il-1b and il-10 cytokine in the intestines, but significantly lowered both levels in the gills. Expression levels of ho-1 were increased significantly in the three organs of fish from the treated group (the gills, the intestines and the spleen).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Roundup exposure also induced differences in gene expression. It is not clear if alterations might impair normal organ functioning and immune defenses.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product was used. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	Based on conservative modelling assumptions, the worst-case concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L. Test concentration is exceeding this by far.
3 Have parameters influencing the endpoints been considered adequately?	
Concluding weight of evidence/proposed action	In vitro studies could be sensitive biomarkers to evaluate the effect of exposure to pollutants chronically present in the environment. Nevertheless, the study investigated a formulated commercial product, probably containing POEA.
Type of information (Critical, supporting, low weight)	**supporting**

Consideration/concluding score	**UBA2**

Sandrini, et al (2013)

	Sandrini, J. Z., Rola, R. C., Lopes, F. M., Buffon, H. F., Freitas, M. M., Martins, C. D., & da Rosa, C. E.	**2013**	**Effects of glyphosate on cholinesterase activity of the mussel *Perna perna* and the fish *Danio rerio* and *Jenynsia multidentata*: In vitro studies.**	**Aquatic toxicology), 130, 171.**

Reliability	
Purpose of the study Description of endpoints	Although glyphosate is not classified as an acethylcholinesterase inhibitor, some studies have reported reduction in the acethylcolinesterase activity after in vivo exposure to both its pure form and its commercial formulations. Considering this controversy, the objective of the present study was to investigate, in vitro, the effects of glyphosate exposure on cholinesterase activity of the brown mussel *Perna perna* and of two fish species: zebrafish *Danio rerio* and onesided livebearer *Jenynsia multidentata*.
Test compound, application procedure, exposure period	Pure glyphosate, no further information.
Experimental approach, Statistics, test environment	For in vitro assays, samples (n = 3–5) of brain (fish), muscle (fish and mussel) and gills (mussel) were homogenized. The enzyme activity was determined using DTNB (5,5_-dithio-bis 2-nitrobenzoicacid) and acetylthiocoline iodide (mussel – 1 mM; fish – 0.75 mM) as substrates (Ellman et al., 1961).
Test organisms	Brown mussel *Perna perna* and of two fish species: zebrafish *Danio rerio* and onesided livebearer *Jenynsia multidentata*.
Biological effects	Cholinesterase from all the fractions analyzed for the mussel *P. perna* had higher sensitivity to glyphosate (IC50 values ranging from 0.62 to 0.81 mM) when compared to cholinesterase of *D. rerio* (IC50 values ranging from 2.43 to 6.67 mM) and *J. multidentata* (IC50 values ranging from 4.26 to 8.43 mM).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*D. rerio* and *P. perna* are usually used as models in toxicological studies, and *J. multidentata* is found naturally close to agricultural areas in Southern Brazil, being currently exposed to herbicides, including glyphosate.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Further investigations are necessary to relate results obtained here to physiological conditions.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Inhibition seems to be dependent of relative high concentrations of glyphosate.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to predicted environmental concentrations?	IC50 values reported were greater than the concentrations encountered in environment.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Pure glyphosate was able to inhibit cholinesterase activity in vitro in a concentration-dependent manner for all species and fractions tested. Inhibition seems to occur at relative high concentrations of glyphosate.
Type of information (Critical, supporting, low weight)	**supporting**

Consideration/concluding score	**UBA2**

Syedkolaei, et al. (2013)

BVL Nr.:	**Syedkolaei, S. J. G., Shiri, N., Mirvaghefi, A. R., Rafiee, G. R., & Makhdomi, C.**	**2013**	**Toxicity evaluation of Malathion, Carbaryle and Glyphosate in common carp fingerlings (*Cyprinus carpio*, Linnaeus, 1758), translation from arabic**	**Journal of Veterinary Research, 68(3), 257-267.**

Reliability	
Purpose of the study Description of endpoints	The objectives of this study were to determine the 96h-LC50-value of the common carp subjected to glyphosate stress and the effect of its sub-lethal concentrations on the AChE activity of different tissues (brain, muscle, gill and liver) hematological and biochemical parameters.
Test compound, application procedure, exposure period	Common carp was subjected to Roundups at 0 (control), 3. 5, 7 and 14 ppm for 16 days, and the AChE activity is verified in tissues of gill, muscle, brain and liver.
Experimental approach, Statistics, test environment	Fish behavior and acute toxicity of glyphosate (roundups), AChE activity assay; Hematological indices; Serum enzyme assay
Test organisms	*Cyprinus carpio*
Biological effects	The 96h-LC50 of Roundup to *C. carpio* was found to be 22.19 ppm. After 5 days, a significant decrease was observed in the AChE activity of muscle, brain and liver tissues. A time- and dose-dependent increase in mean cell hemoglobin (MCH) and mean cell volume (MCV) was observed. In contrast, a significant decrease was found in the quantities of hemoglobin (Hb), hematocrit (HCT) and red (RBC) and white (WBC) blood cell count. Also, the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) in Roundup treated groups were significantly higher than the controlled group at experimental periods.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Since biochemical parameters were assessed, the study has limited value to conclude on the relevance on the population level.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Biochemical parameters not part of traditional ERA. It seems that the changes in hematological and biochemical parameters could be used as efficient biomarkers in order to determine Roundups toxicity in aquatic environment.

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Roundup as a glyphosate-based formulation has been utilized as one of the most commonly applied herbicides. The study could not be taken into account as critical information for the assessment of the active substance glyphosate itself.
2 Do the tested concentrations relate to predicted environmental concentrations?	Glyphosate concentrations ranged from 3.5 mg/L in water of experimental tank to 14 mg/L. These concentrations are exceeding the environmental concentrations by far.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Biochemical parameters are sensitive biomarkers, showing a dose-response relationship between Roundup concentrations and AChE activities in the different tissues in *C. carpio*.

Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2**

Erhunmwunse, et al. (2013)

BVL Nr.: 2716832	**Erhunmwunse, N. O., Alohan, F. I., Enuneku, A., Tongo, I., Ainerua, M., & Idugboe, S.**	**2013**	**Pathological alterations in the liver of post-juvenile African Catfish *(Clarias gariepinus)* exposed to sublethal concentrations of the herbicide Glyphosate.**	**Journal of Natural Sciences Research, 3(15), 87-91.**

Reliability	
Purpose of the study Description of endpoints	In this study, histopathological alterations in the liver of post-juvenile African catfish *Clarias gariepinus* exposed to varying concentration of the commonly used herbicide glyphosate (IPA 360 g/L) were used as biomarkers of effects. Alterations were both concentration and time specific.
Test compound, application procedure, exposure period	Commercial formulation of glyphosate (360 g/l-41 w.wt IPA) at four nominal concentrations of 72, 54, 32 and 18 mg/L were used for sub-lethal test and two replicate tests each were done along-side the main experiment; static renewal assay
Experimental approach, Statistics, test environment	Cellular rupture, aggregation of inflammatory cells, vacuolar degeneration in the hepatocytes, focal areas of necrosis, and rupture of blood vessels that resulted in haemorrhage were characteristic of Glyphosate-exposed liver.
Test organisms	post juvenile *Clarias gariepinus* of both sexes
Biological effects	Liver tissues of post juvenile African catfish *C. gariepinus* exposed to different concentrations of Glyphosate herbicides showed extensive degeneration of histological structure. The nature of the liver damage was concentration dependent.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Histopathological biomarkers allow examining specific target organs, including gills, kidney and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The study can not be taken into account as critical information or the assessment of the active substance glyphosate itself.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation of glyphosate
2 Do the tested concentrations relate to predicted environmental concentrations?	Based on modelling assumptions, the concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.Test concentration is exceeding this by far.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	The observed alterations in the liver of *C. gariepinus* provide evidence to use pathological change in fish as an indicator for monitoring the effect of exposure to low levels of toxicants, which are capable of altering the physiological profile of an organism.
Type of information (Critical, supporting, low weight)	**supporting**

Consideration/concluding score	**UBA2**

Moreno, et al. (2014)

BVL Nr.: 2716708	**Moreno, N. C., Sofia, S. H., & Martinez, C. B.**	**2014**	**Genotoxic effects of the herbicide Roundup Transorb®and its active ingredient glyphosate on the fish *Prochilodus lineatus*.**	**Environmental toxicology and pharmacology, 37(1), 448-454.**

Reliability	
Purpose of the study Description of endpoints	The purpose of this study was to compare the genotoxicity of the active ingredient glyphosate with the formulated product RT, in order to clarify whether the surfactant of the RT formula may exert toxic effects on the DNAmolecule, since its composition has not been revealed yet.
Test compound, application procedure, exposure period	Roundup Transorb®(RT) or only to the active ingredient glyphosate (GLY): Fish were exposed to the commercial formulation of Roundup Transorb® (480 g glyphosate/L, Monsanto doBrazil Ltda) at two nominal concentrations: 1 mg/L(RT1) and 5 mg/L(RT5) or only to clean water (negative control or NC) for 6, 24 and 96 hours. In another set of experiments fish were exposed to glyphosate (Sigma–Aldrich; CAS no. 1071-83-6) at two nominal concentrations of 0.48 mg/L (GLY 0.48) and 2.40 mg/L (GLY 2.4), or only to clean water (negative control or NC), for 6, 24 and 96 hours.
Experimental approach, Statistics, test environment	Comet assay
Test organisms	Juveniles of *Prochilodus lineatus*
Biological effects	Erythrocytes of fish exposed to both concentrations of RT (1 and 5 mg/L) and glyphosate (0.48 and 2.4 mg/L) showed DNA damage after 96 h, while gill cells did not.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	For an evaluation of sublethal effects their population relevance has to be clarified.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to predicted environmental concentrations?	Based on modelling assumptions, the concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.
3 Have parameters influencing the endpoints been considered adequately?	Blood and gill cells of *P. lineatus* can respond differently to DNA damage, reinforcing the importance of using different tissues as complementary tools for detecting genotoxicity in fish.
Concluding weight of evidence/proposed action	Glyphosate and RT cause damage to the DNA molecule of *P. lineatus* at concentrations higher than those predicted in the environment. It is evident that further studies are needed to determine if the changes on biochemical traits are translated to sublethal long-term effects. Glyphosate and the tested product caused damage to the DNA nucleoids of P. lineatus at concentrations higher than those predicted in the environment.

	Erythrocytes exposed to both concentrations of the formulated product (1 and 5 mg/L) and the active substance glyphosate (0.48 and 2.4 mg/L) showed DNA damage after 96 h. Nevertheless, the study shows slight deficiencies, as it is unclear why the negative control showed increase in damaged nucleotids in gill cells (24.3 after 6h and 45 after 96h). This observation is further seen in the positive control, when DNA damage increases after 96 h only. Further studies might demonstrate the sensitivity of the test system is time dependent. Therefore, further studies would be needed to determine if the changes on DNA strands are reproducible. Furthermore, effects observed in the given experimental setup are not transferable into reproductive success of fish as demonstrated by three regulatory studies, conducted according to internationally agreed test guidelines, where at comparable exposure concentrations not adverse effects on reproductive success could be observed.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Webster, et al. (2013)

BVL Nr.:	**Uren Webster, T. M., Laing, L. V., Florance, H., & Santos, E. M.**	**2013**	**Effects of glyphosate and its formulation, Roundup®, on reproduction in zebrafish (*Danio rerio*).**	**Environmental science & technology.**

Reliability	
Purpose of the study Description of endpoints	Authors hypothesized that the mechanisms of toxicity resulting in effects on reproduction might include oxidative stress and disruption of steroid biosynthesis, and to investigate this transcript profiling of a suite of genes involved in these processes in the gonads were conducted. They aimed to investigate the reproductive effects of Roundup and glyphosate in fish and the potential of associated mechanisms of toxicity.
Test compound, application procedure, exposure period	1. The treatment groups consisted of 3 concentrations of Roundup; 0.01, 0.5, and 10 mg/L glyphosate acid equivalent (using Roundup GC liquid glyphosate concentrate containing 120 g/L glyphosate acid, Monsanto, Cambridge, U.K.), 2. 10 mg/L glyphosate (analytical grade; Molekula, Wimborne, U.K.) 3. control group.
Experimental approach, Statistics, test environment	OECD guidelines for fish reproductive tests, 21 d, Transcript Profiling and Histological Analysis
Test organisms	Colonies of 4 male and 4 female adult (20 week old) WIK strain zebrafish
Biological effects	10 mg/L glyphosate reduced egg production but not fertilization rate. Both 10 mg/L Roundup and glyphosate increased early stage embryo mortalities and premature hatching. Exposure during embryogenesis alone did not increase embryo mortality, suggesting that this effect was caused primarily by exposure during gametogenesis. Transcript profiling of the gonads revealed 10 mg/L Roundup and glyphosate induced changes in the expression of cyp19a1 and esr1 in the ovary and hsd3b2, cat, and sod1 in the testis.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Results show that high concentrations of Roundup and glyphosate induce developmental toxicity in zebrafish.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to predicted environmental concentrations?	No: Based on conservative modelling assumptions, the worst-case concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Results demonstrate that the tested chemicals cause reproductive toxicity in zebrafish, although only at concentrations unlikely to occur in the environment.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Gholami-Seyedkolaei, et al. (2013)

BVL Nr.:	**Gholami-Seyedkolaei, S. J., Mirvaghefi, A., Farahmand, H., & Kosari, A. A.**	**2013**	**Effect of a glyphosate-based herbicide in *Cyprinus carpio*: Assessment of acetylcholinesterase activity, hematological responses and serum biochemical parameters**	**Ecotoxicology and Environmental Safety 98(2013), 135–141**

Reliability	
Purpose of the study Description of endpoints	The objectives of this study were to determine the 96h-LC50-value of the common carp subjected to glyphosate stress and the effects of its sub-lethal concentrations on the AChE activity of different tissues (brain, muscle, gill and liver), hematological and biochemical parameters.
Test compound, application procedure, exposure period	Common carp was subjected to Roundups at 0(control), 3.5, 7 and 14 ppm for 16days,and the AChE activity is verified in tissues of gill,muscle,brain and liver.
Experimental approach, Statistics, test environment	Fish behavior and acute toxicity of glyphosate (Roundups), AChE activity assay; Hematological indices; Serum enzyme assay
Test organisms	*Cyprinus carpio*
Biological effects	The 96h-LC50 of Roundups to *C. carpio* was found to be 22.19 ppm. After 5 days, a significant decrease was observed in the AChE activity of muscle, brain and liver tissues. A time- and dose-dependent increase in mean cell hemoglobin (MCH) and mean cell volume (MCV) was observed. Incontrast, a significant decrease was found in the quantities of hemoglobin (Hb), hematocrit (HCT) and , red (RBC) and white (WBC) blood cell count. Also, the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) in Roundups treated groups were significantly higher than the controlled group at experimental periods.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Since biochemical parameters were assessed, the study has limited value to conclude on the relevance on the population level.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Biochemical parameters are not part of traditional ERA. It seems that the changes in hematological and biochemical parameters could be used as efficient biomarkers in order to determine Roundups toxicity in aquatic environment.

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Roundups as a glyphosate-based formulation has been utilized as one of the most commonly applied herbicides. The study could not be taken into account as critical information on the ERA of the active substance glyphosate itself. Nevertheless it shows that the commercial formulation can have sublethal effects.
2 Do the tested concentrations relate to predicted environmental concentrations?	Glyphosate concentrations ranged from 3.5 mg/L in water of experimental tank to 14 mg/L. These concentrations are exceeding the environmental concentrations by far.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Biochemical parameters are sensitive biomarkers, showing a dose-response relationship between Roundup concentrations and AChE activities in the different tissues in *C. carpio.*
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2**

Gholami-Seyedkolaei, S. J. et al. (2013)

BVL Nr. 2716776	**Gholami-Seyedkolaei, S. J. et al.**	**2013**	**Optimization of recovery patterns in common carp exposed to roundup using response surface methodology: Evaluation of neurotoxicity and genotoxicity effects and biochemical parameters**	

Reliability	
Purpose of the study Description of endpoints	The objective of this study was to evaluate the affecting variables on the recovery improvement of *C. carpio* under Roundups stress by statistical optimization using central composite rotatable design (CCRD) of RSM.
Test compound, application procedure, exposure period	Round-ups, (Monsanto) containing isopropylammoniumsalt of glyphosate at 480 g/l as the active ingredient (equivalent to 360 g glyphosate/L) and polyethoxyleneamine (POEA) as surfactant w as used..
Experimental approach, Statistics, test environment	The sub-lethal toxicity bioassay of Roundup in common carp was investigated. After exposure for 16 days to Roundups, some the fishes were introduced to herbicide-free water.The effects of recovery parameters including time, temperature, water exchange rate, and salinity on the levels of biomarkers of genotoxicity (DNA damage), neurotoxicity and the serumalanine (ALT) and aspartate (AST) aminotransferase in plasma were studied.
Test organisms	Common carp (*Cyprinus carpio* L.)
Biological effects	The minimum levels of DNA damage were time of 20 d, temperature of 20 °C, WER of 25 and water salinity of 6 ppt.

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	The study investigates the main, quadratic and interaction effects of four different recovery parameters including time, temperature, WER and salinity on the neurotoxicity, genotoxicity and aminotransferase characteristic of common carps exposed to Roundups herbicide.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative?	Commercial formulation
2 Do the tested concentrations relate to predicted concentrations?	Predicted environmental concentrations might be lower

3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Recovery trend of fishes exposed to Roundups. It is evident that further studies are needed to determine if the changes on biochemical traits are translated to sublethal long-term effects.
Type of information (Critical, supporting, low weight)	low weight
Consideration/concluding score	UBA3

De Souza Filho, et al. (2013)

BVL Nr.: 2716707	**De Souza Filho, J., Sousa, C. C. N., Da Silva, C. C., De Sabóia-Morais, S. M. T., & Grisolia, C. K.**	**2013**	**Mutagenicity and Genotoxicity in Gill Erythrocyte Cells of *Poecilia reticulata* Exposed to a Glyphosate Formulation.**	**Bulletin of environmental contamination and toxicology, 91(5), 583-587.**

Reliability	
Purpose of the study Description of endpoints	The aim of this study was to get more information on the genotoxic risks to aquatic organisms of a more recent glyphosate formulation, Roundup Transorb.
Test compound, application procedure, exposure period	Transorb [64.8 %glyphosate m/v—648 g L-1; equivalent by N-(phosphonomethyl glycine acid) 480 g L-1 and inert ingredients 594 g L-1] formulation was obtained in a local market Brazil.
Experimental approach, Statistics, test environment	*Poecilia reticulata* were exposed to herbicide Roundup Transorb_ for micronucleus test, nuclear abnormalities and comet assay. The exposure-concentrations were based on LC50–96 h following 0, 1.41, 2.83, 4.24 and 5.65 µL /L for 24 h.
Test organisms	Adult *Poecilia reticulata* fish (0.293 ± 0.13 g, 3.58 ± 1.1 cm),
Biological effects	CA and MN demonstrated that R. Transorb herbicide showed a significant mutagenic and genotoxic effect on erythrocyte gill cells of *P. reticulata*, when exposed at concentrations of 1.41, 2.83, 4.24 and 5.65 µl/L, and the causes of these effects are more related to compounds present in the herbicide formulation than its active ingredient.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect? Is the ecotoxicological manifestation level appropriate for the assessment?	For a reasonable assessment of sublethal and / or specific biological effects i (eg. genotoxic or endocrine effects) its relevance to changes in the structures of populations has to be clarified.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product containing POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	Effect could be attributed to a combination of compounds contained in the formulation with the active ingredient glyphosate.
Concluding weight of evidence/proposed action	The commercial formulation containg POEA shows a gradual increase in the number of damaged cells, indicating a concentration-dependent effect and that this herbicide was mutagenic and genotoxic to *P. reticulata*. The 96h LC50 for Roundup®Transorb in the absence and presence of predator stress were 3.76 mg ae/L and 3.39 mg ae/L, respectively.

	The 10-day LC50 value for Roundup was significantly lower, 2.12 mg ae/L and 1.91 mg ae/L in the absence and presence of predator stress, respectively. Lower concentrations of Roundup (1, 2 and 3 mg ae/L) induced the formation of micronuclei (MN) in the erythrocytes in a concentration-dependent manner. Presence of predator stress seemed to increase the toxicity and genotoxicity of Roundup®; but these effects were not statistically significant.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Akinsorotan, et al. (2013)

BVL Nr.: 2716465	**Akinsorotan, A., Zelibe, S., & Olele, N.**	**2013**	**Acute Toxicity And Behavioural Changes On African Catfish (*Clarias gariepinus*) Exposed To Dizensate (Glyphosate Herbicide).**	**International Journal of Scientific & Engineering Research 4(3).**

Reliability	
Purpose of the study Description of endpoints	This study determines the 96h LC50 of glyphosate herbicide to adult of *C. gariepinus* and reports the effects on the physiology and survival of fish.
Test compound, application procedure, exposure period	Aquatic herbicide Dizensate at concentrations of 9.6 mg/L, 14.4 mg/L, 19.2 mg/L, 21.6 mg/L and 24 mg/L. The exposure lasted for 96 hours.
Experimental approach, Statistics, test environment	Dizensate herbicide formulation and concentration of 9.6 mg/L, 14.4 mg/L, 19.2 mg/L, 21.6 mg/L and 24 mg/L were introduced. The LC50 was determined graphically using logarithm transformation at the end of the exposure period. All data obtained were subjected to Analysis of Variance (ANOVA) and multiple range test using the SAS.
Test organisms	African Catfish (*Clarias gariepinus*) adults (mean wt., 215g)
Biological effects	Mean mortality was 0, 17, 33, 50 and 83 % in the concentration of 19.2, 28.8, 38.4, 48.0 and 57.6 mg/L respectively, while there was no mortality in the control treatment. The four days median lethal concentration (96h-LC50) was determined as 43.65 mg/L.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation.
2 Do the tested concentrations relate to predicted environmental concentrations?	Aquatic herbicide used within water ecosystems, Predicted environmental concentrations might be lower in EU; (no overspray of wetlands in the European Union)
3 Have parameters influencing the endpoints been considered adequately?	Physicochemical parameter were measured.
Concluding weight of evidence/proposed action	The study was carried out to determine the toxicity of dizensate herbicide to the most cultivable fish species in Nigeria. The four days median lethal concentration (96h LC50) was determined as 43.65mg/l.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

de Castilhos Ghisi, et al. (2012)

BVL Nr.: 2716829	**de Castilhos Ghisi, N., & Cestari, M. M**	**2012**	**Genotoxic effects of the herbicide Roundup® in the fish *Corydoras paleatus* (Jenyns 1842) after short-term, environmentally low concentration exposure.**	**Environmental Monitoring and Assessment, 185(4): 3201-3207.**

Reliability	
Purpose of the study Description of endpoints	In this study, authors hypothesized that short-term exposure to Roundup® may affect the DNA of this bioindicator fish species, even at an environmentally realistic concentration.
Test compound, application procedure, exposure period	Roundup® Fish responses to different exposure times (3, 6, and 9 days) were tested in semi-static bioassays, whereby one third of the water was replaced every 48 h.
Experimental approach, Statistics, test environment	A bioassay was carried out on 20 animals in the 5.1± 0.7-cm size group, which were placed in 15-L aquariums containing a concentration of 6.67 µg/L of Roundup® (48 % glyphosate, corresponding to 3.20 µg/L glyphosate). This evaluation was made for three genetic biomarkers: piscine micronucleus test (PMT) and the comet assay with blood cells and hepatic cells.
Test organisms	*Corydoras paleatus*
Biological effects	The PMT did not reveal any differences between the control and treatment groups at different exposure times. In contrast, the comet assay showed a significant increase in the rate of DNA damage in C. paleatus blood and hepatic cells after exposure to Roundup®.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Neotropical fish
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	-/-
3 Is the ecotoxicological manifestation level appropriate for the assessment?	-/-.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The toxicological effects of glyphosate were tested using its commercial form (i.e., as Roundup® rather than pure glyphosate)
2 Do the tested concentrations relate to predicted environmental concentrations?	The herbicide concentration used in this experiment is environmentally realistic and was chosen based on the literature.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Authors conclude that short-term exposure of the pesticide Roundup® is genotoxic to the bioindicator fish species *C. paleatus*, even at a relatively low concentration. However, this low concentration tested did not result in clastogenic and/or aneugenic effects. Moreover, the European Community limit (i.e., 0.10 µg/L of pesticide for surface waters) is below the concentration that was tested in this experiment.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Vera-Candioti, et al (2013)

BVL Nr.:	**Vera-Candioti, J., Soloneski, S., Larramendy, M.L.**	**2013**	**Evaluation of the genotoxic and cytotoxic effects of glyphosate-based herbicides in the ten spotted live-bearer fish *Cnesterodon decemmaculatus* (Jenyns, 1842).**	**Ecotoxicology and environmental safety, 89(1): 166–173.**

Reliability	
Purpose of the study Description of endpoints	Characterization of the lethal and sub-lethal toxicity of Panzer and Credit, two glyphosate-based herbicides, on *C. decemmaculatus* exposed under laboratory conditions. Endpoint: LC50 estimation, induction of micronuclei (MN) and alterations in the erythrocyte:erythroblast ratio were employed as biomarkers of genotoxicity and cytotoxicity, respectively.
Test compound, application procedure, exposure period	Two commercialformulations of glyphosate namely Panzer (Dow Agrosciences) and Credit (Nufarm S.A.) containing isopropylaminesalt of glyphosateat 480g/L as active ingredient (equivalent to 48% of glyphosate) were used.
Experimental approach, Statistics, test environment	Determination of LC50, Determination of MN frequency, Analysis of erythrocytes:erythroblasts ratio
Test organisms	*Cnesterodon decemmaculatus*
Biological effects	The LC_{50} 96 h in the current study were 15.68 and 91.73 mg/L for Panzers and Credits, respectively. Both 3.9 and 7.8 mg/L of Panzers increased MN frequency at 48 and 96h of treatment.When fish were exposed to Credits, an increased frequency of MN over control values was found after 96 h for all concentrations assayed, but not after 48 hours.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product containing surfactants.
2 Do the tested concentrations relate to predicted environmental concentrations?	-/-
3 Have parameters influencing the endpoints been considered adequately?	The surfactant POEA is the principal toxic component in the glyphosate-based formulation Roundup.
Concluding weight of evidence/proposed action	In terms of cytotoxic effects neither Panzer nor Credit were capable to induce alterations in erythrocyte:erythroblast ratios, at least within the concentration ranges used.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Guilherme, et al . (2012)

BVL Nr.:	**Guilherme, S., Santos, M.A., Barroso, C., Gaivão, I., Pacheco, M.**	**2012**	**Differential genotoxicity of Roundup® formulation and its constituents in blood cells of fish (*Anguilla anguilla*): considerations on chemical interactions and DNA**	**Ecotoxicology, 21(5): 1381-1390.**

			damaging mechanisms.	

Reliability	
Purpose of the study Description of endpoints	The present study addressed the herbicide Roundup, evaluating the relative contribution of the active ingredient (glyphosate) and the surfactant (polyethoxylated amine; POEA) to the genotoxicity of the commercial formulation on *Anguilla anguilla*.
Test compound, application procedure, exposure period	Roundup-Ultra (isopropylammonium salt of glyphosate at 485 g L-1 as the active ingredient (equivalent to 360 g/L or 30.8 % of glyphosate) and POEA (16 %) as surfactant. Glyphosate (Sigma-Aldrich, POEA (solution at 785 g/L)
Experimental approach, Statistics, test environment	Fish were exposed to equivalent concentrations of Roundup (58, 116 µg/), glyphosate (17.9, 35.7 µg/L) and POEA (9.3, 18.6 µg/L), during 1 and 3 days. The comet assay was applied to blood cells, either as the standard procedure, or with an extra step involving DNA lesion-specific repair enzymes in an attempt to clarify DNA damaging mechanisms.
Test organisms	*Anguilla anguilla* average weight 0.25 ± 0.02 g
Biological effects	Mean values of genetic damage indicator (GDI) measured by the standard (alkaline) comet assay displayed significantly higher values in comparison with the control. Both components contributed to the overall genotoxicity of the pesticide formulation. The analysis of oxidatively induced breaks suggested that oxidation of DNA bases was not a dominant mechanism of damage.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Unclear. Relevance to fish populations by the assessed parameters should be clarified.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The major purpose was to improve the knowledge on the DNA damaging.
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	The analysis of oxidatively induced breaks suggested that oxidation of DNA bases was not a dominant mechanism of damage. The experiment was conducted using the commercial formulation Roundup® Ultra, distributed by Bayer Crop- Science (Portugal), containing isopropylammonium salt of glyphosate at 485 g/L as the active ingredient (equivalent to 360 g/Lor 30.8 % of glyphosate) and POEA (16%) as surfactant. Fish were exposed to equivalent concentrations of the Roundup product (58, 116 µg/L), glyphosate (17.9, 35.7 µg/L) and POEA (9.3, 18.6 µg/L) for 1 and 3 days. The comet assay was applied to blood cells, either as the standard procedure, or with an extra step involving DNA lesion-specific repair enzymes in an attempt to clarify DNA damaging mechanisms. Mean values of genetic damage indicator displayed significantly higher values in comparison to the control. Both components of the formulated product seem to contribute to the effect on genetic structure of the formulation Roundup® Ultra. Roundup® Ultra displayed levels of damage at both tested concentrations and exposure times. Nevertheless, fish exposed to the highest concentration of glyphosate for 3 days recovered from the damage detected after 1 day exposure. The ability of the test organisms to recover from the observed effects was evaluated by adding repair enzymes to the cells. At the highest tested concentration of glyphosate, a repair of DNA damage was seen. Hence, the observed time-related disappearance of DNA damage could be a result of the

	intervention of a DNA repair system. Effects observed in the given experimental setup are not directly transferable into reproductive success of fish as demonstrated by three regulatory studies, conducted according to internationally agreed test guidelines, where at comparable exposure concentrations not adverse effects on reproductive success could be observed.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Guilherme, et al . (2012)

BVL Nr.:	**Guilherme, S. et al.**	**2012**	**DNA damage in fish (*Anguilla anguilla*) exposed to a glyphosate-based herbicide - Elucidation of organ-specificity and the role of oxidative stress**	

Reliability	
Purpose of the study Description of endpoints	The present study addressed the herbicide Roundup, evaluating the relative contribution of the active ingredient (glyphosate) and the surfactant (POEA) to the genotoxicity of the commercial formulation on *Anguilla anguilla*.
Test compound, application procedure, exposure period	Roundup-Ultra (isopropylammonium salt of glyphosate at 485 g/L (equiv. to 360 g/L or 30.8 % of glyphosate) and POEA (16 %) as surfactant. Glyphosate (Sigma-Aldrich, POEA (solution at 785 g/L)
Experimental approach, Statistics, test environment	Fish were exposed to equivalent concentrations of Roundup (58, 116 µg/L), glyphosate (17 and 35. µg/L) and POEA (9 and 18 µg/L), during 1 and 3 days. The comet assay was applied to blood cells, either as the standard procedure, or with an extra step involving DNA lesion-specific repair enzymes in an attempt to clarify DNA damaging mechanisms.
Test organisms	*Anguilla anguilla* average weight 0.25 ± 0.02 g
Biological effects	Mean values of genetic damage indicator (GDI) measured by the standard (alkaline) comet assay displayed significantly higher values in comparison with the control. Both components contributed to the overall genotoxicity of the pesticide formulation. The analysis of oxidatively induced breaks suggested that oxidation of DNA bases was not a dominant mechanism of damage.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Unclear. Relevnce to fish populations by the assessed parameters should be clarified.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The major purpose was to improve the knowledge on the DNA damaging.
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	The analysis of oxidatively induced breaks suggested that oxidation of DNA bases was not a dominant mechanism of damage.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Guilherme, et al. (2014)

BVL Nr.:	**Guilherme, S., Santos, M. A., Gaivão, I., & Pacheco, M**	**2014**	**DNA and chromosomal damage induced in fish (*Anguilla anguilla* L.) by aminomethylphosphonic acid (AMPA)—the major environmental breakdown product of glyphosate.**	**Environmental Science and Pollution Research, 1-10.**

Reliability	
Purpose of the study Description of endpoints	The main goal of the present research was to assess the genotoxic potential of AMPA, as the major breakdown product of glyphosate, in blood cells of *Anguilla anguilla* L, following short-term exposures (1 and 3 days) to environmentally realistic concentrations (11.8 and 23.6 µg/L).
Test compound, application procedure, exposure period	AMPA and all chemicals needed to perform the comet assay and the ENAs test were obtained from the Sigma-Aldrich. For each test condition, 1- and 3-day exposures were carried out. The experiment was carried out in 1-L aquaria, in a semistatic mode. Fish were exposed to 11.8 and 23.6 µg/L AMPA.
Experimental approach, Statistics, test environment	Comet assay , ENA assay
Test organisms	*A. anguilla L.*) with an average weight 0.25±0.02 g (glass eel stage)
Biological effects	Mean values of genetic damage indicator (GDI) measured by the standard (alkaline) comet assay in blood cells of A. anguilla exposed to 11.8 and 23.6 µg/L aminophosphoric acid (AMPA; A1, A2) showed significant increases. the lack of a significant GDI increase for the lower concentration (A1) after 3 days, it can be suggested that fish had the capacity to adapt to the genotoxic stimulus.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Risk posed to fish populations by the assessed parameters may be clarified . High concentrations of glyphosate-based herbicide used in agricultural fields can cause effects on population level, when reproductive traits are affected.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Effects on biochemical responses like changes on chromosomal damage are currently considered as supporting information on commercial products in the field of environmental risk assessment. Nevertheless, it is evident that further studies are needed to determine if the changes on biochemical traits are translated to sublethal long-term effects. Mean values of genetic damage indicator measured by comet assay in blood cells of A. anguilla exposed to 11.8 and 23.6 µg/L

	aminophosphoric acid showed significant increases after 1 day. After 3 days, no effects were observed for the tested lower concentration. Possibly, tested fishs had the capacity to use successfully DNA repair mechanisms. No significant alterations were found in erythrocytic nuclear abnormalities (ENA) assays following the first day of exposure; considering the 3-day exposure, a significant increase for the higher concentration of AMPA was observed. Based on modelling assumptions, the concentrations expected in surface waters exposed to drift are 0.041 mg AMPA/L. Effects observed in the given experimental setup are not transferable into reproductive success of fish as demonstrated the regulatory study, conducted according to internationally agreed test guidelines, , where at comparable exposure concentrations not adverse effects on reproductive success could be observed.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UAB2**

Guilherme, S. et al. (2014)

BVL Nr. 2716777	**Guilherme, S. et al.**	**2014**	**Are DNA-damaging effects induced by herbicide formulations (Roundup® and Garlon®) in fish transient and reversible uponcessation of exposure?**	
Reliability				
Purpose of the study Description of endpoints	The study intended to shed light on the ability of fish to recover from the DNA damage induced by short-term exposures to Roundup® and Garlon® (triclopyr-based) upon the exposure cessation.			
Test compound, application procedure, exposure period	Roundup®Ultra, distributed by Bayer CropScience (containing isopropylammonium salt of glyphosate at 485 g/L as the active ingredient (equivalent to 360 g/L or 30.8% w/v of glyphosate) and POEA (16% v/v) as surfactant . exposure for 3 days, recovery for 1, 7 and 14 days (post-exposure period).			
Experimental approach, Statistics, test environment	The experiments was carried out in 1-L aquaria, in a semi-static mode, comet assay.			
Test organisms	European eel (*Anguilla anguilla*)			
Biological effects	GDI parameter revealed a decrease of the non-specific DNA damage induced by Roundup®after 14days in herbicide-free water, while GDIFPG and GDIEndoIII failed to express a complete recovery of DNA stability.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	yes			
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Question remains whether a single exposure can lead to long-term genome-destabilizing effects and cause stable genotoxic lesions.			
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd			
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?	Roundup®,containing surfactant system with POEA has limited validity for other formulations.			
2 Do the tested concentrations relate to predicted environmental concentrations?	Fish were exposed to 116 µg/L Roundup®, which is assumed to display a realistic worse case concentration.			

3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Roundup®: a recovery was observed when considering non-specific DNA damage on day 14 post-exposure. Effects on biochemical responses like changes on chromosomal damage are currently considered as supporting information on commercial products in the field of environmental risk assessment. Nevertheless, it is evident that further studies are needed to determine if the changes on biochemical traits are translated to sublethal long-term effects. The study investigated the ability of fish to recover from the DNA damage induced by short-term exposures to Roundup® and Garlon® (triclopyr-based). Roundup®Ultra, distributed by Bayer CropScience (containing isopropylammonium salt of glyphosate at 485 g/L as the active ingredient (equivalent to 360 g/L or 30.8% w/v of glyphosate) and POEA (16% v/v) as surfactant). Exposure for 3 days, recovery for 1, 7 and 14 days (post-exposure period). Fish were exposed to 116 µg/L Roundup®, which is assumed to display a realistic worse case concentration. A decrease of the non-specific DNA damage induced by Roundup® after 14days in herbicide-free water could be demonstrated, while a complete recovery of DNA stability could not be shown. A recovery was observed when considering non-specific DNA damage on day 14 post-exposure. Effects of commercial formulations of glyphosate on responses like changes on chromosomal damage in the field of environmental risk assessment are currently considered as supporting information, especially since this evaluation aims at assessing the risk of non target organisms exposed to glyphosate. . Nevertheless, it is evident that further studies with the respective formulated product are needed to determine if the changes on biochemical traits are translated to sublethal long-term effects.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UAB2**

Armiliato,et al (2014)

BVL Nr.:	**Armiliato, N., Ammar, D., Nezzi, L., Straliotto, M., Muller, Y. M., & Nazari, E. M.**	**2014**	**Changes in Ultrastructure and Expression of Steroidogenic Factor-1 in Ovaries of Zebrafish *Danio rerio* Exposed to Glyphosate.**	**Journal of Toxicology and Environmental Health, Part A, 77(7), 405-414.**

Reliability	
Purpose of the study Description of endpoints	The aim of this study was to evaluate the toxicity of glyphosate on ovaries of zebrafish (*Danio rerio*).
Test compound, application procedure, exposure period	Glyphosate, Monsanto do Brasil Ltda
Experimental approach, Statistics, test environment	The research article presents effects of glyphosate on morphological and endocrine traits in fish ovaries. Fish were exposed for 15 days, N=18 per triplicate for the test item and the control group. Organisms per replicate: 1.
Test organisms	Adult females of the zebrafish *Danio rerio*.
Biological effects	Damage due to 65 µg glyphosate/L related to structure and ultrastructure of oogonia and oocytes was assessed, and also the expression of the ovarian steroid hormone regulator SF-1: No apparent change in general morphology towards ovaries, significant increase in diameter of oocytes was observed, immunohistochemitry and immunoblotting revealed greater expression of SF1 in oocytes.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	The concentration tested produced sublethal effects, which are recognizable using molecular and ultrastructural tools.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Glyphosate induces subcellular and molecular impairments that may affect reprodution in femal fish. No dose-response endpoints or NOEC can be extracted from this article, because one singleconcentration was tested.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	No details on the composition and the purity of the test item are given.
2 Do the tested concentrations relate to predicted environmental concentrations?	65 µg glyphosate/L. This concentration displays the range of the max. permissible concentration in Brazilian inland waters.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	No assessment possible due to lack of information regarding the formulation tested.
Type of information (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA 3**

Glusczak, L., et al. (2011)

BVL Nr.:	**Glusczak, L., Loro, V.L., Pretto, A., Moraes, B.S., Raabe, A., Duarte, M.F., de Sousa Valladão, D.M.**	**2011**	**Acute exposure to glyphosate herbicide affects oxidative parameters in Piava (*Leporinus obtusidens*).**	**Archives of environmental contamination and toxicology, 61(4): 624-630.**
Reliability				
Purpose of the study Description of endpoints	The study aimed to investigate the effects of commercial formulation on oxidative stress parameters in piava (*Leporinus obtusidens*) as a complementary study concerning the toxicity of this herbicide.			
Test compound, application procedure, exposure period	Commercial formulation (480 g/L acid equivalent, 692 g/L inert ingredients; Monsanto Company, St. Louis, MO, USA). Herbicide was added to the water only at the beginning of the experiment.			
Experimental approach, Statistics, test environment	Teleostean fish (*Leporinus obtusidens*) were exposed to glyphosate formulation at 0 (control), 3, 6, 10 or 20 mg/L for 96 hours. The effects of the herbicide on plasmatic metabolic parameters, thiobarbituric acid reactive substances (TBARS), catalase activity, protein carbonyl, and mucusm layer parameters were studied.			
Test organisms	Teleostean fish (*Leporinus obtusidens*)			
Biological effects	Plasmatic glucose and lactate levels increased but protein levels showed reduction after herbicide exposure. TBARS levels in brain showed a reduction at all tested concentrations. However, liver demonstrated increased TBARS levels at all tested concentrations, whereas in white muscle TBARS production did not change after exposure to herbicide. Fish exposed to all concentrations of glyphosate showed increase in liver catalase activity and protein carbonyl. Herbicide exposure increased protein and carbohydrate levels of the mucus layer at all tested concentrations.			

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	High concentrations of glyphosate-based herbicide used in agricultural fields can cause changes in oxidative stress parameters in Piava.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial glyphosate herbicide formulation.
2 Do the tested concentrations relate to predicted environmental concentrations?	Based on modelling assumptions, the concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L. Test concentration is exceeding this by far.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	High concentrations of glyphosate-based herbicide used in agricultural fields can cause changes in oxidative stress parameters in Piava.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Penders, E.J.M., et al. (2012)

BVL NR. 2716394	**Penders, E.J.M.,Spenkelink, A., Hoogenboezem, W., Rotteveel, S.G.P., Maas, J.L., Alink, G.M.**	**2012**	**Genotoxic Effects in the Eastern Mudminnow (*Umbra pygmaea*) After Prolonged Exposure to River Rhine water, as Assessed by Use of the In Vivo SCE**	

Reliability	
Purpose of the study Description of endpoints	This study investigated (i) whether new data corroborate that in vivo genotoxicity of River Rhine water is at present lower than in 1978, (ii) whether the Comet assay is a suitable alternative to the SCE assay, and (iii) whether further prolonged exposure results in a further increase in in vivo genotoxicity.
Test compound, application procedure, exposure period	Exposure to River RhineWater fo 11 and 42 days.
Experimental approach, Statistics, test environment	Eastern mud minnows were exposed to River Rhine water for 11 and 42 days in flow-through aquaria, and gill cells were used for the SCE test and the Comet assay.
Test organisms	Eastern mud minnows (*Umbra pygmaea*)
Biological effects	After 11 days of exposure to River Rhine water, there was a significant increase in the number of Sister Chromatid Exchanges per chromosome compared to the control (P = 0.007).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant	River Rhine water used for the study contains a variety

for the substance being assessed?	of compounds of concern.
2 Do the tested concentrations relate to predicted environmental concentrations?	Average Concentration of glyphosate only 60-80 µg/L
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	The SCE assay points at increased DNA damage upon exposure of the fish to River Rhine water. The data corroborate that in vivo genotoxicity of River Rhine water is at present lower than in 1978.
Type of information (Critical, supporting, low weight)	The paper deals with the River Rhine water impact of DNA damage. The test substance River Rhine was described in detail as a mixture of pollutants, but can not be consultated for the risk assessment of the active ingredient 'glyphosate'.
Consideration/concluding score	**UBA3**

Fuzinatto, C.F., et al. (2013)

BVL Nr. 2716421	**Fuzinatto, C.F.,Flohr, L., Melegari, S.P., Matias, W.G.**	**2013**	**Induction of micronucleus of Oreochromis niloticus exposed to waters from the Cubatão do Sul River, southern Brazil**	

Reliability	
Purpose of the study Description of endpoints	The genotoxic potential of complex environmental mixtures taken from the Cubatão do Sul River on the fish species *Oreochromis niloticus* (Nile tilapia), by analyzing the frequency of micronucleus.
Test compound, application procedure, exposure period	Cubatão do Sul River water were tested from 4 sites.
Experimental approach, Statistics, test environment	Fish exposure assays, the samples were collected over 280 days. Erythrocyte collection, Micronucleus assay.
Test organisms	*Oreochromis niloticus* (Nile tilapia),
Biological effects	Please refer to paper

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	dn
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Pollution of aquatic environments, especially surface water from rivers that have potentially genotoxic substances, is a critical problem.
Type of information (Critical, supporting, low weight)	The paper deals with the genotoxic potential of river water. The test substance displays a mixture of pollutants, but can not consultated for the risk assessment of the active ingreient 'glyphosate' itself.
Consideration/concluding score	**UBA3**

El-Shebly, A.A., El-kady, M.A.H. et al (2008)

BVL Nr.: 2716721	**El-Shebly, A.A., El-kady, M.A.H.**	**2008**	**Effects of Glyphosate Herbicide on Serum Growth Hormone (GH) Levels and Muscle Protein Content in Nile Tilapia (*Oreochromis niloticus* L.)**	

Reliability	
Purpose of the study Description of endpoints	Changes in serum concentrations of growth hormone (GH) and muscle protein content in *O. niloticus* treated with different concentrations of glyphosate herbicide under laboratory conditions were evaluated.
Test compound, application procedure, exposure period	Roundup (Glyphosate 48% isopropylamine salt of glyphosate)
Experimental approach, Statistics, test environment	Nile tilapia *Oreochromis niloticus* (mean initial weight of 57.61 ± 4.8 g and initial length 14.87±1.7 cm) were exposed to different concentrations of Roundup: 0 (control), 1, 3, and 5 mg/L for 96 h (short-term) of exposure.
Test organisms	Nile tilapia (*Oreochromis niloticus*) of both sexes
Biological effects	Fish exposed to glyphosate concentrations of 3 and 5 mg/L showed rapid respiration and increased rate of gill cover movements, slowdown of reflexes and swimming movements, fish floated at the surface of water gasping for more oxygen and very rapid movements in various directions of the aquaria. The mucous secretion appeared to increase and accumulated on the gills.
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Endpoints (serum GH levels and muscle protein) analysed, nevertheless, the commercially formulated product was used, possibly containing POEA nd therefore it is of limited validity for the risk assessment of the active ingredient itself.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The conclusion from this study is only valid for glyphosate formulations that contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	Predicted environmental concentrations might be lower.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Authors observed that Roundup even used at low-concentrations affected the growth of .*O niloticus*.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Kaya, I. et al. (2012)

BVL Nr.: 2716729	**Kaya, I. et al.**	**2012**	**Investigation of effects on plasma nitric oxide, malondialdehyde and total sialic acid levels of glyphosate in Kars Creek transcaucasian barb (*Capoeta capoeta* [guldenstaedt, 1773]) in Turkey.**	

Reliability	
Purpose of the study Description of endpoints	In this study, *Capoeta capoeta* was used to determine plasma total sialic acid (TSA), nitric oxide (NO) and malondialdehyde (MDA) levels in fishes when glyphosate was applied.
Test compound, application procedure, exposure period	Korfosat 48 SL, commercial formualtion 0.01 and 0.02 mg/L glyphosate (N-phosphono-methyl glycine, 480 mg/L)
Experimental approach, Statistics, test environment	3 groups were constituted: Fishes in group I in normal water environment, group II and group III fishes sequentially were waited in water environments with 22±2 °C, 7.4 pH in-cluding 0.01 and 0.02 mg/L glyphosate (N-phosphono-methyl glycine, 480 mg/L, Korfosat 48 SL, alkaline chlorine protection, Izmit, Turkey) for 10 days. Nitric oxide levels were appointed. Plasma TSA analyses were performed. ANOVA.
Test organisms	*Capoeta capoeta*
Biological effects	With contamination of glyphosate (0.01 and 0.02 mg/L) in aquatic environment, it was observed that plasma TSA and MDA levels related to lipid peroxidation of *Capoeta capoeta* were significantly changed ($p<0.001$). NO levels were statistically insignificant ($p> 0.05$).

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Adult fishes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Unclear. Relevance of the assessed parameters to fish populations should be clarified .
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The conclusion from this study is only valid for the specific glyphosate formulation.
2 Do the tested concentrations relate to predicted environmental concentrations?	Predicted environmental concentrations seem to be realistic.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Korfosat 48 SL, a commercial formualtion elevated biochemical parameters related to lipid peroxidation.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Shiogiri, N.S. et al. (2012)

BVL Nr. 2716745	**Shiogiri, N.S. et al.**	**2012**	**Acute exposure of a glyphosate-based herbicide affects the gills and liver of the Neotropical fish, *Piaractus mesopotamicus***	

Reliability	
Purpose of the study Description of endpoints	The aim of this study was to determine the acute toxicity of Roundup® Ready (RR) in *Piaractus mesopotamicus*, and evaluate the effects on the morphology of the gills and liver of exposed surviving fish.
Test compound, application procedure, exposure period	Roundup® Ready, Fish were exposed to 3.0, 3.5, 4.0 and 4.5 mg/L.
Experimental approach, Statistics, test environment	Acute toxicity tests and LC50;48h determination, Gill and liver morphological analyses, Gill Na+/K+-ATPase (NKA) activity.
Test organisms	*Piaractus mesopotamicus*,
Biological effects	Gill histopathology was rare, and the activity of Na+/K+- ATPase enzyme did not change, suggesting normal function. the damage to the liver was classified as moderate to severe. Wandering swim patterns, dark color and irregularly aquatic surface respiration characterize the behavior of fish exposed to glyphosate levels ranging from 3.5 to 4.5 mg/L.
Biological Relevance	
1 Is an appropriate test species/ life-stage(s)studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Histopathological changes in the liver revealed moderate to severe and irreparable damage, which may affect liver function, but it is not clear if alterations might impair the population level.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The conclusion from this study is only valid for glyphosate formulations that contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	Predicted environmental concentrations might be lower.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	The overall toxicity of commercial formulations of glyphosate partly depends on the toxicity of its associated compounds, results provide evidence for the moderate toxicity of glyphosate in the RR formulation, but severe level of damage in the liver of fish.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Uchida, M. et al. (2012)

BVL Nr. 2716750	**Uchida, M. et al.**	**2012**	**Toxicity evaluation of glyphosate agrochemical components using Japanese medaka (*Oryzias latipes*) and DNA microarray gene expression analysis**	
Reliability				
Purpose of the study Description of endpoints	The study investigated toxicological effects of the major components of a glyphosate-based agrochemical: glyphosate and fatty acid alkanolamide (DA), first by performing acute toxicity test using toxicologically more sensitive juvenile medaka to determine LC50-values for each of the chemicals, and subsequently by gene expression analysis with medaka DNA microarray and the chemical-exposed adult medaka liver samples.			
Test compound,	N-(phosphonomethyl) glycine (a.k.a. glyphosate, > 99.3% purity: Cas No. 1071-			

application procedure, exposure period	83-6); The surfactant component of a herbicide used in this study was fatty acid alkanolamide (DA).
Experimental approach, Statistics, test environment	Acute toxicity test for juvenile medaka, microarray analysis.
Test organisms	juvenile Japanese medaka (*Oryzias latipes*)
Biological effects	96 h-LC50 of glyphosate, DA, and Roundup® herbicide were > 160 mg/L, 8.5 mg/L and 76.8 mg/L, respectively. No statistically significant ($p < 0.05$) differential expression was observed in the liver samples exposed to glyphosate. Authors observed significant ($p < 0.05$) expression changes (expression ratio of exposed to control > 2 or < 0.5) in 78 genes (44 genes were up-regulated and the 34 genes were downregulated) by the exposure of DA, and furthermore, 138 genes (125 genes were up-regulated and 13 genes were down-regulated) were observed to be affected by the exposure to the glyphosate/DA mixture.
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Not relevant
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, glyphosate as active ingredient was tested.
2 Do the tested concentrations relate to predicted environmental concentrations?	Predicted environmental concentrations might be lower.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	No statistically significant gene expression change was detected in adult medaka liver tissues that were exposed to glyphosate alone, showing that the surfactants in formulations play a significant role.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Bayir, M. et al. (2013)

BVL Nr. 2716762	**Bayir, M. et al.**	**2013**	**Alterations in Fatty Acids of Polar Lipids in *Salmo trutta* on Long-term Exposure to a Glyphosate-Based Herbicide (Roundup®)**	

Reliability	
Purpose of the study Description of endpoints	The effects of sublethal doses (10 and 20 mg/L) of Roundup® on fatty acid pattern in muscle and liver of brown trout were investigated.
Test compound, application procedure, exposure period	20 fish per group were exposed to a range of concentrations of Roundup®Ultra MAx including 450 g/L glyphosate for 30 days under flow-through conditions. Sublethal glyphosate concentrations were selected to be 10 mg/L (group b) and 20 mg/L (group c).
Experimental approach, Statistics, test environment	Lipid fraction separation, Lipid and fatty acid analysis: SPSS version 10.0, ANOVA
Test organisms	Brown trout (*Salmo trutta*)
Biological effects	It was found that the formulation Roundup caused statistically significant differences in the fatty acid composition of phospholipids in the muscle and liver of *Salmo trutta*.
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes

2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Fatty acid metabolism might have indirect effect on the some enzyme dependent dexocification systems, but is currently not related to potential population effects.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	no
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercially formulated product, possibly with POEA and therefore of limited validity for the risk assessment of the active ingredient itself.
2 Do the tested concentrations relate to predicted environmental concentrations?	Predicted environmental concentrations might be lower.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Roundup effects fatty acid metabolism at sublethal concentrations.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Harayashiki, C.A.Y. et al. (2013)

BVL Nr. 2716780	**Harayashiki, C.A.Y. et al.**	**2013**	**Toxic effects of the herbicide Roundup in the guppy *Poecilia vivipara* acclimated to fresh water**	

Reliability	
Purpose of the study Description of endpoints	In the present study, reproductive (sperm quality) and biochemical parameters were evaluated in adult guppies.
Test compound, application procedure, exposure period	The effects of Roundup on sperm quality and biochemical parameters (AChE, GST, ACAP and LPO) were evaluated in *P. vivipara* acclimated to fresh water and exposed (96h) to environmentally realistic concentrations of glyphosate (130 and 700 µg/L) as the commercial formulation Roundup.
Experimental approach, Statistics, test environment	Reproductive (sperm quality) and biochemical parameters (acetylcholinesterase and glutathione S-transferase activity, lipoperoxidation, and antioxidant capacity against peroxyl radicals) were evaluated.
Test organisms	*Poecilia vivipara*
Biological effects	A decrease in the integrity of sperm plasma membrane was observed in fish exposed to any of the concentrations of Roundup tested. Exposure to Roundup did not alter the biochemical parameters analyzed, though differences between genders were observed and deserve further investigations.
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Reproduction is considered one of the most relevant biological functions related to long-term stability of fish populations.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The conclusion from this study is only valid for glyphosate formulations that contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	Roundup at 130 and 700 µg/L for 96 h. These concentrations can be considered as being sublethal *to P. vivipara* and of ecotoxicological interest.
3 Have parameters influencing the endpoints been	nd

considered adequately?	
Concluding weight of evidence/proposed action	Exposure to Roundup did not alter the biochemical parameters analyzed, though differences between genders were observed and deserve further investigations. Findings from the present study suggest that exposure to environmentally relevant concentrations of Roundup may negatively affect at long-term the reproduction of *P. vivipara*, with consequent changes in fish populations inhabiting environments contaminated with the herbicide.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Nwani, C.D. et al. (2013)

BVL Nr. 2716799	**Nwani, C.D. et al.**	**2013**	**Investigation on Acute Toxicity and Behavioral Changes in *Taliapia zillii* due to glyphosate-based herbicide, ForceUp**	

Reliability	
Purpose of the study Description of endpoints	A 96 h semi-static acute toxicity bioassay was carried out to determine the LC_{50} value and behavioural responses of commercial formulation on *Tilapia zillii*.
Test compound, application procedure, exposure period	Commercial formulation (400 g/L glyphosateisopropylamine salt) with trade name 'Forceup'; In definitive test, a set of 10 fish specimen were randomly exposed to glyphosate (Forceup) herbicide (108, 216, 324, 432 and 540 mg/L).
Experimental approach, Statistics, test environment	Acute toxicity bioassay: The experiment was set in triplicate and mortality was recorded up to 96h at every 24h interval to obtain LC_{50} values. The LC_{50} of Forceup was determined following the probit analysis method described by Finney (1971).
Test organisms	*Tilapia zillii*
Biological effects	The LC_{50} values of different concentrations Forceup in *T. zillii* were found to be 477, 296, 253 and 211 mg/L respectively for 24, 48, 72 and 96 h exposure time.
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation with unkown surfactant system and limited validity for other surfactant systems.
2 Do the tested concentrations relate to predicted environmental concentrations?	Predicted environmental concentrations might be far lower.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	The commercial formualtion shows relatively low toxcity compared to other formulations. The induction of nuclear lesions was concentration and duration dependent with the record of highest frequency at 24.4 mg L_1 at 96 h.
Type of information (Critical, supporting, low weight)	supporting
Consideration/concluding score	UBA2

Okonkwo, F.O. et al. (2013)

BVL Nr. 2716800	**Okonkwo, F.O. et al.**	**2013**	**Toxicological Studies on the Short Term Exposure of *Clarias albopunctatus* (Lamonte and Nichole 1927) to Sub-lethal Concentrations of Roundup**	

Reliability	
Purpose of the study Description of endpoints	This study aims to ascertain the short-term toxicity in the common catfish, *Clarias albopunctatus* exposed to sub-lethal concentrations of Roundup, in an attempt to mimic contaminations of fresh water bodies that suround (rice) farms.
Test compound, application procedure, exposure period	The fish were divided into 3 groups of 12 fish each and treated as follows: Group 1 (control) was exposed to tap water only; while Groups 2 and 3 were exposed to 5 ppm and 15 ppm Roundup, respectively.
Experimental approach, Statistics, test environment	Static design, serum determination
Test organisms	*Clarias albopunctatus* (Lamonte and Nichole. 1927) mean weight 42.5±1.7 g.
Biological effects	The results showed that concentrations of bilirubin increased significantly ($p<0.05$) in a dose- and time-dependent manner. Alanine and aspartate aminotransferases and alkaline phosphatase were also significantly ($p<0.05$) elevated in the test fish. Similarly, serum concentrations of creatinine and urea were significantly ($p<0.05$) elevated in a dose-dependent manner. Histomorphologic studies of the liver and gills showed marked destruction of their architecture in the test fish, thus corroborating the data from the biochemical analysis.

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The gills perform vital functions as gas exchange, ion osmoregulation and nitrogen excretion and are particularly sensitive to changes in environmental conditions. Overtime negative consequences for fish population may be possible.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation containing POEA was used.
2 Do the tested concentrations relate to predicted environmental concentrations?	Predicted environmental concentrations can be considered to be realistic.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Short term exposure of *Clarias albupunctatus* to sub-lethal concentrations of Roundup was found to be toxic to the fish.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Yusof, S. et al. (2008)

BVL Nr. 2716821	**Yusof, S. et al.**	**2008**	**Effect of glyphosate-based herbicide on early life stages of Java medaka (*Oryzias javanicus*): A potential tropical test fish**	

Reliability	
Purpose of the study Description of endpoints	Early life stages of Java medaka (*Oryzias javanicus*) for assessing the ecological risk posed by pollutants in the aquatic environments.
Test compound, application procedure, exposure period	Roundup (Monsanto)
Experimental approach, Statistics, test environment	Java medaka adults were cultured in the laboratory and the fertilized eggs of the F2 generation were exposed to different concentrations (100, 200, 300, 400 and 500 ppm) until they hatched. The survival and hatching rates of the embryos, changes in the heart rate and morphological impairments were recorded.
Test organisms	Tropical fish Java medaka (*Oryzias javanicus*)
Biological effects	50% of the embryos exposed to 100 ppm glyphosate died after 16 days of exposure. Several developmental abnormalities were observed in pre-hatch Java medaka embryos when exposed to different concentrations of glyphosate.

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Developmental abnormalities like absence of pectoral fin(s) and cornea, permanently bent tail, abdominal enlargement and cell disruption in the fin, head and abdomen are severe developmental impairments which make negative consequences for fish population be possible.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation: The study could not be taken into account as critical information on the ERA of the active substance glyphosate itself. Nevertheless it shows that the commercial formulation can have severe effects.
2 Do the tested concentrations relate to predicted environmental concentrations?	Predicted environmental concentrations might be lower.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Results show that high concentrations of Roundup and glyphosate induce developmental toxicity in Java medaka.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Marques, A. et al. (2014)

BVL Nr. 2716854	**Marques, A. et al.**	**2014**	**Progression of DNA damage induced by a glyphosate-based herbicide in fish (*Anguilla anguilla*) upon exposure and post-exposure periods Insights into the mechanisms of genotoxicity and DNA repair**	

Reliability

Purpose of the study Description of endpoints	The major purpose of this work was to improve the knowledge on the progression of DNA damage upon exposure and post-exposure periods in association with processes of oxidative DNA damage repair.
Test compound, application procedure, exposure period	Fish were exposed for 3 days to Roundup® (58 and 116 µg/L) and allowed to recover in herbicide-free water for 1, 7 and 14 days.
Experimental approach, Statistics, test environment	DNA damage in liver cells was evaluated by the comet assay improved with nucleoid digestion by endonucleases FPG (formamidopyrimidine DNA glycosylase) and EndoIII (endonucleaseIII) to detect oxidized purines and pyrimidines, respectively. In order to estimate the capacity of oxidative DNA damage repair, an in vitro BER assay was carried out with liver cell extracts, using substrate cells treated with paraquat (a standard inducer of oxidative damage).
Test organisms	Eels *Anguilla anguilla*
Biological effects	Roundup® showed to induce DNA damage (measured both as GDIFPG and GDIEndoIII) in hepatic cells of *A. anguilla* exposed to two environmentally realistic concentrations. After transfer to herbicide-free water, fish were able to reverse the genetic damage.

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	The genetic damage measured by the comet assay showed to be a transient manifestation.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Endpoints like DNA damage at environmentally realistic concentrations reflect important information for the ERA.

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation: The study could not be taken into account as critical information in the ERA of the active substance glyphosate itself. Nevertheless it shows that the commercial formulation can have severe effects.
2 Do the tested concentrations relate to predicted environmental concentrations?	Predicted environmental concentrations can be considered realistic.
3 Have parameters influencing the endpoints been considered adequately?	nd

Concluding weight of evidence/proposed action	Oxidative damage was implicit an important mechanism of genetic damage, which showed to be transient, since DNA integrity returned to the control levels on the first day after cessation of exposure. The study indicated that concentrations of 8.1 mg/ L and 16.3 mg/L commercial glyphosate containing formulation provoke non-significant induction of nuclear lesions; whereas at concentrations of 24.4 mg/L after 96h significantly higher nuclear lesions induction were observed. Roundup® showed to induce DNA damage (measured both as GDIFPG and GDIEndoIII) in hepatic cells of *A. anguilla* at two concnetrations of a glyphosate containing products . After transfer to herbicide-free water, fish were able to reverse the genetic damage.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Prévot d'Alvise, N. et al. (2013)

BVL Nr. 2716857	**Prévot d'Alvise, N. et al.**	**2013**	**Acute toxicity of a commercial glyphosate formulation on European sea bass juveniles (*Dicentrarchus labrax* L.): gene expressions of heme oxygenase-1 (ho-1), acetylcholinesterase (ache) and aromatases (cyp19a and cyp19b)**	

Reliability	
Purpose of the study Description of endpoints	The main objectives of this work were to determine, for the first time, the acute toxicity (LC50 96-h) of Roundup on marine fish and to evaluate the expression of four stress-responsive genes in fish.
Test compound, application procedure, exposure period	Juveniles (*Dicentrarchus labrax* L.) were exposed to a sublethal concentration (35% of the LC50, i.e. 193 mg/L) of Roundup for 96hours.
Experimental approach, Statistics, test environment	The analysis of ho-1 mRNA levels in four tissues (liver, gills, gonads and brain) was used to show the effect of the potential oxidative stress induced by a short-term exposure to herbicide whereas AChE expression was used to evaluate the overall neurotoxicity of the herbicide, and aromatase genes to assess the alteration of the endocrine system.
Test organisms	European sea bass (*Dicentrarchus labrax* L.)
Biological effects	The LC50 96-h value of Roundup was 529 mg/L. Results showed that ho-1 mRNA levels in liver and gills significantly decreased in fish exposed to 193 mg/L of Roundup, whereas in brain and gonads, ho-1 mRNA level was not altered. Results showed that AChE and cyp19b gene transcriptions significantly increased in brain of sea bass, whereas aromatase gene expression (cyp19a) in gonads was not significantly altered.
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes, marine fish species *Dicentrarchus labrax* in juvenile stage. The juveniles could be considered most sensitive stages.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Survival of fish population due to a disturbance of the nervous system can be assumed if effects do not have a transient nature.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Biochemical parameters are currently not part of the traditional ERA.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation probably containing POEA with limited validity for other formulations.
2 Do the tested concentrations relate to predicted environmental concentrations?	Predicted environmental concentrations might be lower.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Results showed complex tissue-specific transcriptional responses after 96h of exposure to a sublethal concentration.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Schneider, S. et al. (2012)

BVL Nr. 2716713	**Schneider, S, Krueger, H, Claude, J, Ross, T, Gallagher, S, Springer, T, Jaber, M**	**2013**	**Glyphosate: Fish Short-Term Reproduction Assay (FSTRA) with the Fathead Minnow (*Pimephales promelas*).**	

Reliability

Purpose of the study Description of endpoints	The objective of this short-term reproduction assay was to determine if glyphosate might impact the hypothalamus-pituitary-gonadal (HPG) endocrine axis resulting in the disruption of reproduction in fish. This study was conducted by Wildlife International, Ltd. for the Joint Glyphosate Task Force at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland.
Test compound, application procedure, exposure period	Breeding groups of fathead minnows (*Pimephales promelas*) were exposed to glyphosate under flow-through conditions at mean measured concentrations of 0.046, 0.23, 1.2, 6.2 and 33 mg a.i./L for 21 days.
Experimental approach, Statistics, test environment	The study was conducted based on procedures outlined in the U.S. EPA Series 890 – Endocrine Disruptor Screening Program Test Guidelines, OPPTS Number 890.1350: Fish Short-Term Reproduction Assay; and the OECD Guidelines for Testing of Chemicals, Guideline 229: Fish Short Term Reproduction Assay Endpoints that were evaluated for endocrine disruption of the reproductive system included fecundity, fertility, secondary sex characteristics (including tubercle and fatpad scores), gonadosomatic index (GSI), histopathology of gonads, as well as plasma vitellogenin. Other endpoints included survival, general observations of health, weight, and length.
Test organisms	Fathead Minnow (*Pimephales promelas*)
Biological effects	No treatment related effects on survival, cumulative no. of eggs produced, the no. of eggs / female reproductive day, or the percent fertility (the no. of fertile eggs of the total no. collected). None of these variables showed any statistically significant decrease in any treatment group in comparison to the negative control during the test ($p > 0.05$). No treatment-related effects on growth among male or female fish during the 21-day test. There were no statistically significant effects on total length and wet weights among males or females in any treatment group in comparison to the negative control ($p > 0.05$). There were no treatment-related effects on tubercle scores, GSI, or VTG among male and female fish during the 21-day test.

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	-/-
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	yes

Concluding weight of evidence/proposed action	There were no apparent effects on survival, growth, reproduction, secondary sex characteristics, GSI, VTG or gonad histopathology in male or female fish exposed to glyphosate for 21 days. Based on the endpoints evaluated, glyphosate does not appear to impact the function of the hypothalamus-pituitary-gonadal (HPG) endocrine axis in fathead minnows.
Type of information (Critical, supporting, low weight)	supporting
Consideration/concluding score	**UBA2**

B.9.13 9.2 Invertebrates (KIIA 8.16)

Dutra et al. (2011)

glyphecotox_121	**Dutra, B.K., Fernandes, F.A., Failace, D.M., Oliveira, G.T.**	**2011**	**Effect of Roundup®(R) (glyphosate formulation) in the energy metabolism and reproductive traits of *Hyalella castroi* (Crustacea, Amphipoda, Dogielinotidae)**	**Ecotoxicology 20: 255-263**

Reliability	
Purpose of the study Description of endpoints	The objective of this investigation was to examine the effects of Roundup® on the biochemical composition, levels of lipoperoxidation, Na^+/K^+ATPase activity and reproductive traits in the *Hyalella castroi*.
Test compound, application procedure, exposure period	Roundup®
Experimental approach, Statistics, test environment	In the laboratory, the animals were kept in aquariums under controlled conditions for 7 days, and after this period they were exposed to 0.36, 0.52, 1.08 and 2.16 mg/L of glyphosate for 7 days. After the period of exposure, the animals were immediately frozen for determination of glycogen, proteins, lipids, triglycerides, cholesterol, levels of lipoperoxidation, and Na^+/K^+ATPase activity. The number of reproductive pairs, ovigerous females and eggs in the marsupium (brood pouch) was counted in each day.
Test organisms	*Hyalella castroi*
Biological effects	All concentrations of Roundup® induced significant decreases in all biochemical parameters and Na^+/K^+ATPase activity, and significant increase in lipoperoxidation levels. No mating pairs, ovigerous females, or eggs in the marsupium were observed in the groups treated with the pesticide; these animals did not pair in the laboratory during all time of treatment. Survival rate 48% at 2.16 mg/L glyphosate.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Physiologigal parameters and reproductive parameters
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Animals did not pair in the laboratory during all time of treatment→ changes in trophic structure of limnic environments
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Survival rate 48% at 2.16 mg/L glyphosate.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation. The conclusion from this study is only valid for glyphosate formulations that contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	Higher than predicted environmental concentrations: Based on modelling assumptions, the concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Pyhsiological study, including survival EC50 approx. at 2.16 mg/L glyphosate.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Achiorno et al. (2008)

glyphecotox_110	**Achiorno, C.L., de Villalobos, C., Ferrari, L.**	**2008**	**Toxicity of the herbicide glyphosate to *Chordodes nobilii* (Gordiida, Nematomorpha)**	**Ecotoxicology (2011) 20:255–263**

Reliability	
Purpose of the study Description of endpoints	The objective of this study is to evaluate the effect of different concentrations of glyphosate (technical grade and formulated product) on *Chordodes nobilii* (Gordiida, Nematomorpha).
Test compound, application procedure, exposure period	Gyphosate, technical grade, 95% (w/v) (Gly), Roundup®, 35.2% (w/v) (formulated Gly),
Experimental approach, Statistics, test environment	Bioassays were performed with embryos and larvae (preparasitic stages), and adults (postparasitic stage). Test organisms were exposed for a short period of time to concentrations ranging between 0.1 and 8 mg a.i./L (technical and formulated).
Test organisms	*Chordodes nobilii.*
Biological effects	Embryo development was not inhibited, decrease in the infective capacity of larvae, adults exposed for 96 h to 1.76 mg/L formulated Gly showed a mortality of 50%.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	No traditional test species, but the comparison between the POEA surfactant, technical grade glyphosate and the formulated product Roundup touches the key concern on the use of the herbicide glyphosate.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Adults exposed for 96 h to 1.76 mg /L formulated Gly showed a mortality of 50%.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Mortality tested
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product for mortality parameter
2 Do the tested concentrations relate to predicted environmental concentrations?	Exceed predicted environmental concentrations. Based on modelling assumptions, the concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	The minimum concentration tested (0.1 mg a.i./L Gly) decreased larval infectivity. This value is below the guidance level for glyphosate in freshwater systems (0.24 mg/L Gly), established to protect the aquatic biota in Argentina.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Brausch, J.M., Smith, P.N. (2007)

glyphecotox_113	**Brausch, J.M., Smith, P.N.**	**2007**	**Toxicity of three polyethoxylated tallowamine surfactant formulations to laboratory and field collected fairy shrimp, *Thamnocephalus platyurus***	**Archives of Environmental Contamination and Toxicology 52 (2):217-221. DOI 10.1007/s00244-006-0151-y.**

Reliability	
Purpose of the study Description of endpoints	The objective of this study was to evaluate the toxicity(48-h LC_{50}) of three POEA surfactants to a freshwater macroinvertebrate potentially exposed to POEA as it enters the environment.
Test compound, application procedure, exposure period	POEA surfactant formulations (98.6%, 99.8%, and 99.4% pure for T-5, T-10, and T-15, respectively)
Experimental approach, Statistics, test environment	Three different POEA formulations were used for testing with average oxide: tallowamine ratios of 5:1 (SurfonicT-5 Surfactant), 10:1 (SurfonicT-10), and 15:1 (SurfonicT-15). Serial dilutions of a stock solution with nominal concentrations of 0.01, 0.1, 1, 10, 100, 1,000, 10,000 µg/L for all formulations of POEA were used.. Each formulation was tested on three different strains of shrimp consisting of five acute toxicity tests and replicated three times for a total of 15 toxicity tests per formulation.
Test organisms	*Thamnocephalus platyurus* (Crustacea, Anostraca)
Biological effects	All POEA formulations were found to be extremely toxic to *T. platyurus* with 48-h LC50 concentrations as low as 2.01 µg/ L for 15:1. POEA toxicity increased as the tallowamine chain length was reduced, whereas the oxide chain length appeared to only slightly increase toxicity.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	three strains of *T. platyurus*
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	-/-
3 Is the ecotoxicological manifestation level appropriate for the assessment?	-/-
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	POEA
2 Do the tested concentrations relate to predicted environmental concentrations?	-/-
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	POEA was very toxic to *T. platyurus* with average 48-h LC50s of 2.01, 2.70, and 5.17 µg/L for POEA surfactants having an oxide:tallowamine ratio of 15:1, 10:1, and 5:1, respectively.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Brausch et al. (2007)

glyphecotox_114	**Brausch, J.M., Blake, B., Smith, P.N.**	**2007**	**Acute and Sub-Lethal Toxicity of Three POEA Surfactant Formulations to** *Daphnia magna.*	**Bulletin of Environmental Contamination and Toxicology. Volume: 78 Issue: 6 Pages: 510-514**

Reliability	
Purpose of the study Description of endpoints	In this study, *Daphnia magna* was used to examine the lethal and sub-lethal toxicity of three POEA formulations consisting of 5:1, 10:1, and 15:1 average oxide:tallowamine.
Test compound, application procedure, exposure period	POEA formulations consisting of 5:1, 10:1, and 15:1 average oxide:tallowamine.
Experimental approach, Statistics, test environment	48h , test conc: 0,01- 10 µg/L
Test organisms	*Daphnia magna*
Biological effects	All formulations inhibited growth at concentrations between 100 and

	500 µg/L. The formulation consisting of 10:1 was the most acutely toxic with a 48-h LC50 value of 97.0 µg/L and 15:1 was least toxic at 849.4 µg/L.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	surfactant
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	POEA was very toxic to *Daphnia magna* POEA 15:1= 0.85 mg/L, POEA 10:1=0.097 mg/L POEA 5:1= 0.18mg/L
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Iummato, M.M. et al. (2013)

glyphecotox_8212716785	**Iummato, M.M. et al.**	**2013**	**Evaluation of biochemical markers in the golden mussel *Limnoperna fortune*i exposed to glyphosate acid in outdoor microcosms**	**Ecotoxicology and Environmental Safety 95 (2013) 123 13129**
Reliability				
Purpose of the study Description of endpoints	In the study,specimens of *L. fortunei* were exposed to environmentally relevant concentrations of glyphosate in microcosms simulating a representative ecologicalenvironment. The experimental design aimed to recreate the impact of a time period of exposure to glyphosate (26 days) similar to the elapsed time between two applications of the pesticide in an agricultural scheme.			
Test compound, application procedure, exposure period	technical grade glyphosate acid			
Experimental approach, Statistics, test environment	Outdoor microcosms treated with nominal glyphosate concentrations of 1, 3and 6mgL. At the end of the experiment (26 days), catalase (CAT), superoxide dismutase (SOD), glutathione-S- transferase (GST), acetylcholinesterase (AChE), carboxylesterases (CES) and alkaline phosphatase (ALP) activities, and lipid peroxidation levels were analyzed.			
Test organisms	*Limnoperna fortunei*			
Biological effects	GST and ALP activities and lipid peroxidation levels showed a significant increase with respect to controls in the mussels exposed to glyphosate (up to 90, 500 and 69 percent,respectively).CES and SOD activities showed a significant decrease in glyphosate exposed bivalves with respect to controls (up to 48 and 37percent,respectively).CATandAChE did not show differences between exposed and no exposed bivalves.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	yes			
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Risk posed to fresh water bivalve mollusc populations by the assessed parameters may be clarified.			
3 Is the ecotoxicological manifestation level	*L. fortunei* showed significant alterations in			

appropriate for the assessment?	biochemicalparameters after 26 days of the application event.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	The increase in lipid peroxidation levels and the decrease in SODand CES activities observed in L. fortunei indicate that glyphosate had adverse effects on the metabolism the bivalve.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Le et al. (2010)

glyphecotox_122	**Le, T.H., Lim, E.S., Lee, S.K., Choi, Y.W., Kim, Y.H., Min, J.**	**2010**	**Effects of glyphosate and methidathion on the expression of the Dhb, Vtg, Arnt, CYP4 and CYP314 in** *Daphnia magna*	**Chemosphere 79: 67-71**

Reliability	
Purpose of the study Description of endpoints	In this study, the expression of five stress responsive genes was quantified and analyzed using a semiquantitativeRT-PCR to study the changes in their expression in *Daphnia magna* after exposure to known pesticides, glyphosate and methidathion.
Test compound, application procedure, exposure period	Glyphosate , FLUKA, probably technical, not stated
Experimental approach, Statistics, test environment	Standard US EPA protocol (2002) to determine the lethal endpoint caused byGlyphosate, concentrations: 190, 202, 214, and 234 mg/L, for 24 h probit method
Test organisms	*Daphnia magna*
Biological effects	LC_{50}=234 mg/L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Not clarified, probably glyphosate technical.
2 Do the tested concentrations relate to predicted environmental concentrations?	-/-
3 Have parameters influencing the endpoints been considered adequately?	-/-
Concluding weight of evidence/proposed action	Tested substance not specified
Type of information (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA3**

Magbanua, F.S. et al. (2013)

glyphecotox_6922716484	**Magbanua, F.S., Tounsend, C.R., Hageman, K.J., Lange, K., Lear, G., Lewis, G.D., Matthaei, C.D.**	**2013**	**Understanding the combined influence of fine sediment and glyphosate herbicide on stream periphyton communities**	**water research 47 (2013) 5110 - 5120**

Reliability	
Purpose of the study Description of endpoints	Determination whether (i) increasedl evels of sediment and glyphosate had individual and/or additive combined effects on invertebrates and leaf breakdown, (ii) increased sediment reduced the toxicity of glyphosate (antagonistic multiple stressor interaction) or (iii) sediment-adsorbed glyphosate prolonged the effects of exposure (synergistic interaction).
Test compound, application procedure, exposure period	128 circular, flow-through stream mesocosms: glyphosate herbicide in 96 mesocosms using four levels of sediment (0, 25, 75 and 100% surface cover) crossed with four of glyphosate (0, 50, 200 and 370 µg/L), with 6 replicates of each treatment combination in a full factorial repeated-measures design
Experimental approach, Statistics, test environment	A 28-day experiment in outdoor stream mesocosms simulated effects of varying levels of fine sediment and a glyphosate-based herbicide on macroinvertebrates and ecosystem function (leaf breakdown) in a fully factorial repeated-measures design.
Test organisms	Invertebrate community
Biological effects	Glyphosate addition negatively affected only two taxa and two invertebrate trait categories, indicating that glyphosate entering streams as a result of herbicide addition to farmland is less likely to be problematic for invertebrates than fine sediment from catchment erosion. No significant overall sediment-by-glyphosate interaction was detected for invertebrate taxonomic or trait compositions or for any common individual taxon, trait or community metric, indicating that the two stressors were acting additively (i.e. without interaction), rather than synergistically or antagonistically.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Population effects were caused by fine sediment. Glyphosate produced few individual effects.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Prbably commercial formualtion
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	Population, community and ecosystem variables responded differently to multiple stressors, highlighting the value of combining structural, functional taxonomic and trait data to assess the effects of anthropogenic stressors.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Bringolf et al. (2007)

glyphecotox_119	**Bringolf, R.B., Cope, W.G., Mosher, S., Barnhart, M.C., Shea, D.**	**2007**	**Acute and chronic toxicity of glyphosate compounds to glochidia and juveniles of** *Lampsilis siliquoidea* **(Unionidae)**	**Environmental Toxicology and Chemistry Volume: 26 Number: 10 Pages: 2094-2100**

Reliability		
Purpose of the study Description of endpoints	The toxicity of several forms of glyphosate, its formulations, and a surfactant (MON 0818) used in several glyphosate formulationswas determined for early life stages of Lampsilis siliquoidea, a native freshwater mussel.	
Test compound, application procedure, exposure period	Roundup®, its active ingredient, the technical-grade isopropylamine (IPA) salt of glyphosate, IPAalone, and MON 0818 (the surfactant in Roundup® formulations)	
Experimental approach, Statistics, test environment	Acute and chronic toxicity tests were performed with a newly established American Society of Testing and Materials (ASTM) standard guide for conducting toxicitytests with freshwater mussels.	
Test organisms	*Lampsilis siliquoidea* (Unionidae)	
Biological effects	EC_{50} values 48h (mg/L)acute Glyphosate technical >200 (glochidia) Glpyhosate IPA=5 (glochidia) Aquastar® >148(glochidia) Roundup®=2.9(glochidia) MON0818 =0.5(glochidia)	EC_{50} values 28days (mg/L)chronic Glyphosate technical = not applicable Glpyhosate IPA=4.8 Aquastar® =43.8 Roundup®=3.7 MON0818 =1.7
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?	Acute and chronic toxicity tests were performed with a newly established American Society of Testing and Materials (ASTM) standard guide for conducting toxicitytests with freshwater mussels.	
2 Is the magnitude of effects of significance to cause a (population) relevant effect?		
3 Is the ecotoxicological manifestation level appropriate for the assessment?		
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?	See above	
2 Do the tested concentrations relate to predicted environmental concentrations?	Based on modelling assumptions, the concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.	
3 Have parameters influencing the endpoints been considered adequately?	yes	
Concluding weight of evidence/proposed action	MON 0818 was most toxic of the compounds tested and the 48-h median effective concentration (0.5 mg/L) for L. siliquoidea EC50 values are taken into account.	
Type of information (Critical, supporting, low weight)	**Critical**	
Consideration/concluding score	**UBA1**	

Tsui, M.T.K., Chu, L.M. (2004)

glyphecotox_018	**Tsui, M.T.K., Chu, L.M.**	**2004**	**Comparative toxicity of glyphosate-based herbicides: aqueous and sediment porewater exposures**	**Arch. Environm. Contam. Toxicol.46, 316-323**
Reliability				

Purpose of the study Description of endpoints	In this study, the water-only acute toxicity of three formulations based on glyphosate (Rodeo, Roundup® Biactive, and Roundup®) were compared using a water-column organism (cladoceran: Ceriodaphnia dubia) and a benthic organism (amphipod: Hyalella azteca). In addition, Roundup® Biactive® and Roundup® were spiked into a clean sediment which was amended with appropriateamounts of peat moss to study the effect of different organic carbon levels (0, 0.4, 1.2, and 2.1%) on their sediment toxicity, with C. dubia exposed to overlying water or porewater prepared from the contaminated sediments.
Test compound, application procedure, exposure period	Rodeo (i.e., isopropylamine salt of glyphosate 53.8%) Roundup® (i.e., isopropylamine salt of glyphosate 41%, 0-20 POEA) Roundup®Biactive® (i.e., isopropylamine salt of glyphosate 41%, surfactant)
Experimental approach, Statistics, test environment	USEPA guideline 2000
Test organisms	*Ceriodaphnia dubia, Hyalella azteca*
Biological effects	The concentration units for the glyphosate-based herbicides were based on the acid equivalent concentration (of glyphosate acid) throughout the study.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulations probably showing differences between the inclsion of toxic and less toxic surfactants.
2 Do the tested concentrations relate to predicted environmental concentrations?	
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	EC50 values are taken into account
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Chen et al. (2004)

glyphecotox_120	**Chen, C.Y., Hathaway, K.M., Folt, C.L.**	**2004**	**Multiple stress effects of Vision (R) herbicide, pH, and food on zooplankton and larval amphibian species from forest wetlands**	**Environmental Toxicology and Chemistry 23 (4):823-831**

Reliability	
Purpose of the study Description of endpoints	As part of a multiple-tier research program, interactions of the herbicide Visiont (glyphosate) with two stressors, pHand food level, were examined. Effects of the formulated product Vision were tested at two test concentrations (0.75 and 1.50 mgacid equivalent/L), two pH levels (pH 5.5 and 7.5), and under high and low food concentrations.
Test compound, application procedure, exposure period	Glyphosate (356 g acid equivalent/L) in the form of an isopropylamine salt as well as polyethoxylated tallowamine surfactant (MON 0818) at a concentration equivalent to 15% by volume.
Experimental	S. vetulus, survival, reproduction, and development time were measured; SAS

approach, Statistics, test environment	LIFETESTt (SAS, Ver 8,Cary, NC, USA). Pairwise comparisons of survival responses in each treatment were made using a log-rank test.
Test organisms	*Simocephalus vetulus*
Biological effects	Between 0.75 to 1.5 mg a.e./l
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*Simocephalus vetulus*,
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Survival and reproduction
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Vision®Commercial formulation containing POEA. Commercial formulation. The conclusion from this study is only valid for glyphosate formulations that contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	EC50 values are taken into account.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Mensah et al. (2011)

glyphecotox_123	**Mensah, P.K., Muller, W.J., Palmer, C.G.**	**2011**	**Acute toxicity of Roundup® herbicide to three life stages of the freshwater shrimp *Caridina nilotica* (Decapoda: Atyidae)**	**Physics and Chemistry of the Earth, Parts A/B/C 36 (14–15):905-909**

Reliability		
Purpose of the study Description of endpoints	The toxicity of the herbicide Roundup® was assessed using three different life stages of the freshwater shrimp Caridina nilotica, a prevalent species in South African freshwater ecosystems.	
Test compound, application procedure, exposure period	Roundup® active ingredient: 360 g glyphosate (glycine) a.e./L (contains 480 g isopropylamine salt of glyphosate/L, registered and distributed by Monsanto South Africa (Pty) Ltd.),	
Experimental approach, Statistics, test environment	ANOVA, Concentrations used were 0,1.7, 2.6, 4.1, 6.4 and 8 mg/L for the neonates (<7 days post hatching(dph)); 0, 1.7, 2.6, 4.1, 6.4, 8 and 10 mg/L for juveniles (>7 dphand < 20 dph); 0, 5.4, 8.4, 13.1, 20.5, 32 and 50 mg/L for adults(>40 dph).	
Test organisms	*Caridina nilotica (*Decapoda: Atyidae) is the most common of four indigenous freshwater caridean species found in the South Africa	
Biological effects	LC50 mg/L 48h neonates = 4.45 LC50 mg/L 48h juvenile = 9.39 LC50 mg/L 48h adults=37.12	LC50 mg/L 96h neonates = 2.54 LC50 mg/L 96h juvenile = 6.96 LC50 mg/L 96h adults=25.507
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?		mortality

3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation containing POEA,Roundup®. Commercial formulation. The conclusion from this study is only valid for glyphosate formulations that contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	
3 Have parameters influencing the endpoints been considered adequately?	
Concluding weight of evidence/proposed action	The neonates of *C. nilotica* were found to be most sensitive with a mean 96 h $LC_{50}$of 2.5 mg/L,
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Zeynep SARIGÜL11 (2009)

glyphecotox_124	**Zeynep SARIGÜL11**	**2009**	**Acute Toxicity of the Herbicide Glyphosate on** *Daphniamagna**	**JOURNAL OF AGRICULTURAL SCIENCES 2009, 15 (2) 204-208**

Reliability	
Purpose of the study Description of endpoints	In this study, median lethal concentrations (LC50) of herbicide Roundup, which contains 48% glyphosate, on Daphnia magna for 24 and 48 hours were determined.
Test compound, application procedure, exposure period	Roundup®
Experimental approach, Statistics, test environment	The experiment has been conducted with the method of static bioassay on two series; five different concentrations (0.0115; 0.018; 0.021; 0.028; 0.032) and one control group have been used. The LC50 values have been calculated with the method of probit analysis.
Test organisms	***Daphniamagna***
Biological effects	Experimental results showed that the concentration of the glyphosatewhich killed 50 % of Daphnia magna was 0.019 mg/L (95% confidence interval=0.012 mg/L–0.024 mg/L) for 24 hours, but the concentration of the glyphosate which killed 50 % of Daphniamagna was 0.012 mg/L (%95 confidence interval=0.001 mg/L-0.016 mg/L) for 48 hours
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative for the substance being assessed?	Commercial formulation. The conclusion from this study is only valid for glyphosate formulations that contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	Oxygen low
Concluding weight of evidence/proposed action	EC50 values taken in to account for the formulation

Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Conners, D.E., Black, M.C. (2004)

glyphecotox_325	**Conners, D.E., Black, M.C.**	**2004**	**Evaluation of lethality and genotoxicity in the freshwater mussel** *Utterbackia imbecillis* **(Bivalvia : Unionidae) exposed singly and in combination to chemicals used in lawn care**	**Archives of Environmental Contamination and Toxicology 46 (3):362-371. DOI 10.1007/s00244-003-3003-z.**

Reliability	
Purpose of the study Description of endpoints	In this study, we evaluated the lethal and genotoxiceffects of chemicals used in lawn care on an early life stage offreshwater mussels (Utterbackia imbecillis).
Test compound, application procedure, exposure period	glyphosate isopropylamine salt (Roundup; 18.0% active ingredient; Monsanto Company)
Experimental approach, Statistics, test environment	Johnson et al. (1993).
Test organisms	Gravid adult *U. imbecillis* mussels (average length 54.7 mm, averageheight 26.9 mm)
Biological effects	LC_{50} 18.3 mg/L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*U. imbecillis* mussels
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Parameter observed mortality
3 Is the ecotoxicological manifestation level appropriate for the assessment?	mortality

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation. Commercial formulation. The conclusion from this study is only valid for glyphosate formulations that contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	Predicted environmental concentrations might be lower (for one indication per area).
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	LC 50 taken into account
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Frontera et al. (2011)

glyphecotox_378	**Frontera, J.L., Vatnick, I., Chaulet, A., Rodriguez, E.M.**	**2011**	**Effects of Glyphosate and Polyoxyethylenamine on Growth and Energetic Reserves in the Freshwater Crayfish** *Cherax quadricarinatus* **(Decapoda,**	**Archives of Environmental Contamination and Toxicology 61 (4):590-598. DOI 10.1007/s00244-011-9661-3.**

			Parastacidae)	

Reliability	
Purpose of the study Description of endpoints	Sublethal effects of a 50-day exposure to glyphosate acid and polyoxyethylenamine (POEA), both alone and in a 3:1 mixture, on the growth and energeticreserves in muscle, hepatopancreas and hemolymph ofgrowing juvenile crayfish were examined.
Test compound, application procedure, exposure period	All stock solutions of glyphosate (as acid) and POEA(99.8% purity; Sigma, St. Louis, Missouri) were preparedweekly by dissolving the appropriate amount of the chemicals in distilled water.
Experimental approach, Statistics, test environment	
Test organisms	*Advanced juvenile C. quadricarinatus*
Biological effects	No mortality was observed in any of the experimentalgroups during the experiment (glyphosate at a concentration of 22.5 mg/l., POEA (polyoxyethylene amine) at a concentrationof 7.5 mg/l.; a mixtureof 15 mg/l (3.75 mg/l POEA and 11.25 mg/l glyphosate and a mixtureof 30 mg/l (7.5 mg/l POEA and 22.5 mg glyphosate). Other physiological parameters like oxygen consumption, glycogen levels or body-weight gain were affected.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	The juveniles could be considered most sensitive stages.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Mortality was not affected
3 Is the ecotoxicological manifestation level appropriate for the assessment?	no
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commerciral formulation
2 Do the tested concentrations relate to predicted environmental concentrations?	Predicted environmental concnetrations might be lower (for one indication per area).
3 Have parameters influencing the endpoints been considered adequately?	
Concluding weight of evidence/proposed action	Physiollogical traits are affected, which might affect fitness. The decrease ofboth glycogen and lipid reserves, as observed in the mixture,could have lead to lower protein levels and decreasedsomatic growth in juvenile crayfish *C. quadricarinatus.*
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Mottiera (2013)

	Mottiera, Bouchartb, Serpentinia, Lebela, Jhac, Costil	**2013**	**Effects pof glyphosate –based herbicides on embryo-larval development and metamorphosis in the Pacific oyster,** *Crassostrea gigas*	**Aquatic Toxicology 128-129 (2013),67-78**

Reliability	
Purpose of the study Description of endpoints	The study aimed to assess the toxicity of glyphosate, its by-product, aminomethylphosphonic acid (AMPA) and two commercial formulations, Roundup Express® (REX) and Roundup Allées et Terrasses® (RAT), containing glyphosate as the active ingredient, on the early life stages of the Pacific oyster, Crassostrea gigas.
Test compound, application procedure, exposure period	Roundup Express® 7.2 g/l Glyphosate +POEA(R_{EX}), Roundup Allées et Terrasses® 4.4 g/L+POEA(R_{AT}) glyphosate (97% purity) , AMPA (97.5%purity)
Experimental approach, Statistics, test environment	For both endpoints, the nominal concentrations correspondingto 0.1, 1, 100 and 10,000 g L−1 of the chemicals (i.e. glyphosateand AMPA) were verified (in duplicate) by ultraperformance liquidchromatography (UPLC) and fluorometric detection (in accordancewith NF ISO 21458) Embryotoxicity bioassay and experimental design: AFNOR procedure (AFNOR XP-T90-382) published in 2009. Regarding the differences between the nominaland measured concentrations, the values of EC50 for both endpointswere re-calculated by considering the measured concentrations.
Test organisms	Pacific oyster, *Crassostrea gigas*
Biological effects	The EC_{50} values were 27.1 and 46.1 mg/Lfor glyphosate and its metabolite, respectively for the parameter development. glyphosate and AMPA LC50 >100mg/L. Rex and Rat were more toxic than the active ingrdient, probably due to the surfactants. EC_{50} development REX= 1.1 mg/L, RAT=2.0mg/L LC_{50} REX 8.5 mg/L , RAT 7.9
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Pacific oyster, *Crassostrea gigas* Embryotoxicity bioassay
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to predicted environmental concentrations?	Predicted environmental concentrations might be lower (for one indication per area).
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	the embryos and 48 h D-shaped larvae were more sensitive than 21 days larvae LC_{50} taken into account.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Dominguez-Cortinas et al. (2008)

glyphecotox_347	**Dominguez-Cortinas, G., Saavedra, J.M., Santos-Medrano, G.E., Rico-Martinez, R.**	**2008**	**Analysis of the toxicity of glyphosate and Faena® using the freshwater invertebrates** *Daphnia magna* **and** *Lecane quadridentata*	**Toxicological & Environmental Chemistry 90 (2):377 - 384**
Reliability				

Purpose of the study Description of endpoints	Therefore, the aim of the present contribution was to perform an ecotoxicologicalassessment of both glyphosate and its commercial formulation Faena using twoooplanktonic invertebrates: the rotifer *Lecane quadridentata*, and the cladoceran *Daphniamagna*.	
Test compound, application procedure, exposure period	Glyphosate and Faena (Sigma Co., St. Louis, MO, USA).	
Experimental approach, Statistics, test environment	Statistica 5.0	
Test organisms	*Daphnia magna* and *Lecane quadridentata*	
Biological effects	LC_{50} 48h *L. quadridentata* Active ingredient =150 Faena®=13.1	LC_{50} 48h *Daphnia magna* Active ingredient=146 Faena®=7.9
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?		mortality
3 Is the ecotoxicological manifestation level appropriate for the assessment?		mortality
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		Active ingredient
2 Do the tested concentrations relate to predicted environmental concentrations?		nd
3 Have parameters influencing the endpoints been considered adequately?		ndn
Concluding weight of evidence/proposed action	The LC_{50} values show that this freshwater rotifer is 11-foldmore susceptible to the commercial formulation (Faena) than to the active ingredient(glyphosate). This effect might be due to the synergistic activity of other components of the industrial formulation that increase the toxicity of the compound. Daphnia magna is almost 20-fold moresusceptible to Faena than to glyphosate. LC_{50} taken into account. EC50 (esterases activity)of glyphosate is 1500-fold smaller than the LC50.	
Type of information (Critical, supporting, low weight)	**supporting**	
Consideration/concluding score	**UBA2**	

Demetrio et al. (2012)

glyphecotox_342	**Demetrio, P.M., Rossini, G.D.B., Bonetto, C.A., Ronco, A.E.**	**2012**	**Effects of Pesticide Formulations and Active Ingredients on the Coelenterate Hydra attenuata (Pallas, 1766)**	**Bulletin of Environmental Contamination and Toxicology 88 (1):15-9. doi:10.1007/s00128-011-0463-0.**

Reliability		
Purpose of the study Description of endpoints	The objective of the study is toassess and compare the acute effects on H. attenuata exposedto the active ingredients and commercial formulations ofglyphosate, cypermethrin, and chlorpyrifos.	
Test compound, application procedure, exposure period	Glyphosate (Technical Grade) were obtained from Gleba S.A. Roundup ®Max (74.4% glyphosate) was obtained from Monsanto S.A.	
Experimental approach, Statistics, test environment	probit model (Finney 1971) with a specific software (Probit USEPA version 1.5)	
Test organisms	*Hydra attenuata*	
Biological effects	LC50 glyphosate a.i (mg/l) =18.2	LC50 RoundupMax® (mg/l) =21.8
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	mortlaity
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Active ingredient versus formulation
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	With glyphosate, higher and significant effects were detected for the formulation at lower concentrations,with a reversal of the behavior at higher concentrations.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Melnichuk et al. (2007)

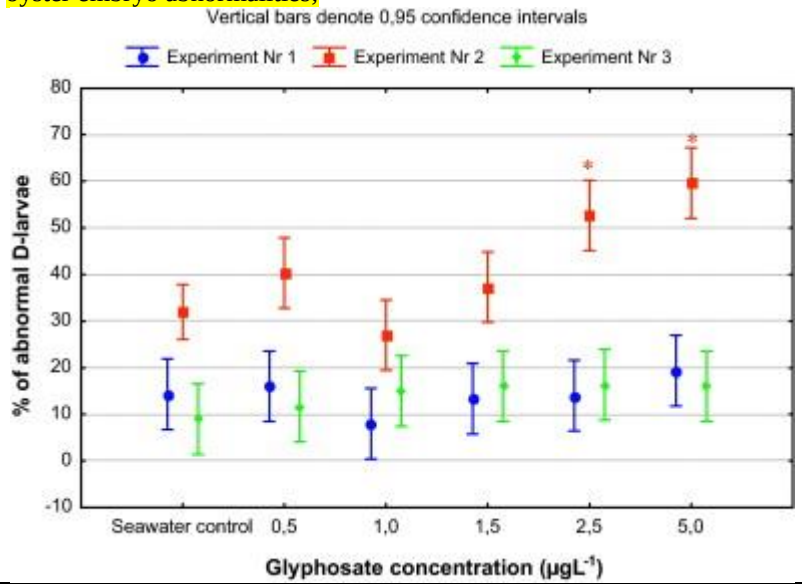
glyphecotox_501	**Melnichuk, S.D., Scherban, Y.P., Lokhanskaya, V.I.**	**2007**	**Effects of Fakel herbicide on vital activity of** *Ceriodaphnia affinis* **in acute and chronic experiments**	**Hydrobiological Journal 43 (6):83-91. doi: 10.1615/HydrobJ.v43.i6.70.**

Reliability	
Purpose of the study Description of endpoints	The aim of this work was to study the influence of Fakel herbicide on the vital activity parameters of *Ceriodaphnia affinis* and to evaluate the toxicity of this herbicide
Test compound, application procedure, exposure period	Fakel herbicide is produced as the 36% (in acid equivalent)aqueous solution of the 48%isopropylamine salt of glyphosate
Experimental approach, Statistics, test environment	concentrations from 0.001 up to 200 mg/dm3 were studied in acuteexperiments and of concentrations from 0.001 up to 10 mg/dm3 – in chronic experiments
Test organisms	*Ceriodaphnia affinis*
Biological effects	LC_{50} Fakel 48h= 13.6 mg/L(dm3)
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Comercial formulation
2 Do the tested concentrations relate to predicted environmental concentrations?	At concentration of 1.0–0.01 mg/dm3, the herbicidereduces productivity of *Ñ. affinis* by 21–23% in each of generations
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Fakel herbicide exerted the greatest inhibitory influence on number of young per broodat concentration of 10 mg/dm3.
Type of information (Critical, supporting, low weight)	**supporting**

Consideration/concluding score	UBA2

Akcha et al. (2012)

glyphecotox_273	Akcha, F., Spagnol, C., Rouxel, J.	2012	Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos	Aquatic toxicology (Amsterdam, Netherlands) 106-107:104-13. doi:10.1016/j.aquatox.2011.10.018

Reliability	
Purpose of the study Description of endpoints	The embryotoxic effects of these herbicides were studied throughvarious embryo-larval bioassays.
Test compound, application procedure, exposure period	Roundup Express®
Experimental approach, Statistics, test environment	Test concentration 0.5; 1.0; 1.5; 2.5; 5.0 µga.s./L; one-way ANOVA
Test organisms	Mature oysters
Biological effects	Significant differences were highlighted in terms of D-larvae abnormalities ($p < 0.001$); exposure at concentrations of 5 µg /L leads to a significant increase in oyster embryo abnormalities; 

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The commercial formulation did not appear to be more toxic than glyphosate – the active substance –
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	nd

Concluding weight of evidence/proposed action	Out of the three bioassays conducted at the laboratory, only one assay revealed glyphosate to have an embryotoxic effect at concentrations of 2.5 µg L−1 ($p < 0.001$) upwards. Taking into account the data from the three bioassays, the main effects ANOVA revealed significant differences between the assays ($p < 0.001$), with assay 2 differing from assays 1 and 3. The potential embryotoxicity of glyphosate was studiesd but the study tested a highest concentration of 5.0 µg L did not reslut in a EC50values. Therefore the estimated EC50 (estimated) for one bioassay resulted in EC50 a.s > 5µg/L.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Cuhra et al. (2013)

	Cuhra, M., Traavik, T., & Bøhn, T.	**2013**	**Clone- and age-dependent toxicity of a glyphosate commercial formulation and its active ingredient in *Daphnia magna*.**	**Ecotoxicology, 22, 251-262.**

Reliability	
Purpose of the study Description of endpoints	Authors tested the acute effects of both glyphosate and a commercial formulation of Roundup (hereafter Roundup), we conducted a series of exposure experiments with different clones and age-classes of *D. magna*.
Test compound, application procedure, exposure period	1.) Aqueous solution of glyphosate-IPA salt, (40%glyphosate) 2.) Roundup Weed & Grass Killer Concentrat (18% glyphosate, 0.73% diquat-dibromide); Duration: Acute toxicity test: 48 h Chronic toxicity test: 55 days
Experimental approach, Statistics, test environment	OECD 202, ISO 6341 (1989), OPPTS 850.1010 (1996); OECSD 211; acute test: Six different clones and of D. magna were exposed to an aqueous solution of glyphosate-IPA salt, Roundup Weed & Grass Killer Concentrate Plus, negative control, *D. magna* of 3 different ages were exposed to both chemicals and a negative control: Chronic test: 0.05, 0.15, 0.45, 1.35, 4.05 mg a.s./L (nominal)
Test organisms	*Daphnia magna*, 5 different clones, < 24 hours old, for testing of age-dependency, 1 and 3 week old specimens.
Biological effects	Acute toxicity testing of juvenile D. magna showed EC_{50} values of 1.4–7.2 mg/l for glyphosate. The EC_{50} values of adults were 31 mg/l for glyphosate. NOEC levels for juvenile size were <0.45 mg/l for glyphosate. NOEC levels for juvenile size were 0.15 mg/l for glyphosate. NOEC levels for fecundity were 0.45 mg/l for glyphosate.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The formulation used in the study contains two active ingredients, diquat dibromide and glyphosate making the data on the formulation not relevant.
2 Do the tested concentrations relate to predicted environmental concentrations?	Based on modelling assumptions, the concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.

3 Have parameters influencing the endpoints been considered adequately?	The duration of the chronic exposure was longer than recommended by the guidelines cited (55 vs. 21 days). Additionally, the exposure concentration was maintained at a constant level by renewal of the test medium very third day.
Concluding weight of evidence/proposed action	Based on conservative modelling assumptions, the worst-case concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L. Putting these results in context results in a margin of safety of around 5. No significant effects on fecundity or abortion rates were seen at concentrations 0.05–0.45 mg/l for glyphosate IPA,
Type of information (Critical, supporting, low weight)	**Critical**
Consideration/concluding score	**UBA1**

Avigliano, et al. (2014)

	Avigliano, L., Fassiano, A. V., Medesani, D. A., de Molina, M. R., & Rodríguez, E. M.	**2014**	**Effects of Glyphosate on Growth Rate, Metabolic Rate and Energy Reserves of Early Juvenile Crayfish, *Cherax quadricarinatus M*.**	**Bulletin of environmental contamination and toxicology, 1-5.**

Reliability	
Purpose of the study Description of endpoints	The objectives of the current study were: (1) to assess the effects of sublethal concentrations of glyphosate on early, sensitive juvenile instars of the freshwater crayfish C. quadricarinatus, in terms of growth, metabolicm rate and energy reserves levels, (2) to determine how glyphosate affects the activity level of key metabolic enzymes, such as pyruvate kinase and (3) to determine the levels of both alanine and aspartate aminotransferase activities (ALAT and ASAT respectively) as indicative of tissue damage. All the experiments were done with pure glyphosate (acid form), in order to extent the results obtained to any glyphosate-based formulation that could be applied in the environment.
Test compound, application procedure, exposure period	A chronic (60-days) bioassay in semistatic conditions, according to standard procedures recommended by the American Public Health Association et al. (2005). At the end of the assay, oxygen consumption, hemolymphatic glucose level and energy reserves (glycogen, protein, and lipids) were determined in all surviving animals, following the methodology used in previous studies made on the same species (Chaulet et al. 2012; Frontera et al. 2011).
Experimental approach, Statistics, test environment	During the assay, both mortality and molting were registered daily. ANOVA
Test organisms	*Cherax quadricarinatus* early juveniles
Biological effects	The highest mortality value (33 %) was seen in animals exposed to 40 mg/L of glyphosate, this value was significantly different from control; A significant decrease in weight gain (35 % lower than control) was seen after the first month of exposure to 40 mg/L of glyphosate. Significant decrease in total protein content in both muscle, at 40 mg/L, and hepatopancreas, at both assayed concentrations. Besides, a significant decrease in total lipid content was observed in muscle. At the 10 mg/L exposure, muscle pyruvate kinase activities were significantly lower (while no differences were seen in the hepatopancreas.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause	Both lipids and proteins are closely involved with the energy available for crustacean growth;

a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Yes; Biochemical parameters not part of traditional ERA.
Environmental Relevance	
1 Is the substance tested representative for the substance being assessed?	yes
2 Do the tested concentrations relate to predicted environmental concentrations?	Based on conservative modelling assumptions, the worst-case concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	Glyphosate is able to reduce growth rates and protein and lipid reserves in chronically exposed early juvenile crayfish at concentrations of at 40 mg/L. Based on conservative modelling assumptions, the worst-case concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.
Type of information (Critical, supporting, low weight)	Supporting information
Consideration/concluding score	UBA2

Mona et al. (2013)

	Mona, M. H., Gaafar, R. M., Helal, I. B., Omran, N. E., & Salama, W. M.	**2013**	**Evaluation of cytotoxic effects of atrazine and glyphosate herbicides on *Biomphalaria glabrata* snails.**	**The Journal of Basic & Applied Zoology, 66(2), 68-75.**

Reliability	
Purpose of the study Description of endpoints	The aim of this work was to evaluate the genotoxic effects in *B. glabrata* snails exposed to herbicides atrazine and glyphosate using SDS–PAGE protein profiles, isozyme assay, and RAPDs.
Test compound, application procedure, exposure period	The snails were separated into 5 groups with 15 snails. The 1st and 2nd groups were exposed to 5 and 0.5 ppm of glyphosate for 3 weeks, respectively. The 3rd and 5th groups were exposed to 10 and 5 ppm of atrazine for 3 weeks, respectively. One negative control group (5th group), exposed only to dechlorinated water was used. The contaminated water was renewed every three days.
Experimental approach, Statistics, test environment	Toxicity experiments, Protein and isozyme analysis, DNA analysis, Statistical and data analysis
Test organisms	Adult *B. glabrata* snails with shell 8-10 mm diameter
Biological effects	The SDS–PAGE results of this study gain and/or loss of some bands and variation in their intensities. Catalase activity was restricted only to the treated groups for 1 and 2 weeks and this may be due to variations of ROS release from hemocytes during this period.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd

3 Is the ecotoxicological manifestation level appropriate for the assessment?	Biochemical parameter were analysed, no lethal or reproductive endpoint, Non-standard test system, no dose response
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The test item description is incomplete.It is not clear if the acitve substance or a commercial formulation was tested.
2 Do the tested concentrations relate to predicted environmental concentrations?	Yes
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Biochemical parameter were analysed, no lethal or reproductive endpoint, Non-standard test system, no dose response
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

dos Santos et al. (2014)

BVL- Nr.: 2716831	**dos Santos, K. C., & Martinez, C. B.**	**2014**	**Genotoxic and biochemical effects of atrazine and Roundup®, alone and in combination, on the Asian clam *Corbicula fluminea*.**	**Ecotoxicology and environmental safety, 100, 7-14.**

Reliability	
Purpose of the study Description of endpoints	The study aimed to evaluate biochemical and genotoxic effects of the herbicides atrazine(ATZ) and Roundups (RD) separately, as well as their mixture, on the fresh water clam Corbicula fluminea after 96 h exposure.
Test compound, application procedure, exposure period	Roundup, not further information. Animals were exposed to 2 and 10 ppb of ATZ, 2 and 10 ppm of RD and the mixtures of both.
Experimental approach, Statistics, test environment	Activities of ethoxyresorufin-O-deethylase (EROD),g lutathione-S-transferase(GST), superoxidedismu-tase (SOD), catalase(CAT), glutathioneperoxidase(GPx) and glutathionereductase(GR), as well as the multixenobiotic resistance mechanism(MXR), reduced glutathione concentrations (GSH) and lipid peroxidation (LPO) were measured in gills and digestive gland. DNA damage was determined in clams hemocytes throughthecometassay.
Test organisms	Adult specimens of *C. fluminea* (Müller,1774;Mollusca,Corbiculidae)
Biological effects	SOD activity increased in the gills of clams exposed to RD10 and in the digestive gland of animals exposed to RD2 and RD10; CATactivitywassignificantly reducedonlyindigestiveglandofclamsexposedRD10; GPX increased in the gills after exposure to ATZ2 and RD10. The exposure to RD10 caused a significant increase in LPO in both gills and digestive gland, While the exposure to ATZ and RD separately did not increase DNA damage.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of significance to cause a (population) relevant effect? 3 Is the ecotoxicological manifestation level appropriate for the assessment?	For an evaluation of sublethal effects ther population relevance has to be clarified.
Environmental Relevance	
1 Is the substance tested representative	Commercial formulation of glyphosate, probably containg POEA.

and relevant for the substance being assessed?	
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	This study showed that both herbicides applied alone revealed to be potentially toxicto *C. fluminea*.
Concluding weight of evidence/proposed action	*C. fluminea* revealed to be a good biomonitor of environments contaminated by the herbicides ATZ and RD; EROD and LPO appeared as potential biomarkers. Mixture effects are complex and variable, either intensifying or mitigating the effects promoted by compounds. The study aimed to evaluate biochemical and genotoxic effects of the herbicides atrazine(ATZ) and Roundups (RD) separately, as well as their mixture, on the fresh water clam after 96 h exposure. Roundup did not increase DNA damage, but induced alterations in biochemical parameter related to oxidative stress (SOD activity increased in the gills of clams exposed to RD10 and in the digestive gland of animals exposed to RD2 and RD10; CAT activity was significantly reduced only in digestive gland of clams exposed RD10; GPX increased in the gills after exposure to ATZ2 and RD10. Exposure to ATZ and RD separately did not increase DNA damage)
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2**

Janssens, et al. (2013)

BVL- Nr.: 2716837	**Janssens, L., & Stoks, R.**	**2013**	**Synergistic effects between pesticide stress and predator cues: Conflicting results from life history and physiology in the damselfly *Enallagma cyathigerum*.**	**Aquatic Toxicology, 132, 92-99.**

Reliability	
Purpose of the study Description of endpoints	In this study, we have investigated potential synergistic effects between pesticide exposure and predator cues on a key life history trait, growth rate, its associated behavioural trait, food intake, and the neglected three types of physiological traits mentioned above.
Test compound, application procedure, exposure period	We tested 0.5 mg/l, 1 mg/l, 2 mg/l, 3 mg/l and 4 mg/l glyphosate. It is not clear, if active substance or pesticides concentrations are described.
Experimental approach, Statistics, test environment	Effects on the stress protein Hsp70, energy reserves (fat, total carbohydrates) and physiological traits related to oxidative stress: the activity of the electron transport system (ETS), the activities of the two key antioxidant enzymes in insects (superoxide dismutase SOD and catalase CAT) and oxidative damage to lipids (lipid peroxidation), a key marker of oxidative stress.
Test organisms	*Enallagma cyathigerum* damselfly larvae: important intermediate predators in aquatic food webs, being predators of small invertebrates (e.g., mosquito larvae) and prey for larger organisms (e.g., fish and dragonfly larvae).
Biological effects	Food intake increased under pesticide exposure. Both stressors additively increased Hsp70 levels. When both stressors were combined, there was a reduction of the antioxidant enzyme superoxide dismutase activity (SOD) and an associated increase of oxidative damage in lipids.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Drift values are likely lower than tested concentrations, The parameter Oxidative stress has limited value to conclude on the relevance on the population level.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Synergistic interactions may have profound fitness implications, nevertheless are not part of the traditional ERA.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Nd, not stated in detail if active ingredient or commercial formulation was used.
2 Do the tested concentrations relate to predicted environmental concentrations?	Based on conservative modelling assumptions, the worst-case concentrations expected in surface waters exposed to lie around 0.1 mg a.i./L.Test concentration is exceeding this by far.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Synergistic interactions were not present for growth rate and food intake, they were identified for antioxidant defence and oxidative damage. Effects on biochemical responses are currently considered as supporting information on commercial products in the field of environmental risk assessment. Nevertheless, it is evident that further studies are needed to determine if the changes on biochemical traits are translated to sublethal long-term effects.
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2**

Kumar, et al. A. (2013)

BVL- Nr.: 2716838	**Kumar, A., Jahavar Ali, A.**	**2013**	**Toxic impacts of two organophosphorus pesticides on the acetylcholinesterase activity and biochemical composition of freshwater fairy shrimp *streptocephalus dichotomus*.**	**International Journal of Pharma and Bio Sciences, 4(2): 966 – 972.**

Reliability	
Purpose of the study Description of endpoints	The sublethal effects of malathion and glyphosate on AChE activity and concentrations of total protein, carbohydrate and lipid in *S. dichotomous* pre adults were studied.
Test compound, application procedure, exposure period	Glyphosate, no further information stated. Toxicity tests were performed to determine the 96 hrs LC50. Preadults were exposed to sub lethal concentrations for a period of 30 days. AChE activity was assayed on '0' and 15th days of exposure (Ellman et al., 1961).
Experimental approach, Statistics, test environment	Fairy shrimps were hatched from cysts by following the procedure of Calleja et al. (1993). The concentrations of biochemical constituents, such as total protein, carbohydrate and lipid were estimated on '0', 15th and 30th days of exposures in the whole body of S. dichotomous by following the methods of Bradford (1976), Hedge and Hofreiter (1962), and Van Handel (1985) and Inouye and Lotufo, (2006) respectively. One way analysis of variance (ANOVA) was performed.
Test organisms	Fairy shrimp, *Streptocephalus dichotomus* preadults
Biological effects	The 96 hrs LC50 values for malathion and glyphosate was determined to be 9.1 ppm and 5.5 ppb, respectively on the fairy shrimp, *Streptocephalus dichotomus* preadults.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	It is not clear why control treatment were signficantly affected as well. Therefore it is not clear if effects can be depicted as treatment releated.
Concluding weight of evidence/proposed action	Since the test compound was not described in detail and further effects in the same range were also observed in the control treatments the study has limited reliability.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Omran, N.E., Salama, W.M. (2013)

BVL- Nr.: 2716710	**Omran, N.E., Salama, W.M**	**2013**	**The endocrine disruptor effect of the herbicides atrazine and glyphosate on *Biomphalaria alexandrina* snails**	**Toxicology and Industrial Health 1–10**

Reliability	
Purpose of the study Description of endpoints	Characterization of the response of the *B. alexandrina* snail and to investigate whether herbicides AZ and GL are able to disrupt the endocrine system of it in terms of steroid levels (T and 17b-E), alteration of CYP1B1, total protein (TP) level, and gonadal structure.
Test compound, application procedure, exposure period	Commercial formulation Herfosate, Percentage of GL iso-propylamine equals 48% w/v, and inert ingredients equal 52% w/v. One liter of herfosate equals 480 g of active ingredient (480 g/l).
Experimental approach, Statistics, test environment	Lethal concentration at 50% and 10% (LC50 and LC10). Mortality was recorded to determine LC50 and LC10 using a Finny program with reliability interval of 95% (Finney, 1971). Experimental design: GL-treated snails: treated for 3 weeks with LC10 (4.2 ppm); Steroid extraction and anlaysis; Determination of CYP4501B1; Histological examination of the ovotestis
Test organisms	*Biomphalaria alexandrina*
Biological effects	LC50: 41.6 ppm; LC10: 4.2 ppm; Level of T, E, and TP contents were significantly decreased in GL-exposed groups; The level of microsomal CYP4501B1-like immunoreactivity increased significantly in GL- and AZ-exposed snails. Histological investigation of the ovotestis showed that GL caused degenerative changes including azoospermia and oocytes deformation.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and	Commercial formulation with unknown surfactant system.

relevant for the substance being assessed?	
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	The data obtained indicated that sublethal doses (LC10) oft he commercial formulation Herfosate for 3 weeks effects the endocrine systems of *B. alexandrina* snails.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Avigliano, L. et al. (2014)

BVL. Nr.: 2716847	**Avigliano, L. et al.**	**2014**	**Effects of glyphosate on egg incubation, larvae hatching, and ovarian rematuration in the estuarine crab *Neohelice granulata***	**Environmental Toxicology and Chemistry, Vol. 33, No. 8, pp. 1879–1884, 2014**

Reliability	
Purpose of the study Description of endpoints	The present study aimed to evaluate the effects of glyphosate (both pure and formulated) on developing embryos, hatched larvae, and ovarian rematuration of N. granulata during the reproductive period.
Test compound, application procedure, exposure period, protocol	glyphosate (as acid, 99.8% purity; Sigma) and Roundup Ultramax (as soluble granules, 67.9% w/w of glyphosate a.e.; Monsanto); The nominal concentrations assayed for pure glyphosate were 2.5 mg/L and 5 mg/L, whereas 2.5 mg/L was used for the Roundup Ultramax treatment.
Experimental approach Statistical design, test environment	Effects on hatching; Effects on ovarian rematuration
Test organisms	Ovigerous *N. granulata* females carrying immature eggs
Biological effects	Because no loss of eggs was verified in any case, 100% of females had normal hatching. A significant reduction in the number of hatched larvae per female was observed in the Roundup treatment. A significantly higher incidence of both hydropsy and hypopigmented eyes was observed for both glyphosate and Roundup groups (2.5 mg/L of glyphosate a.e. in both cases). Pure glyphosate at 2.5 mg/L stimulated ovarian maturation, mainly in terms of a higher gonadosomatic index and a higher percentage of vitellogenic oocytes.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Yes, a significant reduction in the number of hatched larvae per female was observed in the Roundup treatment.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	According to the author glyphosate concentrations used in the present study were similar to some environmental levels reported worldwide, which range from 2.7 mg/L (US) to 7.6 mg/L (Australia).

3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	
Concluding weight of evidence	A significantly lower number of hatched larvae per female was detected in the Roundup Ultramax treatment, a clear embryonic mortality was associated with this formulation, which contained a glyphosate concentration of 2.5 mg/L. Also taking into account that pure glyphosate, at the same concentration, did not significantly reduce the number of hatched larvae, these results indicate that Roundup compounds other than glyphosate may be responsible for the embryonic mortality.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Geret et al. (2011)

BVL. Nr.: 2716775	**F. Geret,T. Burgeot,J. Haure, B. Gagnaire,T. Renault, P. Y. Communal, J. F. Samain**	**2011**	**Effects of Low-Dose Exposure to Pesticide Mixture on Physiological Responses of the Pacific Oyster, *Crassostrea gigas***	**Published online 19 October 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/tox.20764**

Reliability	
Purpose of the study Description of endpoints	The aim of this study was to assess, under controlled conditions, the sublethal effects on Pacific oysters of a mixture of pesticides that is representative of those spread in the largest tonnages in the Marennes-Oleron basin during May and June, which is the period when the oysters are undergoing gametogenesis.
Test compound, application procedure, exposure period, protocol	Mixture of pesticides containing 0.8 µg/L alachlor, 0.6 µg/L metolachlor, 0.7 µg/L atrazine, 0.6 µg/L terbuthylazine, 0.5 µg/L diuron, 0.6 µg/L fosetyl aluminum, 0.05 µg/L carbaryl, and 0.7 µg/L glyphosate for a total concentration of 4.55 µg/L.
Experimental approach Statistical design, test environment	The experimental protocol for this study involved comparing metabolic responses. Several enzyme biomarkers were studied (glutathione S-trans- ferase (GST), metabolizing enzyme of stage II; catalase (CAT), enzyme of antioxidant system; and glutamine synthetase (GS), enzyme of nitrogen metabolism involved in glutamine synthesis in nervous system). Furthermore, Dna damages and disturbances of the immune defenses were analyzed.
Test organisms	Pacific oyster, Crassostrea gigas (‘‘resistant’’ (R) and ‘‘susceptible’’ (S)
Biological effects	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Mixture of pesticides containing sveral substances was tested.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	-/-

Concluding weight of evidence	Low doses and the mixture of pesticides effect the metabolic responses under controlled conditions. Effects on enzyme performances are currently considered as supporting information on commercial products in the field of environmental risk assessment. Nevertheless, it is evident that further studies are needed to determine if the changes on biochemical traits are translated to sublethal long-term effects.
Type of info. (Critical, supporting, low weight)	**low weight for the ERA of the active ingredient glyphosate itself.**
Consideration/concluding score	**UBA3**

Boonsoong & Buliangpoti (2012)

BVL.: 2716764	**Boonsoong & Buliangpoti**	**2012**	**Acute toxicity of Roundup® and carbosulfan to the Thai fairy shrimp, *Branchinella thailandensis***	**Comm Appl. 6~1. Sd, Ghent Unlversily, 7714, 2012**

Reliability	
Purpose of the study Description of endpoints	The objective of this study was to evaluate the acute toxicity of Roundup ® and carbosulfan to *Branchinella thailandensis*.
Test compound, application procedure, exposure period, protocol	Roundup· (lsopropylamine salt 48% w/v); 6 hours, 12 hours, and 24 hours static bioassay
Experimental approach Statistical design, test environment	Median lethal concentration (LC50) values were calculated after probit transformation of resulting data.
Test organisms	naupli fairy shrimp, *Branchinella thailandensis*
Biological effects	Median 24 hours lethal concentrations were 0.319 and -0.702 ppm for Roundup· and carbosuJfan, respectively.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	-/-

Concluding weight of evidence	The commercial formulation Roundup shows a relatively high toxicity (LC50: 0.319 ppm) towards the *Branchinella thailandensis.*
Type of info. (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

B.9.13 9.3 Algae and aquatic plants (KIIA 8.16)

Ray et al. (2008)

glyphecotox_561	**Ray, P., Sushilkumar, Pandey, A.K.**	**2008**	**Deleterious effect of herbicides on waterhyacinth biocontrol agents** *Neochetina bruchi* **and** *Alternaria alternata*	**Biocontrol Science and Technology 18 (5):523-533. Doi 10.1080/09583150802001734.**

Reliability	
Purpose of the study Description of endpoints	Laboratory experiments were conducted to determinethe toxic effect of herbicides on the insect biocontrol agent, the waterhyacinthweevil, Neochetina bruchi Hustache, and phytopathogen, Alternaria alternata,with two commonly used herbicides, glyphosate and 2,4-dichlorophenoxy aceticacid at three recommended doses.
Test compound, application procedure, exposure period	three recommended (labelled) doses, i.e. 0.89, 1.12and 1.34 ppm ai glyphosate
Experimental approach, Statistics, test environment	nd
Test organisms	*nd*
Biological effects	nd
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Waterhycinth in India considered as target species
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Romero et al. (2011)

glyphecotox_578	**Romero, D.M., Rios de Molina, M.C., Juarez, A.B.**	**2011**	**Oxidative stress induced by a commercial glyphosate formulation in a tolerant strain of** *Chlorella kessleri*	**Ecotoxicol Environ Saf 74 (4):741-7. DOI: 10.1016/j.ecoenv.2010.10.034.**

Reliability	
Purpose of the study Description of endpoints	The aim of this work is to study the toxicity of the Herbicide glyphosate and to provide evidence of metabolic altera- Tions related to oxidative stress induced in at olerant strain of *C. kessleri* by exposure to a commercial formulation of glyphosate. For this purpose parameters related

	to metabolic damage were measured.
Test compound, application procedure, exposure period	Commercially available herbicide used in this study was 48%(p/v)Glyphosate (isopropylaminesaltofN-phosphonomethylglycine) ATANORs (Atanor,Munro, Buenos Aires province,Argentina) and the surfactant was alkylaryl polyglycolether 50%IMPACTOs (AGROASISTS.R.L.,Argentina).
Experimental approach, Statistics, test environment	The experimental treatments were prepared according to algal growth inhibition test standards (USEPA, 2002). Concentrationsof 40, 50, 60, and70 mg/L1 of glyphosate
Test organisms	*C. kessleri* (Trebouxiophyceae, Chlorophyta
Biological effects	Algal cell density and dry weight were statistically significant diminished with respect to the control values for concentrations of at least 60 mgL_1 of glyphosate, where the numbe rof cells was approximately one-third that of the control culture(Table 1). The EC50-96 h estimated by LinearInterpolation Methodsoft ware was 55.62 (53.08–57.56)mgL_1.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	no
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product containing surfactant alkylaryl polyglycolether. Although not specified precisely, the tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	Based on EU FOCUS modelling assumptions, the concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	The EC50-96h obtained for *C. kessleri* was higher than those used in risk assessment.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Debenest et al. (2010)

glyphecotox_340	**Debenest, T., Silvestre, J., Coste, M., Pinelli, E.**	**2010**	**Effects of Pesticides on Freshwater Diatoms**	**In Reviews of Environmental Contamination and Toxicology, edited by D. M. Whitacre. Springer New York. pp 87-103. DOI: 10.1007/978-1-4419-1352-4_2.**
Reliability				
Purpose of the study Description of endpoints			Book chapter which provides a broad bibliographical review of articlesthat address the effects of pesticides and certain other xenobiotics on diatoms. In thisreview, we emphasize the following targets of pesticide action: (i) cytology and cellultrastructure, (ii) cell metabolism, and, finally, (iii) effects on community speciescomposition.	
Test compound, application procedure, exposure			nd	

period	
Experimental approach, Statistics, test environment	nd
Test organisms	*nd*
Biological effects	nd
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	No details about Glyphosate in particular.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Inderjit, I., Kaushik, S. (2010)

glyphecotox_412	**Inderjit, I., Kaushik, S.**	**2010**	**Effect of herbicides with different modes of action on physiological and cellular traits of** *Anabaena fertilissima*	**Paddy and Water Environment 8 (3):277-282. DOI: 10.1007/s10333-010-0208-4.**

Reliability	
Purpose of the study Description of endpoints	Comparative study designed to examine toxicity of propanil, pretilchlor and glyphosate on physiological and cellular characteristics of *A. fertilissima.*
Test compound, application procedure, exposure period	nd
Experimental approach, Statistics, test environment	
Test organisms	*A. fertilissima.*
Biological effects	nd
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	According to Algae Base (http://www.algaebase.org/search/species/detail/?species_id=40443) species distribution in North America only.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Romero et al. (2011)

glyphecotox_578	**Romero, D.M., Rios de Molina, M.C., Juarez, A.B.**	**2011**	**Oxidative stress induced by a commercial glyphosate formulation in a tolerant strain of** *Chlorella kessleri*	**Ecotoxicol Environ Saf 74 (4):741-7. DOI: 10.1016/j.ecoenv.2010.10.034.**

Reliability	
Purpose of the study Description of endpoints	The aim of this work is to study the toxicity of the herbicide glyphosate and to provide evidence of metaboli calterations related to oxidative stress induced in a tolerant strain of C. kessleri by exposure to a commercial formulation of glyphosate.
Test compound, application procedure, exposure period	ATANOR, 48% IPA salt
Experimental approach, Statistics, test environment	Parameters related to metabolic damage (biomass, growth rate, chlorophyll content and protein content), lipid peroxidation (malondialdehyde content) and antiox- idant response (catalase and superoxide dismutase activities and reduced glutathione level) were measured.
Test organisms	*Chlorella kessleri*
Biological effects	EC50 = 55.6 mg/L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Although not specified precisely, the tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	The freshwater species *Chlorella* in Europe according to Algae Base((http://www.algaebase.org/search/species/detail/?species_id=40443) species distribution in South America, Asia, in Europe in Romania and Spain. Tolerant strain used .
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Ma et al. (2002)

glyphecotox_476	**Ma, J., Xu, L., Wang, S., Zheng, R., Jin, S., Huang, S., Huang, Y.**	**2002**	**Toxicity of 40 herbicides to the green alga** *Chlorella vulgaris*	**Ecotoxicology and Environmental Safety 51 (2):128-132. DOI 10.1006/eesa.2001.2113**
Reliability				

Purpose of the study Description of endpoints	Work reported effect of 40 hebicieds on the green algae *Chlorella vulgaris*
Test compound, application procedure, exposure period	Glyphosate 95%, technical product.
Experimental approach, Statistics, test environment	Initial cell conc.: 8x 10^{5} /ml, Linear regression for EC_{50} calculation, 5000 lx/cm2, duration 96h
Test organisms	*Chlorella vulgaris*
Biological effects	EC_{50}= 5 mg/L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, technical ingedient.
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Presented EC_{50} values will be taken into account.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Ma et al. (2006)

glyphecotox_474	**Ma, J., Wang, S., Wang, P., Ma, L., Chen, X., Xu, R.**	**2006**	**Toxicity assessment of 40 herbicides to the green alga** *Raphidocelis subcapitata*	**Ecotoxicology and Environmental Safety 63 (3):456-462. DOI 10.1016/j.ecoenv.2004.12.001**

Reliability	
Purpose of the study Description of endpoints	The effects of 40 herbicides with nine modes of action on the green alga *Raphidocelis subcapitata* were studied by 96-h acutetoxicity tests.
Test compound, application procedure, exposure period	Glyphosate 95%, technical product.
Experimental approach, Statistics, test environment	Initial cell conc.: 5x $10^{5/ml}$, Linear regression for EC50 calculation, 5000 lx/cm2, duration 96h
Test organisms	*R. subcapitata*
Biological effects	EC50= 5.5 mg/L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, technical ingedient.
2 Do the tested concentrations relate to predicted environmental concentrations?	nd

3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Presented EC50 values will be taken into account. Freshwater species , taxonomic synonym *Pseudokirchneriella subcapitata*
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Ma, J. (2002)

glyphecotox_471	**Ma, J.**	**2002**	**Differential sensitivity to 30 herbicides among populations of two green algae** *Scenedesmus obliquus* **and** *Chlorella pyrenoidosa*	**Bulletin of Environmental Contamination and Toxicology 68 (2):275-281**

Reliability	
Purpose of the study Description of endpoints	Effect of different herbiceds on the fgreen algae *Scenedesmus obliquus.*
Test compound, application procedure, exposure period	Glyphosate 95%, technical product.
Experimental approach, Statistics, test environment	Initial cell conc.: 4x $10^{5/ml,}$ Linear regression for EC50 calculation, 5000 lx/cm2, duration 96h
Test organisms	*Scenedesmus obliquus and Chlorella pyrenoidosa*
Biological effects	EC_{50}= 56 mg/L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, technical ingredient.
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Presented EC50 values will be taken into account. EC_{50}= 56 mg/L
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Ma et al. (2006)

glyphecotox_473	**Ma, J., Lin, F., Wang, S., Xu, L.**	**2006**	**Toxicity of 21 herbicides to the green alga** *Scenedesmus quadricauda*	**Environmental Contamination and Toxicology 71 (3):594-601. DOI 10.1007/s00128-003-8521-x.**

Reliability	
Purpose of the study	In the present study, 21 herbicides have been tested to examine

Description of endpoints	their effect on the green alga *Scenedesmus quadricauda* and thencompare their differential sensitivity three other green algae, *Scenedesmusobliqnus*, *Chlorella vulgaris* and *Chlorella pyrenoidosa.*
Test compound, application procedure, exposure period	Glyphosate 95%, technical product.
Experimental approach, Statistics, test environment	Initial cell conc.: 4x $10^{5/ml,}$ Linear regression for EC50 calculation, 5000 lx/cm2, duration 96h
Test organisms	***Scenedesmus quadricauda***
Biological effects	EC_{50}= 70mg/L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, technical ingredient.
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Presented EC50 values will be taken into account.Freshwater species occurin g amongst others in Sout Europe. EC_{50}= 70.5 mg/L
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Ma et al. (2001)

glyphecotox_477	**Ma. J., Liang, W., Xu, L., Wang, S., Wei, Y., Lu, J.**	**2001**	**Acute toxicity of 33 herbicides to the green alga** *Chlorella pyrenoidosa*	**Bull Environ Contam Toxicol 66 (4):536-41**

Reliability	
Purpose of the study Description of endpoints	s. above
Test compound, application procedure, exposure period	Glyphosate 95%, technical product.
Experimental approach, Statistics, test environment	Duration 96h, ECso values were calculated using linear regression analysisof transformed pesticide concentration as natural logarithm data versus percent inhibition (Ma et al. 2001), inital cell concentration: $6x10^5$ cells/ml.
Test organisms	***Chlorella pyrenoidosa***
Biological effects	EC_{50}= 3.5mg/L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, technical ingredient.

2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Presented EC50 values will be taken into account. EC_{50}= 3.5 mg/L
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Tsui, M.T.K., Chu, L.M. (2003)

glyphecotox_195	**Tsui, M.T.K., Chu, L.M.**	**2003**	**Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors**	**Chemosphere 52: 1189–1197.**

Reliability		
Purpose of the study Description of endpoints	In this study,the acute toxicity of technical-grade glyphosate acid,isopropylamine (IPA) salt of glyphosate,Roundup® and its surfactant polyoxyethylene amine (POEA) to Microtox bacterium (*Vibrio fischeri*),microalgae (*Selenastrum capricornutum* and *Skeletonema costatum*),protozoa (*Tetrahymena pyriformis* and *Euplotes vannus)* and crustaceans (*Ceriodaphnia dubia* and *Acartia tonsa*) was examined and the relative toxicity contributions of POEA to Roundup® were calculated.	
Test compound, application procedure, exposure period	Glyphosate acid (CAS: 1071-83-6; P97% purity) Polyoxyethylene amine (POEA) (CAS: 61791-26-2;100% a.i.) Roundup® (commercial grade; 41% a.i.) Isopropylamine (IPA) salt of glyphosate (CAS: 38641-94-0; 56.8% a.i.)	
Experimental approach, Statistics, test environment	ASTM (1994), Absorbance at 680nm, The IC50 (or median growth inhibition concentration) and 95% confidence interval were calculated byprobit analysis for the growth inhibition test (Finney,1971).	
Test organisms	Algae, *Selenastrum capricornutum* (UTEX 1648, Freshwater) and *Skeletonema costatum*(UTEX LB2038, Marine)	
Biological effects	Generally,the toxicity order of the chemicals was: POEA> Roundup®_ > glyphosate acid >IPA salt of glyphosate,while the toxicity of glyphosate acid was mainly due to its high acidity. In contrast,microalgae and crustaceans were 4–5 folds more sensitive to Roundup®_ toxicity than bacteria and protozoa. Except photosynthetic microalgae,POEA accounted for more than 86% of Roundup®_ toxicity and the toxicity contribution of POEA was shown to be species-dependent.	
	Selenastrum capricornutum 96 h IC_{50} Glyphosate acid = 24.7 mg AE/l IPA salt of glyphosate= 41.0 mg AE/l POEA=3.92 mg AE/l Roundup®= 1.85 mg AE/l	*Skeletonema costatum* 96 h IC_{50} Glyphosate acid = 2.27 mg AE/l IPA salt of glyphosat= 5.89 mg AE/l POEA =3.35 mg AE/l Roundup®=1.85 mg AE/l
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?		yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?		yes

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Presented EC50 values will be taken into account.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Perez et al. (2011)

glyphecotox_540	**Perez, G.L., Vera, M.S., Miranda, L.A.**	**2011**	**Effects of Herbicide Glyphosate and Glyphosate-Based Formulations on Aquatic Ecosystems**	**In Herbicides and Environment, edited by Kortekamp. Croatia. InTech. Chapter 16. pp 343 - 368.**

Reliability	
Purpose of the study Description of endpoints	Revision of their toxicity to non-target species of algae, aquatic plants, protozoa,crustaceans, molluscs, fish and amphibians. In addition, we describe the importance of eachgroup of organisms in the functioning and health of aquatic ecosystems.
Test compound, application procedure, exposure period	nd
Experimental approach, Statistics, test environment	nd
Test organisms	nd
Biological effects	nd
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Review chapter in book.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Cedergreen, N., Streibig, J.C. (2005)

glyphecotox_319	**Cedergreen, N., Streibig, J.C.**	**2005**	**The toxicity of herbicides to non-target aquatic plants and algae: assessment of predictive factors and hazard**	**Pest Management Science 61 (12):1152-1160. Doi 10.1002/Ps.1117**

Reliability	
Purpose of the study	In this study the toxicity of herbicides to aquatic plants and

Description of endpoints	algae and relate it to environmental herbicide concentrations and exposure scenarios, herbicide formulation and mode of action was evaluated. This was done experimentally for ten herbicides, using the aquatic macrophyte *Lemna minor* L. and the green alga *Pseudokirchneriella subcapitata* (Korshikov) Hindak, supplemented with a database study comprisingalgae toxicity data for 146 herbicides.
Test compound, application procedure, exposure period	Roundup® 360 g/L
Experimental approach, Statistics, test environment	The algae test is described by Arensberg *et al*.and Mayer *et al*.20 and is coherent with the ISO standards. Initial densitiy: 10 000 cells /ml
Test organisms	*P. subcapitata*.
Biological effects	EC_{50} = 270 mg a.s./L EC_{50} formulation = 64.7 mg/L
Test organisms	*Lemna minor* L.
Biological effects	EC_{50} = 46.9 mg a.s./L $EC_{50=}$ formulation = 11.2 mg/L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	Presented EC50 values will be taken into account.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Turgut, C., Fomin, A. (2002)

glyphecotox_128	**Turgut, C., Fomin, A.**	**2002**	**Sensitivity of the rooted macrophyte *Myriophyllum aquaticum* (Vell.) Verdcourt to seventeen pesticides determined on the basis of EC_{50}**	**Bulletin of Environmental Contamination and Toxicology 69 (4):601-608**

Reliability	
Purpose of the study Description of endpoints	Sensitivity of the rooted macrophyte *Myriophyllum aquaticum* (Vell.) to seventeen pesticides was determined on the basis of $EC_{50.}$
Test compound, application procedure, exposure period	Probably commercial product with 36 % a.s, not clarified.
Experimental approach, Statistics, test environment	Liqid growth medium , 5 replicates , 7-8 concentrations

Test organisms	*Myriophyllum aquaticum*	
Biological effects	EC_{50} (mg/L)= 2.0 (fresh weight) EC_{50} (mg/L)= 0.22 (chl a)	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?		yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?		yes
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		Probably commercial product with 36 % a.s
2 Do the tested concentrations relate to predicted environmental concentrations?		yes
3 Have parameters influencing the endpoints been considered adequately?		Sucrose added
Concluding weight of evidence/proposed action	Presented EC50 values will be taken into account. Pigment content was more senstive endpoint than other parameters. The test item is not characterized in the study. In addition, the endpoint of 0.22 mg/L is based on chlorophyll a content, which is not a standard endpoint for aquatic plant risk assessment.	
Type of information (Critical, supporting, low weight)	**supporting**	
Consideration/concluding score	**UBA2**	

Sobrero et al. (2007)

glyphecotox_125	**Sobrero, M.C., Rimoldi, F., Ronco, A.E.**	**2007**	**Effects of the glyphosate active ingredient and a formulation on *Lemna gibba* L. at different exposure levels and assessment end-points**	**Bulletin of Environmental Contamination and Toxicology 79: 537-54**

Reliability		
Purpose of the study Description of endpoints	The sensitivity of a local clone of the macrophyte *Lemna gibba* L. to glyphosate active principle and Roundup® Max formulation was studied in standardized laboratory conditions	
Test compound, application procedure, exposure period	testing both the active ingredient, a.i. (glyphosate acid, technical grade, 95%w/w)and the commercial formulation (Roundup®1Max, 70.7%w/w a.i. as acid),	
Experimental approach, Statistics, test environment	Herbicide phytotoxicity was assessed on growth rate(GR) measured at 2, 5, 7 and 10 days of exposure, and alsoon frond growth (FG), frond number per colony (FNC),total chlorophyll content (TCC) and root length measuredat 7 and 10 days.	
Test organisms	L. gibba	
Biological effects	EC_{50} (mg a.s./L)= 20.5 (growth) EC_{50} (mg/L)= 11.6	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?		yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?		yes
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		yes

2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	Presented EC50 values will be taken into account.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Sura, S. et al. (2012)

2716496	**Sura, S. et al.**	**2012**	**Effects of glyphosate and two herbicide mixtures on microbial communities in prairie wetland**	**J Environ Qual. 41(3):732-43**

Reliability	
Purpose of the study Description of endpoints	The intent of the current study was to provide much needed knowledge regarding the eff ects of this environmentally relevant herbicide mixture on sensitive prairie wetland microbial communities.€ Endpoints for bacteria: pelagic and biofilm bacterial production (H3-thimidine incorporation rate, DAPI cell counts)
Test compound, application procedure, exposure period, protocol	- Glyphosate (Glyphos, Cheminova, 360 g/L) - Mixture 1: 2,4-D, MCPA, clopyralid, dicamba, dichlorprop, mecoprop - Mixture 2: 2,4-D, MCPA, clopyralid, dicamba, dichlorprop, mecoprop, bromoxynil and glyphosate - Glyphosate and mixture 1 applied at = 1000 times the environmentally relevant concentration (ERC values were based on average concentrations detected in wetland ecosystems across the Canadian prairies) => Glyphosate was applied at 225 µg/L and at 2.25, 113 and 225 µg/L in the mixture 2 (refer to paper) - Mixture 2: dose-response approach with the ERCs of each herbicide as the base concentration
Experimental approach Statistical design, test environment	- multitrophic outdoor mesocosm - refer to paper
Test organisms	Pelagic and biofilm microbial commuities
Biological effects	- Glyphosate suppressed algal biomass and production for the duration of the study in pelagic and biofilm communities. - Glyphosate has a higher potential to inhibit primary production and chlorophyll a content in pelagic and attached wetland algal communities than the auxin-type herbicide mixture (mixture 1).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercially formulated product, possibly with POEA.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	No: Based on modelling assumptions, the concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no

Concluding weight of evidence	The results from this study indicate that glyphosate at 225 µg L−1 (GLY) can have an effect on the productivity of aquatic microbial communities over relatively short time periods. Differences in the toxicity of co-formulants preclude making general conclusions across all formulations of glyphosate herbicides.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Annett et al. (2014)

2716470	**Annett, R., Habibi, H.R., Hontela, A.**	**2014**	**Impact of glyphosate and glyphosate-based herbicides on the freshwater environment**	**Journal of Applied Toxicology Volume 34, Issue 5, pages 458–479, May 2014**

Reliability	
Purpose of the study Description of endpoints	The review presents the current exposure data of glyphosate and glyphosate-based herbicides in the aquatic environment and to critically evaluate our current understanding of their effects in aquatic organisms.
Test compound, application procedure, exposure period, protocol	Glyphosate and glyphosate based herbicides
Experimental approach Statistical design, test environment	Identity, Physical and Chemical Properties, Manufacture, Mode of Action Overwiew about cellular reponses, Toxicity to Aquatic Organisms
Test organisms	various
Biological effects	various
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	dn
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Differences in the toxicity of co-formulants preclude making general conclusions across all formulations of glyphosate herbicides.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Comprehensive review article.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Vera, M.S., et al. (2014)

2716497	**Vera, M.S., Juarez, A.B.,**	**2014**	**Comparative Effects of Technical-Grade and a Commercial Formulation of**	

	Pizarro, H.N.		**Glyphosate on the Pigment Content of Periphytic Algae**	
Reliability				
Purpose of the study Description of endpoints	The objective of the study was to explore pos- sible differential effects of the formulation Glifosato Ata-nor and the technical-grade glyphosate acid (activeingredient, a.i.) on the algal fraction of a freshwater peri- phytic community, using a short-term laboratory bioassay.			
Test compound, application procedure, exposure period, protocol	A laboratory bioassay was carried out in 250-ml beakers. Two treatments were used: technical-grade glyphosate acid and Glifosato AtanorÒ (isopropylamine salt of glyphosate 48 % w/v), which were at a concentration of 3 mg active ingredient per liter.			
Experimental approach Statistical design, test environment	The concentrations of chlorophyll a and b and carotenes were determined at 0, 2, 6, 10, 24, 48, 96 and 192 h after herbicide addition.Two-factor analysis of variance			
Test organisms	aquatic plants (*Ceratophyllum* sp.)			
Biological effects	A significant increase in pigment content was observed for both herbicides after a 2-day exposure.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	yes			
2 Is the magnitude of effects of biological significance?	nd			
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Chlorophyll content is not a standard endpoint for aquatic plant risk assessment.			
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?	yes			
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Both herbicides were tested at a nominal glyphosate concentration of 3 mg a.i. per liter, which is exeeded the predicted environmental concentration, but is used for aquatic weed control.			
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes			
Concluding weight of evidence	Results showthat both glyphosate and the commercial formulation Glifosato Atanor stimulate the growth of the auto- trophic fraction in the periphyton			
Type of info. (Critical, supporting, low weight)	**low weight**			
Consideration/concluding score	**UBA3**			

Issa, A.A.E. et al. (2012)

2716786	**Issa, A.A.E. et al.**	**2012**	**Alterations in some metabolic activities of *Scenedesmus quadricauda* and *Merismopedia glauca* in response to glyphosate herbicide**	
Reliability				
Purpose of the study Description of endpoints	The green alga *Scenedesmus quadricauda* and the blue green alga *Merismopedia glauca* were subjected to various concentrations of glyphosate herbicide to determine the effect of glyphosate on the growth and some metabolic activities.			
Test compound, application procedure, exposure period, protocol	Probably commercial product, which is not described further.			

Experimental approach Statistical design, test environment	Photosynthetic pigments extraction, photosynthetic activity, determination of carbohydrate, proteins
Test organisms	The green alga *Scenedesmus quadricauda* and the blue green alga *Merismopedia glauca*
Biological effects	The growth was negatively affected by glyphosate herbicide. The photosynthesis and respiration were increased in the two algae with increasing glyphosate herbicide. Free amino acids were increased by increasing glyphosate.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Differences in the toxicity of co-formulants preclude making general conclusions across all formulations of glyphosate herbicides.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	600, 300 150 ppm. Based on modelling assumptions, the concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.Test concentration is exceeding this by far
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	Glyphosate herbicide inhibited the growth and some metabolic activities in the tested algae, this effect depend on the algal species, the type and concentration of the herbicide.
Type of info. (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Qiu, H. et al. (2012)

2716786	**Qiu, H. et al.**	**2012**	**Physiological and biochemical responses of Microcystis aeruginosa to glyphosate and its Roundup® formulation**	
Reliability				
Purpose of the study Description of endpoints		The purpose of this study was to determine the physiological and biochemical response of M. aeruginosa to glyphosate and its formulation Roundup® as a sole phosphorus source, and to compare the difference between glyphosate and Roundup® on the growth of M. aeruginosa.		
Test compound, application procedure, exposure period, protocol		Ninety-five percent glyphosate was obtained from Shandong Jingbo Agrochemicals, Ltd. Roundup® formulation (containing 41% glyphosate isopropylamine) was obtained from Monsanto Company (America).		
Experimental approach Statistical design, test environment		The physiological and biochemical responses of *Microcystis aeruginosa* (M. aeruginosa) to glyphosate and its formulation in the common herbicide, Roundup®, were compared.		
Test organisms		*Microcystis aeruginosa* PCC7806		
Biological effects		The result suggested that both the cell numbers and Chl-a content increased when the glyphosate concentration increased from 0.01 to 5 mg/L.		
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Differences in the toxicity of co-formulants preclude making general conclusions across all formulations of glyphosate herbicides.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	-/-
Concluding weight of evidence	M. aeruginosa could utilize glyphosate as a sole P-resource, and glyphosate had a positive dosage-response on the cell number and Chl-a content of M. aeruginosa from 0 to 5 mg P L−1. Roundup® showed a hormesis effect on M. aeruginosa growth.
Type of info. (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Sun, K. et al. (2012)

2716812	**Sun, K. et al.**	**2012**	**Ecological risks assessment of organophosphorus pesticides on bloom of *Microcystis wesenbergii***	**International Biodeterioration & Biodegradation Volume 77, February 2013, Pages 98–105**

Reliability	
Purpose of the study Description of endpoints	Hormesis induced by organophosphorus pesticides (OPPs) on *Microcystis wesenbergii* was studied to demonstrate the mechanism of water bloom.
Test compound, application procedure, exposure period, protocol	Glyphosate-isopropylammonium (41%, Roundup, Monsanto Company, Missouri), 1.80- 540 µmol/L
Experimental approach Statistical design,	Acute toxicity test, hormetic effect test, compensatory effect test.
Test organisms	*Microcystis wesenbergii*
Biological effects	The results showed median inhibitory effect concentrations of glyphosate-isopropylammonium on fluorescence of chlorophyll a at 96 h were, 6.84 µmol/L .
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Differences in the toxicity of co-formulants preclude making general conclusions across all formulations of glyphosate herbicides.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Acute toxicity was detected at high concentrations, while pesticide residue in waters were seldom reported to that level
3 Have parameters influencing the endpoints been	nd

considered (e.g. pH, temperature, light conditions)?	
Concluding weight of evidence	Fluorescence of chlorophyll a and the photochemical efficiency were enhanced in the range of natural concentrations. Stimulatingeffect varied among tested parameters; hormetic enhancement on physiological processes might be a factor to induce a water bloom of *M. wesenbergii.*
Type of info. (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

B.9.13 9.4 Sediment-dwelling organisms (KIIA 8.16)

Contardo-Jara et al. (2009)

glyphecotox_326	**Contardo-Jara, V., Klingelmann, E., Wiegand, C.**	**2009**	**Bioaccumulation of glyphosate and its formulation Roundup Ultra in *Lumbriculus variegatus* and its effects on biotransformation and antioxidant enzymes**	**Environ Pollut 157 (1):57-63. DOI: 10.1016/j.envpol.2008.07.027.**

Reliability	
Purpose of the study Description of endpoints	The bioaccumulation potential of glyphosate and the formulation Roundup Ultra, as well as possible effects on biotransformation and antioxidant enzymes in Lumbriculus variegatus were compared by four ays exposure to concentrations between 0.05 and 5 mg L1 pure glyphosate and its formulation
Test compound, application procedure, exposure period	Glyphosate (N-(phosphonomethyl)glycine) was obtained from Dr. Ehrenstorfer (Augsburg, Germany) with 98 0.5% certified purity. The used Roundup Ultra solution (Monsanto Co, St. Louis, MO, USA) contains the monoisopropylamine salt of N-(phosphonomethyl)-glycine (360 g L1) and surfactants of undisclosed chemical composition and concentration (e.g. polyoxyethylene amine.
Experimental approach, Statistics, test environment	The bioaccumulation of glyphosate in *L. variegatus* was studied after four days exposure with renewal of the exposure medium after two days.
Test organisms	*Lumbriculus variegatus*
Biological effects	The bioaccumulation factor (BCF) varied between 1.4 and 5.9 for the different concentrations, and was higher than estimated from log Pow.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance	yes

being assessed?	
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	N d
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Baker, L.F. et al. (2014)

BVL Nr. 2716703	**Baker, L.F. et al.**	**2014**	**The direct and indirect effects of a glyphosate-based herbicide and nutrients on Chironomidae (Diptera) emerging from small wetlands**	**Environmental Toxicology and Chemistry, Vol. 33, No. 9, pp. 2076–2085, 2014**

Reliability	
Purpose of the study Description of endpoints	Replicated, split-wetland experiment was designed to determine the impact of an agricultural-use glyphosate-based herbicide, Roundup WeatherMax, alone and in combination with nutrients, on the abundance and the composition of the Chironomidae assemblage as it emerged from small wetlands.
Test compound, application procedure, exposure period	Roundup WeatherMax; 2 nominal concentrations (2.88 mg acid equivalents/L and 0.21 mg acid equivalents/L) of the glyphosate herbicide Roundup WeatherMax,
Experimental approach, Statistics, test environment	The 24 wetlands used in the present study were located in the Long-Term Experimental Wetlands area, Wetlands were divided with 30-mil (0.76-mm) thick, opaque, high-density polyethylene (HDPE) geomembrane; Quantification of nutrient and glyphosate concentrations; Wetland plant surveys; Chironomidae emergence collection
Test organisms	Chironomidae (Diptera)
Biological effects	emergence of Chironomidae (Diptera) before and after herbicide-induced damage to macrophytes: There were no direct effects of treatment on the structure of the Chironomidae community or on the overall emergence rates. However, after macrophyte cover declined as a result of herbicide application, there were statistically significant increases in emergence in all but the highest herbicide treatment, which had also received no nutrients.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation Roundup WeatherMax
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	Although direct toxicity of Roundup WeatherMax was not apparent, the authors observed longer-term impacts, suggesting that the indirect effects of this herbicide deserve more consideration when assessing the ecological risk of using herbicides in proximity to wetlands.
Type of information (Critical, supporting, low weight)	**supporting**

Consideration/concluding score	**UBA2**

B.9.13 9.5 Microcosm or mesocosm study (KIIA 8.16)

Vera et al. (2010)

glyphecotox_129	**Vera, M.S., Lagomarsino, L., Sylvester, M., Perez, G.L., Rodriguez, P., Mugni, H., Sinistro, R., Ferraro, M., Bonetto, C., Zagarese, H., Pizarro, H.**	**2010**	**New evidences of Roundup® (glyphosate formulation) impact on the periphyton community and the water quality of freshwater ecosystems**	**Ecotoxicology, 19:710-721**

Reliability	
Purpose of the study Description of endpoints	The experiment was carried outover 42 days in ten outdoor mesocosms of different typology: ‘‘clear’’ waters with aquatic macrophytes and/ormetaphyton and ‘‘turbid’’ waters with great occurrence of phytoplankton or suspended inorganic matter.
Test compound, application procedure, exposure period	The herbicide Roundup®was added at 8 mg L-1 of the active ingredient (glyphosate)in in five mesocosms while five were left as controls(without Roundup®addition).
Experimental approach, Statistics, test environment	The ten mesocosms (depth: 1.2 m; area:25 m2), constructed in an area of approximately 1 ha, wereBuilt. The bottom of eachexcavation was covered with soil from places nearby toprovide sediments to each environment (Fig. 1). Finally,they were filled with well water and were left to evolve. Kruskal–Wallisnon-parametric ANOVA
Test organisms	periphyton
Biological effects	Roundup®produced a clear delay in periphytic colonization in treated mesocosms and values of the periphytic mass variables (dry weight, ash-free dry weight and chlorophyll a) were always higher in control mesocosms. Despite the mortality of algae, mainly diatoms, cyanobacteria was favored in treated mesocosms. It was observed that glyphosate produced a long term shift in the typology of mesocosms, ‘‘clear’’ turning to ‘‘turbid’’, which is consistent with the regional trend in shallow lakes in the Pampa plain of Argentina.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	algal groups
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Diatoms (Bacillariophyceae) appeared to be the most affected by the herbicide, Cyanobacteria, on the other hand, emerged enhanced in number in treated mesocosms.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	joint effects
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product with surfactant
2 Do the tested concentrations relate to predicted environmental concentrations?	Might exceed the predicted envirnonmental concnetrations.
3 Have parameters influencing the endpoints been considered adequately?	It is important to point out that the toxicity is produced by the joint effect of both glyphosate and POEA, which is the surfactant of the commercial formulation Roundup®whose toxicity was shown to be higher than glyphosate.

Concluding weight of evidence/proposed action	Changes in community structure
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Perez et al. (2007)

glyphecotox_539	**Perez, G.L., Torremorell, A., Mugni, H., Rodriguez, P., Solange Vera, M., do Nascimento, M., Allende, L., Bustingorry, J., Escaray, R., Ferraro, M., Izaguirre, I., Pizarro, H., Bonetto, C., Morris, D.P., Zagarese, H.**	**2007**	**Effects of the herbicide Roundup® on freshwater microbial communities: a mesocosm study**	**Ecol Appl 17 (8):2310-22**

Reliability	
Purpose of the study Description of endpoints	Effect of the commercial formulation Roundup® using artificial earthen mesocosms.
Test compound, application procedure, exposure period	Roundup®
Experimental approach, Statistics, test environment	The herbicide was added at three doses: a control (without Roundup®) and two treatments of 6 and 12 mg/L of the activeingredient (glyphosate).
Test organisms	Phytoplancton and periphyton community
Biological effects	Roundup® affected the structure of phytoplankton and periphyton assemblages. Total micro- and nanophytoplankton decreased in abundance in treated mesocosms. In contrast, the abundance of picocyanobacteria increased by a factor of about 40. Primary production also increased intreated mesocosms (roughly by a factor of two). Similar patterns were observed in theperiphytic assemblages, which showed an increased proportion of dead : live individuals andincreased abundances of cyanobacteria (about 4.5-fold).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Presented EC_{50} values will be taken into account. 6mg/l elicitated a change in community structure.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Solange Vera et al. (2012)

BVL- Nr.: 2716752	**Marı´a Solange Vera • Eugenia Di Fiori • Leonardo Lagomarsino • Rodrigo Sinistro • Roberto Escaray • Marı´a Mercedes Iummato • Angela Jua´rez • Marı´a del Carmen Rı´os de Molina • Guillermo Tell • Hayde´e Pizarro**	**2012**	**Direct and indirect effects of the glyphosate formulation Glifosato Atanor on freshwater microbial communities**	**Ecotoxicology (2012) 21:1805–1816**

Reliability	
Purpose of the study Description of endpoints	Investigation on the direct and indirect impacts of the commercial formulation on structural features of freshwater microbial communities (phytoplankton, bacterioplankton, periphyton, and zooplankton) through a outdoor microcosms.
Test compound, application procedure, exposure period	Glifosato Atanor ®which is composed of 48 % w/v of glyphosate as isopropylamine salt, surfactants of unknown composition.
Experimental approach, Statistics, test environment	six outdoor artificial small ponds, Three of these microcosms were randomly selected and treated with Glifosato Atanor to obtain a nominal glyphosate concentration of 3.5 mg active ingredient per liter, whereas the remaining three microcosms were used as controls (without herbicide addition).
Test organisms	Periphyton, Zooplankton; Phytoplankton and bacterioplankton
Biological effects	The results showed significant direct and indirect effects of Glifosato Atanor on the microbial communities. A single application of the herbicide caused a fast increase both in the abundance of bacterioplankton and planktonic picocyanobacteria and in chlorophyll a concentration in the water column. An indirect effect of the herbicide on the zooplankton was observed due to the increase in the abundance of the rotifer Lecane spp. as a consequence of the improved food availability given by picocyanobacteria and bacteria. The formulation affected directly a fraction of copepods as a target.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation
2 Do the tested concentrations relate to predicted environmental concentrations?	Based on modelling assumptions, the concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	A single application of Glifosato Atanor caused a fast stimulation of bacterioplankton and planktonic picocyanobacteria as well as of chlorophyll a concentration in the water column, accelerating the eutrophication of the system.
Type of information (Critical, supporting, low weight)	low weight
Consideration/concluding score	**UBA3**

B.9.13 9.6 Summary of the relevant literature on aquatic organisms

Aquatic organisms are potentially exposed to glyphosate containing plant protection products via spray drift, runoff and drainage as a consequence of use near aquatic environments.

Annett et al. (2014) reviews the current literature on concentrations of glyphosate in surface water as well as on potential effects towards aquatic organisms. Due to its herbicidal mode of action, aquatic algae and macrophytes are considered especially vulnerable to the impact of glyphosate.
For the group of algae, a comprehensive database of nearly 30 peer-reviewed papers was submitted by the notifier. The notifier considered five publications (Sobrero et al. 2007; Sanchez et al. 2007; Turgut et al. 2011 and Vera et al. 2010) and considered one publication to be rated in category "Klimisch 2" (Klimisch 1997) and annotated with minimal remarks, whereas the remaining were considered as not acceptable for risk assessment. The submitted publications were also evaluated by the RMS and were assigned to pre-defined categories to characterize their reliability (for classification scheme please refer to **Fehler! Verweisquelle konnte nicht gefunden werden.**). Out of the submitted publications, 15 studies were recognized as supporting information (category UBA2) and are reviewed here. Moreover, after the date of submission, during the administrative process and the public launching period of the evaluation a few more peer-reviewed relevant articles were newly published (mainly period of 2012-2014) and needed to be considered in a revised DAR. Within these references, additional studies focusing on non aquatic organisms have been considered as supporting and critical information for risk assessment. Endpoints deriving out of these publications are listed in the table below.

Regarding the toxicity of glyphosate (technical grade) towards algae, the peer reviewed open literature provides a wide range of EC_{50} and IC_{50} values ranging from 2.3 mg a.s./l for the marine diatom *Skeletonema costatum* (Tsui, 2003), being the most sensitive of the tested species, to 70 mg a.s./L for *Scenedesmus quadricauda* (Ma, 2006). For macrophytes, endpoints similar to algae are reported with IC_{50} and EC_{50} values ranging from 0.22 mg a.s./L for *Myriophyllum aquaticum* (Turgut & Fomin, 2002) to 46.9 mg a.s./L for *Lemma minor* (Cedergreen & Streibig, 2005).

A few higher tier studies focussed on aquatic communities, partly including an assessment of indirect effects. The result of a mesocosm by Perez (2007) indicated an impact on phytoplancton and periphyton community structure (increase of the total micro- and nanophytoplancton abundance; decrease of the picocyanobacteria abundance compared to the control). . Similar changes in phytoplankton and periphyton community structure were observed by Vera et al. (2010). Their results indicated for a commercial Glyphosate product containing a surfactant at a concentration of 8 mg product/L, that despite the mortality of algae (mainly diatoms), cyanobacteria were favored in treated mesocosms. However, it must be considered that in both studies commercial products containing surfactants were used, and therefore the toxicity is determined by the joint effect of both glyphosate and the surfactants of the commercial formulations. Commercial products containing specific formulation ingredients additionally to the active ingredient were shown to be more toxic towards algae than glyphosate acid (Cedergreen & Streibig, 2005; Tsui, 2003).
Based on active substance content, endpoints from the publications Tsui et al. (2003) for algae and Turgut & Formin (2002) for aquatic plants were lower than available endpoints and therefore included in the risk assessment for the active substance. It should be noted that if endpoints were derived from studies performed with glyphosate formulations, these data are only considered in the TER calculation as additional information.

Table B.9.13-3: Effects values of algae and aquatic plants in per-reviewed literature

Species	Substance	Study type	EC50 (mg/L)	Reference (internal tag)
Algae				
Chlorella vulgaris	Glyphosate 95%, technical product.	96h	5	Ma, J.,2002; glyphecotox_476
Raphidocelis subcapitata	Glyphosate 95%, technical product.	96h	5.5	Ma, J.,2002; glyphecotox_474
Scenedesmus obliquus	Glyphosate 95%, technical product.	96h	56	Ma, J., 2002; glyphecotox_471
Scenedesmus quadricauda	Glyphosate 95%, technical product.	96h	70	Ma, J.,2006; glyphecotox_473
Chlorella pyrenoidosa	Glyphosate 95%, technical product.	96h	3.5	Ma. J.,2001; glyphecotox_477
Chlorella kessleri	ATANOR	96h	55.62	Romero, et al., 2011 glyphecotox_578
Pseudokirchneriella subcapitata	Glyphosate 95%, technical product.	48h	270	Cedergreen, N., Streibig, J.C., 2005; glyphecotox_319
	Roundup 360 g/L		64.7	
Periphyton	Commercial product with surfactant	Mesocsm, 42days	8mg/l Changes in community structure	Vera, M.S.,, 2010; glyphecotox_129
Periphyton, Phytoplancton	Roundup®	Mesocsm, 11days	6 mg/L Changes in community structure	Perez, G.L.,2007; glyphecotox_539
Selenastrum capricornutum	Glyphosate acid	96h	24.7 mg a.e./L	Tsui, M.T.K2003 glyphecotox_195
	IPA salt of glyphosate		41.0 mg a.e./L	
	POEA		3.92 mg a.e./L	
	Roundup®		1.85 mg a.e./L	
Skeletonema costatum	Glyphosate acid	96h	2.27 mg a.e./L	Tsui, M.T.K., 2003 glyphecotox_195
	IPA salt of glyphosate		5.89 mg a.e./L	
	POEA		3.35 mg a.e./L	
	Roundup®		1.85 mg a.e./L	
Macrophytes				
Myriophyllum aquaticum	Commercial product, 36% a.s.	14 days	2.0 (fresh weight) 0.22 (chl a)	Turgut & Fomin, 2002, glyphecotox_128
Lemna minor L.	Glyphosate 95%, technical product.	7 days	46.9	Cedergreen, N., Streibig, J.C., 2005; glyphecotox_319
	Roundup 360 g/L		11.2	
Lemna minor L.	Glyphosate 95%, technical product.	10 days	20.5	Sobrero, M.C.,2007; glyphecotox_125
	Roundup1Max, 70.7%w/w a.i. as acid		11.6	

For the group of aquatic invertebrates, a comprehensive database of 42 peer-reviewed papers was submitted by the notifier. The notifier considered three publications (Bringolf *et al.* 2007; Chen et al. 2004 and Mensah et al. 2011) to be rated in category "Klimisch 2" (Klimisch 1997) and annotated with minimal remarks, whereas the remaining publications were

considered to be not reliable to be used in risk assessment. The submitted publications were also evaluated by the RMS and assigned to pre-defined categories to characterize their reliability (for classification scheme please refer to **Fehler! Verweisquelle konnte nicht gefunden werden.**). Out of the submitted publications, 18 studies were classified as supporting information (category UBA2) and are reviewed here (derived endpoints are listed in the table below). Moreover, after the date of submission, during the administrative process and the public launching period of the evaluation a few more peer-reviewed relevant articles were newly published (mainly period of 2012-2014) and needed to be considered in a revised DAR. Within these references, additional studies focusing on non aquatic invertebrates have been considered as supporting and critical information for risk assessment.

Most of the cited studies tested formulated products and not the active ingredient alone.
LC_{50} values obtained from studies investigating the effects of Glyphosate or the Glyphosate IPA salt ranged from 49.3 mg acid equivalents /L for the marine copepod *Acartia tonsa* to 415 mg acid equivalents /L for the cladoceran *Ceriodaphnia dubia* (Tsui, 2003; Le, 2010; Tsui et al., 2004; Dominguez-Cortinas et al., 2008; Bringolf et al., 2007; Mottiera et al., 2013; Frontera, 2011; Dominguez-Cortinas, 2008). The coelenterate *Hydra attenuata* showed an even lower sensitivity towards the active ingredient glyphosate (LC_{50} = 18.2 mg/L). Since coelenterates do not inhabit edge of field surface waters and are not considered representative of other taxionomic groups that do, these toxicity data will not be considered in the risk assessment. However, it is noted that generally these animals are exposed to toxicants in marine aquatic environments to a high extent due their anatomical and physiological structure (Demetrio, 2012). Moreover, sublethal effects were observed at much lower concentrations of glyphosate in comparison to lethal effects (Mottiera, 2013). More data exist on the sensitivity of of marine species, which indicate a higher sensitivity towards marine invertebrates (Akcha, et al. 2012, Kumar et al. 2013).

In general, the formulations are of higher ecotoxicological relevance than the active ingredient glyphosate itself. One of the main commercial formulations is Roundup ®, which contains polyoxyethoxylated alkylamines (POEA) as a surfactant in addition to the active ingredient glyphosate. A few studies investigate the effects of formulations containing the surfactant POEA. These studies have shown that formulations containing POEA are several times more toxic to aquatic invertebrates than the active ingredient glyphosate acid or formulations without POEA (3 to 5 fold more toxic than Roundup®). For more details concerning surfactant ingredients and their toxicity to aquatic organisms please refer to chapter B9.13.16. Based on active substance content, endpoints from the publications Immanuto et al. 2013 and Cuhra et al. 2013 testing chronic effects towards aquatic invertebrates were lower than available endpoints and therefore included in the risk assessment for the active substance. It should be noted that if endpoints were derived from studies performed with glyphosate formulations, these data are only considered in the TER calculation as additional information.

Table B. 9.13-4: Effects values for aquatic invertebrates exposed to glyphosate acid or formulated products with glyphosate. Endpoints published in peer-reviewed open literature

Species	Test item	Study type	LC_{50} (mg a.e./L)	Reference (internal tag)

Species	Test item	Study type	LC_{50} (mg a.e./L)	Reference (internal tag)
Crustaceans				
Daphnia magna	glyphosate acid	48h (mortality)	234	Le, T.H., 2010; glyphecotox_122
C. quadricarinatus	glyphosate acid	50days	>33	Frontera, J.L., 2011; glyphecotox_378
Daphnia magna	glyphosate acid	48h (mortality)	146	Dominguez-Cortinas, G.,2008; glyphecotox_347
	Faena®		7.9	
Lecane quadridentata	glyphosate acid	48h (mortality)	150	
	Faena®		13.1	
Daphnia magna	Glpyhosate IPA	48h (mortality)	4.2	Sihtmäe et al. (2013); BVL Nr.: 2716810
Hyalella castroi	Roundup®	7days (survival estimated)	2.16	Dutra, B.K.,, 2011; glyphecotox_121
Chordodes nobilii	Roundup®	96h (mortality)	1.76	Achiorno, C.L.,2008; glyphecotox_110
Caridina nilotica	Roundup®	48h (mortality)	neonates = 4.45	Mensah, P.K., 2011; glyphecotox_123
			juvenile = 9.39	
			adults=37.12	
		96h (mortality)	neonates = 2.54	
			juvenile = 6.96	
			96h adults=25.507	
Daphnia magna	Roundup®	48h (mortality)	0.019	Sarigül Z., 2009; glyphecotox_124
Simocephalus vetulus	Vision®	48h (mortality)	0.75 to 1.5 a.e.	Chen, C.Y., 2004; glyphecotox_120
Ceriodaphnia affinis	Fakel herbicide	48	13.6	Melnichuk, S.D.,2007; glyphecotox_501
Thamnocephalus platyurus	POEA 15:1	48h (mortality)	2.01	Brausch, J.M., 2007, glyphecotox_113
	POEA 10:1		2.70	
	POEA 5:1		5.17	
Daphnia magna	POEA 15:1	48h (mortality)	0.85	Brausch, J.M., 2007; glyphecotox_114
	POEA 10:1		0.097	
	POEA 5:1		0.18	
Ceriodaphnia dubia	Glyphosate IPA-salt	48h (mortality)	415	Tsui, M.T.K., 2003 glyphecotox_195
	Roundup®		5.4	
	POEA		1.2	
Acartia tonsa	Glyphosate IPA-salt	48h (mortality)	49.3	Tsui, M.T.K., 2003 glyphecotox_195
	Roundup®		1.77	
	POEA		0.57	
Ceriodaphnia dubia	Rodeo®	48h (mortality)	415 a.e.	Tsui, M.T.K., 2004; glyphecotox_018
	Roundup Bioactive®		81.5 a.e.	
	Roundup®		5.7 a.e.	
Hyalella azteca	Rodeo®	48h (mortality)	347a.e.	
	Roundup Bioactive®		120 a.e.	

Species	Test item	Study type	LC_{50} (mg a.e./L)	Reference (internal tag)
	Roundup®		1.5 a.e.	
Nonarthropoda				
Lampsilis siliquoidea	Glyphosate technical	48h (mortality)	>200	Bringolf, R.B.,, 2007; glyphecotox_119
	Glpyhosate IPA		5	
	Aquastar®		>148	
	Roundup®		2.9	
	MON0818		0.5	
Utterbackia imbecillis	Roundup®	24h (mortality)	18.3	Conners, D.E., 2004; glyphecotox_325
Branchinella thai-landensis	Roundup®	24h (mortality)	0.319	Boonsoong & Buliangpoti , 2012, BVL.: Nr.: 2716764
Hydra attenuata	glyphosate (as acid)	96h (mortality)	18.2	Demetrio, P.M., 2012; glyphecotox_342
	RoundupMax® (74.4% glyphosate)		21.8	
Mature oysters	Glyphosate technical		[>0.005] (larval development)	Akcha, F.,2012; glyphecotox_273
Limnoperna fortunei	Glyphosate technical	26d	1 (Glutathione-S-transferase activity)	Immanuto, M.M. et al. 2013
Crassostrea gigas	Glyphosate technical	48h (mortality)	>100	Mottiera, A., 2013
	AMPA		>100	
	Roundup Express®		8.5	
	Roundup Allées et Terrasses®		7.9	
Crassostrea gigas	Glyphosate technical	48h (larval development)	27.1	
	AMPA		46.1	
	Roundup Express®		1.1	
	Roundup Allées et Terrasses®		2.0	

For the group of aquatic vertebrates, a database of more than 60 peer-reviewed publications were submitted by the notifier. The notifier considered seven publications (Filizadeh et al. 2011; Guilherme et al. 2012; Hued et al. 2012; Kelly et al. 2010; Salbego et al. 2010; Benck Soso et al. 2007 and Tierny et al. 2006) and all seven were classified as "Klimisch 3" (Klimisch 1997). The submitted publications were also evaluated by the RMS and were assigned to pre-defined categories to characterize their reliability (for classification scheme please refer to **Fehler! Verweisquelle konnte nicht gefunden werden.**). Out of the submitted publications, 24 studies were classified as supporting information (category UBA2) and are reviewed here. Moreover, after the date of submission, during the administrative process and the public launching period of the evaluation a few more peer-reviewed relevant articles were newly published (mainly period of 2012-2014) and needed to be considered in a revised DAR. Within these references, additional studies focusing on fish have been considered as supporting and critical information for risk assessment.

In the environmental risk assessment of pesticides, the group of aquatic vertebrates is mainly assessed by the results of acute, early life stage or full-life cycle studies performed in the laboratory considering survival, growth and reproduction of individuals as endpoints. This is due to protection of aquatic vertebrates at the individual level (compared to other aquatic organisms that are protected at the population level)
Recent research is focused on endpoints on sub-organismic level, such as indicators of metabolic, haematological and reproduction alterations caused by glyphosate formulations. Various studies deal with sub-lethal endpoints such as histological alterations of gill, liver and further organ tissues, such as neurotoxic endpoints and genetic biomarkers (Guilherme et al., 2010, Salbego et al., 2010; Soso et al., 2007; De Menezes et al., 2011; Kreutz et al., 2011; Cavalcante et al., 2008; Ferreira et al., 2010; Cattaneo et al., 2011; De Menezes et al., 2011).

In a few studies (Evrard et al., 2010; Langiano et al., 2008) histological alterations in the gills, liver, liver gene expressions, methionine metabolism, lipid transport and metabolisms related to oxidative stress were observed. Most of these endpoints measured can be taken as early warning indicators of genotoxic and oxidative stress at the individual level . Some alterations observed in aquatic vertebrate studies like the enhancement of stress related genes and enzymes were of general character being linked to the metabolic response towards abiotic and biotic factors of the experimental environment. Endpoints included in TER calculations if they indicated implications for survival, growth or reproduction, all other endpoints were considered as additional information. In cases where the commercial formulation tested was likely to contain POEA as surfactant strong histologic changes were observed, which might lead to impaired organ functioning (e.g Zhidenko et al. 2007; Ortiz-Ordoñez et al., 2003;),. Based on available studies, the toxicity of the formulation Roundup® could not be compared to that of glyphosate-based formulations not containing POEA since acid equivalents as well as the contents of POEA or other surfactants were not reported. Concerns on side-effects of glyphosate formulations containing POEA as surfactants raised were raised in particular early studies (Folmar et al., 1979, Smith et al., 2004, Haller et al., 2003), whereas technical grade glyphosate has rarely been tested.

Based on active substance content, the endpoint from the publication Webster et al. (2013) testing chronic effects towards fish were lower than available endpoints and therefore included in the risk assessment for the active substance. It should be noted that if endpoints were derived from studies performed with glyphosate formulations, these data are only considered in the TER calculation as additional information.

Several studies investigated changes in the metabolic and enzymatic state in aquatic organisms (Fan, et al. 2013, Sandrini, et al 2013, Syedkolaei, et al. 2013, Gholami-Seyedkolaei, et al. 2013). A few studies associated exposure to commercial formulation with inhibition of AchE activity in brain and/or muscle of aquatic organisms (Cattaneo et al. , 2003, Lajmanovich et al., 2011, Sandrini et al., 2013). These changes in biochemical parameters could be used as biomarkers, because a dose-response association between commercial formulation treatment and enzymtic activity was found in the different tissues.
Available publications studying biochemical, metabolic and histopathological effects were only considered as additional information, since endpoints were not considered to indicate additional implications for growth, survival or reproduction of aquatic vertebrates that are not already covered by the standard risk assessment.

Many tests using fish have been conducted in order to investigate the genotoxic and cytotoxic potentail of glyphosate towards different aquaic organisms (Nwani et a. 2013, Moreno,et al. 2014, De Souza Filho, et al. 2013, de Castilhos Ghisi, et al. 2012, Vera-Candioti, et al 2013, Guilherme, et al. 2012 and 2014). Most of these studies were performed with ecologically realistic concentration of the herbicide. In most cases, again commercial formulations have been used which does not allow to discriminate, which compound of the commercial formulation could be responsible for the observed effects. Nevertheless, it has also been reported that glyphosate itself caused oxidative DNA damage in cells of *A. anguilla* exposed under laboratory conditions (Guilherme et al.,2012).Taking together, these results revealed that both glyphosate itself as well as the formulated products should be carefully monitored considering their potential impact on aquatic biota. Moreover, it seems that a transition from traditional ecotoxicological methods determining acute toxicity with endpoints on mortality and reproduction can be complemented by methods taking into account biochemical parameters. No concluding informations are available at the moment to decide wether alterations might impair normal organ functioning. Therefore, the studies have limited value to conclude on the relevance at a the population level. Nevertheless biochemical biomarkers allow the examination of specific target organs, including gills, kidney and liver and blood that are responsible for vital functions.

Table B. 9.13-5: Effects values for aquatic vertebrates exposed to glyphosate acid or formulated products. Endpoints published in peer-reviewed open literature

Species	Substance	Study type	EC_{50} (mg/L)	Reference
Fish				
Chlorella vulgaris	Roundup®	96h	20-26	Filizadeh, et al. (2011)
Jenynsia multidentata	Roundup Max®	96h	19.2	Hued, A.C. et al. (2012)
Rhamdia quelen	Roundup®	96h	7.3	Kreutz et al. (2008)
Pimephales promelas	Glyphosate a.s,	96h	97	Folmar et al. (1979)
Prochilodus lineatus	Roundup®	96h	14	Langiano et al. (2008)
Goodea Atripinnis	Yerbimat	96h	39	Ortiz-Ordoñez et al. (2003)
Channa punctatus	Roundup®	96h	32	Nwani et a. (2013)
Cyprinus carpio	Roundup®	96h	22	Syedkolaei, et al. (2013)
Danio rerio	Glyphosate	21d	0.5	Webster, et al. (2013)
Clarias gariepinus	Dizensate®	96h	43	Akinsorotan, et al. (2013)
Cnesterodon decemmaculatus	Panzer®	96h	16	Vera-Candioti, et al (2013)
	Credite®		92	

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Tsui, M.T.K., Chu, L.M.; Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors; 2003; Chemosphere 52: 1189–1197.

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Vera-Candioti, J., Soloneski, S., & Larramendy, M. L. (2013). Evaluation of the genotoxic and cytotoxic effects of glyphosate-based herbicides in the ten spotted live-bearer fish *Cnesterodon decemmaculatus* (Jenyns, 1842). Ecotoxicology and environmental safety, 89, 166-173.

Zhidenko, A.A., Kovalenko, Y.M.; The influence of Roundup on the dynamics of histological changes in organs of carps (2007) Hydrobiological Journal 43 (2):93-99

B.9.13.10 Effects on amphibians

For the group of amphibians, a comprehensive database of 85 peer-reviewed papers was collected by the notifier. Out of the submitted, 20 studies were recognized by the notifier as relevant for full evaluation. Further 54 papers were cited in the text and considered supporting the submission, but were not submitted with the application, mostly because of publishing date older than 10 years.

Moreover, further publications have been submitted, but were not assigned to one of the areas of assessment by the notifier. After UBA screening, further 28 papers were identified for the assessment of glyphosate effects on amphibians. Critical and relevant studies are summarized below. Moreover, after the date of submission, during the administrative process and the public launching period of the evaluation a few more peer-reviewed relevant articles were newly published (mainly period of 2012-2014) and needed to be considered in a revised DAR. Within these references, additional studies focusing on non aquatic organsisms have been considered as supporting and critical information for risk assessment.

The following sections summarise the results and discussion as found in the published literature. RMS assessment is given in the final summarising chapter after the results of the studies are presented and discussed.

Howe et al. (2004)

glyphecotox_025	**Howe, C.M., Berrill, M., Pauli, B.D., Helbing, C.C., Werry, K., Veldhoen, N.**	**2004**	**Toxicity of glyphosate-based pesticides to four North American frog species**	**Environmental Toxicology and Chemistry 23 (8):1928-1938**
Reliability				
Purpose of the study Description of endpoints	The study was designed to compare the acute and chronic toxicities of six glyphosate formulations, the technical-grade glyphosate and a polyethoxylated tallow amine surfactant to tadpoles of four species of North American frogs. The sensitivity of different tadpole stages towards glyphosate was examined. Acute studies: Mortality, survival Chronic studies: Forelimb emergence = time to reach Gosner stage 42, Total length, body, length, tail length, visible tail damage and maximum tail height were recorded, snout-vent-length of metamorphs; gonadal histology to determine sex ratios			
Test compound, application procedure, exposure period, protocol	Acute studies static exposure for 96h, expressed as mg test item/L and as formulation glyphosate acid equivalents to enable direct comparisons between the different mixtures of ingredients of the different formulations (FAE: It is assumed 'that the surfactant does not contain glyphosate acid, so the FAE used for the surfactant refers to the calculated amount of glyphosate acid in its formulation equivalent, assuming the surfactant component to be approximately 15%. To calculate the FAE in each glyphosate herbicide formulation, the values published by Giesy et al. (2000, glyphnosubm_050) were followed Thus, for a glyphosate-based formulation of 1.0, the FAE is 0.31, and the surfactant is 0.15. In other words, 1 mg of the formulation is assumed to contain 0.31 mg of glyphosate acid equivalent and approximately 0.15 mg of POEA.' Chronic studies Exposure period 42d, static renewal: weekly spiking of the test items (6 application dates), then rearing in clean water until day 70. Non-GLP			
Experimental approach Statistical design, test environment	Acute studies 20 tadpoles at Gosner-stage 25 were used per treatment-replicates for the formulation comparison and 10 tadpoles at Gosner stage 20 and 25 for the stage-comparison with Roundup Original®, 3 replicates per concentration were tested Test items: Roundup Original®, Glyphosate technical, POEA, Roundup Biactive®, Touchdown®, Glyfos BIO®, Glyphos AU®, Roundup Transorb®; at least four concentrations up to 18 mg FAE/L were tested to determine LC50 and confidence itervals Determination of LC_{50} for 24 and 96h exposure by trimmed Spearman-Karber method Chronic studies test items 1.8 FAE glyphosate technical/L, 0.6 and 1.8 FAE polyethoxylated			

	tallowamine surfactant (POEA)/L, 0.6 and 1.8 FAE Roundup Original/L, 0.6 and 1.8 FAE Roundup Transorb/L
Test organisms	Acute studies *Rana pipiens*, *Rana sylvatica*, *Bufo americanus*, *Rana clamitans* Chronic studies *Rana pipiens*
Biological effects	Acute studies Not all treatment showed sufficient mortality to calculate proper LC50. From the published paper: Table 2. Acute toxicity (median lethal concentration values [LC50] with 95% confidence intervals in parentheses) obtained in 24-h and 96-h exposures of four amphibian species exposed to glyphosate-based herbicides, glyphosate technical material, and polyethoxylated tallowamine surfactant (POEA) at two life stages[a] Earlier stages were slightly less sensitive than Gosner stage 25-individuals Chronic studies 38 % mortality in control aquaria undermines experiment validiy POEA 1.8, Roundup Original ® 0.6 and 1.8 and Roundup Transorb ® showed significant tail damages and reduced tail lengths. NO effects with glyphosate alone. POEA containing formulations showed displaced sex ratios towards intersex individuals. The lengths and percent surviving tadpoles to reach Gosner stage 42 as well as the days to reach stage 42 were significantly altered by most of the treatments except of glyphosate technical. However, results were not strictly dose-dependent.

Species	Gosner stage	Compound	LC50 24 h mg/L	LC50 24 h mg FAE/L	LC50 96 h mg/L	LC50 96 h mg FAE/L
Rana pipiens[b]	25	Roundup Original®	11.9 (11.2–12.6)	3.7 (3.5–3.9)	9.2 (NR)	2.9 (NR)
R. pipiens[c]	20	Roundup Original	>25.8	>8	20.9 (19.8–21.9)	6.5 (6.1–6.8)
R. sylvatica[c]	25	Roundup Original	18.1 (16.7–19.6)	5.6 (5.2–6.1)	16.5 (15.7–17.4)	5.1 (4.9–5.4)
R. sylvatica[c]	20	Roundup Original	>25.8	>8	>25.8	>8
Bufo americanus[c]	25	Roundup Original	13.5 (NR)	4.2 (NR)	<12.9	<4
B. americanus[c]	20	Roundup Original	>25.8	>8	25.8 (NR)	8 (NR)
R. clamitans[d]	25	Roundup Original	6.6 (6.1–7.1)	2.0 (1.9–2.2)	6.5 (6.0–7.0)	2.0 (1.9–2.2)
R. clamitans[c]	20	Roundup Original	>25.8	>8	22.8 (21.2–24.5)	7.1 (6.6–7.6)
R. clamitans[d]	25	Glyphosate technical	>38.9	>17.9	>38.9	>17.9
R. clamitans[d]	25	POEA	1.1 (1.1–1.2)	2.4 (2.2–2.5)	1.1 (1.0–1.1)	2.2 (2.1–2.4)
R. clamitans[d]	25	Roundup Biactive®	>57.7	>17.9	>57.7	>17.9
R. clamitans[d]	25	Touchdown®	>57.7	>17.9	>57.7	>17.9
R. clamitans[d]	25	Glyfos BIO®	>57.7	>17.9	>57.7	>17.9
R. clamitans[d]	25	Glyfos AU®	29.1 (28.1–30.2)	9.0 (8.7–9.4)	28.6 (27.6–29.6)	8.9 (8.6–9.2)
R. clamitans[d]	25	Roundup Transorb®	7.4 (6.9–7.9)	2.3 (2.2–2.4)	7.2 (6.8–7.7)	2.2 (2.1–2.4)

[a] Roundup Original, Roundup Biactive, and Roundup Transorb from Monsanto (St. Louis, MO, USA); Touchdown from Syngenta (Wilmington DE, USA); Glyfos BIO and Glyfos AU from Cheminova (Wayne, NJ, USA). FAE = formulation glyphosate acid equivalents; NR = 95% confidence intervals not reliable.
[b] 2000 Chronic study.
[c] 1994 Study.
[d] 2001 Study.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	It was necessary to measure the developmental endpoints on juvenile tadpole stages. It was proven in this experiment that earlier tadpole stages were less sensitive, which is quite contrary to common expectations that earlier stages should be more sensitive towards chemical stress.
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Yes
3 Is the ecotoxicological manifestation level appropriate for the assessment, e.g. gene induction vs. apical endpoints like growth or reproduction?	The intersex-hypothesis has been intensively criticised by the notifier (weaknesses in histological and statistical analysis), which can be only partly agreed by RMS. There was no statistical procedure described for this endpoint, so that it could be seen as a qualitative measure. Other endpoints have been measured and analysed adequately and are considered by RMS appropriate growth and developmental indicators of toxic stress.
1 Is the substance tested representative and relevant for	Since there was a full-factorial design with glyphosate technical, the POEA surfactants and diverse formulations containing both glyphosate

the substance being assessed?	and surfactants, the study is of high environmental relevance.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	The LC_{50}s of the tested formulations were mainly around 5 mg FAE/L, which is in the range of the relevant aquatic endpoints for the environmental risk assessment of glyphosate.
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	There was a very high control mortality of 38% that was tried to eliminate by the authors by regular water exchange. The ammonia level was presumably too high, as well as the density of tadpoles per area most probably was.
Concluding weight of evidence/proposed action	Important data are presented that prove the high toxic potential of polyethoxylated tallow amines to different species of amphibians. The POEA treatment showed the highest toxicity in the acute tests. Dependent on the formulation that will be assessed by an ERA, the enhanced toxicity by surfactant additives should be considered and could be referenced to this well conducted and informative publication. Can be used for aquatic assessments. The chronic studies have several weaknesses (statistics, control survival rates, dose-response-relationship). Nevertheless, the results indicate several open questions in the assessment of chronic exposure of amphibian to formulated glyphosate products. None of the effects was observed in the treatments with glyphosate alone
Type of information (Critical, supporting, low weight)	**Critical, supporting**
Consideration/concluding score	**UBA1, also for assessment of surfactand effects (POEA)**

Thompson et al. (2004)

glyphecotox_038	**Thompson, D.G., Wojtaszek, B.F., Staznik, B., Chartrand, D.T., Stephenson, G.R.**	**2004**	**Chemical and biomonitoring to assess potential acute effects of Vision (R) herbicide on native amphibian larvae in forest wetlands**	**Environmental Toxicology and Chemistry, Vol. 23, No. 4, pp. 843–849, 2004**

Reliability	
Purpose of the study Description of endpoints	Chemical and biological monitoring studies were conducted following operational forest herbicide spray programs in Ontario, Canada. Magnitude of contamination by a glyphosate herbicide formulation (Vision) was investigated in 51 different wetlands. Wetlands were classified as oversprayed, adjacent, or buffered.
Test compound, application procedure, exposure period	Vision, Glyphosate product identical to Roundup Original, Monsanto. Aerial herbicide treatments of conifer crop trees. Percent mortality at 48 h posttreatment was calculated as response variable.
Experimental approach, Statistics, test environment	Not clear how many sites have replicates within blocks. Larval condition was observed and recorded periodically at approximately 6, 24, 48, and 96 h following herbicide applicationsData for each larval test species were pooled across years and mean mortality rates were calculated for each wetland classification (oversprayed, adjacent, or buffered)
Test organisms	*Rana pipiens* and *Rana clamitans* larvae (Gosner 25)
Biological effects	The mean glyphosate concentrations recorded were: buffered wetlands : 0.03 mg a.e./L adjacent wetlands: 0.18 mg a.e./L.. oversprayed wetlands: 0.33 mg a.e./L; maximum of 1.95 mg a.e./L. Mean mortality rates leopard frog larvae < 15% in all wetland types. No significant differences in mean mortality rates were observed for leopard frog larvae exposed to different glyphosate concentrations under adjacent, buffered, or oversprayed wetland scenarios. Green frog larvae showed higher mean mortality rates of 10, 26, and 36% in

	adjacent, buffered, and oversprayed wetlands, respectively. These differences were not statistically significant. RMS remark: sites/years/blocks were pooled. No separation of different factors possible (time/site/block and glyphosate concentration).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Active ingredient: yes, but applied as formulated product Lead formulation: no (Vision® with 15% POEA)
2 Do the tested concentrations relate to predicted environmental concentrations?	yes (no overspray of wetlands in the European Union, though)
3 Have parameters influencing the endpoints been considered adequately?	Not conclusive (pooling of several monitoring years/no single data available)
Concluding weight of evidence/proposed action	No data available on the variance of larval response allocated to site/year/block factors. Pooling of results on biological and chemical responses over sites and years may lead to misleading interpretation of results. Effects of glyphosate application at site level with direct comparison of sprayed/not sprayed wetland not reported.
Type of information (Critical, supporting, low weight)	**Supporting/low weight**
Consideration/concluding score	**UBA2**

Edge et al. (2011)

glyphecotox_043	**Edge, C.B., Gahl, M.K., Pauli, B.D., Thompson, D.G., Houlahan, J.E.**	**2011**	**Exposure of juvenile green frogs (*Lithobates clamitans*) in littoral enclosures to a glyphosate-based herbicide**	**Ecotoxicology and Environmental Safety 74, 1363–1369 doi:10.1016/j.ecoenv.2011.04.020**

Reliability	
Purpose of the study Description of endpoints	Juvenile green frogs(*Lithobates clamitans)* were exposed to two concentrations (2.16 and 4.27 kg a.e./ha) of a glyphosate formulation (VisionMax®), under typical application scenarios in Canadian forestry. survival, body condition, liver somatic index, observed rate of *Batrachochytrium dendrobatidis* infection.
Test compound, application procedure, exposure period	Enclosures: half of the enclosure was terrestrial and the other half aquatic Each herbicide treatment was comprised of two spray applications. One application was made by spraying the formulated product using a backpack sprayer (Flowmaster, Root-Lowell Manufacturing, Lowell, MI, USA) to one-half of the wetland, while an equivalent second spray application was made directly to the enclosure using a small plant-misting bottle. Environmentally observed concentration (EOC): 0.55 mg a.e./L (upper 99th centile of concentrations measured in Thompson et al., 2004). Predicted maximum environmental concentration(PMEC): 2.89 mg a.e./L
Experimental approach, Statistics, test environment	s.a. Following herbicide applications, animals were counted 1, 4, 7and 14 days after treatment (DAT) to determine survival. On DAT 14, SVL was measured and all animals were weighed.On DAT 14 all animals were euthanized. All animals were dissected,

	livers were removed. Liver somatic index (LSI) was calculated by dividing wet liver mass by wet body mass and multiplying by100. All animals were examined for Bd infection. Differences in arcsinesquareroot transformed proportional survival data/split-plot analysis of variance (ANOVA), with treatment rate as between subject factor,and side (control or treatment) as the within subject factor on DAT 14.
Test organisms	Juvenile green frogs(*Lithobatesclamitans)*
Biological effects	No significant difference in survival between treated and control wetlands. After 14 days, no difference in body condition between wetland sides and no relationship between the measured glyphosate application rate and body condition was observed No significant difference in the number of animals infected with Bd. "marginally significant" negative relationship between the measured glyphosate application rate and the frequency of Bd infection. RMS: Difficulties in the determination of the glyphosate concentrations in the wetlands. Option for use of nominal concentrations discussed.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	VisionMax co-formulation ingredients not known
2 Do the tested concentrations relate to predicted environmental concentrations?	-/-
3 Have parameters influencing the endpoints been considered adequately?	-/-
Concluding weight of evidence/proposed action	Coformulants not known. Glyphosate was not tested alone. Product was applied to soil and water. Exposure pattern not clear
Type of information (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA3**

Wojataszek et al. (2004)

glyphecotox_044	**Wojtaszek, B.F., Staznik, B., Chartrand, D.T., Stephenson, G.R., Thompson, D.G..**	**2004**	**Effects of Vision (R) herbicide on mortality, avoidance response, and growth of amphibian larvae in two forest wetlands**	**Environmental Toxicology and Chemistry, Vol. 23, No. 4, pp. 832–842, 2004**
Reliability				
Purpose of the study Description of endpoints	The effects of Visiont (glyphosate, 356 mg acid equivalents (a.e.)/L) on mortality, avoidance response, and growth of larval amphibians (*Rana clamitans* and *Rana pipiens*) were investigated. In situ enclosures deployed in two forest wetlands of northern Ontario, Canada.			
Test compound, application procedure, exposure period, protocol	Visiont (glyphosate, 356 mg acid equivalents (a.e.)/L) similar formulation to Roundup Original ® with 15% POEA Twenty-four in situ enclosures were positioned at each site. Thirteen enclosures used. The amount of formulated product required to achieve the desired nominal concentrations was based on enclosure volume.			
Experimental approach Statistical design, test environment	General Linear Model procedure (SAS), variance analysis on 96-h mortality least-squares means for each combination of site and species. Preplanned comparisons for differences between mean mortality observed in untreated controls and in			

	replicate enclosures treated at the 1.43 mg a.e./L (RMS: PEC overspray Canada)..
Test organisms	Free-swimming *Rana pipiens* and *Rana clamitans* larvae (Gosner 25 at time of herbicide application)
Biological effects	Table 4. Toxicity of Vision® (Monsanto, Winnipeg, MB, Canada) to *Rana clamitans* and *Rana pipiens* larvae. Ninety-six-hour lethal concentration (LC) point estimates at 10 and 50% mortality were estimated from predictive models derived from analysis of deviance. Ninety-five percent confidence limits about point estimates are given in parentheses. Both experimental sites were located approximately 80 km northeast of Sault Ste. Marie, Ontario, Canada. Site A: 46°53′20″N, 84°7′45″W; Site B: 47°02′04″N, 84°23′06″W

Site	Species	LC10 (mg a.e.[a]/L)	LC10 (mg Vision/L)	LC50 (mg a.e./L)	LC50 (mg Vision/L)
A	*Rana clamitans*	1.78 (0.99, 2.86)	5.74 (3.19, 9.23)	4.34 (3.05, 6.02)	14.0 (9.84, 19.4)
	Rana pipiens	7.31 (3.83, 9.54)	23.6 (12.4, 30.8)	11.47 (9.50, 14.5[b])	37.0 (30.6, 46.8[b])
B	*R. clamitans*	1.20[c] (0.84, 1.60)	3.87 (2.71, 5.16)	2.70 (2.06, 3.67)	8.71 (6.65, 11.8)
	R. pipiens	3.26 (1.66, 3.61)	10.5 (5.36, 11.6)	4.25 (2.45, 7.10)	13.7 (7.90, 22.9[b])

[a] a.e. = glyphosate acid equivalents.
[b] Above range of concentrations tested.
[c] Below expected environmental concentration (EEC) of 1.43 mg a.e./L.

As the aurthors state:'Experimental site and biotic/abiotic factors therein, such as pH and suspended sediments, substantially affected the expression of Vision herbicide toxicity in the amphibian larvae tested.'

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Yes
3 Is the ecotoxicological manifestation level appropriate for the assessment, e.g. gene induction vs. apical endpoints like growth or reproduction?	Yes.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, probably for all formulations containing POEA. Can not be used for the assessment of glyphosate technical/glyphosate acid, although all endpoints are given in acid equivalents.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Comparable high PECsurface water reported in the paper due to watershed overspray practice in Canada.
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	Other parameters influencing the response have been measured, but were not directly considered e.g. in the assessment of difference in toxicity beween sites.
Concluding weight of evidence/proposed action	The study presents acute mortalities for two amphibian species. The data can be employed in the assessment of formulations containing POEA as surfactants. No data for glyphosate technical or glyphosate acid deducible from the tested formulation. Lead formulation for EU renewal of glyphosate approval contains no POEA
Type of information (Critical, supporting, low weight)	**Critical/Supporting**
Consideration/concluding score	**UBA1 for assessment of surfactand effects (POEA)**

Brodman et al. (2010)

glyphecotox_048	**Brodman, R., Newman, W.D.,**	**2010**	**Interaction of an Aquatic Herbicide and Predatory**	**Journal of Herpetology, Vol. 44,**

	Laurie, K., Osterfeld, S,, Lenzo, N.		**Salamander Density on Wetland Communities**	**No. 1, pp. 69–82, 2010 DOI: 10.1670/08-320.1**

Reliability	
Purpose of the study Description of endpoints	Replicated field experiment in constructed ponds to test for both the effects of the glyphosate formulation Accord® and predator (Tiger Salamanders, *Ambystoma tigrinum*) density on amphibians and aquatic invertebrates. Behavior assays of salamander larvae to investigate predator-prey relationships.
Test compound, application procedure, exposure period, protocol	5% herbicide mixture of Accord and 3% Cide-Kick II (aquatic surfactant) Active substance(s): Glyphosate. Surfactant: a nonylphenolpolyethylene NPE-based product (wetting agent Cide-Kick II ®) no a.s. loading reported
Experimental approach Statistical design, test environment	a) Outdoor experimental ponds b) Behavioural assays were conducted ex vivo under laboratory conditions Ponds: 6 x 6 m, volume: 24 m³, depth: 0.67 m; exposure 18th May until end of June 2006 (ca. 1 ½ month), repeated in the year 2007 (started on 14th May, duration not stated); Activity and feeding assays: pyrex containers (7 cm high x 20 cm wide x 20 cm long) Microhabitat assay: plastic containers (26 cm high x 23.5 cm wide x 33 cm long) filled to a depth of 16 cm with dechlorinated tap water. The containers were partitioned into two equal chambers using a plastic mesh with 1.5-cm openings. One chamber had a 2-cm layer of pondweed, leaf litter, and algae, the other chamber was left empty. For the activity, behaviour, feeding and microhabitat assay, samples were collected once a week and assessments were conducted in the afternoon and early evening (13:00 – 20:00h). Snout vent length (SVL) of *A. tigrinum* larvae, amphibian and invertebrate density, species richness and diversity, mortality, metamorphosis, number of movements (position of the head), distance moved by the larvae, feeding activity (predation rate, prey preference), stomach content of dead larvae, behaviour (aggression, percentage of time in vegetation and time separated).
Test organisms	Experimental pond communities containing tadpoles of different species of amphibians: *Ambystoma tigrinum* *Rana pipiens* *Rana clamitans* *Bufo americanus* Aquatic invertebrates as natural inhabitants *Ambystoma tigrinum* larvae (mesocosm study):mean size of 32.1 mm SVL19 (3 size classes "< 25 mm", "25 – 35 mm" and ">35 mm"), age not specified Laboratory assays conducted with larvae of *A. tigrinum*: not precisely stated, for microhabitat assay approximately the same age with SVL differences ranging from 5 – 10 mm.
Biological effects	From the paper:

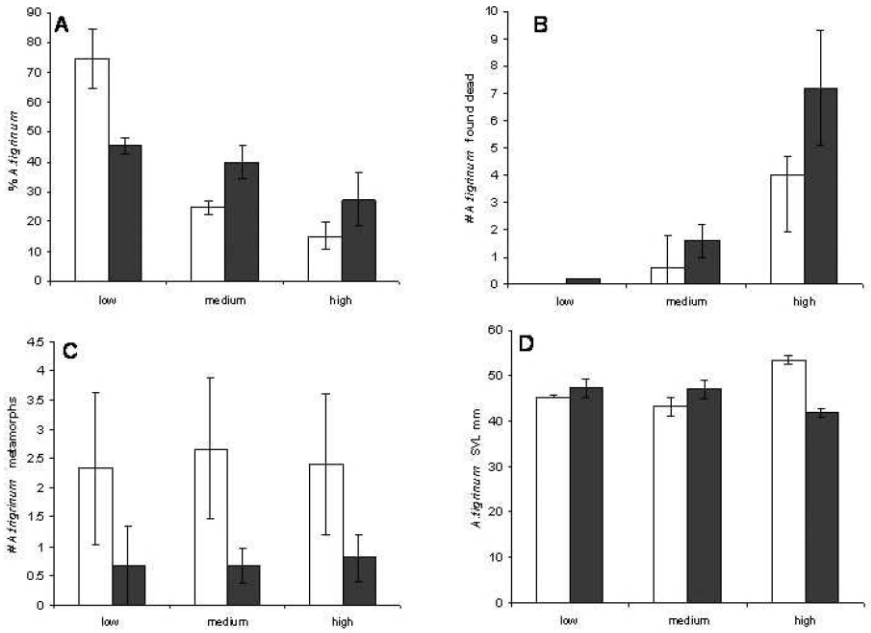

FIG. 1. The effect of herbicide treatment on the mean percentage of *Ambystoma tigrinum* larvae (A) captured, (B) number found dead, (C) number of metamorphs, and (D) SVL (mm) per pond, at three initial salamander densities (low, medium, high). Light bars indicate control ponds, and dark bars indicate herbicide treated ponds. Error bars indicate the standard error of the mean.

Effects of glyphosate formulation on salamander is density dependent. Herbicide treatment and salamander density have an overall effect on tadpoles (interaction between herbicide treatment and initial density also significant). Significant effect of herbicide treatment on the SVL of Northern Leopard Frog tadpoles. SVL and number of metamorphic Northern Leopard Frogs were greater in herbicide-treated ponds.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	No. Formulation with nonylphenolpolyethylene NPE-based surfactant
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Difficult to determine (loading not determined, no analytics)
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	Abiotic parameters (pH, alkalinity, nitrate, nitrite and dissolved oxygen) in outdoor ponds were monitored on week 1, 3, 5 and 7, but not entirely reported
Concluding weight of evidence/proposed action	Formulated product not relevant for current assessment (glyphosae + NPE). Glyphosate was ot tested per se.
Type of information (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA3**

Cauble & Wagner, (2005)

glyphecotox_049	**Cauble, K.,**	**2005**	**Sublethal effects of the**	**Bulletin of**

	Wagner, R.S.		**herbicide glyphosate on amphibian metamorphosis and development**	**Environmental Contamination and Toxicology, 75, 429-435**

Reliability	
Purpose of the study Description of endpoints	Effects of chronic exposure to Roundup ® were investigated at non-acute levels in a static renewal test on *Rana cascadae* larval metamorphosis and development. Larvae were evaluated on a daily basis for 43 days for mortality, feeding behaviour, swimming activity, morphological abnormalities and behavioural alterations.
Test compound, application procedure, exposure period, protocol	Roundup ® (50.2% glyphosate isopropylamine salt), specific product not reported! RMS: Roundup ® Original? Seven larvae per treatment were exposed to five replicates of each treatment of 0, 1, and 2 mg glyphosate/L (nominal) and a control.
Experimental approach Statistical design, test environment	Static renewal (7 day intervals). Duration of study: 43 d chronic 5 replicates per concentration, Organisms per replicate: 7; Feeding: Not stated Larvae were evaluated on a daily basis for 43 days for mortality (time to death), feeding behaviour (feeding or not feeding), swimming activity (high, medium, slow), morphological abnormalities (edema, lesions, bent tail) and behavioural alterations (head out of water, erupted forelimbs, erupted hind limbs, emersion from water). Mean dry mass was compared using Student's t-test; differences among replicates and treatments were evaluated using one-way ANOVA followed by Tukey-Kramer Multiple comparison tests with NCSS as post-hoc.
Test organisms	*Rana cascadae*
Biological effects	Tadpoles were continuously exposed to concentrations of 1 and 2 mg glyphosate a.e./L along with an untreated control in a static renewal system with weekly renewals. Glyphosate concentrations were measured and mean measured levels were similar to nominal concentrations. At the highest concentration tested (1.94 mg glyphosate/L, mean measured), no individuals survived until end of the exposure. The 48 hour LC50 value for *R. cascadae* is reported to be 3.2 mg a.e/L. Exposure to 1 mg glyphosate/L resulted in earlier metamorphosis and smaller size for Roundup ®, when compared to the control.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment, e.g. gene induction vs. apical endpoints like growth or reproduction?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Unfortunately, the product assessed is not specified (Roundup®..). Therefore, no precise assignment is possible.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	Control survival, the loading rate, water quality parameters, and the water temperature during the exposures not reported
Concluding weight of evidence/proposed action	Unfortunately, the precise formulation identification is not possible. Moreover, experimental details are missing (e.g. mortalities in controls). Supporting for fromulations containing POEA (LC50 same range as other

	publications with Glyphosate+POEA formulations).
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Dinehart et al. (2010)

glyphecotox_064	**Dinehart, S.K., Smith, L.M., McMurry, S.T., Smith, P.N., Anderson, T.A., Haukos, D.A.**	**2010**	**Acute and chronic toxicity of Roundup WeatherMAX® and Ignite® 280 SL to larval *Spea multiplicata* and *S. bombifrons* from the Southern High Plains, USA**	**Environmental Pollution 158 (8):2610-2617. DOI 10.1016/j.envpol.2010.05.006.**

Reliability	
Purpose of the study Description of endpoints	Acute and chronic effects of two herbicide formulations (Roundup WeatherMAX®, active ingredient glyphosate; and Ignite 280 SL® (IG), active ingredient glufosinate) to larvae of New Mexico spadefoot toads and Plains spadefoot toads. It was desired to compare for differences between typical populations of croplands and grasslands. Here only setup and results of tests with glyphosate are discussed (not glufosinate). Body weight and survival rates of amphibian larvae
Test compound, application procedure, exposure period, protocol	Roundup WeatherMAX, 48.8 % glyphosate in potassium salt form, 51.2% 'other ingredients' Non-GLP, but ASTM Guideline for acute toxicity tests with aquatic organisms, including amphibians was used. 80% of water was changed after 4 days to maintain normal range ammonia concentrations Acute studies 48h static exposure, 168h post-exposure period Chronic studies Static-renewal exposure for 30 days
Experimental approach Statistical design, test environment	Aquaria of 18.95 L, containing 15 L aged tap water, water quality was monitored Acute study Test concentrations: WM 0.75, 1.5, 2.25, 3, 4.5, 6, 7.5,10 mg glyphosate/L, 9 tadpoles in each of three replicate containers of each treatment Non-normal distribution of weights: Wilcoxon-two-sample test; 48 and 216 hour LC50 by Probit-analysis Chronic study Test concentrations: 2.0 and 2.8 mg acid equivalents of glyphosate T-Tests for weight differences, GLM to analyse survival data-series, percent survival as response variable, treatment, landuse, species as independent variables
Test organisms	larval *Spea multiplicata*, New Mexico spadefoot toads and larval *Spea bombifrons*, Plains spadefoot toads, all at Gosner stages 29-30
Biological effects	Acute studies: Most sensitive LC_{50} 48h = 1.85 mg ae/L and LC_{50} 216h = 1.65 mg ae/L for *S. bombifrons*, no significant differences between the individual origins crop- or grassland nor between the species in survival rates and body weights Chronic studies none of all spadefoots tested chronically survived the longer than 12 days of exposure, while control mortality was very low. The statistical comparison of the factor-combinations did not reveal clear answers. From the published paper (modified): Table 3: Acute toxicity of RoundupWeatherMAX (WM) to larval Spea multiplicata and S. bombifrons (New Mexico and Plains spadefoot, respectively) from playa wetlands embedded in cropland or grassland. Both 48- and 216-h (i.e., including post-exposure mortality) LC values and associated 84% confidence intervals were calculated via probit analysis. From the paper:

		WM LC_{50} values (84% confidence intervals), mg glyphosate acid equivalents (ae)/L[a]	
	n	48-h	216-h
S. bombifrons			
Grass	208	2.03 (1.90–2.16)[Bc*]	1.99 (1.85–2.13)[Bc*]
Crop	175	1.85 (1.62–2.06)[Bc*]	1.65 (1.42–1.87)[Ba*]
S. multiplicata			
Grass	80	2.30 (2.06–2.55)[Bc*]	1.93 (1.68–2.20)[Bc*]
Crop	113	2.11 (1.85–2.41)[Bc*]	2.11 (1.85–2.41)[Bc*]

RMS: The use of the generalised linear statistical method remained unclear, so was the use of the replication in the whole study. It could not be understood if the data was analysed as a time series, since time was not taken as an explanatory factor or as covariable in the analysis.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes, larvae of toads should be more sensitive towards aquatic exposure due to gill breathing
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Yes, survival of juveniles.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, probably for all formulations containing adjuvants of relevant toxicity in similar amounts. Not to be used for the assessment of glyphosate technical/glyphosate acid, although all endpoints are given in acid equivalents.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	The authors state that predicted environmental concentrations of glyphosate were modelled up to 2.8 mg ae/L due to direct overspray.
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	The authors adjust and maintain normal range conditions for the tadpoles, regarding water quality, nutrients and volume of the containers. The systems were stable
Concluding weight of evidence/proposed action	In conclusion, the study presents slightly differing acute mortalities between the species and land use type, which can be assessed togheter with other glyphosate formulations containing surfactants of relevant toxicity like the one tested in this study. No clear interaction between the species, origin from crop- or grassland and treatments on survival could be shown. The chronic data was insufficiently analysed, or it was ambiguously described.
Type of information (Critical, supporting, low weight)	**Supporting/critical**
Consideration/concluding score	**UBA1 for assessment of surfactand effects (POEA)**

Edginton et al. (2004)

glyphecotox_066	**Edginton, A.N., Sheridan, P.M., Stephenson, G.R., Thompson, D.G., Boermans, H.J.**	**2004**	**Comparative effects of pH and Vision (R) herbicide on two life stages of four anuran amphibian species**	**Environmental Toxicology and Chemistry 23/4, 815–822**

Reliability	
Purpose of the study Description of endpoints	Product Vision® and the concurrent factor pH were tested to determine their effects on early life-stage anurans. Mortality and the prevalence of malformations
Test compound, application procedure,	Vision® (glyphosate-based formulation with15% (weight:weight) polyethoxylated tallow amine surfactant blend)

exposure period, protocol	
Experimental approach Statistical design, test environment	Ninety-six-hour laboratory static renewal studies under a central composite rotatable design. Generalized linear models.
Test organisms	Embryonic and larval life stages (Gosner 25) of *Rana clamitans*, *R. pipiens*, *Bufo americanus*, and *Xenopus laevis*
Biological effects	Significant interaction of pH with Vision® concentration in all eight models. The toxicity of Vision® was amplified by elevated pH. Larvae of *B. americanus* and *R. clamitans* were 1.5 to 3.8 times more sensitive than their corresponding embryos, whereas *X. laevis* and *R. pipiens* larvae were 6.8 to 8.9 times more sensitive. From the published paper:

Table 2. Comparative sensitivity of the embryonic and larval stages (Gosner 25) of four anuran species to Vision® (Monsanto Canada, Winnipeg, MB, Canada) at representative pH levels of 6.0 and 7.5. Based on these lethal concentration (LC) estimates, the embryo models produced values greater than those of the larvae and, in general, Vision was more toxic at pH 7.5 than at pH 6.0. The asterisks denote a point estimate at or below the expected environmental concentration of 1.4 mg acid equivalents (a.e.)/L

Species	Life stage[a]	pH	96-h LC10 (mg a.e./L) (95% confidence interval)	96-h LC50 (mg a.e./L) (95% confidence interval)
Xenopus laevis	Embryo	6.0	6.2 (4.7, 7.4)	15.6 (12.7, 23.0)
		7.5	4.0 (3.1, 4.7)	7.9 (7.2, 8.7)
	Larvae	6.0	1.99 (1.7, 2.0)	2.1 (2.0, 2.7)
		7.5	0.85 (0.55, 0.87)*	0.88 (0.84, 0.92)*
Bufo americanus	Embryo	6.0	2.2 (0, 3.8)	4.8 (4.0, 5.7)
		7.5	4.3 (0, 7.5)	6.4 (5.8, 7.0)
	Larvae	6.0	2.1 (1.8, 3.9)	2.9 (2.3, 10.5)
		7.5	1.2 (1.0, 1.4)*	1.7 (1.5, 1.9)
Rana clamitans	Embryo	6.0	2.6 (0, 6.0)	5.3 (3.9, 9.2)
		7.5	2.8 (2.2, 3.8)	4.1 (3.4, 6.4)
	Larvae	6.0	2.1 (1.7, 2.5)	3.5 (3.0, 4.6)
		7.5	0.89 (0.70, 1.1)*	1.4 (1.2, 1.7)*
Rana pipiens	Embryo	6.0	13.1 (12.8–13.3)	15.1 (14.0–17.5)
		7.5	6.7 (6.3–6.9)	7.5 (7.0–9.0)
	Larvae	6.0	1.1 (1.0–1.3)*	1.8 (1.5–2.2)
		7.5	0.83 (0.71–0.92)*	1.1 (0.96–1.14)*

[a] Embryo = Gosner 8 to Gosner 25; larvae = Gosner 25.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Formulated product Vision® contains POEA. Glyphosate alone was not tested.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence/proposed action	Results Vision® can be used for the assessment of glyphosate formulations with POEA
Type of information (Critical, supporting, low weight)	**Critical/Supporting**
Consideration/concluding score	**UBA1 for assessment of surfactand effects (POEA)**

Jayawardena et al. (2011)

glyphecotox_074	**Jayawardena UA, Navaratne AN, Amerasinghe PH, Rajakaruna RS**	**2011**	**Acute and chronic toxicity of four commonly used agricultural pesticides on the Asian common toad, *Bufo melanostictus* Schneider.**	**Journal of the National Science Foundation of Sri Lanka 39: 267-276. doi: 10.4038/jnsfsr.v39i3.3631.**

Reliability	
Purpose of the study Description of endpoints	Acute and chronic effects of the formulation Roundup ® containing the active ingredient glyphosate on juvenile Asian common toads (*Bufo melanostictus*) were tested. Recorded were survival (LC_x), snout-vent length, time to forelimb emergence (TE_{50}), body weight of the tadpoles. A tropical, Sri-Lankan scenario was aimed to be represented by the study.
Test compound, application procedure, exposure period, protocol	Commercial Roundup® formulation with a.i. Glyphosate and possibly containing POEA. Exposure acute study: 9.50, 11.25, 15.00, 18.75 and 25.0 ppm. Exposure in chronic experiment: series of 0.25, 0.50, 0.75 and 1.00 ppm of glyphosate were applied (ppm equals mg a.i./L at an assumed density of the solution of 1). In the chronic study the medium was renewed every week, exposure semi-static. Non-GLP
Experimental approach Statistical design, test environment	2 L glass tanks per treatment and each of 3 egg clutch replicates; Replication was not used in the Probit analysis to find the LCx after an F-test on variance differences. Pearson correlation between growth parameters and treatments. 3 replicates for comparison of body weights, snout-vent lengths and TE50.
Test organisms	20 five-days post-hatch tadpoles per tank; Acute measurements (mortality) at 48 h after exposure. Chronic measurements at 10 days post-hatch, 30 days post-hatch and metamorphic tadpoles.
Biological effects	LC_{50} after 48h: 45.94 mg /L. Not clear if product or active substance are meant. Most sensitive survival endpoint (chronic study): 1 ppm glyphosate treatment to metamorphs. Significant overall impact of glyphosate concentrations on mean body weight, SVL and TE_{50} (chronic study), ANOVA, no post-hoc tests applied or explained in the text. Other malformations not quantitatively analyzed.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes
2 Is the magnitude of effects of biological significance?	Statistics: It remained widely unclear if post-hoc tests after the ANOVA on an overall effect of the test concentrations were applied as indicated by the asterisks in figure 1 vs. no indication in table 3.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	A variety of endpoints was assessed, which is appropriate for refined considerations of the most sensitive and relevant endpoint
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The toxicity of surfactant is know to interfere with the toxicity of the active substance and may contribute majorly to the overall effect of a formulation. Unfortunately, the glyphosate formulation used is not identifiable
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	There was no measurement of the environmental conditions in the test tanks, in particular during the chronic study that lasted at least for about 50 days until metamorphosis (figure 1, TE_{50}). Crowding stress with succeeding experimental duration and toxic metabolism products would probably cause a treatment-related bias in the data. However, mortality and malformation rates of the controls were negligible low

	and statistically significant different from the treatments.
Concluding weight of evidence/proposed action	Many details of the experimental procedures, the use of statistics and the identity of the tested substances are lacking. The study is therefore considered not applicable for a specific use in ERA.
Type of information (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA3**

Jones, et al. (2010)

glyphecotox_075	**Jones, D.K., Hammond, J.I., Relyea, R.A.**	**2010**	**Roundup® and amphibians: The importance of concentration, application time, and stratification**	**Environmental Toxicology and Chemistry, Vol. 29, No. 9, 2016–2025, DOI: 10.1002/etc.240**

Reliability	
Purpose of the study Description of endpoints	Role of application amount, timing, and frequency using outdoor mesocosm communities containing larval amphibians (*Rana sylvatica* and *Bufo americanus*) and using a commercial formulation of the herbicide glyphosate (Roundup Original MAX®) were assessed. Survival day 18.
Test compound, application procedure, exposure period, protocol	Roundup Original MAX®, authors state Glyphosate isopropylamine salt (glyphosate-Ipa), Surfactant reported not to be POEA (*pers. comm.*Monsanto). Purity: 48.7% active ingredient. RMS: surfactant not known/ Formulation not correctly reported? Roundup Original MAX ® is a potassium salt formulation according to MSDS Monsanto Company
Experimental approach Statistical design, test environment	Mesocosm; Duration of study: 18 days 750-L cattle watering tanks filled with 542 L of well water. Addition of the 2 test species was defined as day 0. Total of 12 treatments including 9 treatments with 3 different concentrations of single applications on day 0, on day 7 and on day 14, respectively, 2 treatments with 2 concentrations of multiple applications on day 0, 7 and 14, and 1 control treatment. Test concentrations: 1 x 1 mg a.e./L, 1 x 2 mg a.e./L, 1 x 3 mg a.e./L, 3 x 0.33 mg a.e./L and 3 x 1 mg a.e./L. 4 replicates /20 tadpoles of each of species in every mesocosm. No additional feeding
Test organisms	*Rana sylvatica* (Ranidae; wood frog) Gosner stage 26 *Bufo americanus* (Bufonidae; American toad) Gosner stage 25
Biological effects	Quoted from article

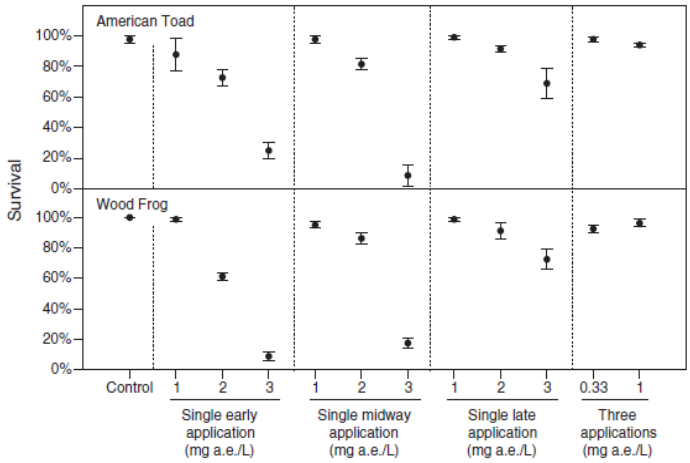

Fig. 1. Survival of American toad and wood frog tadpoles when exposed to varying Roundup Original MAX concentrations (mg a.e. of glyphposate/L) at different times (day 0, 7, or 14). Data points represent mean survival (± standard error) for all four replicates. Survival was recorded on day 18 following experimental takedown.

Exposures of up to 3 mg acid equivalent (a.e.)/L caused substantial amphibian death. However, the amount of death was considerably higher when the herbicide was applied earlier in the experiment than later in the experiment. Single, large applications (at different times) had larger effects on tadpole mortality and growth than multiple, small applications (of the same total amount).
Effects on mass were also dependent from application time and gyphosate concentration.
RMS: From the results
- an overall NOEC of 1 mg a.e./L is postulated
- lowest LC50 is 2.10 mg a.e./L (2.00, 2.19) for *Bufo americanus*
From the published paper (modified):
Table 3. Results of probit analyses used to estimate the LC10, LC50 values (mg a.e. /L)lethal concentrations that cause 10, 50% mortality) for Roundup Original Max® in outdoor mesocosms at three application times. Means are followed by 84% confidence intervals; non-overlapping confidence intervals are significantly different (p<0.05).

Species	Application	LC10	LC50
Wood frog	Early (day 0)	1.45 (1.29, 1.57)	2.10 (2.00, 2.19)
	Midway (day 7)	1.56 (1.07, 1.84)	2.44 (2.15, 2.79)
	Late (day 14)	2.02 (1.47, 2.34)	4.27 (3.47, 7.42)
American toad	Early (day 0)	0.99 (0.42, 1.35)	2.31 (1.86, 3.06)
	Midway (day 7)	1.67 (0.72, 2.01)	2.30 (1.84, 2.89)
	Late (day 14)	1.98 (1.49, 2.28)	3.93 (3.33, 5.83)

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Not clear. No POEA are included in the formulation. Nevertheless, results point at a significant toxicity of the surfactant. The surfactant might belong to the so-called group of POEA-similar surfactant classes. Roundup Original MAX® contains a potassium salt of glyphosate and not the IPA salt as stated in the paper?
2 Do the tested concentrations relate to	-/-

measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence/proposed action	Formulation ingredient undisclosed. No separate test of glyphosate as acid or salt
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Jones, et al. (2011)

glyphecotox_076	**Jones, D.K., Hammond, J.I., Relyea, R.A.**	**2011**	**Competitive stress can make the herbicide Roundup® more deadly to larval amphibians**	**Environmental Toxicology and Chemistry, 30/2, 446-454 DOI: 10.1002/etc.384**
Reliability				
Purpose of the study Description of endpoints	To explore how the natural stress of competition might interact with a glyphosate-based herbicide. Outdoor mesocosms containing three tadpole species exposed to a factorial combination of three glyphosate concentrations (0, 1, 2, or 3mg acid equivalent (a.e.)/L of the commercial formulation Roundup Original MAX1) and three tadpole densities (low, medium, or high). growth, mortality			
Test compound, application procedure, exposure period, protocol	Roundup Original MAX®, authors state Glyphosate isopropylamine salt (glyphosate-Ipa), Surfactant reported not to be POEA (*pers. comm.*Monsanto). Purity: 48.7% active ingredient. RMS: surfactant not known/ Formulation not correctly reported? Roundup Original MAX ® is a potassium salt formulation according to MSDS Monsanto Company Exposure: approx 22-23 days			
Experimental approach Statistical design, test environment	12 treatments including 3 different concentrations of single glyphosate applications and 1 untreated control crossed with 3 tadpole densities (low, medium, or high). Test concentrations: 1, 2 and 3 mg a.e./L glyphosate (a.e. = acid equivalent). 2 replicates per treatment Organisms per replicate: Low density: 20 of each of the three species Medium: 40 of green and gray tree frog and 20 of bullfrog High: 60 of green and gray tree frog and 20 of bullfrog multivariate analysis of variance (MANOVA).			
Test organisms	*Rana catesbeiana* (bullfrog) *Rana clamitans* (green frog) *Hyla versicolor* (gray tree frog) Age of test organisms at study initiation: early stage appr. Gosner 25			
Biological effects	The LC50 values for the tested species reflected a competition effect; LC50 values were similar at low and medium densities, but both were different from the LC50 values at high tadpole density Lowest LC50 reported for Bullfrog at high densities = 1.61 mg a.e./L (1.52, 1.70). From the paper (modified): Table 3. Results of species-specific probit analyses used to estimate the lethal concentrations of Roundup Original MAX1 (Monsanto) that cause 10, 50% death (LC10 and LC50 respectively). Estimates are based on outdoor mesocosm experiments that crossed four concentrations of Roundup (0, 1, 2, or 3 mg acid equivalent/L) with three levels of tadpole competition. Means are followed by			

84% confidence intervals; nonoverlapping confidence intervals are significant. All estimates adjust for low amounts of mortality in the controls.

Species	Competition	LC10	LC50
Gray tree frog	Low	1.41 (0.81, 1.70)	2.04 (1.70, 2.35)
	Medium	1.85 (0.00, 2.20)	2.29 (1.56, 10.8)
	High	1.00 (0.53, 1.29)	1.71 (1.36, 2.07)
Green frog	Low	1.26 (0.42, 1.68)	2.58 (2.07, 3.86)
	Medium	1.84 (1.09, 2.13)	2.35 (1.98, 2.84)
	High	1.58 (1.25, 1.78)	2.18 (1.99, 2.37)
Bullfrog	Low	1.38 (0.69, 1.72)	2.18 (1.77, 2.63)
	Medium	1.63 (0.59, 1.91)	2.12 (1.70, 2.58)
	High	1.18 (1.06, 1.28)	1.61 (1.52, 1.70)

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Not clear. No POEA are included in the formulation. Nevertheless, results point at a significant toxicity of the surfactant. The surfactant might belong to the so-called group of POEA-similar surfactant classes. Roundup Original MAX® contains a potassium salt of glyphosate and not the IPA salt as stated in the paper?
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence/proposed action	Formulation ingredient undisclosed. No separate test of glyphosate as acid or salt
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Lajmanovich et al. (2003)

glyphecotox_078	**Lajmanovich, R.C., Sandoval, M.T., Peltzer, P.M.**	**2003**	**Induction of mortality and malformation in Scinax nasicus tadpoles exposed to glyphosate formulations**	**Bull. Environ. Contam. Toxicol. 70, 612–618 DOI: 10.1007/s00128-003-0029-x**

Reliability	
Purpose of the study Description of endpoints	Tadpoles of *Scinax nascius* were exposed under laboratory conditions to GLYFOS®, a formulation containing glyphosate at nominal test concentrations of 3.07, 3.84, 4.8, 6 and 7.5 mg formulation/L. A negative control (artificial pond water) was prepared in parallel. Ten tadpoles were exposed in three replicates in the control and at each treatment level. All tadpoles were observed at daily intervals for the 96 hour study duration with mortality recorded. At the end of exposure, surviving tadpoles were fixed in formalin solution and examined for morphological changes..
Test compound, application procedure, exposure period, protocol	Glyfos® 48% glyohosate
Experimental approach Statistical design, test	See above

environment	
Test organisms	*Scinax nascius* tadpoles
Biological effects	Larval malformations were minimal at 3.07 mg/L when tadpoles were exposed for one day, whereas an increased malformation was observed at levels of 7.5 mg Glyfos®/L. The 96 hour LC50 value for tadpoles of *Scinax nascius* exposed to Glyfos® was 2.64 mg formulation/L (nominal) with 95% confidence interval of 2.19 to 2.84 mg/L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Glyphos® contains with very high probabiliy POEA
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence/proposed action	The study confirms the relatively high toxicity of glyphosate preparations possibly mediated by POEA surfactants.
Type of information (Critical, supporting, low weight)	**Supporting/critical**
Consideration/concluding score	**UBA1 for assessment of surfactand effects (POEA)**

Lajmanovich et al. (2011)

glyphecotox_080	**Lajmanovich, R.C., Attademo, A.M., Peltzer, P.M., Junges, C.M., Cabagna, M.C.**	**2011**	**Toxicity of Four Herbicide Formulations with Glyphosate on Rhinella arenarum (Anura: Bufonidae) Tadpoles: B-esterases and Glutathione S-transferase Inhibitors**	**Arch Environ Contam Toxicol, 60, 681–689 10.1007/s00244-010-9578-2**

Reliability	
Purpose of the study Description of endpoints	tadpoles *Rhinella arenarum* were exposed to different concentrations of Roundup Ultra-Max (ULT), Infosato (INF), Glifoglex, and C-K YUYOS FAV. Tadpoles were exposed at thefollowing concentrations (acid equivalent [ae]): 0 (control), 1.85, 3.75, 7.5, 15, 30, 60, 120, and 240 mg ae/L for 6–48 h (short-term). Mortality was recorded. Acetylcholinesterase (AChE), butyrylcholinesterase (BChE), carboxylesterase (CbE), and glutathione S-transferase (GST) activities were measured among tadpoles sampled from those treatments that displayed survival rates >85%.
Test compound, application procedure, exposure period, protocol	Roundup Ultramax®: commercial grade, 74.7% a.i. No POEA Infosato, Glifoglex, C-K Yuyos FAV: commercial grade, 48% a.i., each, co-formulants undisclosed 48 h
Experimental approach Statistical design, test environment	Larvae were exposed in glass tanks (12.5 cm diameter × 13.5 cm height) filled with 1 L of DTW (deionised tap water?). Whole tadpoles were homogenized in 0.1% triton X-100, 25 mM Tris-HCL (pH 8.0) Replicates per concentration: 3; Organisms per replicate: 7
Test organisms	*Rhinella arenarum*
Biological effects	Forty-eight-hour LC_{50} for *R. arenarum* tadpoles in the static tests ranged from ULT = 2.42 to FAV = 77.52 mg ae/L. For all CF-GLY, the LC50 values stabilized at 24 h of exposure. Enzyme activity measues possibly biased by high mortalities
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Acute endpoint: yes
2 Is the magnitude of effects of biological significance?	Acute endpoint: yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Acute endpoint: yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Roundup UltraMAX® is stated not to contain POEA. Test results indicate the formulation contains surfactants with toxicity similar to POEA. Other formulation employed with unknown co-formulants. Far lower toxicities than the product above.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	Not conclusively
Concluding weight of evidence/proposed action	Acute endpoints reliable, reporting of experimental details not exaustive, formulations partly unknown
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Relyea (2005)

glyphecotox_083	**Relyea R.A.**	**2005**	**The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities**	**Ecology Letters 9 (10):1157-1171. DOI 10.1111/j.1461-0248.2006.00966.x.**
Reliability				
Purpose of the study Description of endpoints	Effects of glyphosate as Roundup ® on total and functional group based species richness of zooplankton, periphyton, invertebrates and amphibians; biomass of functional groups; abundance of individual species.			
Test compound, application procedure, exposure period, protocol	Amongst three other pesticides, the active ingredient glyphosate was tested using a commercial Roundup® formulation containing polyethoxylated tallowamines as surfactants. A single concentration of 3.8 mg a.i./L was used, corresponding to the maximum recommended application rate of 6.4 mL Roundup® (25.2 % a.i.)/m². Exposure: 13 d approx.. Non-GLP			
Experimental approach Statistical design, test environment	An artificial assemblage of several specimens of limnic vertebrate and invertebrate organisms (Spotted salamander, Diving beetle, Dragonfly, Damselfly, Backswimmer, Water bug, Wood frog, Leopard frog, American toad, Gray tree frog, Spring peeper, Snail, Cladoceran, Copepod) was added to experimental ponds of 1000L volume. Controls and treatments were 6-fold replicated and sampled once on day 13 of the experiment.			
Test organisms	See above			
Biological effects	Effects at 3.8 mg glyphosate/L. Total species richness decreased by 22 % compared to control (statistically different at 5 % error probability). The test item caused a decrease in large herbivore richness. A significant decrease of the abundance of individual species was seen for the copepod *Eurytemora affinis* The amphibian species tested were affected by the Roundup treatment. Effects of glyphosate as Roundup ® on total and functional group based species richness of zooplankton, periphyton, invertebrates and amphibians; biomass of functional groups; abundance of individual species.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	Although the author stated that the species compete with each other at the respective trophic level, the communities were not tested at an equilibrium state. The test item was applied immediately after completion of the experimental setup by introducing the vertebrate specimens to the ponds. It should be seen as a 'combined single species approach' and not as a community level study and could serve as an estimate of acute to subchronic toxicity under realistic exposure conditions.			
2 Is the magnitude of effects of biological significance?	There is no indication of the numbers that form the basis of the statistical analysis, i.e. no recovery rates of the previously introduced individuals were given by the author. It remains unclear why it was necessary to conduct multivariate pre-testing and data conversions.			
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Generally, the abundance of persisting or newly hatched individuals is an appropriate level of investigation for the semi-field ecosystem level.			
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?	The active compound Glyphosate was tested in a formulation possibly containing POEA, representing a common practice of enhancing the surfactant characteristics of a formulation. Does not resemble the lead formulation for EU assessment of reneval of approval for glyphosate as active substance			
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	The tested concentration was deduced by the assumption of direct overspray at the recommended field rate, and is thus considered a realistic and possible worst-case.			
3 Have parameters influencing the endpoints been considered	Obviously, there the author did not measure any environmental parameters, nor considered them fort the interpretation of results. Indirect			

adequately (e.g. pH, temperature, light conditions)?	effects within the newly established community were discussed.
Concluding weight of evidence/proposed action	The study confirms the relatively high toxicity of glyphosate preparations possibly mediated by POEA surfactants. Not relevant for the risk assessment of glyphosate due to weakness in methodological accuracy.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Relyea (2005)

glyphecotox_085	**Relyea R.A.**	**2005**	**The lethal impact of roundup on aquatic and terrestrial amphibians**	**Ecological Applications, 15(4), 2005, pp. 1118–1124 DOI: 10.1890/04-1291**
Reliability				
Purpose of the study Description of endpoints	Aquatic: Communities of three species of North American tadpoles in outdoor pond mesocosms that contained different types of soil and Roundup as a direct overspray. Terrestrial: three species of juvenile (post-metamorphic) anurans to a direct overspray of Roundup in laboratory containers. Aquatic: survival after 21 days exposure Terrestrial: survival after 24h			
Test compound, application procedure, exposure period, protocol	Roundup® "Weed and Grass Killer", concentrated lawn and garden formulation. Active substance(s): Glyphosate isopropylamine salt (glyphosate-IPA), 25.2% glyphosate. Adjuvant/Surfactant: suspected POEA. Aquatic: Experimental units were 1200-L cattle watering tanks filled with 1000 L of well water. Each tank was treated with no soil, sand or loamy soil. After inoculation of mesocosm and addition of test species, ponds were applied with test item or water (control) two day later. Survival was recorded 21 days later at termination of experiments. Terrestrial: Post-metamorphic animals were placed in 10-L plastic tubs that were lined with damp water towels. Subsequently, replicates were treated with glyphosate or water (control) and survival was recorded 24 h later.			
Experimental approach Statistical design, test environment	Aquatic: total of 6 treatments including 3 soil treatments (i.e. no soil, 19 L sand, 19 L loam soil) crossed with 1 glyphosate and 1 control treatment. Test concentrations: 3.8 mg a.s./L (corresponding to 1.6 mL a.s./m2), 5 replicates. Notifier: Correcting for density, nominal tested concentrations become 1.37 mg GlyIPA/L and 4.17 mg GlyIPA/L or 1.01 mg glyphosate a.e./L and 3.09 mg glyphosate a.e./L. RMS: only one concentration tested, though. Recalculation to 3.09 mg glyphosate a.e./L? Terrestrial: 1 glyphosate and 1 control treatment: 1.6 mL a.s./m2, 4 replicates Rate corresponds to 16,000 mL a.s./ha. Approx. 4 to 5 times higher than applied in Europe. Organisms per replicate: Aquatic: 20 tadpoles of each of the 3 species in every mesocosm. Terrestrial: 7 juvenile frogs/toads per experimental unit separated by species.			
Test organisms	Aquatic experiments: *Rana pipiens* (leopard frog), *Bufo americanus* (toad), and *Hyla versicolor* (gray tree frogs). Terrestrial experiments: *Rana sylvatica* (wood frog), *Bufo woodhousii fowleri* (Fowler's toad), and *Hyla versicolor* (gray tree frogs)			
Biological effects	Aquatic: After three weeks, Roundup at tested concentrations resulted in a mortality of 96–100% of larval amphibians (regardless of soil presence). Terrestrial: After one day, Roundup at tested concentrations resulted in a mortality of 68–86% of juvenile amphibians			

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The active compound Glyphosate was tested in a formulation possibly containing POEA. Does not resemble the lead formulation for EU assessment of reneval of approval for glyphosate as active substance
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Terrestrial:1.6 mL a.s./m2. Rate corresponds to 16,000 mL a.s./ha. Approx. 4 to 5 times higher than applied in Europe. Aquatic: overspray scenario, not appropriate for evaluation of intended uses in Europe
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence/proposed action	The study confirms the relatively high toxicity of glyphosate preparations possibly mediated by POEA surfactants.
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Relyea (2012)

glyphecotox_088	**Relyea R.A.**	**2012**	**New effects of Roundup on amphibians: Predators reduce herbicide mortality; herbicides induce antipredator morphology.**	**Ecological Applications 22/2, 634-647 DOI: 10.1890/11-0189.1**
Reliability				
Purpose of the study Description of endpoints	Outdoor mesocosms with simple wetland communities containing leaf litter, algae, zooplankton, and three species of tadpoles (wood frogs *Rana sylvatica* or *Lithobates sylvaticus*, leopard frogs *R. pipiens* or *L. pipiens* and American toads *Bufo americanus* or *Anaxyrus americanus*.			
Test compound, application procedure, exposure period, protocol	Roundup Original MAX® Glyphosate 540 mg a.e./L; Adjuvant/Surfactant: Undisclosed Duration of study: 21 days			
Experimental approach Statistical design, test environment	Factorial combination of herbicide concentrations (0, 1, 2, or 3 mg acid equivalents [a.e.]/L of Roundup Original MAX) crossed with three predator-cue treatments (no predators, adult newts *Notophthalmus viridescens*) or larval dragonflies *Anax junius*).			
Test organisms	*Rana sylvatica* (wood frog), *Rana pipiens* (northern leopard frog), *Bufo americanus* (American toad), early stage appr. Gosner 25			
Biological effects	From the published paper:			

TABLE 2. Estimated LC50 values (i.e., the concentration required to kill 50% of a population) for three species of tadpoles when exposed to a range of concentrations of glyphosphate (as the commercial formulation Roundup Original MAX) in the presence of three caged-predator environments.

Species	Caged predator	LC50	84% CI
Wood frog	no predator	2.95	-
	caged newt	2.63	2.45, 2.84
	caged dragonfly	3.09	2.75, 3.80
Leopard frog	no predator	2.91	2.74, 3.03
	caged newt	3.02	-
	caged dragonfly	3.26	3.02, 3.90
American toad	no predator	2.46	2.27, 2.69
	caged newt	2.44	2.26, 2.64
	caged dragonfly	2.82	2.66, 2.96

Notes: Estimates are followed by 84% confidence intervals; nonoverlapping confidence intervals are significant at approximately $\alpha = 0.05$ (Payton et al. 2003). In two cases, indicated by dashes, the confidence interval could not be estimated due to the distribution of the data.

Interactions between pesticide and predator effects. Surprisingly, in presence of predators, the LC50 increases.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The active compound Glyphosate was tested in a formulation possibly containing surfactants with similarl toxicity as POEA. Does not resemble the lead formulation for EU assessment of reneval of approval for glyphosate as active substance
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Aquatic: overspray scenario, not appropriate for evaluation of intended uses in Europe
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence/proposed action	The study confirms the relatively high toxicity of glyphosate preparations mediated by surfactants.
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Lamanovich et al. (2010)

glyphecotox_448	**Lajmanovich, R.C., Peltzer, P.M., Junges, C.M., Attademo, A.M., Sanchez, L.C., Bassó, A.**	**2010**	**Activity levels of B-esterases in the tadpoles of 11 species of frogs in the middle Paraná River floodplain: Implication for ecological risk assessment of soybean crops**	**Ecotoxicology and Environmental Safety 73 (7):1517-1524. DOI: 10.1016/j.ecoenv.2010.07.047.**

Reliability	
Purpose of the study Description of endpoints	Determination of acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and carboxylesterases (CbEs) activities in 11 anuran species in the Parana River floodplain, Brasil
Test compound, application procedure, exposure period, protocol	-/-
Experimental approach Statistical design, test environment	-/-
Test organisms	-/-
Biological effects	-/-
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	-/-
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	-/-
3 Is the ecotoxicological manifestation level appropriate for the assessment, e.g. gene induction vs. apical endpoints like growth or reproduction?	-/-
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	-/-
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	-/-
Concluding weight of evidence/proposed action	**The study is not relevant for ERA of glyphosate because the substance was not applied nor monitored explicitly. Most likely, the notifier assigned erroneously a high relevance to the study.**
Type of information (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA3**

McDaniel et al. (2008)

glyphecotox_496	**McDaniel, T.V., Martin, P.A., Struger, J., Sherry, J., Marvin, C.H., McMaster, M.E., Clarence, S., Tetreault, G.**	**2008**	**Potential endocrine disruption of sexual development in free ranging male northern leopard frogs (Rana pipiens) and green frogs (Rana clamitans) from areas of intensive row crop agriculture**	**Aquatic Toxicology 88 (4):230-42. DOI: 10.1016/j.aquatox.2008.05.002.**
Reliability				
Purpose of the study Description of endpoints	The occurrence of potential endocrine effects in amphibians inhabiting farm ponds and agricultural drains in intensive row crop agriculture areas of southwestern Ontario was assessed. Effects were compared to amphibians from two agricultural reference sites as well as four non-agricultural reference sites. Blood samples were taken from northern leopard frogs (*Rana pipiens*) and green frogs (*Rana clamitans*) for analysis of circulating sex steroids and vitellogenin-like protein (Vtg-lp), a biomarker of exposure to environmental estrogens. Gonads were histologically examined for evidence of abnormalities.			

Test compound, application procedure, exposure period	A suite of different pesticides were found at the field sites. The applied products are not known
Experimental approach, Statistics, test environment	The relationships between the proportion of males with TOFS/circulating sex steriods and a broad suite of pesticide residues and nutrients concentrations in waterwere explored using Partial Least Squares (PLS). PLSwas used to correlate biological endpoints [independent variable] (Y) with multivariate contaminants components (X), consisting of pesticide residues and nutrient concentrations.
Test organisms	northern leopard frogs *(Rana pipiens)* and green frogs *(Rana clamitans)*
Biological effects	The occurrence of testicular ovarian follicles (TOFS) in male R. pipiens was significantly higher (42%; $p < 0.05$) at agricultural sites. The proportion of testicular oocytes did correlate with a mixture of pesticides and nutrients, particularly atrazine and nitrate.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	not conclusive
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	A mixture was assessed. No allocation of effects to single substances possible. Applied products not known
2 Do the tested concentrations relate to predicted environmental concentrations?	Field assessment. Glyphosate Level of Detection far higher than for all other measured pesticides.
3 Have parameters influencing the endpoints been considered adequately?	The parameters were assessed in the survey only as aggregate exposure of all employed agricultural substances. No further discrimination possible.
Concluding weight of evidence/proposed action	Study is a field survey on the impact of an exposure to the sum of possible substances employed in agricultural management on amphibian biomarkers and development. The investigation of the effects of single substances was not part of the study. The impact of glyphosate cannot be determined.
Type of information (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA3 (monitoring)**

Wagner, et al. (2013)

BVL-Nr. 2716356	**Wagner, N., Reichenbecher, W., Teichmann, H., Tappeser, B., & Lötters, S.**	**2013**	**Questions concerning the potential impact of glyphosate-based herbicides on amphibians.**	**Environmental Toxicology and Chemistry 32, 1688–1700**

Reliability	
Purpose of the study Description of endpoints	The authors review the available data related to potential impacts of glyphosate-based herbicides on amphibians and conduct a qualitative meta-analysis. Response variable in the meta-analysis: unnatural deformity rates of larvae, endocrine disruption in larvae, effects on development, inhibition of specific enzymes, genotoxic effects, effects on embryos, behavioral effects, abiotic interactions, and biotic interactions.
Test compound, application procedure, exposure period	Not applicable, meta analysis of published literature

Experimental approach, Statistics, test environment	Qualitative meta-analysis following vote counting method on how many studies found or did not find significant effects (alpha 0.05) of glyphosate and glyphosate-based herbicides on the response variables (see above). Fisher's exact test to test against the null hypothesis that glyphosate and glyphosate-based herbicides do not have effects on the considered response variable. Analyses were performed with the software R (R Developmental Core Team, 2009). RMS: bias toward the publications of data concluding on effects of the tested substance vs. data showing no effects possible
Test organisms	Data on 37 species were included in the analyses
Biological effects	Glyphosate-based herbicides are classified by the authors as moderately toxic, but glyphosate as slightly toxic to practically nontoxic to amphibian larvae (categories as by USEPA). Salt and acid forms of glyphosate are slightly toxic (10 mg/L < LC50 < 100 mg/L) or even practically nontoxic (LC50 > 100 mg/L) to tadpoles. Some glyphosate-based herbicides (e.g. formulated with aklyamine ethoxylates) have been found to be moderately toxic (1 mg/ L < LC50 < 10 mg/L) or even highly toxic (0.1 mg/L < LC50 < 1 mg/L) to tadpoles.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Meta-analysis included studies with glyphosate alone but also with several different glyphosate-based formulations
2 Do the tested concentrations relate to predicted environmental concentrations?	Several published papers evaluated and compared with different environmental scenarios (i.e. 1) measured during monitoring studies, 2) field measurements after experimental set ups and 3) worst case as predicted by models).
3 Have parameters influencing the endpoints been considered adequately?	Cannot be answered in total, only for the single studies in the meta-analysis
Concluding weight of evidence/proposed action	Maximum Predicted Environmental Concentrations PEC in european surface waters (sw) are predicted excluding the direct application to the water bodies, since this use is not authorized in Europe Please refer to Vol. 3 B 8.6.2 for calualted PECsw values. Most conservative avalable FOCUS Step 1 PECsw value is 104.81µg glyphosate/L. Results support evaluation of published literature by RMS
Type of information (Critical, supporting, low weight)	**Critical/supporting meta-analisys of published data**
Consideration/concluding score	**UBA 1/ UBA 2; also relevant for the evaluation of glyphosate-based formulatios with critical co-formulants**

Dornelles, et al. (2013)

BVL-Nr. 2716830	**Dornelles, M. F., & Oliveira, G. T.**	**2013**	**Effect of Atrazine, Glyphosate and Quinclorac on Biochemical Parameters, Lipid Peroxidation and Survival in Bullfrog Tadpoles (Lithobates catesbeianus).**	**Archives of environmental contamination and toxicology, 1-15.**

Reliability	
Purpose of the study Description of endpoints	Effects of different concentrations of the herbicides atrazine, glyphosate, and quinclorac on biochemical parameters, lipid peroxidation,and survival in tadpoles of *Lithobates catesbeianus* (bullfrog) were investigated. Endpoints: After exposure, gill, liver, and muscle samples were removed from each animal for quantitation of glycogen, total lipids, triglycerides, cholesterol, total proteins, and lipid peroxidation. Survival, weight, size.
Test compound, application procedure, exposure period	On day 8 after the start of the experiment, the herbicides were added to the aquarium water. The introduction of liquid-formulation (atrazine and glyphosate) and powder-formulation herbicides (quinclorac) was performed through the dissolution of agrochemical in distilled water and added only once to the water of aquariums in concentration to be used. The exposure period was 7 days.
Experimental approach, Statistics, test environment	All animals were kept in aquariums under controlled laboratory conditions for 7 days and exposed to commercial formulations of atrazine (5, 10, and 20 lg/L), glyphosate (36, 72, and 144 lg/L), and quinclorac (0.05, 0.10, and 0.20 lg/L) for 7 days thereafter.
Test organisms	Two hundred eighty-eight tadpoles of Lithobates catesbeianus were acquired from a frog farm in the south of Brazil.
Biological effects	Atrazine, glyphosate, and quinclorac exposure induced a significant decrease in levels of glycogen and total lipids in gill, liver, and muscle. Triglycerides levels in the gill increased after exposure to glyphosate, and decreased after exposure to atrazine and quinclorac; their levels in liver and muscle decreased on exposure to all herbicides. Cholesterol and total protein levels decreased in liver and muscle for all three herbicides. All tissues exhibited increased lipid peroxidation after exposure to all herbicides. Exposure to the herbicides tested in this study induced significant changes in biochemical parameters and increased lipid peroxidation levels in tadpoles of L.catesbeianus. No significant differences in tadpole survival, weight, size after exposure to the herbicide compared to control animals.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Cannot be assessed conclusively, in the same test there were no significant differences in tadpole survival, weight, size after exposure to the herbicide compared to control animals.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	A glyphosate-based formulation was tested: 'Roundup Original'commercially avaliable. Most likely with alklyamine ethoxylate (POEA) surfactants
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	Supporting information on biochemical response of tadpoles to environmental relevant concentrations of commercial formulation containing glyphosate and POEA
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA 2 also for assessment of surfactand effects (POEA)**

Moore et al . (2012)

BVL-Nr. 2716841	**Moore LJ, Fuentes L, Rodgers JH Jr, Bowerman WW, Yarrow GK, Chao WY, Bridges WC Jr.**	**2012**	**Relative toxicity of the components of the original formulation of Roundup to five North American anurans.**	**Ecotoxicology and Environmental Safety78, 128-33**

Reliability	
Purpose of the study Description of endpoints	The responses of five North American frog species that were exposed in an aqueous system to the original formulation of Roundup were compared. The formulated mixture of this herbicide as well as its components, isopropylamine (IPA) salt of glyphosate and the surfactant MON 0818 (containing polyethoxylated tallowamine (POEA)) were separately tested in 96h acute toxicity tests with Gosnerstage 25 larval anurans. Endpoint: survival; LC50 values are expressed as glyphosate acid equivalents (ae) or as mg/L for MON0818 concentrations for comparison between the formulation and components.
Test compound, application procedure, exposure period	11 concentrations of theoriginal formulation of Roundup herbicide, nine concentrations of MON0818 and three concentrations of IPA salt of glyphosate
Experimental approach, Statistics, test environment	static (non-renewal) aqueous 96 h laboratory tests . Bioassays performed according to EPA- 821-R-02–012 (U.S. EPA, 2002). Chemical and physical measurements of testing conditions, dilution water, and test solutions were conducted according to the ASTM methods (ASTM, 2003).
Test organisms	*Rana pipiens*, *R. clamitans*, *R. catesbeiana*, *Bufo fowleri*, and *Hyla chrysoscelis* were reared from egg masses
Biological effects	*R.pipiens* was the most sensitive of five species. 96h-LC50 values for formulation tests for the five species ranged from 1.80 to 4.22 mg ae/L, and with 96h-LC50 values after exposure to MON 0818 ranging from 0.68 to 1.32 mg/L. No significant mortality was observed during exposures of 96h for any of the five species exposed to glyphosate IPA salt at concentrations up to 100 times the predicted environmental concentration (PEC).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, tests were performed separately with formulated products containing POEA, glyphosate alone and the surfactant
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	The results fully supports previous RMS evaluation. Surfactant MON 0818 containing POEA drives the toxicity of the herbicide formulations containign glyphosatge for amphibians.
Type of information (Critical, supporting, low weight)	**Critical**

Consideration/concluding score	**UBA 1, also for assessment of surfactand effects (POEA)**

Lajmanovich, et al. (2013)

BVL-Nr.: 2716839	**Lajmanovich, R. C., Junges, C. M., Attademo, A. M., Peltzer, P. M., Cabagna-Zenklusen, M. C., & Basso, A**	**2013**	**Individual and Mixture Toxicity of Commercial Formulations Containing Glyphosate, Metsulfuron-Methyl, Bispyribac-Sodium, and Picloram on *Rhinella arenarum* Tadpoles.**	**Water, Air, & Soil Pollution 224 (3), 1-13**

Reliability	
Purpose of the study Description of endpoints	Effects of four commercial formulations of herbicides (glyphosate [GLY], metsulfuron-methyl [MET], bispyribac-sodium [BIS], and picloram [PIC]) individually, and in three 50:50 mixtures (GLY‑MET, GLY‑BIS, GLY‑PIC) on the common toad Rhinella arenarum (Anura: Bufonidae) tadpoles. Endpoints: survival, enzymatic parameters (glutathione S-transferase (GST), butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) activities), as well as erythrocyte nuclear abnormalities (ENA).
Test compound, application procedure, exposure period	For short-term (48-h) static toxicity tests, commercial formulations of GLY (74.7 % active ingredient [a.i.], N-(phosphonomethyl) glycine; Ultra-Max®, Monsanto Co., Argentina was employed.
Experimental approach, Statistics, test environment	Interactions between herbicides in mixtures were evaluated and classified as additive, synergistic, or antagonistic
Test organisms	Premetamorphic larvae, collected in 2011 from temporary ponds in natural floodplains of the Paraná River (Argentina). The average size (snout–tail tip) was 17±0.5 mm and weight was 0.055±0.009 g; Gosner stages (GS) 29–30 (Gosner 1960).
Biological effects	Toxicity results (48-h LC50) showed that PIC was the most toxic herbicide, followed by BIS, GLY, and MET. LC50 values are reported from the paper:

Table 1 Summary of acute toxicity bioassay (48-h LC_{50}) results of different commercial herbicide formulations: glyphosate (GLY), metsulfuron-methyl (MET), bispyribac-sodium (BIS) and picloram (PIC) on *R. arenarum* tadpoles. GLY and MET, GLY and BIS, and GLY and PIC were tested in a 50:50 mixture

Herbicide treatment	48-h LC_{50} (mg a.i. l^{-1}) (95 % CI)	NOEC and LOEC (mg a.i. l^{-1})	S	AI
GLY	13.20[a] (11.57–15.05)	10 20	–	–
MET	105.56[b] (92.55–120.40)	80 160	–	–
BIS	0.20[c] (0.18–0.24)	0.1562 0.3125	–	–
PIC	0.025[d] (0.022–0.029)	0.0195 0.0390	–	–
GLY and MET	25.62[e] (18.89–34.56)	5 10	2.18	−1.18
GLY and BIS	0.4123[f] (0.3615–0.4703)	0.0781 0.1562	2.08	−1.08
GLY and PIC	0.051[g] (0.045–0.058)	0.0097 0.0195	2.04	−1.04

Values in columns followed by different letters are statistically different at the 5 % level of probability by Duncan's Multiple Range Test

S sum of biological activity, *AI* additive index, *NOEC* no observed effect concentration, *LOEC* lowest observed effect concentration

All commercial herbicide formulations and their mixtures significantly inhibited BChE activity in exposed tadpoles. The AChE activity was also inhibited by all herbicides and their mixtures, except by GLY-BIS. The inhibition of GST activity was only significant for GLY, MET, PIC, and GLY-MET.A significant increase in the frequency of ENA was found for tadpoles exposed either to commercial herbicide formulations or to mixtures, except for GLY.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Survival: yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Survival: yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation of glyphosate, not containing POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	-/-
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	Commercial formulation of glyphosate, not containing POEA. Similar tests supported the conclusions that the surfactant system in UltraMax © is of similar toxicity as POEA, but the present paper reports a much toxicity of the formulated product compared to other results.
Type of information (Critical, supporting, low weight)	**supporting**

Consideration/concluding score	**UBA2**

Meza-Joya, et al.(2013)

BVL-Nr. 2716696	**Meza-Joya, F.L., Ramírez-Pinilla, M.P., Fuentes-Lorenzo, J.L.**	**2013**	**Toxic, cytotoxic, and genotoxic effects of a glyphosate formulation (roundup®SL–cosmoflux®411F) in the direct-developing frog *eleutherodactylus johnstonei*.**	**Environmental and Molecular Mutagenesis 54, 362-373**

Reliability	
Purpose of the study Description of endpoints	The study evaluates the toxic, cytotoxic, and genotoxic effects of a glyphosate formulation (RoundupVR SL–CosmofluxVR 411F) in the direct-developing frog *Eleutherodactylus johnstonei* by estimating the median lethal application rate (LC50), median hemolytic application rate (HD50), and extent of DNA damage using the in vitro and in vivo Comet assays.
Test compound, application procedure, exposure period	RoundupVR SL–CosmofluxVR 411F. This glyphosate formulation is a mixture (55:44:1) of water, RoundupVR SL and the surfactant CosmofluxVR 411F. RoundupVR SL contains 480 g/L of IPA salt of glyphosate or 360 g/L glyphosate acid equivalent (a.e.).
Experimental approach, Statistics, test environment	Ex- situ exposure conditions for terrestrial frogs that mimic the worst-case scenario under field conditions (i.e., direct overspray). Exposure chambers (glass terrariums of 300 cm^2), glyphosate formulation was applied by always spraying 3 mL, which was enough to generate a homogeneous mist into the exposure chambers. Glyphosate formulation is applied at a rate of 23.7 liter per hectare (L/ha) in the Colombian aerial spraying program. Considering that the concentration of active ingredient in the formulation is 158 g a.e./L, the coca eradication application rate is 3.74 kg a.e./ha.
Test organisms	*E. johnstonei* specimens were captured at the "Parque de las Orquideas" located within the Bucaramanga metropolitan area (Santander, Colombia). Neonates used in the toxicity assays were obtained by mating breeding pairs under laboratory conditions. The frogs were fed ad libitum with crickets, spiders, and mosquitoes captured on the UIS campus (Bucaramanga, Colombia). total of 144 Adult males and neonates (eight specimens per treatment) were assayed, whereas only 90 adult females (five specimens per treatment) were used. After 24, 48, 72, and 96 hr, the dead frogs per groups were counted and then the percentage mortalities were computed.
Biological effects	Toxicity results indicated that the application rate [37.4 mg acid equivalent (a.e.)/cm2] equivalent to that used in aerial spraying (3.74 kg a.e./ha) is not lethal in male and female adult frogs, whereas neonates are highly sensitive. Glyphosate formulation at application rates above 5.4 mg a.e./cm2 (in vivo) and concentrations above 95 mg a.e./mL (in vitro) showed clear evidence of cytotoxicity. *Eleutherodactylus johnstonei* Neonates exposed to RoundupVR SL + CosmofluxVR 411F formulation LC 50 24h = 3.1 (2.8–3.6) kg a.e./ha LC 50 48h = 1.8 (1.5–2.0) kg a.e./ha LC 50 96 h 1.2 (1.1–1.4) kg a.e./ha In vivo and in vitro exposure of *E. johnstonei* erythrocytes to the

	glyphosate formulation induced DNA breaks in a dose-dependent manner with statistically significant values (P<0.05) at all doses tested. DNA damage initially increased with the duration of exposure and then decreased, suggesting that DNA repair events were occurring during in vivo and in vitro exposures.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	*Eleutherodactylus johnstonei* neonates and adults were exposed to RoundupVR SL + CosmofluxVR 411F formulation
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	Tested formulation not comparable to lead formulation. No areal spray as intended use in Europe, no glyphosate-based product with Cosmoflux®411F authorized in Europe. However, tested application rates are equal to intended uses in South America.
Type of information (Critical, supporting, low weight)	**Supporting / critical for the assessment of formulations containig the surfactant system Cosmoflux ® 411F**
Consideration/concluding score	**UBA 2**

Lanctôt, et al. (2013)

BVL-Nr. 2716790	**Lanctôt, C., Robertson, C., Navarro-Martín, L., Edge, C., Melvin, S. D., Houlahan, J., & Trudeau, V. L.**	**2013**	**Effects of the glyphosate-based herbicide Roundup WeatherMax® on metamorphosis of wood frogs *Lithobates sylvaticus* in natural wetlands.**	**Aquatic Toxicology 154, 291-303**

Reliability	
Purpose of the study Description of endpoints	The purpose of this study is (1) to determine if an agriculturally relevant exposure to Roundup WeatherMax® influences the development of wood frog tadpoles (*Lithobates sylvaticus*) through effects on the mRNA levels of genes involved in the control of metamorphosis; (2) to compare results to the well-studied Vision® formulation (containing the isopropylamine salt of glyphosate [IPA] and polyethoxylated tallowamine [POEA] surfactant) and to determine which ingredient(s) in the formulations are responsible for potential effects on development Endpoints: Survival, development, growth, sex ratios and mRNA levels of genes involved in tadpole metamorphosis
Test compound, application procedure, exposure period	Roundup WeatherMax® (0.21 mg a.e./L), Vision® (2.89 mg a.e./L), IPA and POEA in concentrations comparable to the content in 2.89 mg a.e./L of Vision® (356 g a.e glyphosate as IPA salt /L formulated product + 15% POEA by weight). Overspray scenario
Experimental approach, Statistics, test environment	Overspray scenario (Vision), realistic worst case for agricultural areas in Southern Ontario (Roundup WeatherMAx)

Test organisms	Wood frog tadpoles (*Lithobates sylvaticus*)
Biological effects	Results show that formulated products (2.89 mg acid equivalent (a.e.)/L) caused 100% mortality after the first pulse. High mortality at concentrations corresponding to 2.89 mg a.e./L for IPA salt. adpoles treated with a lower concentration of Roundup WeatherMax® (0.21 mg a.e./L) as well as Vision® (2.89 mg a.e./L), IPA and POEA had an increased condition factor (based on length and weight measures in the tadpoles) relative to controls at Gosner stage (Gs) 36/38. At Gs42, tadpoles treated with IPA and POEA had a decreased condition factor.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Yes, survival
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Yes, survival
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Glyphosate was tested separately as IPA salt. Roundup WeatherMAX® GLY w/o POEA; w surfactants of POEA similar toxicit, Vision® GLY w POEA
2 Do the tested concentrations relate to predicted environmental concentrations?	partly (not the overspray scenario)
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	Critical regarding the results for GLY. Supporting information regarding the effects of formulated products different from the lead formulation in Europe
Type of information (Critical, supporting, low weight)	**Critical /supporting**
Consideration/concluding score	**UBA 1**

Yadav, et al. (2013)

BVL-Nr. 2716846	**Yadav, S. S., Giri, S., Singha, U., Boro, F., & Giri, A.**	**2013**	**Toxic and genotoxic effects of Roundup on tadpoles of the Indian skittering frog (*Euflictis cyanophlyctis*) in the presence and absence of predator stress.**	**Aquatic Toxicology, 132, 1-8.**

Reliability	
Purpose of the study Description of endpoints	Investigation of the toxic and genotoxic effects of Roundup, a commercial formulation of glyphosate, in the tadpoles of the Indian skittering frog (Euflictis cyanophlyctis).
Test compound, application procedure, exposure period	Not further specified. Roundup (41% isopropylamine salt), manufactured by Monsanto India Ltd., Mumbai
Experimental approach, Statistics, test environment	Roundup at different concentrations (0, 1, 2, 3, 4 and 8 mg acid equivalent (ae)/L), tested in a 2 ×6 factorial design in the presence and absence of predator stress: 5 min prior to the treatments, we placed the beetle in a beaker filled with 200 ml of well water and tadpoles to generate the cue. The water containing the chemical cue from each cup was slowly poured through a fine-meshed net into the centre of each container to minimize the disturbance to the water. The control group received 200 ml of well water only.
Test organisms	skittering frog (*Euflictis cyanophlyctis*). Water beetles belonging to the genera Dytiscidae were collected from the same source to

	generate predator cues.
Biological effects	The 96-h LC50 for Roundup in the absence and presence of predator stress were 3.76 mg ae/L and 3.39 mg ae/L, respectively. The 10-day LC50 value for Roundup was significantly lower, 2.12 mg ae/L and 1.91 mg ae/L in the absence and presence of predator stress, respectively. Lower concentrations of Roundup (1, 2 and 3 mg ae/L) induced the formation of micronuclei (MN) in the erythrocytes of tadpoles in a concentration-dependent manner. Presence of predator stress apparently increased the toxicity and genotoxicity of Roundup; but these effects were not statistically significant.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Yes, survival
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	No verification possible, compound not specifically defined
2 Do the tested concentrations relate to predicted environmental concentrations?	n.a.
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	Low weight because of the unclear identity of the tested compound. Possibly Roundup original with POEA
Type of information (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA 3**

Güngördü, et al. (2013)

BVL-Nr.: 2716836	**Güngördü, A.**	**2013**	**Comparative toxicity of methidathion and glyphosate on early life stages of three amphibian species: *Pelophylax ridibundusPseudepidalea viridis*and*Xenopus laevis***	**Aquatic Toxicology**
Reliability				
Purpose of the study Description of endpoints	In this study, the toxic effects of glyphosate and methidathion were evaluated comparatively on early developmental stages of 3 anurans—2 natural (*Pelophylax ridibundus, Pseudepidalea viridis*) and 1 laboratory species (*Xenopus laevis*). Endpoints: survival, glutathione S-transferase (GST), acetylcholinesterase (AChE), carboxylesterase (CaE), glutathione reductase, lactate dehydrogenase, and aspartate aminotrasferase were assayed in 4-day-old tadpoles after 96-h pesticide exposure.			
Test compound, application procedure, exposure period	The technical formulations of glyphosate (Roundup) and methidathion (Supracide) that were considered in this study were purchased from local agrochemical stores. The active ingredient of Roundup is the isopropylamine salt of glyphosate in commercial solutions at 441 g/L. with POEA The active ingredient of methidathion in a commercial solution of Supracide is 426 g/L.			
Experimental approach, Statistics, test	For the toxicity tests, groups containing 20 tadpoles were randomly			

environment	placed into covered polycarbonate dishes with 50 mL of different concentrations of methidathion (6.4–51.2 mg AI/L for P. viridis, 4.92–51.2 mg AI/L for P. ridibundus and 1.6–32.3 mg AI/L for X. laevis) and glyphosate (18.8–51.2 mg AI/L for P. viridis and P. ridibundus, 12.8–56.2 for X. laevis) solutions. All groups were tested with 4 replicate dishes, containing 20 tadpoles each, using a total of 80 tadpoles. Test solutions were changed every 24 h during the 4-day test periods.
Test organisms	*P. viridis*, *P. ridibundus*, and *X. laevis* tadpoles.
Biological effects	The 96-h LC50 values for methidathion and glyphosate were determined as 25.7–19.6 mg active ingredient (AI)/L for *P. viridis*, 27.4–22.7 mg AI/L for *P. ridibundus*, and 15.3–5.05 mg AI/L for *X. laevis* tadpoles. The GST induction after 3.2 mg AI/L methidathion exposure was determined to be 173%, 83%, and 38% of control, and the AChE inhibition for the same dose was determined to be 86%, 96%, and 30% of control for *P. ridibundus*, *P. viridis*, and *X. laevis*, respectively. Unlike the application of methidathion, all enzyme activities showed statistically significant increases on glyphosate exposure compared to controls. However, these increases in enzyme activities were not shown to be parallel with the increase of concentration.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes, survival
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Possibly Roundup Original with POEA
2 Do the tested concentrations relate to predicted environmental concentrations?	-/-
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	Supporting information for the assessment of effect of glyphosate-based formulated products containing POEA
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA 2**

Edge et al. (2014)

BVL-Nr. 2716474	**Edge, C., Gahl, M., Thompson, D., Hao, C., Houlahan, J.**	**2014**	**Variation in amphibian response to two formulations of glyphosate-based herbicies**	**Environmetal Toxicilogy and Chemistr DOI: 10.1002/etc.2723**

Reliability	
Purpose of the study Description of endpoints	In the present study, the toxicity of 2 formulations of commonly used glyphosate-based herbicides to wood frog (*Lithobates sylvaticus*) larvae were investigated. Goal was to assess the toxicity of each formulation and to determine if the

	toxicity of either herbicide differed among populations.
Test compound, application procedure, exposure period	Roundup Weed and Grass Control is intended for household use; Roundup WeatherMax is intended for large-scale agricultural applications. These formulations differ in the type of glyphosate salt they contain (Roundup Weed and Grass Control contains isopropylamine salt, and Roundup WeatherMax contains potassium salt), the concentration of glyphosate, and the surfactant(s) used in the commercial product.
Experimental approach, Statistics, test environment	Aquatic exposure
Test organisms	Wood frog tadpoles, derived from 4 geographically isolated populations, were exposed to 9 concentrations of each herbicide and compared the results among the populations. 4 separate experiments were conducted chronologically as egg masses were laid. The order of populations was Ohio, Eastern Ontario, New Brunswick, and Central Ontario. Each concentration was replicated 6 times
Biological effects	For Roundup Weed andGrass Control, 96-h LC50 values ranged from 0.085mg a.e./L to 1.1mg a.e./L. In the first population, Ohio, exposed to Roundup WeatherMax, no mortality was observed in any of the exposure concentrations up to 4.0 mg a.e./L. Therefore, the exposure concentrations were doubled for the next population, Eastern Ontario. In the Eastern Ontario experiment, limited mortality was observed at concentrations up to 8.0 mg a.e./L. The exposure concentrations were doubled again for the New Brunswick population. In the New Brunswick exposures, some mortality was observed at concentrations at and above 4.0 mg a.e./L, but complete mortality in all replicates was observed only at the highest concentration, 16 mg a.e./L.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Yes, survival
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Not all tested formulations comparable to the lead formulation
2 Do the tested concentrations relate to predicted environmental concentrations?	-/-
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	Supporting information for the assessment of the tested formulations
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA 2**

Wagner, N., Lötters, S. (2013)

glyphecotox_676	**Wagner, N., Lötters, S.**	**2013**	**Possible correlation of the worldwide amphibian decline and the increasing use of glyphosate in the agrarian industry**	**BfN - Federal Agency for Nature Conservation**
Reli Reliability				
Purpose of the study	1.Review article on the basis of the following questions: Which concentrations of			

Description of endpoints	Glyphosate and AMPA can be found in the environment? What do we know about the impacts of Glyphosate and its formulations on amphibians? Can there be identified effects on amphibians as a result of the use of Glyphosate coupled with biotic or abiotic stressors? What are possible exposure pathways to different amphibian life-stages? Are amphibians differently affected by the cultivation of conventional crops compared with the HR crops especially with respect to different weed management systems? 1. Statistical, macroecological approach concerning the questions: Do the agricultural change in the Americas and the resulting increased use of Glyphosate with amphibian population decline?Are there as yet any 'signs' for negative impacts on amphibian populations in Germany as a result of an increased deployment of Glyphosate in conventional agriculture?
Experimental approach Statistical design, test environment	Literature review, search for published scientific articles, Macroecological approach. Study of correlative allusions between long-term amphibian population trends and agricultural factors.
Test organisms	Most studies used anuran larvae (tadpoles) as test organisms and little is known about effects on terrestrial life-stages as well as the other two amphibian orders (Caudata and Gymnophiona; i.e. salamanders/newts and caecilians).
Biological effects	Reported toxic effects on tadpoles include – besides increased mortality –damages of the gills and different malformations, inhibition of vital enzymes, release of oxidative stress and genotoxic effects. Chronic and delayed effects include delayed but also precipitated time to metamorphosis.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	Amphibian populations in anthropogenically influenced landscapes are usually affected by different stressors and, therefore, herbicide applications should not be regarded separately.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Mortality, damages of the gills and different malformations, inhibition of vital enzymes, release of oxidative stress and genotoxic effects.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Most likely, surfactants are responsible for adverse effects rather than Glyphosate itself.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Sometimes, effects were demonstrated at environmentally relevant concentrations. Max.concentrations Argentina, 0.7 mg a.e./L (a.e. = acid equivalent) in small water bodies next to HR soybean; Canada 1.95 mg a.e./L in a forest pond after aerial applications in at approved rates. Worst-case expected environmental concentration for surface waters from national agencies are 1.44 mg a.e./L for Canada (where aerial applications are approved) and 0.9 mg a.e./L for Germany (drift rate into water during application without buffer strip).
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	The results suppose that land use is more relevant for amphibian populations than pesticide applications including Glyphosate applications.
Concluding weight of evidence/proposed action	Fertilisers and pesticides – can affect amphibian individuals, in agricultural areas, but do not necessarily effect the population level. Authors empahsise that population viability analysis based on sufficient data and/or long-term field monitoring is necessary to investigate potential impacts on amphibian populations. (Tallowamine) surfactants, apparently ranked among the most harmful pesticides for amphibians.

	Nevertheless, risks for amphibian populations due to Glyphosate use cannot be named per se because species, life-stage, formulation and application-specific reactions have been observed.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Wagner, N., Lötters, S. (2013)

BVL. Nr. 2716818	**Wagner, N., Lötters, S.**	**2013**	**Effects of Water Contamination on Site Selection by Amphibians: Experiences from an Arena Approach With European Frogs and Newts**	Arch Environ Contam Toxicol (2013) 65:98–104

Reli Reliability	
Purpose of the study Description of endpoints	the impact of glyphosate isopropylamine salt, a commercial formulation and AMPA on individual residence time in water of European Frogs and Newts were investigated.
Test compound, application procedure, exposure period, protocol	AMPA (99.5 % purity): four concentrations were tested: 0, 5, 50, and 500 lg/L. Roundup LB PLUS : 1 mg a.e./L as a high concentration (again with two lower and more environmentally relevant concentrations of 0.1 and 0.01 mg a.e./L) for both the formulation and the active ingredient.
Experimental approach Statistical design, test environment	Common Frog Experiments: Effects of contamination with AMPA on residence time in water were tested for male frogs; Experiments were performed in arenas with 10 replicate; an arena (1.5 9 1.5 m) was defined by an amphibian drift fence and consisted of a hiding place (leaves and planks) and four artificial pools. Concentrations were renewed every 24 h because AMPA has a half-life of 7–14 days in water depending on local conditions. Newt Experiments. Study design and methods were the same as described for Common frogs, newts were set pairwise in each arena
Test organisms	The following European amphibian species were tested: Common frog (*Rana temporaria*), Palmate newt (*Lissotriton helveticus*), and Alpine newt (*Ichthyosaura alpestris*).
Biological effects	The residence time in water was not significantly affected by concentrations below or slightly above the European Environmental Quality Standards for AMPA or the German ‘‘worst-case’’ expected environmental concentrations for GLY-IS and RU-LB-PLUS.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	All three species tested in these experiments entered water contaminated at concentrations. Whether this may cause chronic effects remains to be studied.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Perception and avoidance (shown by residence time) of contaminated water bodies.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, see above

2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Yes, see above
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	Occasionally, microclimatic cofactors (nightly minimum ground temperature, water temperature) apparently influenced the residence time.
Concluding weight of evidence/proposed action	Water contamination with the tested environmentally relevant concentrations did not seem to lead to avoidance of contaminated pools. Perception and avoidance (shown by residence time) of contaminated water bodies should be investigated by arena approaches; however, to study other (relevant) behavior, such as oviposition site selection, authors suggest to use more sophisticated designs.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Plötner J. & Matschke J 2012

Dok. Nr. 2716826	**Plötner J. & Matschke J**	**2012**	**Akut-toxische, subletale und indirekte Wirkungen von Glyphosat und glyphosathaltigen Herbiziden auf Amphibien – eine Übersicht.**	**Zeitschrift f. Feldherpetologie 19: 1-20 VBS**

Reli Reliability	
Purpose of the study Description of endpoints	Review article describing the effects of glyphosate on amphibians and their habitats. Due to the variety of published data and findings not all aspects could fully taken into account. Auhtors give some basic explanations on the biochemical mechanisms of action of glyphosate in the plant and the resulting toxic effects in the ecosystem.
Test compound	nd
Content	Effects of glyphosate and glyphosate resisdues,biochemical effects of glyphosate containg herbicides, review on acute toxicity, sublethal toxicity and indirect effects, conclusions.
Test organisms	nd
Biological effects	nd
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	nd
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence/proposed action	Authors state that the findings presented to the acute toxic, sublethal and indirect effects of glyphosate herbicides on amphibians illustrate an ecological hazard potential, especially if herbicides are applied in approprate in high concentrations and over extended periods of time.
Type of information (Critical, supporting, low weight)	**Supporting/ low weight**

Consideration/concluding score	**UBA2-3**

Brodeur, J. C., et al. (2014)

Dok. Nr. 2716828	**Brodeur, J. C., Poliserpi, M. B., & Sánchez, M.**	**2014**	**Synergy between glyphosate- and cypermethrin-based pesticides during acute exposures in tadpoles of the common South American Toad *Rhinella arenarum*.**	**Chemosphere, 112, 70-76.**

Reliability	
Purpose of the study Description of endpoints	The aim of the current study was to evaluate the toxicity of equitoxicand non-equitoxic binary mixtures of glyphosate and cypermethrin to tadpoles of the common South American toad, *Rhinella arenarum*.
Test compound, application procedure, exposure period, protocol	Two different commercial formulations of formulations were tested: Glifoglex® and Glifosato Atanor®. Both contain 48% of glyphosate in the form of isopropylamine salt which corresponds to 36% of glyphosate acid equivalent (ae). Cypermethrin formulations tested were Glextrin® and Xiper®
Experimental approach Statistical design, test environment	Individual acute toxicity of glyphosate and cypermethrin formulations: 10 tadpoles having recently reached stage 25 were placed in 70 mL of culture water with or without (controls) test chemicals for 96 h. Acute toxicity of equitoxic and non-equitoxic binary mixtures of glyphosate and cypermethrin.Final nominal concentrations tested were as follows: Glifosato Atanor: 10, 15, 17.5, 20, 22.5 and 25 mg ae /L, Glifoglex 60, 65, 70, 75, 80, 85 and 90 mg ae/ L.
Test organisms	South American Toad *Rhinella arenarum*
Biological effects	Concentrations of glyphosate and cypermethrin causing 50% mortality after 96 h: Glifosato ATANOR: 19.4 mg ae L/L; Glifoglex: 72.8 mg ae /L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Tadpoles of the common South American toad, *Rhinella arenarum*
2 Is the magnitude of effects of biological significance?	Yes, Water quality monitoring programs from agricultural regions of North America indicate that diverse pesticide contaminants are often present at low concentrations throughout the year and that herbicides are commonly detected in 70–90% of the samples.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Although not specified precisely, the tested formulation is likely to contain surfactant. This causes limited validity regarding effects of Glyphosate formulations that contain POEA or other surfactants.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Considering overspray scenarios for surface water ponds yes, nevertheless not necessarily appropriate for drift rates in Europe. But the approach of toxicity testing of non-equitoxic binary mixtures of glyphosate and cypermethrin.
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	Authors emphasize that In the case of glyphosate formulations, toxicity depends greatly on the surfactant employed in the formulation.
Concluding weight of evidence/proposed action	The study shows that glyphosate and cypermethrin-based pesticides exhibit synergic interactions during acute exposures in tadpoles of the common South American toad, *R. arenarum*.

Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA 2**

Bosch et al. (2011)

Dok. Nr. 2716367	**Bosch, B., Manas, F., Gorla, N., Aiassa, D.**	**2011**	**Micronucleus test in post metamorphic *Odontophrynus cordobae* and *Rhinella arenarum* (Amphibia: Anura) for environmental monitoring**	**Journal of Toxicology and Environmental Health Sciences Vo l. 3(6) pp. 155-163, June 2011**

Reli Reliability	
Purpose of the study Description of endpoints	The objectives of this study were to define the the genotoxic effect of cyclophosphamide and glyphosate in a commercial formulation
Test compound, application procedure, exposure period, protocol	C yclophosphamide monohydrate (CAS Nº 6055-19-2), isopropilamine salt of N-phosphomethyl-glycine, glyphosate, (CAS Nº 38641-94-0) Roundup® (glyphosate 48%); Each experimental group consisted of three males and two females; Cyclophosphamidewas used as a positive control (40 mg/L). The groups exposed to the herbicide were kept in Roundup ® solutions prepared in water, at the following concentrations: glyphosate 100 mg (G100), 200 mg (G200), 400 mg (G400) and 800 mg (G800) active ingredient (a.i.)/ L.
Experimental approach Statistical design, test environment	a) Definition of basal MN frequency in peripheral blood Erythrocytes b) proposal of the variation of these species in MN frequency as a biomarker of genotoxic effect c) MN frequency with four different concentrations of glyphosate herbicide in a commercial formulation. d) comparison the sensitivity of both amphibians to these compounds
Test organisms	*Odontophrynus cordobae* (Anura: Cyclorhamphidae) and *Rhinella arenarum* Hensel, 1867 (Anura: Bufonidae), amphibians widely distributed in the agroecosystems of the Province of Córdoba, Argentina.
Biological effects	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Formulated product contains POEA Authors state that an evaluation to pure glyphosate could be added in order to rule out the effect of other components present in the commercial formulation.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	The tested concentrations (> 100 mg a.i /L) are exceedeing the predicted environmental concentrations. The concentrations used in this work were approximately 10 times higher than those utilized in previous studies with glyphosate in pro-metamorphic amphibians.
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	The data can be employed in the assessment of formulations containing POEA as surfactants. No data for glyphosate technical or glyphosate acid deducible from the tested formulation. Lead formulation for EU renewal of glyphosate approval contains no POEA
Concluding weight of evidence/proposed action	High concentrations Roundup ® can lead to basal spontaneous occurrence of MNE in postmetamorphic stages.
Type of information (Critical, supporting, low weight)	**low weight**

Consideration/concluding score	**UBA3**

Berger et al. (2013)

Dok. Nr. 2716471	**Berger, G., Graef, F., Pfeffer, H.**	**2013**	**Glyphosate applications on arable fields considerably coincide with migrating amphibians**	**Scientific Reports Volume:3, Article number: 2622**

Reliability	
Purpose of the study Description of endpoints	Based on a field study from 2006 to 2008 on amphibian activity in a pond-rich agricultural landscape, we quantitatively assessed the temporal exposure of amphibian populations to Glyphosate applied to arable fields, documenting their migrations between terrestrial sites and breeding ponds.
Test compound, application procedure, exposure period, protocol	Pre-sowing or preemerging application in spring was used with maize, siccation for harvest preparation with winter barley. For seed bed preparation in late summer and autumn prior to crop sowing, Glyphosate was applied on the stubbles to all six crops but not to every field. Stubble application was done on about 20% of the fields in 2006 and , 10% in 2007. Of all stubble fields treated, 45% were winter rape and 30% winter rye. The Glyphosate application frequency to crops varied between years. While in late summer and autumn 2006 at least 40% of the fields were treated prior to sowing winter barley, triticale and maize, in 2007 less than 25% or even no application took place.
Experimental approach Statistical design, test environment	Amphibian activity using fence trapping, installation of 49 drift fences consisting of 26 open, 10-m-long cross-shaped fences and 23 enclosures. Enclosures encircled biotopes (wood lots, small water bodies) located at the field edge or completely within fields.
Test organisms	Fire-bellied toad (*Bombina bombina* (Linnaeus, 1761)), moor frog (*Rana arvalis* (Nilsson, 1842)), spadefoot toad (*Pelobates fuscus* (Laurenti, 1768)) and crested newt (*Triturus cristatus* (Laurenti, 1768)).
Biological effects	For pre-sowing/preemerging application in spring and stubble management in late summer and autumn prior to crop sowing, we found coincidences with amphibian populations for almost all fields treated. For siccation with winter barley in summer, seven out of 12 treated fields coincided with amphibians. The proportions of populations coinciding with Glyphosate application to crops differed considerably depending on which of the two DT50-values was used.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Glyphosate formulations, not specified further.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence/proposed action	Depending on a) age- and species-specific main migration periods, b) crop species, c) Glyphosate application mode for crops, and d) the presumed DT50 value (12 days or 47 days) of Glyphosate up to 100% coincidence was calculated. The amphibians regularly co-occur with pre-sowing/ pre-emerging applications to maize in spring and with stubble management prior to crop sowing in late summer and autumn. Siccation treatment in summer coincides only with early pond-leaving juveniles. Authors suggest in-depth investigations of both acute and long-term effects of Glyphosate applications

	on amphibian populations not only focussed on exposure during aquatic periods but also terrestrial life stages.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Edge et al. (2014)

Dok. Nr. 2716474	**Edge, C., Gahl, M., Thompson, D., Hao, C., Houlahan, J.**	**2014**	**Variation in amphibian response to two formulations of glyphosate-based herbicies**	

Reli Reliability	
Purpose of the study Description of endpoints	In this study we investigated the toxicity of two formulations of commonly used glyphosate-based herbicides to wood frog (Lithobates sylvaticus) larvae.
Test compound, application procedure, exposure	Roundup Weed and Grass Control™ (isopropylamine salt) Roundup WeatherMax™ (potassium salt) concentrations 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 mg a.e./L.
Experimental approach Statistical design, test environment	Animals from each population were exposed to nine concentrations and each concentration was replicated six times. We used the formulations RoundUp Weed and Grass Control (5.18 g a.e./L present as the isopropylamine salt) and the formulation Roundup WeatherMax (540 g a.e./L present as the potassium salt) which is designed for agricultural use. 54 experimental units (9 conc. x 6 rep.) for each formulation and a total of 108 experimental units (54 experimental units for each of two different formulations) for each population. To test for differences in % mortality after 96 h between populations we used Analysis of Covariance (ANCOVA).
Test organisms	wood frog (*Lithobates sylvaticus*) larvae.
Biological effects	Roundup Weed and Grass Control: 96 hLC50: 0.085 to 1.1 mg a.e./L (animals survived in the control and all animals died at concentrations at and above 2.0 mg a.e./L). Roundup WeatherMax™ : LC50 for Ohio and Eastern Ontario populations > n 4 and 8 mg a.e./L, respectively. The present study found that Roundup WeatherMax is approximately five times less toxic to larval wood frogs than Roundup Weed and Grass Control.

Table 1: LC10 and LC50 values for four populations of *Lithobates sylvaticus* tadpoles exposed to 9 concentrations of two herbicide formulations. Values are mg acid equivalents/L. 95 % confidence intervals are in parentheses.

	Roundup Weed and Grass Killer		Roundup WeatherMax	
Population	LC10	LC50	LC10	LC50
Ohio	0.90 (0.84, 0.97)	1.10 (1.046, 1.16)		> 4
Eastern Ontario	0.38 (0.31, 0.46)	0.71 (0.63, 0.80)		> 8
New Brunswick	0.33 (0.10, 1.079)	0.58 (0.52, 0.66)	1.06 (0.67, 1.69)	4.94 (4.18, 5.83)
Central Ontario	0.73 (0.64, 0.83)	0.99 (0.92, 1.06)	5.98 (5.28, 6.77)	8.26 (7.71, 8.83)
Replicate 1				
Central Ontario	0.018 (0, 0.77)	0.085 (0.009, 0.80)		
Replicate 2				
Central Ontario	0.41 (0.34, 0.49)	0.70 (0.63, 0.77)		
Replicate 3				

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of	Authors state that the within population variation indicates that in order to

biological significance?	generate the precise estimates of LC50 values necessary to make among population comparisons multiple 'repeats' of toxicity tests are required to capture experimental, and/or small-scale spatial variability.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulations were tested.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	Toxicity values for glyphosate-based herbicides to amphibian larvae are lower at high pH. Differences among populations of amphibians in their response to environmental variables, such as temperature and length of activity season.
Concluding weight of evidence/proposed action	The results of this experiment support prior studies that have found some formulations of glyphosate-based herbicides are toxic to amphibian larvae at concentrations at the upper end of those measured in the environment and that toxicity differs among formulations.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Ruamthum et al. (2011)

Dok. Nr. 2716676	**Ruamthum, W. et al.**	**2011**	**Effect of glyphosate-based herbicide on acetylcholinesterase activity in tadpoles, *Hoplobatrachus rugulosus***	
Reliability				
Purpose of the study Description of endpoints	Authors determine the acute toxicity and changes in activity levels of the enzyme AChE In tadpoles after exposure to a glyphosate-based herbicide to understand the biochemical effects on amphibian tadpoles.			
Test compound, application procedure, exposure period, protocol	glyphosate-based herbicide (glyphosate-isopropylammonium composed of Isopropylamine salt 48% w/v SL and N-(phosponomethyl) glycine 36% w/v; concentrations of 21,24, 27 and 30µl/L.			
Experimental approach Statistical design, test environment	This research used a completely randomized experimental design with 4 replicates of 20 tadpoles each (stages 35-39) for each concentration. Each replicate of tadpoles was placed into a glass container (10x15,20 em); AChE activity was measured by a method modified from Ellman et 01. (1961) and Bullangpoti (2007).			
Test organisms	East Asian Bullfrog, *Hopfobatrachus rugulosus*, a species that is widely distributed in rice fields of Tropical Asia.			
Biological effects	After 24·96h of exposure, acute toxicity LC5o values were 25, 24, 24 and 23 µl/L, respectively. The 96h LC50 was 1.07 times lower than the 24h LC5o. The greatest decrease sin AChE activities occurred in the 30 µl /L glyphosate based-herbicide treatment, in which relative lnhibitions in AChE activities of 27 to 32-fold compared with the control			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				

1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	AChE enzyme actIvity of the tadpoles was influenced by each dose treatment.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	A glyphosate-based formulation was tested, but was not specified further. Toxicity differs among formulations depending on the surfactant system.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	Differences between amphibian species in AChE susceptibility are discussed.
Concluding weight of evidence/proposed action	Supporting information on biochemical response of tadpoles to environmental relevant concentrations of commercial formulation containing glyphosate and probably POEA.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Junges, C.M. et al. (2013)

Dok. Nr. 2716704	**Junges, C.M. et al.**	**2013**	**Effectiveness evaluation of glyphosate oxidation employing the H2O2/UVC process: Toxicity assays with *Vibrio fischeri* and *Rhinella arenarum* tadpoles**	
Reli Reliability				
Purpose of the study Description of endpoints	In this work, the H_2O_2/UVC process was applied to the degradation of a commercial formulation of glyphosate in water. Two different bioassays were used for determining sample toxicity at different stages of mineralization.			
Test compound, application procedure, exposure period, protocol	Commercial herbicide Eskoba® 35.6% (w/v) as acid or 48% as a monoisopropylamine salt (MIPA).			
Experimental approach Statistical design, test environment	Short-term (24 and 48-h) static tests were performed to evaluate the toxicity of the samples collected at different times during the H_2O_2/UVC process. For these assays glass tanks (12.5 cm diameter and 13.5 cm high) with 0.5 L of untreated sample M_0 plus samples M_1, M_2,M_3, $M_{1,E}$, $M_{2,E}$ and$M_{3,E}$, and seven tadpoles were housed per tank. H_2O_2 and distilled water were used as positive and negative controls, respectively.			
Test organisms	luminescence bacterium *Vibrio fischeri* (a traditional assay for evaluating AOPs) and tadpoles of *Rhinella arenarum*, a common anuran that is frequently found in forests, wetlands, agricultural land and urban territories (Prometamorphic tadpoles (Gosner stages 36–38)[30] of *R. arenarum*)			
Biological effects	50mg/L of the commercial formulation caused 100 % mortality after 48 h.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				

1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	nd
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence/proposed action	Ecotoxicity tests were performed for evaluating the effectiveness of de-contamination by AOPs. Results have limited value for ERA.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Schneider, S., et al. (2012)

Dok. Nr. 2716712	**Schneider, S, Krueger, H, Claude, J, Ross, T, Gallagher, S, Springer, T, Jaber, M**	**2012**	**Glyphosate: Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances.**	

Reliability	
Purpose of the study Description of endpoints	An amphibian metamorphosis assay was conducted by Wildlife International, Ltd. for the Joint Glyphosate Task Force at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland from October 24, 2011 to November 14, 2011.
Test compound, procedure, exposure period,	African clawed frog (Xenopus laevis) tadpoles were exposed to glyphosate at mean measured concentrations of 0.13, 0.79, 4.3, 20 and 90 mg a.e./L for 21 days under flow through conditions.
Experimental approach Statistical design, test environment	Endpoints that were evaluated to determine if the test substance impacted the HPT axis included survival, gross morphological abnormalities, developmental stage, wet weight, body length, snout-to-vent length, normalized hind-limb length, and thyroid gland histology.
Test organisms	African clawed frog (*Xenopus laevis*) tadpoles
Biological effects	There were no treatment-related effects on survival, stage, or normalized hind limb length during the 21-day test. Histopathologic analysis showed no treatment-related changes in the thyroid glands of *Xenopus laevis* tadpoles when compared to negative control animals. There was a slight increase in wet weight in the 90 mg a.e./L treatment group and in snout-to-vent length in the 4.3 and 90 mg a.e./L treatment groups at the end of the 21-day test.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	nd

Concluding weight of evidence/proposed action	Authors observed no effects observed on normalized hind-limb length, stage, or thyroid histology. Glyphosate was not found to interfere with the normal function of the hypothalamus.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Brodeur, J.C. et al. (2012)

Dok. Nr. 2716716	**Brodeur, J.C. et al.**	**2012**	**Evidence of Reduced Feeding and Oxidative Stress in Common Tree Frogs (*Hypsiboas pulchellus*) from an Agroecosystem Experiencing Severe Drought**	**Journal of Herpetology, Vol. 46, No. 1, pp. 72–78, 2012**

Reliability	
Purpose of the study Description of endpoints	Authors evaluated the impacts of the herbicide glyphosate and subsequent intense drought on the Tree Frog. Endpoints determind were organismic indices (stomach content index, hepatosomatic index, body fat index, gonadosomatic index, condition factor) as well as biomarkers of oxidative stress, exposure to contaminants (hepatic gluthatione-S-transferase activity), and genotoxicity (frequency of micronuclei).
Test compound, application procedure, exposure period, protocol	October: the herbicide glyphosate was applied, GM soybean was sowed. Spring of 2008: the intense drought prevented the plant from developing, and no more pesticides were applied.October sampling is considered to be pre-application of glyphosate. November: application of glyphosate. January:drought was at its peak December 2008 and 6 January 2009: no rainfall
Experimental approach Statistical design, test environment	Anuran Sampling, Organ Indices and Body Condition; Enzymes, Glutathione, and Protein Assays; two-way analysis of variance (ANOVA) followed by a Holm-Sidak test for multiple comparisons.
Test organisms	Common Tree Frog, *Hypsiboas pulchellus*
Biological effects	No significant differences were observed in the parameters measured when comparing frogs sampled before, 2, and 15 days after glyphosate exposure. Anurans sampled in the same site two months later, when a drought was at its peak, presented a decrease in stomach content and hepatosomatic index, as well as an increase in hepatic catalase activity, hepatic GSH content and micronuclei frequency in peripheral circulating erythrocytes.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Applied products not known.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered	The dry weather and the absence of rainfalls in the weeks following the application of glyphosate may have unusually limited the dispersion and

adequately (e.g. pH, temperature, light conditions)?	impacts of the herbicide in the environment.
Concluding weight of evidence/proposed action	Authors observded that adult H. pulchellus were not affected by glyphosate because no significant difference was observed when comparing animals sampled before) and after the application. As drought maybe influenced the results it is stated that aquatic and terrestrial anuran species may be more exposed or be more vulnerable than the arborescent species studied here, and more research should be conducted before generalizing results to other circumstances or anuran species.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Fuentes, L. et al. (2014)

Dok. Nr. 2716850	**Fuentes, L. et al.**	**2014**	**Role of sediments in modifying the toxicity of two roundup formulations to six species of larval anurans.**	
Reli Reliability				
Purpose of the study Description of endpoints	The objective of this laboratory study was to discern the role that sediments may have in altering the toxicity of the 2 formulations to 6 species of larval anurans.			
Test compound, application procedure, exposure period, protocol	Roundup® and Roundup WeatherMAX®; Exposure concentrations were arrayed according to range-finding tests and were between 0.3 and 7.0 milligrams of acid equivalents per liter (mg ae/L) for wateronly tests and between 1.0 and 10.0 mg ae/L for water-sediment tests.			
Experimental approach Statistical design, test environment	96-h static,non-renewal aqueous exposures with and without sediment. Toxicity tests were performed using EPA-821-R-02-012 methodology; Data were analyzed using SAS® 9.1, Probit regression was used to determine no observed effect concentrations (NOEC), lowest observed effect concentrations (LOEC), lethal concentration (LCx) and 95% confidence intervals (CI).			
Test organisms	Six species of anurans (*Bufo fowleri, Hyla chrysoscelis, Rana catesbeiana, R. clamitans, R. sphenocephala, and R. pipiens*); Gosner stage 25			
Biological effects	LC50 values ranged from 1.80 to 4.22 mg ae/L in water-only exposures and from 4.37 to 8.26 mg ae/L in water-sediment exposures of the original formulation (Table 1). LC50 values ranged from 1.33 to 3.26 mg ae/L and 2.94 to 4.56 mg ae/L in water-only and water-sediment exposures of Roundup WeatherMAX®, respectively.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes
2 Is the magnitude of effects of biological significance?				yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?				
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation tested.			
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	The PEC for label rates directly after application into a 15.2 cm (6 inch) deep body of water is explicitly prohibited by the product label (0.55, 1.11, and 2.76 mg ae/L) and displays a worse case scenario of inadvertent, direct applications.			
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature,	Calculated NOEC values in the present study were based on laboratory tests and do not take into account the presence of competing ligands and factors affecting the rate of dissipation in actual field			

light conditions)?	applications that would further increase the MOS.
Concluding weight of evidence/proposed action	If used according to label instructions, the original formulation of Roundup® and Roundup WeatherMAX® herbicides should pose minimal risk to Anuran amphibians in actual field applications
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Thibodeau, J. et al. (2012)

Dok. Nr. 2716814	**Thibodeau, J. et al.**	**2012**	**Oxidation of retinoic acids in hepatic microsomes of wild bullfrogs *Lithobates catesbeianus* environmentally-exposed to a gradient of agricultural contamination**	
Reli Reliability				
Purpose of the study Description of endpoints	The main objective of the present study was to evaluate whether the complex mixtures of waterborne agricultural contaminants affect RA (retinoic acid) metabolism in indigenous amphibians. The formation of metabolites was compared between sampling sites and sexes to gain a better understanding of RA metabolism in bullfrogs in the context of agricultural contamination.			
Test compound, application procedure, exposure period, protocol	glyphosate was detected only at the RN site: 0.22 µg/l in surface waters of the sub-watersheds sampled in 2007. (High agricultural activity was representedby Rivie`re Noire (RN) catchment.)			
Experimental approach Statistical design, test environment	Microsome preparation; Partial characterization of the enzymatic RA oxidation Activity; Enzymatic assays and HPLC analysis of the reaction Products; Inhibitor studies; Plasma 13cis-4-oxo-RA analysis			
Test organisms	wild bullfrogs *Lithobates catesbeianus*			
Biological effects	Female bullfrogs associated with the most contaminated site tend to have an altered liver RA oxidation.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	yes			
2 Is the magnitude of effects of biological significance?	nd			
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd			
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?	Agricultural contaminants probably a mixture out of different commercial formulations.			
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes			
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	nd			
Concluding weight of evidence/proposed action	Long-term objective of these investigations is the development of in vitro assays and non-destructive biomarkers associated with impacts on RA metabolism and signalling in an environmental context.			
Type of information (Critical, supporting, low weight)	**low weight**			
Consideration/concluding score	**UBA3**			

Paetow, L.J., McLaughlin, J.D. (2013)

Dok. Nr. 2716803	**Paetow, L.J., McLaughlin, J.D.**	**2013**	**Mortality of American Bullfrog Tadpoles Lithobates catesbeianus Infected by *Gyrodactylus jennyae* and Experimentally Exposed to *Batrachochytrium dendrobatidis***	

Reli Reliability	
Purpose of the study Description of endpoints	Examination of the combined effects of a keratinophilic chytrid fungus in conjunction with another potential pathogen on the survival of bullfrog tadpoles exposed to pesticides.
Test compound, application procedure, exposure period, protocol	Nominal chemical exposure concentrations were 21 µg of atrazine, and 1.5 mg acid equivalents (a.e.) of glyphosate, per liter of water. In the herbicide exposed animals, the two acute exposures to *Bd* occurred at 14 and 16 d after the start of the pesticide exposures, and each *Bd* exposure lasted 24 h. Each replicate consisted of six tadpoles.
Experimental approach Statistical design, test environment	As the goal was to determine whether the pesticide exposures enhanced susceptibility to infection by *Bd*, no pesticide-only exposures were included in the design.
Test organisms	American Bullfrog Tadpoles *Lithobates Catesbeianus* Gosner stages 26–37; Batrachochytrium dendrobatidis is a keratinophilic chytrid fungus that infects cutaneous epithelial cells of adult amphibians and the keratinized mouthparts of larval stages.
Biological effects	The concentrations of glyphosate measured at 12 h and at 14 d postaddition of the pesticides to the aquariums were both 2.1 mg/L a.e., which was 1.4 times higher than the nominal concentration of 1.5 mg/L a.e.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	no
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Probably commercial formulation, which is not specified further.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	nd
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence/proposed action	There was no significant difference in mortality between tadpoles exposed only to *Bd* and tadpoles additionally exposed to atrazine or glyphosate, which suggests that the pesticide exposures did not alter the immune system or enhance the virulence of the pathogens in this experiment.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Lanctot, C. et al. (2014)

Dok. Nr. 2716790	**Lanctot, C. et al.**	**2014**	**Effects of glyphosate-based herbicides on survival, development, growth and sex ratios of wood frog (*Lithobates sylvaticus*) tadpoles. II: Agriculturally relevant exposures to Roundup WeatherMax® and**	**Aquatic Toxicology 154 (2014) 291–303**

			Vision® under laboratory conditions	
Reliability				
Purpose of the study Description of endpoints	The purpose of this study is (1) to determine if an agriculturally relevant exposure to Roundup WeatherMax® influences the development of wood frog tadpoles through effects on the mRNA levels of genes involved in the control of metamorphosis; (2) to compare results to the well-studied Vision® formulation (containing the isopropylamine salt of glyphosate [IPA] and polyethoxylated tallowamine [POEA] surfactant) and to determine which ingredient(s) in the formulations are responsible for potential effects on development; (3) to compare results to recent field studies that used a similar experimental design.			
Test compound, application procedure, exposure period, protocol	Tadpoles were exposed to an agriculturally relevant application (i.e., two pulses) of Roundup WeatherMax® and Vision® as well as the active ingredient (IPA) and the POEA surfactant of Vision®.			
Experimental approach Statistical design, test environment	Survival, development, growth, sex ratios and mRNA levels of genes involved in tadpole metamorphosis were measured. Treatments were added to aquaria twice (pulse exposure) to reflect application patterns used for agricultural practices, except for POEA.			
Test organisms	wood frog (*Lithobates sylvaticus*) tadpoles			
Biological effects	Results show that Roundup WeatherMax® (2.89 mg (a.e.)/L) and (1.43 mg/L) POEA caused 100% mortality after the first pulse. By the end of the experiment (after 2 pulses), survival was 78% in control, 86% in Roundup WeatherMax® (0.21 mg a.e./L), 68% in Vision® (2.89 mg a.e./L), 86% in IPA (2.89 mg a.e./L) and 70% in POEA pulse (1.43 mg/L). Significant differences between control and treatment group were only detected for the IPA (2.89 mg a.e./L) treatment concerning the Sex ratios (increase in the proportion of females to 55.2%). Analysis of mRNA levels in the brain and tail give evidence of altered mRNA levels of thyroidand stress-related genes after exposure to an environmentally realistic concentration of Roundup WeatherMax® (0.21 mg a.e./L).			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	yes			
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Agriculturally relevant two-pulse exposure to the predicted maximum environmental concentration (USA) of Roundup WeatherMax® (2.89 mg a.e./L) is extremely toxic to L. sylvaticus tadpoles under laboratory conditions, causing complete mortality after the first pulse exposure.			
3 Is the ecotoxicological manifestation level appropriate for the assessment, e.g. gene induction vs. apical endpoints like growth or reproduction?	mRNA levels of genes involved in the control of metamorphosis were effected at low exposure concentrations (0.21 mg a.e./L), but concentrations were not high enough to translate into clear phenotypic or developmental changes.			
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?	yes			
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Roundup WeatherMax® with 0.21 and 2.89 mg a.e./L. According to the author the lower concentration represents the environmentally realistic concentration (ERC), in wetlands located in agricultural areas in Southern Ontario. The higher concentration is the predicted maximum environmental concentration (PMEC) in typical agricultural use patterns.			
3 Have parameters influencing the endpoints been considered	Pulse exposure to POEA (1.43 mg/L) did not cause a greater disruption of growth, development, or mRNA abundance of thyroid-dependent genes			

adequately (e.g. pH, temperature, light conditions)?	than the active ingredient (IPA). Thus, results suggest that POEA is not the only ingredient in glyphosate-based formulations responsible for the disruption of genes involved in metamorphosis of L. sylvaticus tadpoles.Authors discussed the differences between laboratory and field studies and conclude that the effects of are exaggerated in laboratory studies.
Concluding weight of evidence/proposed action	Pulse exposures to Roundup WeatherMax® at a much lower and environmentally relevant concentration (0.21 mg a.e./L) did not affect the survival to metamorphic climax (Gs42) of wood frog tadpoles. Authors conclude that agriculturally relevant exposure to Roundup WeatherMax® and chronic exposure to POEA (1.43 mg/L) is highly toxic to L. sylvaticus tadpoles under laboratory conditions. Survival, growth and mRNA results also indicate that Roundup WeatherMax® has greater toxicity than Vision® formulation containing the POEA surfactant.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Katzenberger, M. et al. (2014)

Dok. Nr. 2716787	**Katzenberger, M. et al.**	**2014**	**Swimming with Predators and Pesticides: How Environmental Stressors Affect the Thermal Physiology of Tadpoles**	**PLOS one May 2014 | Volume 9 | Issue 5 | e98265**

Reli Reliability	
Purpose of the study Description of endpoints	Study on the thermal physiology of grey treefrog tadpoles by determining whether exposures to predator cues and an herbicide (Roundup) can alter their critical max. temperature (CTmax) and their swimming speed across a range of temperatures, which provides estimates of optimal temperature (Topt) for swimming speed and the shape of the thermal performance curve (TPC).
Test compound, application procedure, exposure period, protocol	Commercial formulation Roundup, which is not explicitely stated in chapter Materials and Methods.
Experimental approach Statistical design, test environment	The experiment used a completely randomized, 2x2 factorial design comprised of the presence or absence of predator cues crossed with the presence or absence of an herbicide (nominal concentrations of 0 or 2 mg active ingredient per liter (a.e./L).
Test organisms	grey treefrog tadpoles (*Hyla versicolor*)
Biological effects	The herbicide had no effect on CTmax value. Combining predator cues and the herbicide produced an intermediate Topt that was 0.5C higher than the control.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	Thermoregulation, and morphological changes are countered by other phenotypic changes that might impair swimming ability.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	no
Environmental Relevance	
1 Is the substance tested representative and relevant for	Probably commercial formulation Roundup,

the substance being assessed?	which is not specified further.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Concentrations resemble predicted maximum environmental concentration in USA.
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence/proposed action	Environmental contamination influence thermal performance. Roundup itself not beeing in mixture with other stressors did not have any effect on CTmax estimates of tadpoles.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Hanlon, S.M., Parris, M.J. et al. (2013)

Dok. Nr. 2716779	**Hanlon, S.M., Parris, M.J.**	**2013**	**The interactive effects of chytrid fungus, pesticides, and exposure timing on gray treefrog (*Hyla versicolor*) larvae**	**Environmental Toxicology and Chemistry, Vol. 33, No. 1, pp. 216–222, 2014**

Reli Reliability	
Purpose of the study Description of endpoints	To test the hypothesis that pesticides alter host– pathogen relationships in amphibians, gray treefrog tadpoles were exposed to pathogenic fungus, the pesticides Sevin or Roundup.
Test compound, application procedure, exposure period, protocol	Water-soluble formulations of Roundup (Pro Concentrate1; 50.0% glyphosate potassium salt; Monsanto) probably containing polyethoxylated tallowamine.
Experimental approach Statistical design, test environment	1.5-L containers into which tadpoles were placed. Pesticide concentrations were confirmed from the stock solutions; Survival analysis;Usage of qPCR to confirm Bd infection in experimental subjects.
Test organisms	gray treefrog (*Hyla versicolor*) tadpoles larval development: 1) early (Gosner 26), 2) mid (Gosner 30–31), and 3) late (Gosner 35–36).; pathogenic fungus *Batrachochytrium dendrobatidis* (Bd);
Biological effects	All treatments reduced survival compared with Bd-unexposed subjects in no-pesticide treatments. Roundup increased survival in Bd-exposed tadpoles compared with those exposed to Bd alone.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested relevant for the substance being assessed?	Commercial formulation
2 Do the tested concentrations relate to predicted environmental concentrations?	Concentrations were chosen because they represent a worst-case Scenario in US (i.e., direct application of a pesticide into a water body, but not 100% lethal) for environmental exposure.
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	Timing of exposure may influence the effect(s) of environmental perturbations.

Concluding weight of evidence/proposed action	Roundup ameliorated the effects of Bd, illustrating the complexity of interactions of multiple perturbations on amphibians. Previous research has shown that exposure to multiple stressors usually results in combined effects that reduce fitness (e.g., growth, survival) more so than exposure to individual pressures.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Hanlon, S.M. et al. (2013)

Dok. Nr. 2716778	**Hanlon, S.M. et al.**	**2013**	**Mouthparts of Southern Leopard Frog, *Lithobates sphenocephalus*, Tadpoles not Affected by Exposure to a Formulation of Glyphosate**	

Reliability	
Purpose of the study Description of endpoints	This study evaluated the impacts of Roundup ® on tadpole mouthpart damage as a mechanism for reduced growth and developmental rates *in Lithobates sphenocephalus* (Southern leopard frog) tadpoles.
Test compound, application procedure, exposure period, protocol	Roundup ® ProConcentrate (50.0 % glyphosate potassium salt; Monsanto, St. Louis, MO, USA) at 2.5 (high), 0.25 (mid), 0.025 (low) mg/L, and water control. Contains probably the surfactant polyethoxylated tallow amine.
Experimental approach Statistical design, test environment	Tadpoles (N = 40, 10 per treatment group) were placed into 1.5 L plastic containers filled with 1 L of aged tap water. Water changes were performed every 3 days, at which time respective concentrations were reapplied. Deformities were assessed in 10 zones of the oral disc; Multivariate analysis of variance; calculation of body condition (=mass/snout-ventlength) as an indicator of the overall growth of individuals.
Test organisms	*Lithobates sphenocephalus* (Southern leopard frog) tadpoles.
Biological effects	Authorsdid not find evidence that Roundup ® damages larval mouthparts, nor was there a significant relationship between mouthpart damage and either body condition or developmental rate.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	There was no effect of Roundup ® on mouthpart damage.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative for the substance being assessed?	Commercial formulation Roundup ®
2 Do the tested concentrations relate to environmental concentrations ?	Concentrations were chosen because they represent a range of environmentally realistic concentrations ranging from a scenario of exposure via direct application or overspray (US) to concentrations that are more likely to be observed in natural settings from spray drift or runoff.
3 Have parameters influencing the endpoints been considered?	nd
Concluding weight of evidence/proposed action	Documenting the absence of an effect of Roundup ® on tadpole mouthparts is essential to understand the mechanisms through which pesticides may or may not negatively affect amphibian life histories. Authors conclude that more research is necessary to elucidate

	the mechanisms by which pesticides affect amphibians throughout larval development.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Figueiredo, J., Rodrigues, D.J. (2014)

Dok. Nr. 2716772	**Figueiredo, J., Rodrigues, D.J.**	**2014**	**Effects of four types of pesticides on survival, time and size to metamorphosis of two species of tadpoles (*Rhinella marina* and *Physalaemus centralis*) from the southern Amazon, Brazil**	Published by the British Herpetological Society; Volume 24 (January 2014), 7–15

Reliability	
Purpose of the study Description of endpoints	The study addresses the acute and chronic effects of four types of pesticides on tadpoles of two species of anurans from southern Amazonia.
Test compound, application procedure, exposure period, protocol	Glyphosate 480 (Roundup), Agripec® (concentration: 480 g/L). The concentrations of glyphosate applied to *R. marina* were 8, 16, and 24 mg/L.
Experimental approach Statistical design, test environment	Acute toxicity experiments: The LC5096 values were determined using the nonparametric statistics Trimmed Spearman-Karber method; Chronic toxicity experiments; ANOVA with Scott-Knott grouping a posteriori For each individual that reached metamorphosis, total length, body length, body height, body width, tail height, tail muscle height, oral disc width and intraocular distance, according to Altig & McDiarmid (1999) were measured.
Test organisms	*Rhinella marina* (Linnaeus, 1758) and *Physalaemus centralis* (Bokermann, 1962); (1960), point they were used in the experiments: stage 25 of Gosner
Biological effects	*Rhinella marina*: LC_{50}96h: 32.00 mg/L. *Physalaemus centralis*: LC_{50}96h: 19.70 mg/L. The time to metamorphosis was not affected by any concentration of any pesticide used in the chronic toxicity test with *R. marina* ($p>0.05$). At concentrations of 8.00 mg/L and -16.00 mg/L of glyphosate, *R. marina* showed a longer time to metamorphosis but, due to large variations between individuals, this was not statistically significant when compared with control.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative for the substance being assessed?	Commercial formulation. Differences in the toxicity of co-formulants preclude making general conclusions across all formulations of glyphosate herbicides.
2 Do the tested concentrations relate to environmental conce	-/-
3 Have parameters influencing the endpoints been considered adequately?	-/-
Concluding weight of evidence/proposed action	LC50 values for the formulation is determinded. No separate test of glyphosate as acid or salt performed. Differences in the toxicity of co-formulants preclude making general conclusions across all formulations of glyphosate herbicides.

Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Edge, C. et al. (2012)

Dok. Nr. 2716770	**Edge, C. et al.**	**2012**	**Laboratory and field exposure of two species of juvenile amphibians to a glyphosate-based herbicide and *Batrachochytrium dendrobatidis***	**Science of the Total Environment 444 (2013) 145–152**

Reliability	
Purpose of the study Description of endpoints	The primary goal of this study is to assess whether exposure to glyphosate-based herbicide commonly used in agriculture(Roundup WeatherMax™) has negative effects on semi-aquaticjuvenile amphibians.
Test compound, application procedure, exposure period, protocol	Roundup WeatherMax™ (540 g a.e./L, Monsanto, Winnipeg, MB, CAN), four herbicide concentrations: 0, 2.16, 4.32 and 8.64 kg a.e./ha.
Experimental approach Statistical design, test environment	Field experiment 16-day exposure: enclosures (0.8 m×2 m, 1.6 m2) enclosure surrounded only littoral vegetation and nowater; to each enclosure using a separate small handheld plant misting bottle for each application rate. To achieve uniform coverage of the ground and vegetation within the enclosure with the herbicide, 16 mL of diluted herbicide was applied to each enclosure. Laboratory experimental setup: exposure to the herbicide and to Bd.
Test organisms	green frogs (*Lithobates clamitans*), northern leopard frogs (*Lithobates pipiens*)
Biological effects	Roundup WeatherMax has limited effects on juvenile amphibian survival. LSI, body condition and disease incidence.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	-/-
3 Is the ecotoxicological manifestation level appropriate for the assessment?	-/-
Environmental Relevance	
1 Is the substance tested representative for the substance being assessed?	Commercial formulation. Differences in the toxicity of co-formulants preclude making general conclusions across all formulations of glyphosate herbicides.
2 Do the tested concentrations relate to environmental concentrations ?	yes
3 Have parameters influencing the endpoints been considered?	-/-
Concluding weight of evidence/proposed action	In this experimnatl setup exposure to Roundup WeatherMax poses a negligible risk of acute mortality or sublethal effects on LSI, body condition or the incidence of chytridiomycosis.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Edge, C. et al. (2012)

Dok. Nr.	**Edge, C. et**	**2012**	**A silviculture application of the glyphosate-**	**Environ.**

2716719	**al.**		**based herbicide Visionmax to wetlands has limited direct effects on amphibian larvae**	**Toxicol. Chem. 31, 2012**

Reliability	
Purpose of the study Description of endpoints	Study was designed to assess the potential risk of the formulation VisionMAXTM (Monsanto) to amphibian larvae at a concentration at the upper end of those measured in the environment and at a concentration expected under a worstcase direct overspray scenario.
Test compound, application procedure, exposure period, protocol	VisionMAX(540 g a.e./L); The experimental treatments were direct application: aqueous glyphosate concentration of 550 µg a.e./L and 2,880 µg a.e./L. The experimental designed employed wetlands split in half with an impermeable plastic barrier and caged juveniles on either side.
Experimental approach Statistical design, test environment	10 semipermanent or permanent wetlands (<1 ha), Each wetland was split in half using an impermeablemplastic barrier; analysis of variance (RM-ANOVA). To characterize the length of the exposure, we calculated the time to 50% dissipation (DT50) of glyphosate in the water column using a first-order reaction rate. Parameters observed: Amphibian growth, Amphibian survival.
Test organisms	Free-swimming larval green frogs (*Lithobates clamitans*); free-swimming larval green frogs (*L. clamitans*)
Biological effects	On day after treatment mean aqueous concentrations were 776 µg a.e./L in the treated sides of wetlands receiving the low treatment level and 3100 µg a.e./L receiving the high treatment level. results show no statistically significant deleterious effects of this herbicide at either the high or the low treatment level on any of the response parameters that were measured.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative for the substance being assessed?	Commercial formulation. Differences in the toxicity of co-formulants preclude making general conclusions across all formulations of glyphosate herbicides.
2 Do the tested concentrations relate to environmental concentrations ?	These two target concentrations represent a probabilistic estimate of the highest concentrations likely to be observed in forestry wetlands under an inadvertent overspray scenario (low) and a reasonable worstcase overspray scenario (high).
3 Have parameters influencing the endpoints been considered?	Interspecific competition was discussed, nevertheless environmetal conditions during the study phase are not stated.
Concluding weight of evidence/proposed action	As one wetland was assigned both as the control and the other side as the treatment the integrity of the impermeable barriers might be compromised. Nevertheless, leakage was demonstrated to be two orders of magnitude below the lowest concentrations. It seems that trend lower toxicity can being observed in field studies compared to laboratory and mesocosm studies.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Navarro-Martín, L. et al. (2014)

Dok. Nr. 2716856	**Navarro-Martín, L. et al.**	**2014**	**Effects of glyphosate-based herbicides on survival, development, growth and sex ratios of wood frogs (Lithobates sylvaticus) tadpoles. I: Chronic laboratory exposures**	**Aquatic Toxicology 154 (2014) 278–290**

			to VisionMax®	
RelaReliability				
Purpose of the study Description of endpoints	Developmental rates, growth, and the expression of genes involved in the thyroid and stress axes were measured at different developmental stages to assess the effects of VisionMax®on *L. sylvaticus* development and metamorphosis at the molecular level. In addition, sex ratios and gonadal morphology were analyzed to study any possible effects ofVisionMax® on *L. sylvaticus* gonadal differentiation.			
Test compound, application procedure, exposure period, protocol	VisionMax®(VM®; 540 g acid equivalents (a.e.)/L as the potassiumsalt). Tadpoles Gosner stage 25 to 42. Concentrations of VisionMax® ranging from 0.021 to 2.9 mg acidequivalents/L.			
Experimental approach Statistical design, test environment	Partial water renewal three times a week (6 L/aquarium). Glyphosate concentrations were measured with LC/MS.; Morphometrics, tissue collection, sex ratios and gonadalhistology; RNA isolation and cDNA synthesis; Analysis of mRNA levels by real time RT-PCR.			
Test organisms	wood frogs (*Lithobates sylvaticus*) tadpoles			
Biological effects	Survival in the VM®(2.9) was 77%, significantly lower than in the control. Animals that reached (Gs42) ranged between 78% and 88.0% in all treatments with no significant differences observed except for VM®(2.9). Tadpoles exposed to VM®(2.9) weighed 1.3-times more than control tadpoles. Treated tadpoles had longer tails at Gs35 and Gs42, although significant differences were detected only at Gs42. No significant changes in sex ratios were observed between treat-ments and control group. An increase in brain trβand dio3 mRNA levels between all stages studied.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?		yes		
2 Is the magnitude of effects of biological significance?		yes		
3 Is the ecotoxicological manifestation level appropriate for the assessment?		No LC50 value determined, probably a surrogate could be derived.		
Environmental Relevance				
1 Is the substance tested representative for the substance being assessed?	Commercial formulation. Differences in the toxicity of co-formulants preclude making general conclusions across all formulations of glyphosate herbicides.			
2 Do the tested concentrations relate to environmental concentrations ?	The highest concentration chosen was based on the predicted max. environmental concentration (PMEC), based on max. label rates (4.3 kg a.e./ha) used for typical agricultural and forestry use patterns.			
3 Have parameters influencing the endpoints been considered?	-/-			
Concluding weight of evidence/proposed action	Results indicate that chronic exposures to VisionMax® in the laboratory have direct effects on the metamorphosis of *L.sylvaticus* tadpoles by decreasing development rates and meta-morphic success. Analysis of mRNA levels indicates that exposure to VisionMax® affects key genes linked to the hormonal cascade inducing metamorphosis in developing anuran tadpoles. Further studies are needed to determine if the changes of mRNA levelsof these key genes are translated to sublethal long-term effects as metamorphs reach adult stages.			
Type of information (Critical, supporting, low weight)	Supporting information for the assessment of effects of glyphosate-based formulated products in case of worse case, chronic exposure.			
Consideration/concluding score	**UBA2**			

Relyea RA. et al. (2009)

Dok. Nr.	**Rick A. Relyea**	**2009**	**A cocktail of contaminants: how mixtures of pesticides at low concentrations affect aquatic communities.**	**Oecologia (2009) 159:363–376**

RelReliability				
Purpose of the study Description of endpoints	Study on how a single application of five insecticides (malathion, carbaryl, chlorpyrifos, diazinon, and endosulfan) and five herbicides (glyphosate, atrazine, acetochlor, metolachlor, and 2,4-D) at low concentrations (2–16 p.p.b.) affected aquatic communities composed of zooplankton, phytoplankton, periphyton, and larval amphibians (gray tree frogs, Hyla versicolor, and leopard frogs, Rana pipiens).			
Test compound, application procedure, exposure period, protocol	technical grade Glyphosate, nominal concentrations of 10 p.p.b			
Experimental approach Statistical design, test environment	Outdoor mesocosms, Examination of each pesticide alone, a mix of insecticides, a mix of herbicides, and a mix of all ten pesticides; multivariate analysis.			
Test organisms	Aquatic communities composed of zooplankton, phytoplankton, periphyton, and larval amphibians (gray tree frogs, *Hyla versicolor*, and leopard frogs, *Rana pipiens*)			
Biological effects	Glyphosate application did not significantly changes in the survival, mass at metamorphosis, and time to metamorphosis of leopard frog tadpoles (Rana pipiens) or gray tree frog tadpoles (Hyla versicolor) in outdoor mesocosms.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	yes			
2 Is the magnitude of effects of biological significance?	yes			
3 Is the ecotoxicological manifestation level appropriate for the assessment?	-/-			
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?	Glyphosate 98 %			
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes			
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	Temperature, pH, and dissolved oxygen were analysed.			
Concluding weight of evidence/proposed action	Author concludes that when pesticides were combined, the mix of herbicides had no negative effect on the survival and metamorphosis of amphibians, but the mix of insecticides and the mix of all ten pesticides eliminated 99% of leopard frogs.			
Type of information (Critical, supporting, low weight)	**low weight**			
Consideration/concluding score	**UBA3**			

Bernal et al. (2009)

Dok. Nr.	**M. H. Bernal a , K. R. Solomon b & G. Carrasquilla**	**2009**	**Toxicity of Formulated Glyphosate (Glyphos) and Cosmo-Flux to Larval and Juvenile Colombian Frogs 2. Field and Laboratory Microcosm Acute Toxicity**	**Journal of Tox. and Environ.l Health, Part A: Current Issues, 72:15-16, 966-973**
Reliability				

Purpose of the study Description of endpoints	The study was conducted to compare the toxicity of the mixture of Glyphos and Cosmo-Flux to larval amphibians as reported under laboratory conditions to responses under similar field conditions where sediments and suspended particles are present in shallow water systems.
Test compound, application procedure, exposure period, protocol	Glyphos and Cosmo-Flux
Experimental approach Statistical design, test environment	Animals were placed in outdoor microcosms made from polyethylene plastic fish ponds (2.07 m in diameter, 37 cm high) in an experimental area. After up to 100 tadpoles of each frog species were placed in the microcosms, they were sprayed with the coca mixture at concentrations greater and less than the normal application rate (3.69 kg glyphosate a.e./ha).
Test organisms	Gosner stage 25 tadpoles of *Rhinella granulosa*, *R. marina*, *Hypsiboas crepitans*, and *Scinax ruber;*
Biological effects	Mortality at 96 h in the control microcosms was between 0 and 16% and LC50 values were between 8.9 and 10.9 kg glyphosate a.e./ha (equivalent to initial concentrations of 5963 to 7303 mg glyphosate a.e./L). Mortality >LC50 was only observed in the tested species when the application rate was >2- fold the normal application rate.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation containing POEA. Results Glyphos and Cosmo-Flux can be used for the assessment of glyphosate formulations with POEA.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence/proposed action	The study presents the mortality data for the terrestrial stages of eight species of frogs are as nominal application rate (kg ae glyphosate / ha) in order to allow a comparison with field application rates. Mortality at the rate of 3.69 kg ae /ha, which corresponds to the maximum annual application rate was between 15% and 35% and the LC50 values 3-8-fold higher than the application rate . With a application rate of 1.85 kg ae / ha, which corresponds to the simple application rate of an application, the mortality rates were in were 3 of 8 experiments between 15% and 35% and the LC50 values 3-8 times higher than that application rate. The authorization procedure currently has no harmonized models and evaluation approaches, but would currently –with derivation of safety factors- not provide adequate protection of amphibians.
Type of information (Critical, supporting, low weight)	**Critical**
Consideration/concluding score	**UBA1-2**

B.9.13 10.1 Summary of the relevant literature on amphibians

Since the 2001 EU glyphosate evaluation, a number of acute and chronic amphibian toxicity studies with glyphosate and commercial glyphosate-based formulations have been published. For this review of the literature, acute studies are considered to be 96 hours or less. Chronic studies did mostly evaluate lethality, though, coupled with the investigations of glyphosate formulations on weight and/or performance at metamorphosis. Only few studies assessed the toxicity of glyhosate formulation on terrestrial stages of amphibians.

Most of the acute and chronic amphibian study results are from laboratory toxicity tests. However, some of the studies were performed in the field using in situ enclosures or field mesocosms to assess impacts under representative natural conditions, and chemical and biological monitoring studies conducted under conditions directly relevant to product use. Studies were evaluated based on criteria of reliability and relevance/adequacy for risk assessment.

A significant amount of research exists on the toxicity of glyphosate and several glyphosate-based formulations for amphibian.
Acute studies with glyphosate acid and glyphosate IPA for sensitive Gosner stage 25 tadpoles show medial lethal values comparable to values obtained with fish in regulatory studies and in the literature. The LC_{50} values for amphibian exposed to glyphosate and its salts range from >17.9 to >466 mg a.s./L (see table below).

Table B.9.13-76: Effect values reported in peer reviewed literature for amphibians: glyphosate acid and salts of glyphosate

Species	Substance	Study duration (h)	LC_{50} (mg a.s./L)	Reference
Amphibians				
Crinia insignifera tadpole	Glyphosate acid	96	103.2	Bidwell and Gorrie 1995 glyphnosubm_023
Crinia insignifera adult	Glyphosate acid	96	75.0	Bidwell and Gorrie 1995 glyphnosubm_023
Litoria moorei tadpoles	Glyphosate acid	48	81.2	Mann and Bidwell 1999 glyphnosubm_024
Litoria moorei tadpoles	Glyphosate acid	48	121.0	Mann and Bidwell 1999 glyphnosubm_024
Crinia insignifera adult	Glyphosate acid	48	83.6	Mann and Bidwell 1999 glyphnosubm_024
Rana clamitans	Glyphosate IPA	96	>17.91	Howe et al., 2004 glyphecotox_025
Lymnodynastes dorsalis tadpoles	Glyphosate IPA	48	>400.0	Mann and Bidwell 1999 glyphnosubm_024
Litoria moorei tadpoles	Glyphosate IPA	48	>343.0	Mann and Bidwell 1999 glyphnosubm_024
Crinia insignifera tadpole	Glyphosate IPA	48	>466.0	Mann and Bidwell 1999 glyphnosubm_024

Species	Substance	Study duration (h)	LC_{50} (mg a.s./L)	Reference
Amphibians				
Heleioporus eyrei tadpole	Glyphosate IPA	48	>373.0	Mann and Bidwell 1999 glyphnosubm_024

The effects of different glyphosate-based formulations on amphibian survival have been evaluated on almost 30 species of amphibians (e.g. Howe et al., 2004; Cauble and Wagner, 2005; Dinehart et al., 2010; Edginton et al., 2004; Jayawardena et al., 2001; Jones et al., 2010 and 2011; Relyea 2012, Moore et al., 2012; Meza-Joya et al. 2013).The literature review by Wagner et Lötters . 2013 provide a literature study of correlative allusions between long-term amphibian population trends and agricultural factors.

The medial lethal concentration for amphibian exposed to formulation of glyphosate containing specific surfactant classes are far lower than for glyphosate acid or its salts (see Table B.9.13-5). The surfactants displaying a high toxicity in glyphosate-based formulations belong usually to the classes of polyoxyethoxylated alkylamines (POEA; e.g. ethoxylated tallow- and cocoamines), or are e.g. fatty nitrogen derivate etheramine. For the implications resulting from these obervations, please see chapter B.9.13 16.1 (Surface active substances in glyphosate-based formulations).

Table B.9.13-5: Effect values reported in peer reviewed literature for amphibians: glyphosate formulations and surfactants; GLY: glyphosate; POEA: polyoxyethoxylated alkylamine; w: with; w/o: without

Species	Substance	Study duration (hours or days)	LC_{50} (mg a.e./L)	Reference
Amphibians				
Rana pipiens; Gosner 25	Roundup Original GLY w POEA	96 h	2.9	Howe et al., 2004 glyphecotox_025
Rana sylvatica, Gosner 25		96 h	5.1	
Bufo americanus; Gosner 25		24 h	4.2	
		48 h	<4.0	
Rana clamitans Gosner 25		48 h	2.0	
Rana clamitans Gosner 25	POEA	48 h	2.2	Howe et al., 2004 glyphecotox_025
Rana clamitans Gosner 25	Roundup Bioactive ® GLY w/o POEA	48 h	> 17.9	Howe et al., 2004 glyphecotox_025
Rana clamitans Gosner 25	Touchdown ® GLY w/o POEA	48 h	> 17.9	Howe et al., 2004 glyphecotox_025
Rana clamitans Gosner 25	- Glyfos BIO ® - GLY w/o POEA	48 h	> 17.9	Howe et al., 2004 glyphecotox_025
Rana cascadae tadpole	Roundup Original? GLY w POEA	48 h	3.2	Cauble and Wagner, 2005 glyphecotox_049
Spea bombifrons Gosner 29	Roundup WeatherMAX® GLY w/o POEA; w surfactants of POEA similar toxicity	48 h	1.9	Dinehart et al., 2010 glyphecotox_064
Spea multiplicata Gosner 29		48 h	2.1	
Xenopus laevis Gosner 25	Vision® GLY w POEA	96 h	0.9	Edginton et al., 2004 glyphecotox_066 [a)]
Bufo americanus Gosner 25		96 h	1.7	
Rana clamitans Gosner 25		96 h	1.4	
Rana pipiens Gosner 25		96 h	1.1	
Scinax nascius Gosner 25	Glyfos® GLY w POEA?	96 h	2.6 [b)] 1.3	Lajmanovich et al., 2003 glyphecotox_078
Rhinella arenarum; Gosner 25	Glifosato ATANOR® Glifoglex ®	96h	19.4 72.8	Brodeur, J. C., et al. 2014; Dok. Nr. 2716828
Rhinella arenarum Gosner 25	Roundup UltraMAX® GLY w/o POEA; w surfactants of POEA similar toxicity	48 h	2.4	Lajmanovich et al. 2011 glyphecotox_080
Lithobates sylvaticus	Roundup Weed and Grass Killer®	96h	0.70-1.10	Edge et al. 2014
	Roundup Weather-Max®	96h	4.94-8.26	

Species	Substance	Study duration (hours or days)	LC_{50} (mg a.e./L)	Reference
Amphibians				
Rana pipiens	Roundup®	96h	1.8	Fuentes, L. et al.2014
Rana sphenocephala	Roundup Weather-Max®	96h	1.33	
Rhinella marina Gosner 25 *Physalaemus centralis Gosner 25*	Roundup Original? GLY w POEA	96h	32.00 19.70	Figueiredo, J., Rodrigues, D.J. (2014)
Rana sylvatica Gosner 25	Roundup Original MAX® GLY w/o POEA w surfactants of POEA similar toxicity	21 d	2.9	Relyea, 2012 glyphecotox_088
Rana pipiens Gosner 25		21 d	2.9	
Bufo americanus Gosner 25		21 d	2.5	
Rana sylvatica Gosner 26	Roundup Original MAX®, GLY w/o POEA; w surfactants of POEA similar toxicity	18 d	2.1	Jones et al., 2010 glyphecotox_075 [c)]
Bufo americanus Gosner 26		18 d	2.3	
Rana catesbeiana Gosner 25	Roundup Original MAX®, GLY w/o POEA; w surfactants of POEA similar toxicity	23 d	2.2	Jones et al., 2011 glyphecotox_076 [d)]
Rana clamitans Gosner 25		23 d	2.6	
Hyla versicolor Gosner 25		23 d	2.0	

a) Values reported for test series with pH 7.5; lower toxicity at pH 6.0
b) Value refer to mg formulation/L
c) Values reported for early application day 0; lower toxicity with split applications
d) Values reported for low animal density (single species); higher toxicity if kept at higher densities

Comparable to the findings regarding glyphosate as salt or acid, also the range in LC_{50} values reported for tadpoles when exposed to formulations of glyphosate is comparable to the range of LC_{50} values reported for fish. A first mechanistic explanation proposed why fish and tadpoles have very similar acute sensitivities to the surfactants that are added to glyphosate-based formulations relates to the toxic mode of action of surfactants. Increasing the permeability of cell membranes, addition of surfactants result in loss of osmotic or ionic stability at the gill. Consequently, the mode of action of surfactants to aquatic organisms could explain why the range of sensitivities for amphibians and fish in acute tests are similar when exposed to comparable glyphosate-based formulations.

It should be noted here that only few data assessing the effect of glyphosate and glyphosate-based products on terrestrial stages of amphibians are available. Berger et al. 2013 could show

that amphibians regularly co-occur with pre-sowing/ pre-emerging applications in spring and with stubble management prior to crop sowing in late summer and autumn. Moreover siccation treatment in summer coincides with early pond-leaving juveniles. Therfore, it is recommended that investigations on amphibian populations should not only focus on exposure during aquatic periods but also consider terrestrial life stages.

Studies with other products (Brühl et al. 2013; Belden et al. 2010) have shown that terrestrial stage of amphibians do experience environmental concentration far higher than a medial lethal rate (LR_{50}) at authorized field uses. Therefore, if the acute risk for the aquatic stages of amphibian seem to be covered by a proper assessment of the risk to fish, this is not the case for the terrestrial stages. The risk assessment for bird and mammals has long been taken also as protective for amphibian in terrestrial environments. Since it has been shown by Brühl et al. (2013) that juvenile amphibian exposed to other products die at authorized field rates - and for some products even at 1/10 of field rates - the conclusions of the risk assessment for birds and mammals for a specific product do not cover necessarily the risk of exposed terrestrial amphibian stages.

Further studies evaluated were performed in the field in controlled enclosures (Thompson et al., 2004; Edge et al., 2011, 2012; Wojtaszek et al., 2004) employing specific formulations for forest applications with overspray scenarios for surface water ponds. These studies were not performed with dose-response design and report amphibian survival rates and other parameters at given concentrations supposed to be environmentally relevant. Since exact exposure scenario was not always quantifiable and mean lethal concentrations mostly not reached in the chosen study design, these results are not directly utilizable for the ecotoxicological assessment of glyphosate formulations for amphibians. Bernal et al. (2009) presents mortality data derived from an outdoor microcosm for the terrestrial stages of eight amphibian species in order to allow a comparison with field application rates. For the tested glyphosate-based formulations (containing POEA) the observed LC_{50} values were 3-8-fold higher than the max. annual application rate.

Regarding chronic toxicity enpoints that do not relate merely to long-term effects on amphibian survival rate, Cauble and Wagner (2005) studied the effects of glyphosate formulations on larval methamorphosis. In glyphosate treatments (1 mg a.e./L), there are indications of earlier metamorphosis and smaller size of *Rana cascadae* when compared to the control.

Also Howe et al. (2004) monitored in lab studies several chronic enpoints (e.g. forelimb emergence, tail damage and maximum tail height, snout-vent-length of metamorphs; gonadal histology to determine sex ratios). Significant tail damages and reduced tail lengths were recorded in treatments with the Roundup Original ® formulation and in treatments with the surfactant POEA. No effects on chronic endpoints were determined when the amphibian were exposed to glyphosate alone. POEA containing formulations showed displaced sex ratios towards intersex individuals. Again, this was not observed in treatment with glyphosate technical (Moore et al. 2012, Lajmanovich, et al. 2013) However, results were not always strictly dose-dependent.

Furthermore research was performed on the biochemical responses of tadpoles to environmental relevant concentrations of commercial formulations and pesticide mixtures (Ruamthum et al. 2011; Wagner et Lötters, 2013; Brodeur, et al. 2014; Katzenberger, et al.,

2014; Hanlon et al. 2013; Navarro-Martín, L. et al. 2014). Effects on biochemical responses like changes on mRNS level, activity levels of the enzyme or thermal performances are currently considered as supporting information on commercial products in the field of environmental risk assessment. Nevertheless, it is evident that further studies are needed to determine if the changes on biochemical traits are translated to sublethal long-term effects.

The studies by Cauble and Wagner (2005) and Howe et al. (2004) have been criticized by the Notifier as regards to experimental and/or reporting deficiencies. Not all critical points are shared by RMS. RMS believes that the findings pointing at chronic toxicity of surfactants in glyphosate-based formulations are not exaustively resolved by a critique of the study set up. Even if the cited studies suffer from experimental difficulties, the results indicate effects of ethoxylated surfactants on amphibian metamorphosis. The implications of these findings for the potential registration of glyphosate-based formulation with surfactants of significant toxicity are discussed in chapter B.9.13.16.

The lead formulation for the assessment of glyphosate as active substance does not contain surfactants of overt toxicity.

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B.9.13.11 Bees

For a detailed description and evaluation of acceptability and validity of the study please refer to Vol. 3, chapter B.9.4.

B.9.13.12 Effects on other arthropod species (KIIA 8.16)

For the group of terrestrial non-target arthropods (NTA), a database of 31 publications was collected by the notifier. The notifier considered none publications to be rated to be acceptable for risk assessment. The submitted publications were also evaluated by zRMS and have been assigned according to an UBA screening. 11 studies were recognized as information with low weight (category UBA3) and 7 publications (Bueno et al., 2011; Benamu et al., 2010; Evans et al., 2010; Michalkova et al., 2009; Schier A., 2006, Renaud et al., 2004; Santos et al., 2010) have been considered as supportive information (UBA2). Moreover, after the date of submission, during the administrative process and the public launching period of the evaluation a few more peer-reviewed relevant articles were newly published (mainly period of 2012-2014) and needed to be considered in a revised DAR. Within these references, additional studies focusing on arthropod have been considered as supporting information for risk assessment.

The following sections summarise the results and discussion as found in the published literature. View of the RMS will be given in the final summariesing chapter after the results of the study are presented and discussed.

Addison, P.J., Barker, G.M. (2006)

glyphecotox_266	**Addison, P.J., Barker, G.M.**	**2006**	**Effect of various pesticides on the non-target species** *Microctonus* **hyperodae, a biological control agent of** *Listronotus bonariensis*	**Entomologia Experimentalis Et Applicata 119 (1):71-79**

Reliability	
Purpose of the study Description of endpoints	Four experiments were conducted to investigate the effects of various pesticides that are commonly used in the pastoral environments of L. Bonariensis and M. hyperodae
Test compound, application procedure, exposure period	glyphosate (Roundup®, Monsanto Co., St. Louis, MO, USA), or the adjuvant Silwett L-77m(Pulse, EI DuPont de Nemours and Co. Inc., Wilmington, DE, USA) were mixed with water according to their label recommendations
Experimental approach, Statistics, test environment	Field experiment
Test organisms	*Microctonus hyperodae*
Biological effects	Silwett L-77, an organo-silicone copolymer penetrant and surfactant, was the only treatment to significantly increase M. hyperodae mortality compared to that of the water-treated controls. The herbicidal products had no demonstrable effect.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	no
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	no
3 Is the ecotoxicological manifestation level appropriate for the assessment?	no
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Species are not relevant for central zone, no data presented for glyphosate
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Albajes et al. (2011)

glyphecotox_274	**Albajes, R., Lumbierres, B., Pons, X.**	**2011**	**Albajes, R., Lumbierres, B., Pons, X.**	**Biological Control 59 (1):30-36. DOI 10.1016/j.biocontrol.2011.03.008.**

Reliability	
Purpose of the study Description of endpoints	The study aimed to compare arthropod densities in GMcorn plots treated with a broad-spectrum herbicide or with a conventional selective pre-emergence treatment.
Test compound, application, exposure, protocol	MON 78044 at 3 l/ha
Experimental approach, Statistical design, test environment	Field experiment, Each year, the number of predators and main herbivore prey (leafhoppers, aphids and phytophagous thrips) were counted: ANOVA
Test organisms	Orius spp., Nabis sp.
Biological effects	Authors conclude that no significant changes in heteropteran predator densities may be expected from moderate alterations in weeds arising from the deployment of herbicide- tolerant corn varieties and that leafhoppers are probably the herbivore prey that most influences Orius spp. densities in corn in our study area.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	no
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	no significant changes observed, GM corn tested
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Bueno et al. (2011)

glyphecotox_305	**Bueno, A.F., Bueno, R.C.O.F., Parra, J.R.P., Vieira, S.S.**	**2011**	**Effects of pesticides used in soybean crops to the egg parasitoid** *Trichogramma pretiosum*	**Ciencia Rural 38 (6):1495-1503**

Reliability	
Purpose of the study Description of endpoints	This research aimed to study the effects of different insecticides, herbicides and fungicides on eggs, larvae and pupae of *Trichogramma pretiosum*
Test compound, application procedure, exposure period, protocol	glyphosate 960 grams ha-1 (Gliz® 2000 milliliters ha-1); glyphosate 972 grams ha-1 (Roundup® Ready® 2000 milliliters ha-1); glyphosate 960 grams ha-1 (Roundup® Transorb® 1500 milliliters ha-1); glyphosate 960 grams ha-1 (Roundup® Original® 2000 milliliters ha-1);

Experimental approach, Statistical design, test environment	Laboratory: Cardboard squares (1cm2) with approximately 250 *A. kuehniella* eggs each were offered for 24 hours to recently emerged *T. pretiosum* females in vials. Then, these cards were transferred to vials and kept until the time after parasitism was sprayed with the treatments that were: 72 hours (eggs), 144 hours (larvae), 192 hourspupae) (MANZONI et al., 2007).
Test organisms	*Trichogramma pretiosum*
Biological effects	glyphosate 960.0 (Gliz® and Roundup® Transorb®), was classified as harmless to all imature *T. pretiosum* stages.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	Reduction of parasitism viability comparared to the untreated was 100 % for Roundup® Ready.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial products
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Glyphosate (Roundup® Ready) 972 grams ha-1 were classified as harmfull for T. pretiosum eggs and harmless to the other parasitoid stages . Glyphosate 960 (Roundup® Original) was classified as slightly harmful for eggs and harmless for pupae of the parasitoid .
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Lipok, J. (2009)

glyphecotox_462	**Lipok, J.**	**2009**	**Dual action of phosphonate herbicides in plants affected by herbivore-Model study on black bean aphid Aphis fabae rearing on broad bean Vicia faba plants**	**Ecotoxicology and Environmental Safety 72 (6):1701-1706. DOI 10.1016/j.ecoenv.2009.03.007.**

Reliability	
Purpose of the study Description of endpoints	The paper describes the sensitivity of blackaphid *A.fabae* towards tested herbicides as the nutrients and the influence of herbicide-treated plants of broad bean *V. faba* L. on the host plant choice and population development of black aphid *A.fabae*. The combined effect of sublethal doses of herbicides and presence of aphids on the growth of broad bean plants was also investigated.
Test compound, application procedure, exposure period, protocol	Each of four tested compounds was studied at three concentrations: 15, 1.5 and 0.015mM using two means of treatment. Pure glyphosate (N-phosphonomethylglycine) was obtained by the author via laboratory procedure: from commercial Roundup®s 360 SL(Monsanto, MO,U SA) formulation by dissolving in water and maintaining the pH of the solution to 1.5–2.0 with hydrochloric acid. This resulted incrystallisation of the pure herbicide. Its structure and purity were confirmed using 1H, 13C and 31P NMR spectroscopy. The retention ime of this substance

	incapillary electrophoresis was the same as the retention time of glyphosate standard obtained from Monsanto.
Experimental approach, Statistical design, test environment	The experimental system was composed of phosphonate herbicides, broad bean *Vicia faba* (L.) plants and blackbean aphid *Aphis fabae (Scopoli*). Two mean of herbicide application, namely standard spraying and direct introduction of the herbicide into stem via glass capillary, were examined.
Test organisms	*Aphis fabae* , *Vicia faba*
Biological effects	Reaction of aphids towards artificial diet supplemented with herbicides The insects, which were settled on the artificial diet supplemented with glyphosate, tended to escape from the membranes or were dead at the second or fourth day of experiment. Studies on aphids cultured on artificial diet supplemented with herbicides revealed that influence negatively the insect development most likely exhibiting weak insecticidal activity.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Authors state that the herbicides decreased the rate of growth and development of the aphid populations, most probably by exhibiting weak insecticidal activity.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Active ingredient
2 Do the tested concentrations relate to predicted environmental concentrations?	15, 1.5 and 0.015mM
3 Have parameters influencing the endpoints been considered adequately?	
Concluding weight of evidence/proposed action	Number of aphids accompanied with treated plants cannot be used for risk assessment, no effects observed.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Evans et al. (2010)

glyphecotox_147	**Evans, S.C., Shaw, E.M., Rypstra, A.L.**	**2010**	**Exposure to a glyphosate-based herbicide affects agrobiont predatory arthropod behaviour and long-term survival**	**Ecotoxicology 19: 1249-1257**

Reliability	
Purpose of the study Description of endpoints	Study quantifies the effects of a commercial formulation of a glyphosate-based herbicide on the activity of three predatory arthropod species that inhabit agricultural fields in the eastern United States. Authors measured the survival of the most common species.
Test compound, application procedure, exposure period, protocol	commercially formulated herbicide solution (Buccaneer Plus) containing 41% (480 g/l) glyphosate (N-(phosphonomethyl)glycine) isopropylamine salt and 59% other ingredients, including a polyethoxylated tallowamine (POEA) surfactant
Experimental approach, Statistical design, test environment	We tested the reactions of the wolf spider, Pardosa milvina, to either direct application (topical) or contact with a treated substrate (residual). We quantified the reactions of a larger wolf spider, Hogna helluo, and a ground beetle, Scarites quadriceps, to a compound (topical plus residual) exposure.

Test organisms	wolf spider *Pardosa milvina*, wolf spider, *Hogna helluo*, ground beetle, *Scarites quadriceps*
Biological effects	Exposure of terrestrial arthropods to glyphosate-based herbicides affects their behaviour and long-term survival.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*Pardosa spp.*
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	Results suggest that herbicides can affect arthropod community dynamics separate from their impact on the plant community and may influence biological control in agroecosystems.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Activity metrics recorded,
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	commercially formulated product containing POEA
2 Do the tested concentrations relate to predicted environmental concentrations?	12 g/l of the glyphosate salt, higher than the expected drift rates
3 Have parameters influencing the endpoints been considered adequately?	Laboratory approach
Concluding weight of evidence/proposed action	No endpoints on mortality. Tested concentrations higher thatn expected drift rates. But authors demonstrate that arthropod predators inhabiting agroecosystems around the world exhibit subtle shifts in behaviour and reproduction during or after exposure to herbicide.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Griesinger et al. (2010)

glyphecotox_148	**Griesinger, L.M., Evans, S.C., Rypstra, A.L.**	**2010**	**Effects of a glyphosate-based herbicide on mate location in a wolf spider that inhabits agroecosystems**	**Chemosphere 84: 1461 - 1466**

Reliability	
Purpose of the study Description of endpoints	The aim of this study was to examine effects of a commercial formulation of a glyphosate-based herbicide on the ability of males to find females.
Test compound, application procedure, exposure period, protocol	Roundup®_ II Original. This herbicide is manufactured by Monsanto, St Louis, MO, USA (United States Patent US4528023). As provided, this herbicide contains 41% (480 g L_1) glyphosate (N-(phosphonomethyl)glycine) isopropylamine salt and 59% other ingredients, including a polyethoxylated tallowamine (POEA) surfactant. For the pitfall experiment, we diluted it with distilled water to a concentration of 2.5% (12 g L_1 of the glyphosate salt).
Experimental approach, Statistical design, test environment	Field experiment , Pitfall experiment, In one pair of treatments we applied 5 lL of either distilled water or herbicide solution to the filter paper inside the vial with the female. In another two treatments, we applied 0.926 mL of either distilled water or herbicide solution to the ring of filter paper surrounding the cup.

Test organisms	wolf spider, *Pardosa milvina*
Biological effects	Traps with herbicide on the filter paper inside with the female captured fewer males.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	wolf spider, *Pardosa milvina*
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	Our experiments suggest that a commercial formulation of a glyphosate-based herbicide affects mate location in a wolf spider that is common in agroecosystems where these chemicals are routinely applied. however, the circumstances under which these effects influence population viability, community structure, and/ or the food web remain to be explored
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Reduction of the efficacy of natural infochemicals important to mate location in *Pardosa milvina*has probably minor impact on population and communities of spiders.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product containing POEA
2 Do the tested concentrations relate to predicted environmental concentrations?	Drift rates are predicted to be lower.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Behavioral study , with POEA containing product. Tested concentrations probably higher than drift rates.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Michalková, V., Pekár, S. (2009)

glyphecotox_506	**Michalková, V., Pekár, S.**	**2009**	**How glyphosate altered the behaviour of agrobiont spiders (Araneae: Lycosidae) and beetles (Coleoptera: Carabidae)**	**Biological Control 51 (3):444-449**

Reliability	
Purpose of the study Description of endpoints	Aim of the study was to assess the effect of the Roundup® residues on the predatory, defensive, locomotory and reproductive behaviour of epigeic spiders and carabid beetles
Test compound, application procedure, exposure period, protocol	Roundup® Biaktiv (Monsanto; glyphosate, IPA 480 g l_1). The formulation wasdiluted in water using a rate (1:25) recommended for use in cereals. A piece (5 _ 5.5 cm) of the filter paper (Whatman 2R/80 g) wasdipped into the solution and gave rise to two different residues: freshand 1-day old, thepapers were rolled to form a tube and inserted into 10 ml glass tube.Inside of the paper roll a spider or a beetle was kept for 2 h to maximiseits contact with the residues.
Experimental approach, Statistical design, test environment	Locomotory and reproductive behaviour of epigeic spiders and carabid beetles. Specimens of *Pardosa* Agricola (Araneae: Lycosidae) and *Poecilus cupreus* (Coleoptera: Carabidae) were exposed for 2 h to the fresh and 1-day old residues of Roundup® Biaktiv (Monsanto, IPA 480 g/l).
Test organisms	*Pardosa* and *Poecilus*
Biological effects	Capture and consumption of flies by *Pardosa* spiders did not differ between spiders exposed to any herbicide residues and the control surface, *Pardosa* spiders ran slightly slower after being exposed to herbicide residues but the difference was not significant, But *Poecilus* beetles exposed to both types of herbicide residues moved significantly slower than those exposed to the control surface, no effects on avoidance and defence, no qualitative difference in mating behaviour.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*Pardosa* and *Poecilus* are standard test species in RA
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	Roundup® Bioaktiv thus appears to be harmless to lycosid spiders and only slightly harmful to carabid beetles. The biological control potential of both predators should not be reduced directly by the application of Roundup® Bioaktiv.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?	Recommended in field rate was used.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	*Pardosa* and *Poecilus* are standard test species in RA, predation rate, locomotion speed, avoidance, defence and mating behavior nor standard parameters. Biological control potential of the two species should not be directly reduced following herbicide application in the field.
Type of information (Critical, supporting, low weight)	**supporting**

Consideration/concluding score	**UBA2**

Schier, A. (2006)

glyphecotox_595	**Schier, A.**	**2006**	**Field study on the occurrence of ground beetles and spiders in genetically modified, herbicide tolerant corn in conventional and conservation tillage systems**	**Journal of Plant Diseases and Protection. Special Edition XX:101-113**

Reliability	
Purpose of the study Description of endpoints	The objective of this study was to analyse and compare the impact on weed control and arthropod abundance of conventional and conservation tillage methods under different herbicide regimes. The study was conducted between 2002 and 2005 on continuously planted Roundup® Ready® (RR) corn.
Test compound, application procedure, exposure period, protocol	MON 78044 (Glyphosate, 360g/l) , Roundup® Ready®
Experimental approach, Statistical design, test environment	field experimental design, Pitfall traps were used to survey populations of soil dwelling arthropods
Test organisms	soil dwelling arthropods
Biological effects	The results of this multi year study indicate that the combination of conserva-tion tillage and herbicide tolerant corn has a positive impact on biodiversity
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	soil dwelling arthropods
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	no
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product with unknown surfactant chemistry included. Therfore limited validity for other products and the active substance glyphosate itself.
2 Do the tested concentrations relate to predicted environmental concentrations?	Not stated.
3 Have parameters influencing the endpoints been considered adequately?	Field study with climatic extremes and uncertainties.
Concluding weight of evidence/proposed action	Field study with climatic extremes and uncertainties. The results of this multi year study indicate that total abundance of ground beetles and spiders were not affected due to reduced soil tillage combined with glyphosate treatment.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Ainsworth, N. (2003)

glyphecotox_272	**Ainsworth, N.**	**2003**	**Integration of herbicides with arthropod biocontrol agents for weed control**	**Biocontrol Science and Technology 13 (6):547-570. Doi 10.1080/0958315031000151819.**

Reliability	
Purpose of the study Description of endpoints	This literature review first considers the direct toxic effects of herbicides and surfactants on biocontrol agents.
Test compound, application procedure, exposure period, protocol	nd
Experimental approach, Statistical design, test environment	nd
Test organisms	arthropods
Biological effects	Glyphosate had low, if any, direct toxicity to several biocontrol agents (Ding et al ., 1998;Boersma & Ireson, 1999; Lindgren et al. , 1999; Hayes, 2000b). However, Searle et al. (1990) reported some toxicity to mites, which increased when extra surfactant was added.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	No information relevant for risk assessment
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Renaud et al. (2004)

glyphecotox_570	**Renaud, A., Poinsot-Balaguer, N., Cortet, J., Le Petit, J.**	**2004**	**Influence of four soil maintenance practices on Collembola communities in a Mediterranean vineyard**	**Pedobiologia 48 (5-6):623-630..**

Reliability	
Purpose of the study Description of endpoints	Influence of a) postemergence herbicide with glyphosate; (b) postemergence and pre-emergence herbicides with glyphosate, terbuthylazine, diuron and oryzalin; (c) natural flora and (d) tillage to a depth of10–15 cm was studied.
Test compound, application procedure, exposure period, protocol	Not stated
Experimental approach, Statistical design, test environment	Vineyard called ‘‘le Domaine de Donadille’’ situated at Rodilhan, The vineyard was planted with 15–20 year old Syrah variety vine plants in a silt-clay soil. Sampling took place between December 2000 and June

	2002; no samples were taken in summer due to drought. On each sampling date, six soil samples were taken from the central inter-row space of each treatment plot.
Test organisms	Collembola
Biological effects	The postemergence herbicide glyphosate treatment practice and the natural flora practice practice favoured the development of epigeic and hemiedaphic species,due to preservation of the weed cover. C. denticulate and *L. cyaneus* were favoured in tillage practice.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Abundance and species diversity were assessed.
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	no
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Probably commercial product, no information
2 Do the tested concentrations relate to predicted environmental concentrations?	Glyphosate (15 l /ha)
3 Have parameters influencing the endpoints been considered adequately?	Wheatear and rainfall influence was discussed.
Concluding weight of evidence/proposed action	Total abundance was highest in natural flora practice and in the practice with a postemergence herbicide. Glyphosate treatment weed cover was preserved.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Wrinn et al. (2012)

glyphecotox_650	**Wrinn, K.M., Evans, S.C., Rypstra, A.L.**	**2012**	**Predator cues and an herbicide affect activity and emigration in an agrobiont wolf spider**	**Chemosphere. doi: 10.1016/j.chemosphere.2011.12.030.**

Reliability	
Purpose of the study Description of endpoints	Exploration how Buccaneer Plus, a common herbicide similar to Round-up (active ingredient glyphosate), affected the interactions between intraguild predators.
Test compound, application procedure, exposure period, protocol	BuccaneerPlus, also known as Roundup® II original, created by the Monsanto Company, St. Louis, Missouri, USA (United States Patent US4528023). This herbicide contains the active ingredient glyphosate (480 g /L) in the form of isopropylamine salt, and an added polyethoxylated tallowamine (POEA) surfactant. diluted it to 2.5%, which was within the manufacturer's recommended levels of 0.625–5%, spray rate of 127.4 mL m_2 (or 15.3 kg a.i. ha_1 of glyphosate), which was the minimum necessary to gain a complete and uniform coverage of the areas for the laboratory container with filter paper.
Experimental approach, Statistical design, test environment	Arthropods were collected within 3 d after herbicide application in the field, and were not used in experiments until 2 months after the date of last herbicide application. Laboratory arena for exposing Pardosa milvina to herbicide and/or predator cues. Filter paper pieces with herbicide or water are alternated by those with predator cues (*Hogna helluo or Scarites quadriceps*) or blank paper.

Test organisms	The focal species for these experiments was *Pardosa* milvina (Araneae: Lycosidae), a numerically dominant, epigeal generalist arthropod predator in agricultural fields throughout eastern North America
Biological effects	Predator cues and herbicide led to a decrease in movement by *P. milvina*. However, although *H. helluo* cues alone decreased movement, S. quadriceps cues only decreased movement when combined with herbicide.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*Pardosa milvina*
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product with POEA
2 Do the tested concentrations relate to predicted environmental concentrations?	Tested concentration probably higher than the expected drift rate. application rate was higher than that which would likely be found in a real situation
3 Have parameters influencing the endpoints been considered adequately?	spray rate was not properly controlled
Concluding weight of evidence/proposed action	Authors conclude that predation risk andmherbicide application likely interact to affect the movement of a major arthropod predator.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Benamu et al. (2010)

glyphecotox_146	**Benamu, M.A., Schneider, M.I., Sanchez, N.E.**	**2010**	**Effects of the herbicide glyphosate on biological attributes of Alpaida veniliae (Araneae, Araneidae), in laboratory**	**Chemosphere 78 (7):871-6.**

Reliability	
Purpose of the study Description of endpoints	The purpose of this study was to address the effects of glyphosate on some biological attributes of *A. veniliae*, in laboratory.
Test compound, application procedure, exposure period, protocol	ANOVA, Glifoglex 48 (48% glyphosate, Gleba SA, Buenos Aires, Argentina) was used in toxicity bioassays. Fresh solutions with 192 mg L_1 a.i. (maximum field registered nominal concentration) (CASAFE, 2007).
Experimental approach, Statistical design, test environment	Solutions were prepared using acetone (Analytical Grade) as solvent to assure the evaporation of herbicide solution, considering that spiders avoid feeding on wet preys. The exposure route was by ingestion ‘‘through the treated prey” and the chronic toxicity was analyzed. The prey (*M. domestica* adults) was treated by dipping during 20 s according to Schneider et al. (2009), and dried under fume cupboard.
Test organisms	Arthropod predator *Alpaida veniliae* (Araneae, Araneidae) is one of the most abundant orb web weaver spiders of Argentinia.
Biological effects	Results of this study showed no lethal direct effects of Glifoglex_ on this spider, but it is the first report in literature about sublethal effects of this herbicide on a spider’s biological attributes. Negative effects on prey consumption, web building, fecundity, fertility and developmental time of progeny were observed.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	web weaver spiders of Argentinia
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	Sublethal effects of glyphosate in the laboratory on prey consumption, web building, fecundity, fertility and developmental time of progeny of A. veniliae. Females poorly fed will be affected in their survival, fecundity and fertility, therefore, natural populations of this spider would be seriously affected in its capacity to grow and persist in natural conditions
3 Is the ecotoxicological manifestation level appropriate for the assessment?	prey consumption, web building, fecundity, fertility and developmental time of progeny were analysed, no lethal or reproductive endpoint.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?	In Off field expected drift values are lower.
3 Have parameters influencing the endpoints been considered adequately?	Deficiencies are discussed, parameter (sublethal effects)not reliable for RA, argentinian species.
Concluding weight of evidence/proposed action	Authors conclude that sublethal effects are relevant from an ecological point of view, since the reduction of the arthropod performance may create risks to arthropod biodiversity conservation in agroecosystems.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Castilla et al. (2010)

glyphecotox_311	**Castilla, A.M., Dauwe, T., Mora, I., Malone, J., Guitart, R.**	**2010**	**Nitrates and Herbicides Cause Higher Mortality than the Traditional Organic Fertilizers on the Grain Beetle,** *Tenebrio molitor*	**Bulletin of Environmental Contamination and Toxicology 84 (1):101-105. DOI 10.1007/s00128-009-9883-5.**

Reliability	
Purpose of the study Description of endpoints	The present laboratory study determined mortality of adult beetles (Tenebrio molitor) rates of under different pesticide treatments (a mixture of glyphosate and 2,4-D)
Test compound, application procedure, exposure period, protocol	Mixture of two types of herbicides: 1 L of the isopropylamine salt of Glyphosate (Lograd o, Masso Division Agro), 36% p/v (360 g/L), and 100 cm3 of2,4-D (Agrodan), 80%, in 4 L of water.
Experimental approach, Statistics, test environment	Beetles were placed in manufactured soft aluminium open boxes (16 9 11 9 3 cm).
Test organisms	Grain Beetle, *Tenebrio molitor*
Biological effects	Using a binary mixture makes it difficult to deduce the individual effect of each herbicide to the insect.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The individual effect s of each herbicide to the insect cannot be assigned.
Environmental Relevance	

1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Herbicide mixture, deficiencies in test design,
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Bernard et al. (2010)

glyphecotox_296	**Bernard, M.B., Cole, P., Kobelt, A., Horne, P.A., Altmann, J., Wratten, S.D., Yen. A.L.**	**2010**	**Reducing the Impact of Pesticides on Biological Control in Australian Vineyards: Pesticide Mortality and Fecundity Effects on an Indicator Species, the Predatory Mite *Euseius victoriensis* (Acari: Phytoseiidae)**	**Journal of Economic Entomology 103 (6):2061-2071. Doi 10.1603/Ec09357.**

Reliability	
Purpose of the study Description of endpoints	Laboratory bioassays on detached soybean, *Glycine max* (L.) Merr., leaves were used to test pesticides on a key Australian predatory mite species Euseius victoriensis (Womersley) in "worst-case scenario" direct overspray assays
Test compound, application procedure, exposure period, protocol	Glyphosate (360 g/liter) Roundup® (Nufarm Australia) 2.187 g /L in 400 ml
Experimental approach, Statistics, test environment	Zero- to 48-h-old juveniles, their initial food, and water supply were sprayed to runoff with a Potter tower; Cumulative mortality was assessed 48 h, 4 d, and 7 d after spraying., Fecundity was assessed for 7 d from start of oviposition
Test organisms	*Euseius victoriensis*
Biological effects	Glyphosate had no signiÞcant effects on mortality (Tukey b; Table 2), or fecundity compared with the control
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Australien mite
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	No effects
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?	Infield concentrations used.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Test species not relevant for Europe, no effects detected
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Santos et al. (2010)

glyphecotox_591	**Santos, M.J.G., Soares, A.M.V.M., Loureiro, S.**	**2010**	**Joint effects of three plant protection products to the terrestrial isopod *Porcellionides pruinosus* and the collembolan *Folsomia candida***	**Chemosphere 80 (9):1021-1030.**

Reliability	
Purpose of the study Description of endpoints	Determination of the effects of 3 products on the avoidance response pattern of *P. pruinosus* and in the reproductive output of F. *candida*; secondly to

	predict the response patterns for mixture exposures using the CA and IA conceptual models for the two test-species.
Test compound, application procedure, exposure period, protocol	Commercial formulations: (ROUNDUP®_ with 360 g AI/L, and which contains glyphosate-isopropylammonium (45%), surfactant (16%) and water 42.5%)), The nominal concentrations: 0.5 to 54.5 mg kg_1 dry soil in the avoidance experiment and between 0.1 and 2mg kg dry soil in the reproduction test;
Experimental approach, Statistics, test environment	The avoidance tests conducted with *P. pruinosus* were performed based on a methodology by Loureiro et al. (2005), consisting in exposing 10 isopods in a plastic box (14.3 cm _ 9.3 cm _ 4.7 cm height) divided in two sections, one with the control soil and the other with the test soil. After 24 and 48 h the number of animals in each side of the test-box was counted and mortality was registered. The experimental procedure for the reproduction test with the springtail F. candida was performed accordingly to the ISO 11267 protocol (ISO, 1999).
Test organisms	terrestrial isopod *Porcellionides pruinosus* and the collembolan *Folsomia candida Miguel* J.
Biological effects	The exposure resulted in a clear avoidance response in the higher concentrations (73% avoidance at 17.4 mg kg) although a small decrease in the degree of avoidance response was reflected in the highest concentration. EC_{50} values (mg kg dry soil) and 95% confidence intervals (CI) for the effects of m single exposure on the reproductive output of *Folsomia candida* exposed for 28 d on LUFA 2.2 soil= 0.33 (mg kg dry soil (0.18–0.48). For the effect of single exposure pesticide on the avoidance behaviour of *Porcellionides pruinosus* exposed for 48 h on LUFA 2.2 soil the AC50 = 40 mg /kg dry soil

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes, standard test species
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The conclusion from this study is only valid for glyphosate formulations that contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	0,33 mg/kg dry soil corresponding to approximately 250 g /ha in the top 5 cm soil 40 mg/kg dry soil corresponding to approximately 30kg/ha
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	ER_{50} reproduction at approx.12xPEC AC_{50} for avoidance at approx 10xPEC
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

El Sebai, O.A., El-Tawil, M.F. et al. (2012)

BVL. Nr: 2716720	**El Sebai, O.A., El-Tawil, M.F.**	**2012**	**Side-Effect of Certain Herbicides on Egg Parasitoid *Trichogramma evanescens* (West.) (Hymenoptera: Trichogrammatidae)**	
Reliability				

Purpose of the study Description of endpoints	The objective of this study was to evaluate the side-effects of some herbicides used to control weedsassociated with local crops on the biological aspects oft he bioagent egg parasitoid *T. evanescens.*
Test compound, application procedure, exposure period, protocol	commercial herbicide: Glyphosate (Baron®) 48% SL (Elhelb) Cards with 200 S. cerealella fresh eggs were dipped in the tested concentrations (300-3600ppm);
Experimental approach, Statistics, test environment	Each treated card was placed individually in a small Petri-dish (5 cm diameter) containing 30 T. evanescens adult wasps (<24 h old). The eggs were checked daily to determine the tested biological parameters (% parasitism, % adult emergence, sex ratio of the emerged wasps, developmental period and % mortality of the T. evanescens developmental stages).
Test organisms	Adult wasps of *Trichogramma evanescens*
Biological effects	Fresh S. cerealella eggs exposed to low concentrations of glyphosate had a high parasitismviability (85.5%) and high percent of T. evanescens adult emergence (91.14%), that was closely similar to the untreated 89.88% and 95.59%, respectively. the highest concentration of glyphosate had a relatively slight effect on the parasitism (47 and 27.17%). values for the emerged adults were (72.67, 66.44 and 20.44%) for eggs treated with glyphosate 3, 5 and 8 days post parasitism, respectively compared to a significant high emergence rate (96%) for the untreated.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	The tested formulation had low impact on parasitism at concentrations probalby higher that expected to occur in the off field.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation
2 Do the tested concentrations relate to predicted environmental concentrations?	Concentrations are displaying expected off field drift rates. Appliacation rate in field are not covered.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Treated *S. cerealella* eggs exposed to low concentrations of glyphosate had a high parasitism viability (85%) and higher percent of *T. evanescens* adult emergence (91%), what was closely similar to the untreated 89% and 95%, respectively.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Menezes, C.W.G. et al. (2012)

BVL. Nr: 2716796	**Menezes, C.W.G. et al.**	**2012**	**Reproductive and toxicological impacts of herbicides used in Eucalyptus culture in Brazil on the parasitoid *Palmistichus elaeisis* (Hymenoptera: Eulophidae)**	**Weed Research Vol. 52, Issue 6, pages 520–525, December 2012**
Reliability				
Purpose of the study Description of endpoints	This research evaluated the biological and toxicological impact of herbicides used in eucalyptus plantations in Brazil on the reproduction, development and			

	survival of the parasitoid *P. elaeisis.*
Test compound, application procedure, exposure period, protocol	Commercial formulation Scout ® (Glyphosate (720 g/ kg); The herbicides were applied at the recommended field rate per hectare, assuming that each pupa received a dose equivalent to 50% of its body surface as calculated by its mean surface area. Sixty *T. molitor* pupae at 48 h old were sprayed.
Experimental approach, Statistics, test environment	Treatment consisted of ten replicates, with one pupa of T. molitor and six 72-h-old females of P. elaeisis. The longevity of P. elaeisis adult females exposed to T. molitor pupae sprayed with herbicides was evaluated daily. The life cycle (egg to adult), % parasitism, % emergent progeny, no. of individuals emerged, No.of males and females, sex ratio, width of the head capsule and body length of parasitoids emerging from each pupa of *T. molitor* were obtained from parasitised pupae. The width of the head capsule was obtained. The sex ratio was calculated as the proportion of females.
Test organisms	*Palmistichus elaeisis* is endoparasitoid of Lepidoptera and Coleoptera Pupae and alternative prey insect *Tenebrio molitor*
Biological effects	Scout ® did not affect the duration of the parasitoid life cycle, survival of the parasitoid over time. The herbicide glyphosate showed intermediate harm to P. elaeisis compared to other herbicides. Significant effects coulb be observed for the paramters: length of femals head capsule and female length.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	no
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	The commercial formulation did not reduce the parasitism and emergence of *P. elaeisis.*
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Rittman, S. et al. (2013)

BVL. Nr: 2716808	**Rittman, S. et al.**	**2013**	**Glyphosate-Based Herbicide Has Contrasting Effects on Prey Capture by Two Co-Occurring Wolf Spider Species**	**J Chem Ecol. 2013 Oct;39(10):1247-53.**
Reliability				
Purpose of the study Description of endpoints	Authors tested the hypothesis that the presence of a commercial formulation of a glyphosate-based herbicide would affect the prey capture behavior of two wolf spiders.			
Test compound, application procedure, exposure period, protocol	Commercial formulation (Buccaneer Plus ®, Monsanto containing 41 % (480 g/l) glyphosate isopropylamine salt and 59 % other ingredients, including a polyethoxylated tallowamine (POEA) surfactant. Dilution with distilled water to a concentration of 2.5 % (12 g/l of the glyphosate salt) .			
Experimental approach, Statistics, test environment	Experiments were conducted in circular plastic arenas (15 cm diam., 10 cm high) with a Plaster of Paris base.			

Test organisms	wolf spiders, *Tigrosa helluo* and *Pardosa milvina* (Araneae, Lycosidae),
Biological effects	Herbicide did not affect the timing of prey capture for Pardosa but it did cause them to use more lunges in the process. herbicide altered the nature of the predatory event; Pardosa was more likely to take three or more lunges in their attempts to capture crickets when herbicide was included in the arena.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	no
3 Is the ecotoxicological manifestation level appropriate for the assessment?	no
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation.
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	The commercial formulation affects the predatory interactions of both *Tigrosa and Pardosa* differently. Pesticide treatment can have selective implications for animals living in agricultural systems.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

B.9.13 12.1 Summary of the relevant literature on on other arthropod species

For the group of terrestrial non-target arthropods (NTA), a database of 31 publications was collected by the notifier. The notifier considered none of the publication as reliable to be used in the risk assessment.

The submitted publications were also evaluated by the RMS and were assigned to pre-defined categories to characterize their reliability (for classification scheme please refer to **Fehler! Verweisquelle konnte nicht gefunden werden.**). From this screening, 11 studies were classified as information with low weight (category UBA3) and 10 publications (Bueno et al., 2011; Benamu et al., 2010; Evans et al., 2010; Michalkova et al., 2009; Schier, 2006, Renaud et al., 2004; Santos et al., 2010, El Sebai, et al. (2012), Menezes et al. (2012), Rittman et al. (2013) have been classified as supporting information (UBA2). Moreover, after the date of submission, during the administrative process and the public launching period of the evaluation a few more peer-reviewed relevant articles were newly published (mainly period of 2012-2014) and needed to be considered in a revised DAR. Within these references, additional studies focusing on arthropod have been considered as supporting information for risk assessment.

Arthropods in their natural environment can be exposed directly to pesticides after the application due to residues on food or due to contact with contaminated surfaces (such as plants, soil, surrounding substrate). Indirect effects on beneficial arthropod communities take place within treated areas and are principally due to vegetation changes subsequent to herbicide application. These vegetation changes, mainly decomposition of plant cover, might result in a drastic reduction of the habitats of beneficial and other non-target arthro-

pod communities and a loss of their refuges from predators. In a multiyear study using pitfall trapping to collect mobile arthropod species on the soil surface, the combination of conservation tillage and herbicide treatment had less impact on biodiversity than conventional ploughing (Schier, 2006). However, conservation tillage without the use of glyphosate is not practiced, due to the upcoming weed pressure on culture crops. When collembolan populations were assessed in field plot experiments in Mediterranean vineyards (Renaud et al. 2004) the result suggested apparently that plant protection products containing glyphosate favored the occurrence of epigeic and hemiedaphic species due to the preservation of decaying organic material on the soil surface compared to tillage practice. RMS considers it misleading to confuse the effects of tillage practice vs. non-tillage practice with the effects of an application of glyphosate without proper negative control. In a laboratory study it could be shown that reproductive capacity of the collembolan species *Folsomia candida* was not influenced by the application of glyphosate containing plant protection product when applied at relevant environmental concentrations (Santos et al., 2010).

Risk analysis is currently based on so-called beneficial arthropods which are important in the biological control of agronomic pests, typically through predation or parasitism including beetles, mites, wasp and spider. Tests are performed on glass plates or on extended laboratory tests with a 2 dimensional exposure on leaf substrates testing the formulated product for the determination of the median lethal dose (LD_{50}) and/or median effect on reproduction. Thereby test species were selected more for practical reasons because of their utility in agricultural production and feasibility in experimental setups than on the basis of their ecological relevance. At the same time effects on various developmental stages of arthropods, physiology, and behavior or prey consumption are not given consideration in traditional risk assessment. Bueno et al., (2011) could show that glyphosate containing products can be harmfull towards egg stages of *Trichogramma*, whereas at other parasitoid stages the same product was harmless. Sublethal effects of glyphosate were assessed in the laboratory on prey consumption, web building, fecundity, fertility and developmental time of progeny of spiders (Benamu et al., 2010, Rittmann et al. 2013, Evans et al., 2010). The authors concluded that the exposure to glyphosate containing products can affect the behavior of the animals and their capacity to grow and persist in agroecosystems. In contrast, short term exposure of spiders and carabid beetles did not affect mating or avoidance of the arthropods, but slightly slower movement (Michalkova et al., 2009).

These effects together with the indirect effects of herbicide treatment on the vegetation of their habitat receive less attention even though they might have implications for the success of survival and reproduction.

References

Benamu, M.A., Schneider, M.I., Sanchez, N.E.; Effects of the herbicide glyphosate on biological attributes of *Alpaida veniliae* (Araneae, Araneidae), in laboratory (2010): Chemosphere 78/7, 871-6. DOI: 10.1016/j.chemosphere.2009.11.027.

Bueno, A.F., Bueno, R.C.O.F., Parra, J.R.P., Vieira, S.S. (2008): Effects of pesticides used in soybean crops to the egg parasitoid *Trichogramma pretiosum.* Ciencia Rural 38/6, 1495-1503

Evans, S.C., Shaw, E.M., Rypstra, A.L. (2010): Exposure to a glyphosate-based herbicide affects agrobiont predatory arthropod behaviour and long-term survival. Ecotoxicology 19, 1249-1257

Michalková, V., Pekár, S. (2009): How glyphosate altered the behaviour of agrobiont spiders (Araneae: Lycosidae) and beetles (Coleoptera: Carabidae). Biological Control 51/3, 444-449

Renaud, A., Poinsot-Balaguer, N., Cortet, J., Le Petit, J.(2004): Influence of four soil maintenance practices on Collembola communities in a Mediterranean vineyard. Pedobiologia 48/5-6, 623-630. DOI 10.1016/j.pedobi.2004.07.002.

Santos, M.J.G., Morgado, R., Ferreira, N.G.C., Soares, A.M.V.M., Loureiro, S.(2011): Evaluation of the joint effect of glyphosate and dimethoate using a small-scale terrestrial ecosystem. Ecotoxicology and Environmental Safety 74/7, 1994-2001. DOI: 10.1016/j.ecoenv.2011.06.003.

Schier, A. (2006): Field study on the occurrence of ground beetles and spiders in genetically modified, herbicide tolerant corn in conventional and conservation tillage systems. Journal of Plant Diseases and Protection. Special Edition XX:101-113

B.9.13.13 Effects on earthworms

Among soill organisms, eathworms are standard organsims in the ERA as the have a potential role in the formation and maintenance of fertile soils. Besides laboratory studies submitted for the application for Renewal of Approval (AIR 2) following international guidelines, additional 21 references "open literature" has been submitted dealing with soil organsims in gerneral. Within these references 5 studies (Casabe et al., 2007; . Correia et al., 2012; Kaneda et al., 2009; Verrel et al., 2004 and Yasmin et al., 2003) focusing on earthworms have been considered to represent supporting information for risk assessment. Moreover, after the date of submission, during the administrative process and the public launching period of the evaluation a few more peer-reviewed relevant articles were newly published (mainly period of 2012-2014) and needed to be considered in a revised DAR. Within these references, additional studies focusing on earthworms have been considered as supporting information for risk assessment.
The following sections summarise the results and discussion as found in the published literature. RMS conclusions are given in the final summarising chapter after the results of the study are presented and discussed.

Kaneda et al. (2009)

glyphecotox_419	**Kaneda, S., Okano, S., Urashima, Y., Murakami, T., Nakajima, M.**	**2009**	**Effects of herbicides, glyphosate, on density and casting activity of earthworm, Pheretima (Amynthas) carnosus**	**Japanese Journal of Soil Science and Plant Nutrition 80:469-476, inc. English translation**
Reliability				
Purpose of the study Description of endpoints	Direct effects of herbicide application on the mortality, behavior, and body weight of earthworms were studied in a pot test.			
Test compound, application procedure, exposure period, protocol	Roundup® (ingredient: 41% glyphosate isopropylamine salt.			
Experimental approach Statistical design, test environment	herbicide was applied several years, The pplication amount was 0.33 L of a 100-fold dilution per square meter, as recommended bythe manufacturer, throughout the test period. The relationship between the earthworm habitat density and the amount of castings produced on the surface was evaluated via simple linear regression			
Test organisms	*Pheretima (Amynthas) carnosus*			
Biological effects	It is considered that herbicide application in no-tillage field did not directly affect the mortality and behavior of Pheretima (Amynthas) carnosus, but instead affected the casting production rate indirectly via changes in soil moisture and litter amount.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				yes
3 Is the ecotoxicological manifestation level appropriate for the assessment, e.g. gene induction vs. apical endpoints like growth or reproduction?				yes
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				The conclusion from this study is only valid for glyphosate formulations

	that contain POEA
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence/proposed action	**Not considered because of deficiencies in translation**
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Casabe et al. (2007)

glyphecotox_309	**Casabe, N., Piola, L., Fuchs, J., Oneto, M.L., Pamparato, L., Basack, S., Gimenez, R., Massaro, R., Papa, J.C., Kesten, E.**	**2007**	**Ecotoxicological Assessment of the Effects of Glyphosate and Chlorpyrifos in an Argentine Soya Field**	**Journal of Soils and Sediments 7 (4):232-239. DOI 10.1065/jss2007.04.224.**

Reliability	
Purpose of the study Description of endpoints	Authors performed field-laboratory study on an Argentinem soya field sprayed with glyphosate and chlorpyrifos under controlled conditions. GLY reduced cocoon viability, decreasing the number of juveniles. Moreover, earthworms avoided soils treated with GLY and a reduction in the feeding activity under laboratory and field conditions.
Test compound, application procedure, exposure period, protocol	Roundup® 1440 g a.s./ha, inc. analytic
Experimental approach Statistical design, test environment	In laboratory assays, *Eisenia fetida* Andrei were exposed to soil samples (0–10 cm depth) collected between the rows of soya. Endpoints linked to behavior and biological activity (reproduction, avoidance behavior and bait-lamina tests) and cellular/subcellular assays (Neutral Red Retention Time – NRRT; DNA damage – Comet assay) were tested.
Test organisms	*Eisenia fetida* Andrei
Biological effects	behavior and biological activity (reproduction, avoidance behavior and bait-lamina tests) and cellular/subcellular assays (Neutral Red Retention Time – NRRT; DNA damage – Comet assay) were tested.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The conclusion from this study is only valid for glyphosate formulations that contain POEA.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Detailed study on the toxicity of this special commercial formulation,nevertheles with limited value for other formulations, as toxicity of the glyphosate-based herbicides to non-target organisms vary within a wide

	range, depending on the surfactant system in the product.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Correia, F.V., Moreira, J.C. (2010)

glyphecotox_171	**Correia, F.V., Moreira, J.C.**	**2010**	**Effects of glyphosate and 2,4-D on earthworms (Eisenia foetida) in laboratory tests**	**Bull. Environ.Contam.Toxicol. DOI10.1007/s00128-010-0089-7**

Reliability	
Purpose of the study Description of endpoints	Long-term exposure (56 days) to soil contaminated with glyphosate demonstrated a toxic effect on normal development and reproduction of *Eisenia foetida*, indicating that this substance may have significant toxic effects on soil biota. Study describes results of a 56d Reproduction test with *Eisenia fetida* Andrei. Earthworms kept in glyphosate treated soil were classified as alive in all evaluations, but showed gradual and significant reduction in mean weight (50%) at all test concentrations.
Test compound, application procedure, exposure period, protocol	Glyphosate 99,7% from SIGMA Aldrich
Experimental approach Statistical design, test environment	1, 10, 100, 500, 1000 mg/kg Soil representative for Brazil
Test organisms	*Eisenia foetida*
Biological effects	Morphological abnormalities like elevating the body, coiling, and curling were observed in all specimens exposed to the highest concentrations of glyphosate (1000 mg/kg).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Reduction in mean weight (50%) at all test concentrations.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	**Study will be considered.**
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Verrell, P., Van Buskirk, E. (2004)

glyphecotox_640	**Verrell, P., Van Buskirk, E.**	**2004**	**As the worm turns: Eisenia fetida avoids soil contaminated by Glyphosate-based herbicide**	**Bulletin of Environmental Contamination and Toxicology 72 (2):219-224. DOI 10.1007/s00128-003-9134-0.**

Reliability	
Purpose of the study	Laboratory acute experiments designed to test acute effects on E. fetida .

Description of endpoints	Exposure to nominal concentration influences the activity of worms, as they emerged onto the surface within 2 h in all seven replicates exposed to nominal concentrations.
Test compound, application procedure, exposure period, protocol	Ortho Ground clear vegetation Killer (5% glyphosate as IPA salt)
Experimental approach Statistical design, test environment	Not similar to standard Nominal to 1/ 10.000, no statistics
Test organisms	*Eisenia foetida*
Biological effects	After 48 h animals were found to be buried in the soil. Authors suggest that acute exposure to concentrations of Groundclear recommended for application may comprise the survival of earthworms even though is direct toxicity appears low.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	Study will be considered. No GLP, no OECD, no standard method, but results and conclusion shown credibly. Detailed study on the toxicity of this special commercial formulation,nevertheles with limited value for other formulations, as toxicity of the glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant system in the product.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Santos, M.J.G. et al. (2012)

glyphecotox_8992716863	**Santos, M.J.G. et al.**	**2012**	**Pesticide application to agricultural fields: effects on the reproduction and avoidance behaviour of *Folsomia candida* and *Eisenia andrei***	**Ecotoxicology (2012) 21:2113 132122**
Reliability				
Purpose of the study Description of endpoints	The objective of this work was to assess the impact of pesticide application to non-target soil organisms simulating what happens following pesticide application in agricultural fields and thus obtaining higher realism on results obtained.			
Test compound, application procedure, exposure period, protocol	Montana (30.8 % a.i. glyphosate)			
Experimental approach Statistical design, test environment	3 commercial formulations containing the insecticides chlorpyrifos and endosulfan and the herbicide glyphosate were applied to a Mediterranean agricultural field. The soil was collected after spraying and dilution series were prepared with untreated soil to determine the impact of the pesticides			

	on the avoidance behaviour and reproduction of the earthworm *Eisenia andrei* and the collembolan *Folsomia candida*.
Test organisms	*Folsomia candida* and *Eisenia andrei*
Biological effects	Glyphosate did not seem to affect either earthworms or collembolans in the recommended field dose. No mortality was recorded in all reproduction tests performed.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	The FOCUS guidance documents (FOCUS 2000) were used for calculation of the plateau concentration and annual cumulative application dose, taking into account crop interception levels, soil density and pesticide degradation rates
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	Glyphosate did not seem to affect either earthworms or collembolans in the recommended field dose. Detailed study on the toxicity of this special commercial formulation,nevertheles with limited value for other formulations, as toxicity of the glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant system in the product.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Yasmin, S., D'Souza, D. (2003)

glyphecotox_304	**Yasmin, S., D'Souza, D.**	**2003**	**Effect of Pesticides on the Reproductive Output of Eisenia fetida**	**Journal of Agricultural and Food Chemistry 51 (15):4268-4272. Doi 10.1021/Jf034018f.**

Reliability	
Purpose of the study Description of endpoints	Effects of glyphosate on growth and reproduction of the earthworm species, *Eisenia fetida* was tested.
Test compound, application procedure, exposure period, protocol	Glycel 41% S.L. 2 mg /kg soil and 8 mg/kg soil
Experimental approach Statistical design, test environment	similar to standard procedure, no statistic
Test organisms	*Eisenia fetida*
Biological effects	Earthworm weight was significantly reduced due to its exposure to 8mg /kg soil glyphosate In contrast, glyphosate did not have a significant effect on the reproduction of *E. fetida.*
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	No GLP, no OECD, no standard method, but results and conclusion shown credibly. Detailed study on the toxicity of this special commercial formulation,nevertheles with limited value for other formulations, as toxicity of the glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant system in the product.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Zhou, C.F. et al. (2013)

glyphecotox_8952716859	**Zhou, C.F. et al.**	**2013**	**Subacute toxicity of copper and glyphosate and their interaction to earthworm (Eisenia fetida)**	**Environmental Pollution 180 (2013) 71-77**
Reliability				
Purpose of the study Description of endpoints	In order to explore the interactions between GPS and Cu, subacute toxicity tests of Cu and GPS on soil invertebrate earthworms (Eisenia fetida) were conducted.			
Test compound, application procedure, exposure period, protocol	To examine the toxicity interaction between Cu and GPS, earthworms were exposed to soils spiked with Cu concentrations at 50 or 200 mg kg and GPS concentrations at 0, 25, 50, 100, or 200 mg/ kg respectively			
Experimental approach Statistical design, test environment	Toxicity tests on earthworms were referred to OECD Guidelines (OECD, 2004			
Test organisms	*Eisenia fetida*			
Biological effects	The earthworms in all treatments survived after 28 days of exposure.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?			yes	
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?			nd	
3 Is the ecotoxicological manifestation level appropriate for the assessment?			yes	
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?			yes	
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?			nd	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?			nd	
Concluding weight of evidence	During the 28 d exposures, therewas no significant difference in weight or the cocoon production between the			

	control group and the GPS group. Our results showed that GPS had very low toxicity to the earthworms, which is consistent with other studies.
Type of info. (Critical, supporting, low weight)	**Supporting evidence**
Consideration/concluding score	**UBA2**

Zhou, C.F. et al. (2012)

glyphecotox_7052716498	**Zhou, C.F., Wang, Y.-J., Yu, Y.-C., Sun, R.-J., Zhu, X.-D., Zhang, H.-L., Zhou, D.-M.**	**2012**	**Does glyphosate impact on Cu uptake by, and toxicity to, the earthworm Eisenia fetida?**	**Ecotoxicology (2012) 21:2297 132305**

Reliability	
Purpose of the study Description of endpoints	A laboratory experiment was conducted to investigate the interactions between GPS and copper (Cu) on the acute toxicity of soil invertebrate earthworm (Eisenia fetida), which was exposed to aqueous solutions for 48 h with different mixing concentrations of Cu and GPS (technical-grade Gly acid).
Test compound, application procedure, exposure period, protocol	Glyphosate (95 % Technical, Sigma)
Experimental approach Statistical design, test environment	The mortality rates, Cu uptake by earthworm, and some biomarkers such as superoxide dismutase (SOD) activity, glutathione (GSH) content, and acetylcholinesterase (AchE) activity were measured.
Test organisms	*Eisenia fetida*
Biological effects	The mortality rates and whole-worm metal burdens increased significantly with the increasing Concentration in solution. GPS could significantly reduce the acute toxicity of Cu to earthworms. The mortality rates decreased sharply and the uptake of Cu was nearly halted in the presence of GPS. In addition, the SOD activity, GSH content, and AchE activity almost declined to the levels of the control.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Toxicity of GPS to earthworms was not observed in this study
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	nd
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	Toxicity to earthworms was not observed in this study.
Type of info. (Critical, supporting, low weight)	Supporting evidence
Consideration/concluding score	UBA2

Moreno et al. (2009)

glyphecotox_517	**Moreno et al.**	**2009**	**Rainfed olive farming in south-eastern Spain: Long-term effect of soil management on biological indicators of soil quality**	**Agriculture Ecosystems & Environment 131 (3-4):333-339. DOI 10.1016/j.agee.2009.02.011.**
Reliability				
Purpose of the study Description of endpoints			The elimination of weeds with herbicides reduced the microbial functional diversity in covered soil but did not affect the other microbiological parameters.	
Test compound, application procedure, exposure period, protocol			Field study design lasting over 40 years	
Experimental approach Statistical design, test environment			Field study design lasting over 40 years No statistics	
Test organisms			Bacterial 16S rRNA soil DNA extracts	
Biological effects				
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?				nd
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				not assessable
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?				not assessable
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?				not assessable
Concluding weight of evidence			**Supporting evidence**	
Type of info. (Critical, supporting, low weight)				
Consideration/concluding score			**UBA2**	

Negga et al. (2011)

glyphecotox_523	**Negga et al.**	**2011**	**Exposure to Mn/Zn ethylene-bis-dithiocarbamate and glyphosate pesticides leads to neurodegeneration in *Caenorhabditis elegans***	**NeuroToxicology 32 (3):331-341. DOI: 10.1016/j.neuro.2011.02.002.**

Reliability	
Purpose of the study Description of endpoints	Toxicology studies determining whether exposure to our pesticides of interest could induce regionally specific neurodegeneration.
Test compound, application procedure, exposure period, protocol	Touchdown Hitech, formulation with [52.3% glyphosate] from Syngenta AG, Wilmington, DE. Exposure 30 min an 24h.
Experimental approach Statistical design, test environment	No ecotoxicological standard methods
Test organisms	*Caenorhabditis elegans* (N2) and NW1229worms
Biological effects	Studies demonstrate that *C. elegans* are vulnerable to glyphosate-containing herbicides and Mn/Zn-EBDC-containing fungicides at environmentally relevant concentrations, suggesting that these worms are a valuable and viable model system for future testing involving these pesticides.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	Study indicates that *C. elegans* are vulnerable to glyphosate-containing herbicides at environmentally relevant concentrations in terms of neurotoxicity. Toxicity of glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant system in the product.
Type of info. (Critical, supporting, low weight)	**Not relevant** for ERA
Consideration/concluding score	**UBA3**

Piola, L. et al. (2013)

glyphecotox_696 2716489	**Piola, L., Fuchs, J., Oneto, M.L., Basack, S., Kesten, E., Casabe, M.**	**2013**	**Comparative toxicity of two glyphosate-based formulations to *Eisenia andrei* under laboratory conditions**	**Chemosphere 91 (2013) 545 13551**
Reliability				

Purpose of the study Description of endpoints	Study was conducted to evaluate the comparative toxicity of two glyphosate-based products: Roundup FG (monoammonium salt, 72% acid equivalent, glyphosate- A) and Mon 8750 (monoammonium salt, 85.4% acid equivalent, glyphosate-B
Test compound, application procedure, exposure period, protocol	1. Roundup FG (monoammonium salt, soluble granules, 72% p/p acid equivalent (ae); GLY-A) 2. Mon 8750 (monoammonium salt, soluble granules, 85.4% p/p ae; GLY-B)
Experimental approach Statistical design, test environment	Lethal toxicity, Sublethal toxicity, Comet assay, NRRT assay.
Test organisms	*Eisenia andrei*
Biological effects	Median lethal concentration (LC50) showed that glyphosate-A was 4.5-fold more toxic than glyphosate-B. Sublethal concentrations caused a concentration-dependent weight loss, consistent with the reported effect of glyphosate as uncoupler of oxidative phosphorylation. Glyphosate- A showed deleterious effects on DNA and lysosomal damage at concentrations close to the applied environmental concentrations (14.4 lg ae cm2). With glyphosate-B toxic effects were observed at higher doses, close to its LC50, suggesting that the higher toxicity of formulate A could be attributed to the effects of some of the so-called ‘‘inert ingredients’’, either due to a direct intrinsic toxicity, or to an enhancement in the bioavailability and/or bioaccumulation of the active ingredient.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	nd
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd

Concluding weight of evidence	Results highlight the importance of ecotoxicological assessment not only of the active ingredients, but also of the different formulations. Median lethal concentration showed that formulations can be multiple times more toxic than glyphosate itself, and differences among the toxicity of formulations exist,. Formulations at sublethal concentrations can cause weight loss, and effects on DNA and lysosomal damage. Results highlight the importance of ecotoxicological assessment not only of the active ingredients, but also of the different formulations.
Type of info. (Critical, supporting, low weight)	Supporting evidence
Consideration/concluding score	UBA2

Jeffrey et al. (2010)

	Jeffrey D. Weidenhamer & Ragan M. Callaway	**2010**	**Direct and Indirect Effects of Invasive Plants on Soil Chemistry and Ecosystem Function**	**Journal of Chemical Ecology 36 (1):59-69. DOI 10.1007/s10886-009-9735-0.**

Reliability	
Purpose of the study Description of endpoints	The literature review indicates that invasive species can alter the biogeochemistry of ecosystems, that secondary metabolites released by invasive species may play important roles in soil chemistry as well as plant-plant and plantmicrobe interactions.
Test compound, application procedure, exposure period, protocol	nd
Experimental approach	Review article
Test organisms	
Biological effects	Herbicides used to control invasive species can impact plant chemistry and ecosystems in ways that have yet to be fully explored.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Yes, the literature review indicates that invasive species can alter the biogeochemistry of ecosystems
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	nd
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	Herbicides used to control invasive species can impact plant chemistry and ecosystems in ways that have yet to be fully explored.
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA 2 relevant**

Druart et al. (2010)

glyphecotox_353	**Druart et al.**	**2010**	**Towards the development of an embryotoxicity bioassay with terrestrial snails: Screening approach for cadmium and pesticides**	**Journal of Hazardous Materials 184 (1-3):26-33. DOI 10.1016/j.jhazmat.2010.07.099.**

Reliability	
Purpose of the study Description of endpoints	Description of the method to assess the embryotoxicity of chemicals on *Helix aspersa*. This terrestrial gastropod is already the subject of a standardized test with snail eggs.
Test compound, application procedure, exposure period, protocol	Roundup® Biovert 360 (360 g/l glyphosate; Monsanto Europe S.A.),
Experimental approach Statistical design, test environment	Yes, EC50=18 mg /L; POEA influence
Test organisms	Helix aspersa
Biological effects	Glyphosate and its formulations or its associated adjuvants was toxic to snail embryos at lower concentrations than the recommended application

	concentrations for agriculture. The authors hypothesized that the surfactant polyoxyethylene amine (POEA, also called MON 818) contained in Roundup®, improved the transfer of glyphosate, by interacting with the plasma membrane. Another hypothesis is that the POEA is in fact the compound mainly responsible for the toxicity of Roundup® and could even be more toxic than the Roundup® itself . It appears necessary to assess the risk of the final product (which will be applied to crops) and not only of the active ingredient individually.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	POEA influence
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The conclusion from this study is only valid for glyphosate formulations that contain POEA
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Lower concentrations than the recommended application concentrations for agriculture were used
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	
Concluding weight of evidence	**Relevant information about formulations containing POEA.**
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA 2**

Muangphra, P., Kwankua, W., Gooneratne, R. (2012)

BVL Nr.: 2716390	**Muangphra, P., Kwankua, W., Gooneratne, R.**	**2012**	**Genotoxic Effects of Glyphosate or Paraquat on Earthworm Coelomocytes**	**Environmental ToxicologyVolume 29, Issue 6, Article first published online: 30 MAY 2012**
Reliability				
Purpose of the study Description of endpoints	The genotoxicity of glyphosate or paraquat as reflected by chromosomal aberrations, DNA damage, and cytoskeleton damage as measured by pinocytic adherence activity in P. peguana earthworm coelomocytes is evaluated.			
Test compound, application procedure, exposure period, protocol	Commercial herbicide formulations, active ingredient 36% (w/v)] (both from World Crop International Company, Bangkok, Thailand) no further specification. Filter papers were moistened with 3 mL of different concentrations of the herbicides: 997, 2991, 4985, 6979, 9164 mg glyphosate/L, equivalent to exposure of glyphosate at 47, 141, 235, 329, and 432 µg cm–2			
Experimental approach Statistical design, test environment	Acute and sublethal Toxicity Studies, Micronucleus Test, Comet Assay			
Test organisms	*Pheretima peguana*			
Biological effects	Glyphosate did not cause any significant DNA damage as compared with the controls (Table III) in the Comet assay. The LC50 of glyphosate at 48 h to *P. peguana* was 251.45 µg cm–2, respectively.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	The product did not cause clastogenic effects, but induced aneugenic effects on coelomcytes including a marked chromosomal loss during anaphase at high concentration (LC50) .
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Commercial product with unknown composition was tested.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product with unknown composition was tested.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	The effective concentration of glyphosate in the field would be much lower because of the binding of some of the glyphosate by soil organic matter.
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	The genotoxicity of glyphosate containing formulations reflected by chromosomal aberrations, DNA damage, and cytoskeleton damage as measured by pinocytic adherence activity in *P. peguana* earthworm coelomocytes is evaluated. Commercial herbicide formulations with active ingredient 36% (w/v)] was used with no further specification. Glyphosate did not cause any significant DNA damage as compared with the controls (Table III) in the Comet assay. The LC50 of glyphosate at 48 h to *P. peguana* was 251.45 µg cm–2, respectively. The product did not cause clastogenic effects, but induced aneugenic effects on coelomcytes including a marked chromosomal loss during anaphase at high concentration (LC50) .
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA 2**

Wang, Y. et al. (2012)

BVL-Nr.: 2716753	**Wang, Y. et al.**	**2012**	**Toxicity assessment of 45 pesticides to the epigeic earthworm Eisenia fetida**	**Chemosphere. 2012 Jul;88(4):484-91. doi: 10.1016/j.chemosphere.2012.02.086. Epub 2012 Mar 28.**

Reliability	
Purpose of the study Description of endpoints	This study was conducted to investigate comparative toxicity of 45 pesticides, including insecticides, acaricides,fungicides, and herbicides, toward the epigeic earthworm *Eisenia fetida.*
Test compound, application procedure, exposure period, protocol	Active ingredients were used instead of commercial formulations.
Experimental approach Statistical design, test environment	Contact toxicity test, Soil toxicity test
Test organisms	The oligochaete *E. fetida*
Biological effects	Glyphosate showed moderate toxicity LC_{50} 14d (95% CI) 328 µg/ cm2
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	The effective concentration of glyphosate in the field would be much lower because of the binding of some of the glyphosate by soil organic matter.
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	Authors highlight that results from single-pesticide experiments did not actually reflect field situations in which multiple pesticides or pesticide mixtures are used (Zhou et al., 2011).
Type of info. (Critical, supporting, low weight)	supporting
Consideration/concluding score	UBA2

Negga et al. (2011)

BVL-Nr.: 2716392	**Negga, R., Stuart, J.A., Machen, M.L., Salva, J., Lizek, A.J., Richardson, S.J., Osborne, A.S.**	**2011**	**Exposure to Glyphosate- and/or Mn/Zn-Ethylene-bis-Dithiocarbamate-Containing Pesticides Leads to Degeneration of ?-Aminobutyric Acid and Dopamine Neurons in *Caenorhabditis elegans***	

Reliability	
Purpose of the study Description of endpoints	Determination of the vulnerability of DAergic and/or GABAergic neurons *in C. elegans* to exposure to either acute or chronic TD

	and/or MZ.
Test compound, application procedure, exposure period, protocol	TouchDown
Experimental approach Statistical design, test environment	Transgenic *C. elegans* strains were either acutely or chronically treated with two agrochemicals.
Test organisms	*Caenorhabditis elegans* and *Escherichia coli* Strains
Biological effects	Changes inneuronal morphology were only observable at the LC75 (10% glyphosate).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Model organsims representing nematodes and bacteria
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	In vitro experiments examinat-ing neuronal effects
3 Is the ecotoxicological manifestation level appropriate for the assessment?	In vitro experiments examinat-ing neuronal effects
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation, probably containing surfactants.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Concentrations are within the environmentally relevant concentrations.
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	The commercial formulation induces changes in neuron morphology in the model organism *C. elegans.*
Type of info. (Critical, supporting, low weight)	supporting
Consideration/concluding score	UBA2-3

Zhou, C.F., et al. (2014)

BVL-Nr.: **2716499**	**Zhou, C.F., Wang, Y.-J., Sun, R.-J., Liu, C., Fan, G.-P., Qin, W.X., Li, C.C., Zhou, D.-M.**	**2014**	**Inhibition effect of glyphosate on the acute and subacute toxicity of cadmium to earthworm *Eisenia fetida***	**Environmental Toxicology and Chemistry**Volume 33, Issue 10, pages 2351–2357

Reliability	
Purpose of the study Description of endpoints	The acute and subacute toxicities of cadmium (Cd) to earthworm Eisenia fetida in the presence and absence of glyphosate were studied.
Test compound, application procedure, exposure period, protocol	Glyphosate (95% technical; Sigma); Earthworms were then exposed to the solution containing different concentrations of Cd (0 mmol/L, 0.1 mmol/L, 0.2 mmol/L, and 0.4 mmol/L). Glyphosate was added to each Cd concentration, and the glyphosate-to-Cd molar ratios were adjusted to 0, 1, 2, 5, and 10, respectively.
Experimental approach Statistical design, test	Acute toxicological tests of Cd and glyphosate on earthworm were performed following the Organisation for Economic Cooperation and Development

environment	(OECD) guidelines.
Test organisms	earthworm *Eisenia fetida*
Biological effects	Although Cd is highly toxic to *E. fetida*, the presence of glyphosate markedly reduced the acute toxicity of Cd to earthworm; both the mortality rate of the earthworms and the accumulation of Cd decreased with the increase of the glyphosate/Cd molar ratio.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Model organsims *Eisenia fetida*
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Not applicable
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Not applicable
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Concentrations are most likely exceeding the environmentally relevant concentrations.
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	Glyphosate is a strong chelating agent for heavy metals. The results of the study indicated that the Cd availability and, consequently, toxicity were reduced by the presence of glyphosate.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	UBA2

Buch, A.C. et al. (2012)

BVL-Nr.:	**Buch, A.C. et al.**	**2012**	**Toxicity of three pesticides commonly used in Brazil to *Pontoscolex corethrurus* (Müller, 1857) and *Eisenia andrei* (Bouché, 1972)**	**Applied Soil Ecology, Volume 69, July 2013, Pages 32–38**
2716767				

Reliability	
Purpose of the study Description of endpoints	The experiment was performed to evaluate the sensitivities of *E. andrei* and *P. corethrurus* to three frequently used pesticides in Brazil – carbendazim, carbofuran and glyphosate.
Test compound, application procedure, exposure period, protocol	The nominal concentrations of glyphosate – Pica-Pau® 480 SC (480 g L−1 a.i.) – used for both the avoidance and mortality tests were 0, 7, 14, 21, 30 and 47 mg a.i. kg−1 TAS, based on application rates used in citrus, cotton, rice and maize production.
Experimental approach Statistical design, test environment	Avoidance test (ISO 17512-1 (ISO, 2007), Acute test (ISO 11268-1 (ISO, 1993)), Data analysis
Test organisms	*Pontoscolex corethrurus* (Müller, 1857) and Eisenia andrei (Bouché, 1972)
Biological effects	No earthworm mortality was observed in any of the treatments for any of the three pesticides after 48 h. Avoidance behavior to glyphosate was significant only at the highest dose, i.e., 47 mg a.i. kg−1 ($p < 0.05$ level) for *P. corethrurus* and at 30 mg

	a.i. kg−1 or higher for *E. andrei*. Even the highest concentrations of glyphosate (47 mg a.i. kg−1) did not lead to significant mortality (3–4%) of either *E. andrei* or *P. corethrurus*.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Model organsims and potential tropical alternative test species.
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	no
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	The conservative worst-case approach of the ERA reaches maximal concentration of 6 mg/kg.
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	For the pesticides tested in the present experiment there were no important and very few significant differences between both species in their sensitivity to the three pesticides used. The commercial formualtion was found to be non-toxic for both species at the concentrations used.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	UBA2

Fusilero, M.A. et al. (2013)

BVL-Nr.: 2716774	**Fusilero, M.A. et al.**	**2013**	**Weed management systems and other factors affecting the earthworm population in a banana plantation**	**European Journal of Soil Biology Volume 56, May–June 2013, Pages 89–94**
Reliability				
Purpose of the study Description of endpoints	This research was conducted to compare the effects of two contrasting weed management systems, conventional farmers' manual practice and the application of herbicides, on earthworm populations. The study also aimed to examine other factors in the banana field that could influence earthworms such as soil organic matter, amount of rainfall and ground cover			
Test compound, application procedure, exposure period, protocol	Glyphosate is a systemic broad spectrum herbicide, not further specification. Earthworm populations were monitored in plots receiving either a cyclical course of two applications of paraquat (480 mL ha_1) at monthly intervals followed by one of glyphosate (1680 mL/ha) or manual weeding using slashing, mechanical cutting and scraping.			
Experimental approach Statistical design, test environment	Field experiment: 2 areas located in an upland dominated by deep, fine textured and slightly acid soils (vertisoils). The clay soils are classified to be Tugbok clay loam reddish brown residual soil (Typic Tropudalf). It is prismatic and slightly compact clay, hard and brittle when dry, but elastic when moist and has a pH of 5-7.			
Test organisms	Earthworms			
Biological effects	There were no significant differences in earthworm counts between manual and chemical plots both in areas with 15% (15_ inclination) site (F = 0.48, P ¼=0.49) and 25% (25% inclination) site (F =0.44, P = 0.51) during the 2-year period. Instead, variation in			

	earthworm counts could be attributed to differences in rainfall, organic matter, organic mulch and soil cover.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Earthworms with different taxonomy
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Not applicable
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Not applicable
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Unknown commercial formulation tested
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	Yes in detail: Weed management effects, Rainfall effects, Effect of ground cover, Organic matter effects, Effect of soil pH
Concluding weight of evidence	The use of commercial preparation of herbicides paraquat and glyphosate under the tested field conditions did not affect the earthworm population. Other factors found to affect the population were ground cover, rainfall and soil organic matter.
Type of info. (Critical, supporting, low weight)	supporting
Consideration/concluding score	UBA2

García-Torres, T. et al. (2014)

BVL-Nr.: 2716851	**García-Torres, T. et al.**	**2014**	**Exposure Assessment to Glyphosate of Two Species of Annelids**	**Bulletin of Environmental Contamination and Toxicology August 2014, Volume 93, Issue 2, pp 209-214**
Reliability				
Purpose of the study Description of endpoints	The aim of this work was to study the response of *E. fetida* and *Octolasion tyrtaeum* to the application of glyphosate in soil. While *E. fetida* is internationally recognized for its use in ecotoxicological tests, O. tyrtaeum is found in many Argentine agricultural ecosystems.			
Test compound, application procedure, exposure period, protocol	The following nominal exposures were established: 5, 50, 500, 5,000 and 50,000 mg kg-1 of glyphosate in soil, and a control without glyphosate. The source was glyphosate, purity 95 % (Sinochem Qingdao Co., Ltd., Qingdao, CN). The exposure period was 28 days. Following the exposure of adults, cocoons were counted and placed in the original concentrations of glyphosate in soil, or control soil, for 42 days.			
Experimental approach Statistical design, test environment	Mortality, biomass, fecundity and viability of cocoons was studied.			
Test organisms	*E. fetida* and *Octolasion tyrtaeum*			
Biological effects	Following the first week, there were no survivors of *E. fetida* at the maximum concentration tested, while at lower concentrations, survival was greater than 90 % for both species during the 28 days of exposure. Fertility losses were10%, 20 % and 80 % at nominal glyphosate concentrations of 50, 500 and 5,000 mg/kg, respectively.			

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Model organsim
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	no
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Yes, LOAEL for acute toxicity and reproduction endpoints were determined.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	The conservative worst-case approach of the ERA reaches maximal concentration of 6 mg/kg.
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	Results revealed that the species *O. tyrtaeum* was more sensitive to exposure to glyphosate than *E. fetida*, decreasing mortality and biomass. In addition, there were adverse effects in adult fecundity and viability of cocoons by exposure to high concentrations of glyphosate. This study demonstrated adverse effects at concentrations which exceed the predicted concentrations of 6 mg/kg.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	UBA2

B.9.13 13.1 Summary of the relevant literature on earthworms

Among soil organisms, earthworms are standard organisms in the risk assessment as they have an important role in soil formation and nutrient cycling. Besides laboratory studies submitted for the application for the renewal of approval of the active substance glyphosate following international guidelines, additional 21 references have been submitted dealing with soil organisms in general. Moreover, after the date of submission, during the administrative process and the public launching period of the evaluation a few more peer-reviewed 28 relevant articles were newly published (mainly period of (2012-2014) and needed to be considered in a revised DAR.Within all these references, studies focusing on earthworms have been considered as supporting information for risk assessment.

Acute earthworm risk assessment was exclusively based on mortality endpoints. It is noted that effects on behaviour might also have negative consequences for worms, e.g.–when worms move to the surface of contaminated soil- exposure to predators or to detrimental light. It could be shown that the activity of worms was influenced by the exposure to environmentally relevant concentration of commercial formulation of glyphosate (Verrel and Buskirk, 2004). The worms emerged onto the surface within 2 h after exposure. Nevertheless, after 48 h animals were found to be buried in the soil again. Authors concluded that acute exposure to the glyphosate containing plant protection product may compromise the survival of earthworms even though its direct toxicity appears low (Verrel & Buskirk, 2004). In general, it seems important to assess not only the active ingredients, but also the product

toxicity in order to detect effects of the so-called inert ingredients, which can have a direct intrinsic toxicity or can enhance in the bioavailability (Piola et al. 2013).

Effects on reproduction were examined by Casabé et al. (2007), Kaneda et al. (2009) and Yasmin et al. (2006) using commercial formulations with the recommended application rates. The tested products did not seem to affect earthworms reproduction (Zhou et al. 2012 and 2013, Santos et al. 2012, Fusilero, M.A. et al. 2013, García-Torres, T. et al. (2014) at least when the recommended field dose was tested.However, it can not be excluded that with repeated applications of glyphosate containing plant protection products during the season or year by year will have negative effects on the biotic soil community. It is considered that herbicide application did not directly affect the mortality or reproduction but instead the biological activity of the animals.

In a reproduction test with *Eisenia fetida*, which was conducted with the active substance glyphosate itself (Correia et al., 2010), earthworms were kept in treated soil and were classified as alive after the evaluation period, but showed significant reduction in mean weight at all test concentrations. Moreover morphological abnormalities like elevating the body, coiling, and curling were observed in all specimens exposed to the highest concentrations of glyphosate (1000 mg/kg). Further behavioural abnormalities were described in terms of reduced casting production (Kaneda et al., 2009), reduced cocoon viability, a reduction in the feeding activity (Casabé et al., 2007) or reduced body weight (Yasmin et al., 2006). However, the test rates were similar or above the one tested in the officially submitted studies, so that the outcome of the risk assessment for earthworm did not change.

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Chui-Fan Zhou , Yu-Jun Wang , Cheng-Cheng Li , Rui-Juan Sun , Yuan-Chun Yu , Dong-Mei Zhou (2013) Subacute toxicity of copper and glyphosate and their interaction to earthworm (Eisenia fetida). Environmental Pollution 180 (2013) 71-77

B.9.13.14 Effects on soil non-target micro-organisms

For the group of soil non-target micro-organisms, a database of 99 publications was collected by the notifier. The notifier considered 21publications to be necessary to be described in the literature review. After the date of submission, during the administrative process and the public launching period of the evaluation a few more peer-reviewed relevant articles were newly published (mainly period of (2012-2014) and needed to beconsidered in a revised DAR.The submitted publications were also evaluated by zRMS and have been assigned according to an UBA screening. Most of the studies submitted by the notifier dealt with the rhizobia of glyphosate-resistant crops and were therefore not assignable for ERA in the European Union. However, after screening 34 studies were recognized as informative with low weight (category UBA3), 42 publications have been considered as supportive information (UBA2) and only one publication from Cycon & Kaczynska (2004) has been classified as UBA1 (critical data, high weight of evidence in risk assessment). In this study, performed according to the OECD guidelines 216 and 217, the authors applied glyphosate at the field rate of 4.5 mg/ kg of soil (PEC) as well as at a 5-fold higher concentration (22.5 mg/ kg of soil). After 1, 7, 14 and 28 days of incubation, soil respiration rates (SIR – Substrate Induced Respiration) and the amounts of nitrate did not significantly differ from control soil.

Accinelli et al. (2002)

glyphecotox_265	**ACCINELLI C., SCREPANTI C., DINELLI G., VICARI A.**	**2002**	**SHORT-TIME EFFECTS OF PURE AND FORMULATED HERBICIDES ON SOIL MICROBIAL ACTIVITY AND BIOMASS**	**Intern. J. Environ. Anal. Chem., (82): No. 8–9, pp. 519–527**
Reliability				
Purpose of the study Description of endpoints	Investigate short-time effects of glyphosate and gluphosinate-ammonium on soil microbial activity. Pure and formulated herbicides were tested. Endpoints: soil respiration & soil dehydrogenase activity (DH)			

Test compound, application procedure, exposure period, protocol	Glyphosate: Roundup® Bioflow (31% a.i. SL) Gluphosinate-ammonium: Basta (11.33% a.i. SL) 2, 20 and 200 mg a.i. g/ soil = 1X, 10X and 100X (multiple value with respect to the recommended agricultural rate) RQ: a soil layer of 1 cm was considered 20-days incubation period. Non-GLP
Experimental approach Statistical design, test environment	Three-way ANOVA was employed to test at, each time interval, the significance of soil microbial activity and biomass C in soil samples receiving separately different application rates of the six pure and formulated herbicides with respect to the untreated soil
Test organisms	Sandy loam: from Experimental Farm of the University of Bologna at Ozzano (Bologna, Italy), from the top 20 cm of a field with no previous pesticide history.
Biological effects	Both pure and formulated glyphosate and glufosinate-ammonium determined a rapid and significant increase of soil respiration compared with the untreated soil.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	These results further support the absence of adverse effects of glyphosate and glufosinate-ammonium on soil microbial population (as previously reported by other authors) The paper focuses on ecosystem function and do not inform on ecosystem structure diversity
Type of info. (Critical, supporting, low weight)	Supporting information
Consideration/concluding score	**UBA2**

Araujo et al. (2003)

glyphnosubm_151	**Araujo, A.S.F., Monteiro, R.T.R., Abarkeli, R.B..**	**2003**	**Effect of glyphosate on the microbial activity of two Brazilian soils**	**Chemosphere 52 (5):799-804. Doi 10.1016/S0045-6535(03)00266-2.**

Reliability	
Purpose of the study Description of endpoints	Study in vitro, changes in the microbial activity of 2 typical Brazilian soils, with and without applied glyphosate. Endpoints: soil respiration (evolution of CO2), fluorescein diacetate (FDA), plate counts of bacteria, actinomycetes and fungi
Test compound, application procedure, exposure period,	Glyphosate (technical glyphosate) 2.16 mg glyphosate kg/soil

protocol	32 days; No-GLP
Experimental approach Statistical design, test environment	Comparison of soils with 11 years of application of glyphosate with soils without reported history of glyphosate Soils were sampled from surface layer up to a depth of 10 cm.
Test organisms	- Microcosms - 2 types of soil (Hapludult and Hapludox Brazilian soils) with different histories of glyphosate application
Biological effects	increase of 10–15% in the CO_2 evolved and a 9–19% increase in FDA hydrolyses in the presence of glyphosate. Community shift: number of actinomycetes and fungi had increased while the number of bacteria showed a slight reduction. Long-term effects of repeated application (six and eleven years) showed an increase in the microbial activity compared to soils with no reported application of glyphosate, showing that repeated application lead to increased microbial activity due the utilization of glyphosate as an available substrate.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	-
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	Glyphosate was biodegraded by soil microorganisms with the formation AMPA, and that the herbicide had positive effect on the soil microbial activity in short- and long-term. Detection of a community shift, was not discussed.
Type of info. (Critical, supporting, low weight)	Supporting information
Consideration/concluding score	**UBA2**

Cycon, M., Kaczynska, A. (2004)

glyphecotox_331	**Cycon, M., Kaczynska, A.**	**2004**	**Effects of selected pesticides on soil microbial activity in nitrogen and carbon transformation**	**Pestycydy 1/2:113-120**

Reliability	
Purpose of the study Description of endpoints	Investigate the effects of selected fungicides (dithianon, procymidone), herbicides (glyphosate, Hnuron) and insecticides (lambda-cyhalothrin, diazinon) on microbial activity measured by SIR and the level of nitrification in sandy-loam soil during the 28d. Endpoints: soil microbial activity (SIR: Substrate-Induced Respiration) and nitrogen transformation
Test compound, application	Glyphosate : 360 g dm-3

procedure, exposure period, protocol	Used concentrations [mg/ kg of soil]: PEC: 4.5 and 5xPEC: 22.5 The OECD Guidelines No 216 and 217
Experimental approach Statistical design, test environment	Soil was collected from the top 20 cm layer from an agricultural plot in Pszczyna, South of Poland ANOVA + Turkey HSD (post hoc comparison)
Test organisms	Refer to paper
Biological effects	Application of above-mentioned pesticides at their recommended field rates did not have any effect on soil microbial activity and nitrogen transformation
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Application of glyphosate at recommended field rates did not have negatively effect on soil microbial activity measured by SIR and nitrogen transformation 28 days treatment. However, it is impossible to draw a general conclusion regarding the effect of glyphosate on soil microorganisms because a number of factors influence on the activity of this agrochemical in soil ecosystem, therefore estimation of two parameters only may be not adequate in some situations
Type of info. (Critical, supporting, low weight)	Critical data, high weight of evidence in RA
Consideration/concluding score	**UBA1**

Gomez et al. (2009)

glyphecotox_391	**Gomez, E., Ferreras, L., Lovotti, L., Fernandez, E.**	**2009**	**Impact of glyphosate application on microbial biomass and metabolic activity in a Vertic Argiudoll from Argentina**	**European Journal of Soil Biology 45 (2):163-167**

Reliability	
Purpose of the study Description of endpoints	To evaluate the effect of increasing doses of glyphosate on biomass, metabolic activity and metabolic quotient of soil microbiota under controlled conditions in a soil with a long history of glyphosate. Endpoints: carbon from microbial biomass (C-MB), microbial respiration rate (MR), metabolic quotient (qCO2), and dehydrogenase activity (DA) at day 4 and day 45
Test compound, application procedure, exposure period, protocol	Commercial formulation of glyphosate (48%) 0.48, 0.96, 1.92 and 3.84 L a.i ha-1 Analysis of repeated measures; Means comparisons Duncan test
Experimental approach Statistical design, test environment	25°C and 75% of water holding capacity.

Test organisms	Vertic Argiudoll (Argentina)
Biological effects	C-MB: significantly lower in the highest doses at day 4 and 45 MR: significant differences over the time but not between doses qCO2: significant differences between doses after both 4d and 45d DA: significantly higher in the treatments with glyphosate at day 4.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Refer to paper
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Refer to paper
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Refer to paper
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Refer to paper
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	The results of this study demonstrate an initial inhibitory effect that affected the microbial cells, which showed to be temporary, indicating that no harmful effects should be expected in the short-term when glyphosate is applied at doses equivalent or higher than those usually applied in the field.
Type of info. (Critical, supporting, low weight)	Supporting information
Consideration/concluding score	**UBA2**

Haney et al. (2002)

glyphecotox_400	**Haney, R.L., Senseman, S.A., Hons, F.M.**	**2002**	**Effect of Roundup® ultra on microbial activity and biomass from selected soils**	**Journal of Environmental Quality 31 (3):730-735**
Reliability				
Purpose of the study Description of endpoints			To determine the effect of Roundup® Ultra on soil microbial biomass and activity Endpoints: C- and N-mineralization and soil microbial biomass	
Test compound, application procedure, exposure period, protocol			Roundup® Ultra [Monsanto, St. Louis, MO]; (480 g a.i. L-1) 234 mg active ingredient kg-1 soil based on an assumed 2-mm glyphosate–soil interaction depth	
Experimental approach Statistical design, test environment			Refer to paper	
Test organisms			Nine soils from Georgia and Texas were used Variation in pH, soil organic C, clay content	
Biological effects			Cumulative C- mineralization and-mineralization increased for all treatments with RU Strong linear relationships between C & N mineralized (slope= 3) Glyphosate C to N ratio of 3:1 => strongly suggest that RU was the direct cause of the enhanced microbial activity	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes
2 Is the magnitude of effects of biological significance, e.g. is a very small				yes

statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Roundup® Ultra appeared to be rapidly degraded by soil microbes regardless of soil type or organic matter content, and increased their population and activity even at high application rates, without adversely affecting microbial activity
Type of info. (Critical, supporting, low weight)	Supporting information
Consideration/concluding score	**UBA2**

Haney et al. (2002)

glyphecotox_401	**Haney, R.L., Senseman, S.A., Krutz, L.J., Hons, F.M.**	**2002**	**Soil carbon and nitrogen mineralization as affected by atrazine and glyphosate**	**Biology and Fertility of Soils 35 (1):35-40**

Reliability	
Purpose of the study Description of endpoints	Atrazine alone and atrazine plus glyphosate were added to soil to determine their effect on soil microbial activity Endpoints: C and N mineralization (Cmin, Nmin)
Test compound, application procedure, exposure period, protocol	Roundup® Ultra (480 g active ingredient l–1) + Atrazine (1/2 w/w) 2× (188 mg kg^{-1}), 4× (376 mg kg^{-1}) and 6× (564 mg kg^{-1}) assuming a 2-mm soil penetration depth for glyphosate 56 days of incubation
Experimental approach Statistical design, test environment	Refer to paper
Test organisms	Weswood silt loam
Biological effects	Atrazine plus glyphosate stimulated microbial activity more than atrazine alone. The addition of glyphosate with atrazine significantly increased C mineralization in all treatments compared with atrazine alone
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	Refer to paper

Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Hart, M.M et al. (2009)

glyphecotox_404	**Hart et al.**	**2009**	**Separating the effect of crop from herbicide on soil microbial communities in glyphosate-resistant corn**	**Pedobiologia 52 (4):253-262**

Reliability	
Purpose of the study Description of endpoints	To examine the effect of both the transgenic corn and the use of glyphosate on two groups of rhizosphere microbes, denitrifying bacteria and fungi. Endpoints: qPCR, t-RFLP based on DNA
Test compound, application procedure, exposure period, protocol	Roundup®(1.8kgha-1 atrazine) conventional herbicides : isoxaflutone + atrazine (79 + 800 g aiha-1)
Experimental approach Statistical design, test environment	Fully factorial, field study where the effects of crop type and herbicide treatment on microbe numbers and diversity were separated. Measurement of the numbers and community composition of two soil rhizosphere microbes to determine if their communities were affected by: (1) glyphosate-resistant corn versus conventional corn and (2) glyphosate vs conventional herbicides (isoxaflutole & atrazine).
Test organisms	Experimental field located at the Elora Research Station of the University of Guelp (Canada) Conostogo silt loam soil
Biological effects	we found neither crop type (transgenic or conventional) nor herbicide (glyphosate or conventional) affected rhizosphere denitrifying or fungal communities. results showed that seasonality was a significant determinant of denitrifier and fungal abundance
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	na
Concluding weight of evidence	Neither GR corn nor glyphosate had significant impacts on the denitrifying bacteria and fungi in this study
Type of info. (Critical, supporting, low weight)	Supporting information
Consideration/concluding score	**UBA2/3**

Kyaw, K.M., Toyota, K. (2007)

glyphecotox_446	**Kyaw, K.M., Toyota, K.**	**2007**	**Suppression of nitrous oxide production by the herbicides glyphosate and propanil in soils supplied with organic matter**	**Soil Science and Plant Nutrition 53 (4):441-447**
Reliability				
Purpose of the study Description of endpoints	Investigate the impact of two herbicides, a commercial formulation of glyphosate (Roundup®) and propanil (DCPA), on nitrous oxide (N2O) production and soil respiration in two different soils (Tyatkone and Miura) amended with rice straw and chitine; Endpoints: N2O production rates			
Test compound, application procedure, exposure period, protocol	Roundup® (41% a.i., 59% water and surfactant, Nissan Chemical, Tokyo, Japan) Application: 2 L a.i. ha^{-1}, 10 cm soil layer 6-week incubation			
Experimental approach Statistical design, test environment	Refer to paper			
Test organisms	Refer to paper			
Biological effects	Application of glyphosate AND propanil: Suppress cumulative N2O production in both types of amended soils Decrease N_2O production in rice straw amended soil (< 25%)			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				
3 Is the ecotoxicological manifestation level appropriate for the assessment?				yes
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?			The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.	
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?				
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?			no	
Concluding weight of evidence			herbicides used in this study had no severely adverse effects on the overall soil microbial community.	
Type of info. (Critical, supporting, low weight)			**low weight in RA, not assignable**	
Consideration/concluding score			**UBA3**	

Lupwayi, N.Z., et. al (2004)

glyphecotox_467	**Lupwayi et. al**	**2004**	**Soil microbial biomass and diversity after herbicide application**	**Canadian Journal of Plant Science 84 (2):677-685**
Reliability				
Purpose of the study Description of endpoints	Greenhouse and field experiments were conducted to investigate the effects of herbicides on soil microbial biomass, bacterial diversity and community structure. Endpoints: Microbial biomass: microbial C			

	Bacterial diversity: Biolog method Community structure: specific patterns of substrate utilization by bacteria (CLPP) => Shannon index, Evenness
Test compound, application procedure, exposure period, protocol	Glyphosate IPA (900 g a.i. /ha) Glufosinate ammonium (500 g a.i. /ha) 0, 1, 2, 3 and 4 wk after treatment
Experimental approach Statistical design, test environment	Refer to paper
Test organisms	Gray Luvisolic soil
Biological effects	Microbial C increased/Shannon index was lower In all experiments, examination of microbial community structure revealed herbicide-induced shifts in microbial composition even when diversity indices among treatments were not different
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	Herbicides applied once at recommended rates did not have significant or consistent effects on microbial C or diversity indices. However, shifts in microbial community structures were sometimes evident where microbial C and diversity were not different..Such shifts can lead to successions in the microbial communities that could have longterm effects on soil biological processes. Therefore, it is important to incorporate measures of microbial diversity and composition in herbicide ERA studies.
Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Malkomes, H.-P. (2007)

glyphecotox_481	**Malkomes, H.-P.**	**2007**	**Influence of differently formulated glyphosate herbicides and a herbicidal reference compound on microbial activities in soil**	**Nachrichtenbl. Deut. Pflanzenschutzd. 59 (6):124-132**
Reliability				
Purpose of the study Description of endpoints	Investigate under laboratory conditions the effects of differently formulated glyphosate herbicides on biomass-related microbial activities and carbon and nitrogen mineralization in two soils with and without lucerne meal amendment. Endpoints: dehydrogenase, substrate-induced short-term respiration			
Test compound, application procedure, exposure period, protocol	Basamid Granulat: 0,24 g/kg Dazomet: 0,23 g/kg Herbogil Liquide 7,86 µl/kg Dinoterb : 1,96 mg/kg Roundup® Ultra:6,67 µl/kg Roundup® Ultragran 4 kg/ha			

	Touchdown 7,28 µl/kg Glyphosat (Isopropylamin-Salz) 2,4 mg/kg Glyphosat (Na-Salz) 2,4 mg/kg Glyphosat (-Trimesium) 2,4 mg/kg
Experimental approach Statistical design, test environment	Refer to paper
Test organisms	
Biological effects	The various glyphosate treatments (formulation, dosage) only sometimes had small effects on the endpoints. Only the nitrogen mineralization was increased for some time by the higher dosage whereas the relation of carbon to nitrogen mineralization was diminished. Sodium and isopropylamine salts of glyphosate sometimes acted little stronger than the trimesium compound When the soil was stressed by a preceding fumigation no further additional effects occurred by glyphosate.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no

Concluding weight of evidence	Independently from the tested formulations the overall effects of field-related dosages of glyphosate induced only relatively small effects on the investigated microbial activities in the soil.
Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Mijangos, I., et al. (2009)

glyphecotox_508	**Mijangos, et al.**	**2009**	**Effects of glyphosate on rhizosphere soil microbial communities under two different plant compositions by cultivation-dependent and -independent methodologies**	**Soil Biology & Biochemistry 41 (3):505-513**

Reliability	
Purpose of the study Description of endpoints	Studyon the short-term effects of glyphosate on rhizosphere soil microbial communities under two different plant compositions (triticale versus a mixture of triticale and pea) by cultivation-dependent (Biolog Ecoplates) and –independent (PCR-DGGE) methodologies Endpoints: potentially mineralizable nitrogen, ammonium content, community-level physiological profiles using Biolog EcoplatesTM, DNA microbial biomass and genotype diversity by means of PCR-DGGE
Test compound, application procedure, exposure period, protocol	Roundup® Plus 15 and 30 days
Experimental approach	Factorial treatments that included two different compositions of forage plant

Statistical design, test environment	species (triticale versus a mixture of triticale and pea) and two concentrations of glyphosate (50 and 500 mg active ingredient kg-1 soil, as a commercial formulation, Roundup® Plus)
Test organisms	pot study carried out with soil collected from the top layer (0–30 cm) of natural grassland located in Derio (Basque Country, northern Spain)
Biological effects	15 days: stimulation of the activity and functional diversity (glyphosate acting as an available source of C, N and P.) 30 days: inconsistent response to glyphosate addition Shift in the carbon utilization pattern as a result of herbicide treatment, which again suggests a non-target effect of glyphosate on the rhizosphere soil microbial community BiologTM was more sensitive than PCR-DGGE to detect changes in soil microbial communities

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	Glyphosate was quickly used by soil microorganisms as a source of nutrients which resulted in a stimulation of the activity and functional diversity of the cultivable portion of the heterotrophic soil microbial community. effects on the rhizosphere soil microbial community which were, interestingly, more enhanced in triticale than in ‘‘triticaleþ pea’’ pots. Biolog was more sensitive to detect changes in soil microbial communities induced by glyphosate and plant composition than PCR-DGGE.
Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Ratcliff et al. (2006)

glyphecotox_560	**Ratcliff, A.W., Busse, M.D., Shestak, C.J.**	**2006**	**Changes in microbial community structure following herbicide (glyphosate) additions to forest soils**	**Applied Soil Ecology 34 (2-3):114-124**

Reliability	
Purpose of the study Description of endpoints	To examine changes in community structure by PLFA and C utilization analyses, supported by a coarse-level comparison of bacteria and fungi by epifluorescent microscopy and traditional culturing techniques. Our objective was to determine whether glyphosate results in short-term changes, either deleterious or beneficial, in forest soil microbial communities Endpoints: Total and culturable bacteria, fungal hyphal length, bacterial:fungal

	biomass, carbon utilization profiles (BIOLOG), bacterial and fungal phospholipid fatty acids (PLFA)
Test compound, application procedure, exposure period, protocol	Roundup® Field rate of 5 kg a.i. ha_1 and 100x field rate 1, 3, 7 and 30 days
Experimental approach Statistical design, test environment	Factorial treatments including 3 levels of glyphosate (0, 50, and 5000 mg a. i./kg soil) and four sampling dates (1, 3, 7, and 30d)
Test organisms	Clay loam and a sandy loam forest soil (0 to 15 cm depth from two ponderosa pine) plantations in northern California
Biological effects	Endpoints not affected at field rate application High concentration of glyphosate (100x field rate) altered the bacterial community in both soils: Increase of generalist bacteria Community shifted from fungal dominance to equal ratio

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	
Concluding weight of evidence	No major changes in microbial community structure assessed by C utilization, PLFA, and standard cultural and microscope methods were detected in forest soils following the addition of the recommended field-rate concentration of glyphosate commercial formulation of glyphosate has a benign affect on community structure when applied at the recommended field rate, and produces a non-specific, short-term stimulation of bacteria at a high concentration.
Type of info. (Critical, supporting, low weight)	Supporting information
Consideration/concluding score	**UBA2**

Zabaloy et al. (2008)

glyphecotox_663	**Zabaloy, M.C., Garland, J.L., Gomez, M.A.**	**2008**	**An integrated approach, to evaluate the impacts of the herbicides glyphosate, 2,4-D and metsulfuron-methyl on soil microbial communities in the Pampas region, Argentina**	**Applied Soil Ecology 40 (1):1-12.**

Reliability	
Purpose of the study Description of endpoints	Investigate the impact of postemergence herbicides on soil microbial communities Endpoints: - culturable aerobic heterotrophic bacterial (AHB) density - substrate-induced respiration (SIR) - dehydrogenase activity (DHA)

	- fluorescein diacetate (FDA) hydrolysis - functional richness (biolog)
Test compound, application procedure, exposure period, protocol	glyphosate (N-(phosphonomethyl)glycine), soluble concentrate (48% a.i.) Application: 10X recommended field rate: 150 mg a.i. kg-1 Incubation: 3 weeks
Experimental approach Statistical design, test environment	Refer to paper
Test organisms	Typic Argiudoll, Typic Haplustoll and Petrocalcic Paleustoll (Argentina)
Biological effects	(1) early stimulation of SIR and AHB; (2) dissimilar response in the soils for FDA and DHA (3) transient increase in functional richness.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	The addition of these herbicides at a dose 10 times higher than the normal field application rates caused minor changes to soil microbial activity, bacterial density and functional richness. The specific changes varied among herbicides, with the effects of glyphosate most pronounced.
Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Lancaster et al. (2006)

glyphecotox_449	**Lancaster, S.H., Haney, R.L., Senseman, S.A., Hons, F.M., Chandler, J.M.**	**2006**	**Soil microbial activity is affected by Roundup® WeatherMax and pesticides applied to cotton (Gossypium hirsutum)**	**J Agric Food Chem 54 (19):7221-6**
Reliability				
Purpose of the study Description of endpoints	Evaluate the influence of glyphosate-based cotton pest management systems on soil microbial activity. Endpoints: C and N mineralization Soil microbial biomass (chloroform-fumigation-incubation method)			
Test compound, application procedure, exposure period, protocol	Roundup®: WeatherMAX, Monsanto Co., St. Louis, MO Application rate: 152.7 µg a.i./kg soil trifluralin, aldicarb, and mefenoxam + pentachloronitrobenzene with or without glyphosate (applied as Roundup® WeatherMax). 1 control and 1 treatment with only Roundup® WeatherMax			
Experimental approach Statistical design, test environment	Refer to paper			
Test organisms	Weswood clay loam collected from a bermuda grass pasture and a fallow field			

	previously planted with cotton
Biological effects	Soils treated with glyphosate alone exhibited greater cumulative C mineralization 30 days after treatment than all other treatments The addition of Roundup® WeatherMax reduced C mineralization in soils treated with fluometuron, aldicarb, or mefenoxam + PCNB formulations. These results indicate that glyphosatebased herbicides alter the soil microbial response to other pesticides
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	For all variables measured, pasture soil exhibited greater microbial activity and biomass than cultivated soil. Cumulative C mineralization after 30 days was greater in soils treated only with glyphosate as compared to all other treatments. Nitrogen mineralization was greater in soils that had been treated with applications that included glyphosate as compared with soils that were not treated with glyphosate. Soil microbial biomass C increased relative to non-treated soils when glyphosate was applied alone. Soil microbial biomass N was not affected. soil microbial biomass measurements using the fumigation-incubation method are less sensitive than C and N mineralization measurements for detecting the influence of microbial activity
Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Bennicelli et al. (2009)

glyphecotox_294	**Bennicelli, R.P., Szafranek-Nakonieczna, A., Wolinska, A., Stepniewska, Z., Bogudzinska, M.**	**2009**	**Influence of pesticide (glyphosate) on dehydrogenase activity, pH, Eh and gases production in soil (laboratory conditions)**	**International Agrophysics 23 (2):117-122**

Reliability	
Purpose of the study Description of endpoints	Determinate dehydrogenase activity (DHA) and soil gases (CO_2, N_2O) emission in soils enriched with glyphosate (1 µg and 10 µg g-1 of pesticide doses) during time (42 days), under lab conditions at 20°C. Endpoints: dehydrogenase activity (DHA)
Test compound, application	Glyphosate (Product ?)

procedure, exposure period, protocol	0, 1 and 10 µg g-1 of soil
Experimental approach Statistical design, test environment	Refer to paper
Test organisms	Mollic Gleysols, Eutric Fluvisols and Terric Histosols taken from surface layer (0-20 cm)
Biological effects	The decrease of DHA activity was observed that depended on the pesticide dose; Increase of the N2O concentration with growth of pesticide dose
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	
Concluding weight of evidence	1. Glyphosate caused an inhibition of DHA activity in all investigated soils up to 21st day. 2. CO2 formation increased in the case of Terric Histosols and Eutric Fluvisols, but decreased in the case of Mollic Gleysols. 3. Glyphosate caused an increase of N2O concentration in all investigated soils. 4. Eh, pH and CO2 concentration had high correlations with DHA activity.
Type of info. (Critical, supporting, low weight)	Supporting information
Consideration/concluding score	**UBA2**

Lancaster et al. (2010)

glyphecotox_450	**Lancaster, S.H., Hollister, E.B., Senseman, S.A., Gentry, T.J.**	**2010**	**Effects of repeated glyphosate applications on soil microbial community composition and the mineralization of glyphosate**	**Pest Manag Sci 66 (1):59-64**
Reliability				
Purpose of the study Description of endpoints	study the effect of one, two, three, four or five applications of glyphosate on soil microbial community composition and glyphosate mineralization and distribution of 14C residues in soil. Endpoints: - fatty acidmethyl esters - sequencing of 16S rRNA bacterial genes - cumulative percentage 14Cmineralized - Incorporation of 14C residues into soil microbial biomass			
Test compound, application procedure, exposure period, protocol	Glyphosate-isopropylammonium 480 g AE L^{-1} SL (Roundup® WeatherMAX; Monsanto Company, St Louis, MO) Applied in 3 mL solution (giving 33% v/v water content) at a rate of 49 µg AE g−1 soil to the soil surface			
Experimental approach Statistical design, test environment	At 2, 4, 6 and 8 weeks after the initial glyphosate applications, an additional 49 µg AE g−1 soil was added in 0.5 mL solution, to create soil samples that received one, two, three, four or five applications of glyphosate.			

	Each treatment was replicated 4 times Endpoints measured 3, 7 and 14 days after the final glyphosate application to each treatment (DAA).
Test organisms	Weswood silt loam with no record of glyphosate application during previous 2 years
Biological effects	- Increase of gram-negative bacteria FAMES - Increase of the abundance of the gram-negative Burkholderia ssp sequences - Decrease of the cumulative percentage 14C mineralized 14 DAA when glyphosate was applied 4 or 5 times - Incorporation of 14C residues into soil microbial biomass was greater following five glyphosate applications than following the first application 3 and 7 DAA

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Refer to paper
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Refer to paper
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes

Concluding weight of evidence	Changes in the dissipation or distribution of glyphosate following repeated applications of glyphosate may be related to shifts in the soil microbial community composition
Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Ruzkova et al. (2011)

glyphecotox_583	**Ruzkova, M., Ruzek, L., Vorisek, K., Vrablik, P., Musilova, D.**	**2011**	**Microbiological characterization of land set-aside before and after Roundup® desiccation**	**Plant Soil and Environment 57 (2):88-94**

Reliability	
Purpose of the study Description of endpoints	The changes in the biological parameters under different soil management (chemical vs biological) are described. Endpoints: microbial biomass, available organic carbon, basal respiration, metabolic quotient, biomass-specific available organic carbon, arylsulfatase activity, soil organic matter carbon and total nitrogen
Test compound, application procedure, exposure period, protocol	Roundup® Biaktiv (5 l/ha)
Experimental approach Statistical design, test environment	Refer to paper
Test organisms	loamy luvic chernozem developed on carbonate loess with a 200 mm thick layer of arable top-soil. Formerly used in arable system until 1995, then changed into a land set-aside
Biological effects	Repeated Roundup® desiccation caused a strong (highly significant) decrease of arylsulfatase activity (–28%), however highly significant increase of

	microbial biomass (+69%) and nitrate-nitrogen ratio (+86%) (=>decreased immobilization nitrates by the plants!!)
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	Refer to paper
Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Widenfalk et al. (2008)

glyphecotox_649	**Widenfalk, A., Bertilsson, S., Sundh, I., Goedkoop, W.**	**2008**	**Effects of pesticides on community composition and activity of sediment microbes - responses at various levels of microbial community organization**	**Environmental Pollution 152 (3):576-584**

Reliability	
Purpose of the study Description of endpoints	To assess whether sediment microbes were affected by exposure to the pesticides captan, glyphosate, isoproturon and pirimicarb at environmentally relevant and high pesticide concentrations, at both community and subcommunity (‘‘species’’) levels Endpoints: community-level: bacterial activity, fungal and total microbial biomass sub-community level: PLFA, 16S rRNA genotyping, T-RFLP
Test compound, application procedure, exposure period, protocol	Glyphosate N-(phosphono-methyl) glycine 150 and 150.000 µg/kg dw
Experimental approach Statistical design, test environment	microcosms
Test organisms	sediment from lake Erken, Sweden (relatively unaffected by agricultural activities)
Biological effects	- Community-level: endpoints were not affected by pesticide exposure (bacterial activity was quantified too late? bacterial activity usually shows an almost instantaneous response to pesticide exposure…) - Sub-community level: significant shifts in bacterial community composition (as T-RFLP) at environmentally relevant concentrations => certain groups of bacteria were stimulated at low exposure concentrations?
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental	yes

concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	The study showed that community-level end points failed to detect these changes, underpinning the need for application of molecular techniques in aquatic ecotoxicology.
Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Liphadzi, K.B., et al. (2005)

glyphecotox_461	**Liphadzi, K.B., et al.**	**2005**	**Soil microbial and nematode communities as affected by glyphosate and tillage practices in a glyphosate-resistant cropping system**	**Weed Science 53 (4):536-545**

Reliability	
Purpose of the study Description of endpoints	Determine the response of soil microbial and nematode communities to different herbicides and tillage practices under a glyphosate-resistant cropping system. Endpoints: - soil microbial biomass (SMB) carbon determination - substrate-induced respiration (SIR) - BIOLOG substrate utilization - nematode populations
Test compound, application procedure, exposure period, protocol	Glyphosate: ? Application rate: 1.12 kg ai ha-1, when weeds were 10-20 cm tall All glyphosate treatments received a second glyphosate application approximately 2 wk after the first application
Experimental approach Statistical design, test environment	Conventional herbicides: - tank mixture of cloransulam plus S-metolachlor plus sulfentrazone for soybean - commercially available mixture of acetochlor and atrazine
Test organisms	Report to paper
Biological effects	- SMB carbon, SIR, and BIOLOG substrate utilization were not altered by glyphosate Nematode community response to the glyphosate treatment was similar under both conventional tillage and no-till environments. Total nematode densities were similar with the glyphosate and conventional herbicide treatments
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	Soil health in a glyphosate-resistant cropping system was similar to that of cropping systems that used conventional herbicides.

Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Busse et al. (2001)

glyphnosubm_155	**Busse, M.D., A.W. Ratcliff, C.J. Shestak, and R.F. Powers**	**2001**	**Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities.**	**Soil Biology and Biochemistry 33:1777-1789.**

Reliability	
Purpose of the study Description of endpoints	Assess direct and indirect effect of glyphosate on soil microbial communities from pine plantation. Endpoints: Lab: soil bioassay at high concentrations Field: microbial biomass, respiration, metabolic diversity
Test compound, application procedure, exposure period, protocol	Refer to paper
Experimental approach Statistical design, test environment	Refer to paper
Test organisms	3 types of soil: clay, Fe, Al oxide content (northern California)
Biological effects	Microbial respiration was unchanged at expected field concentrations and stimulated at conc. 100-fold greater
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no

Concluding weight of evidence	Long-term, repeated applications had minimal effects on seasonal microbial characteristics, which was more a function of time of year and site. Tests in artificial media are of limited relevance for glyphosate Field rate applications of glyphosate should have little or no effect on soil microbial communities in ponderosa pine plantations.
Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Gorlenko, M.V. et al. (2012)

2716722	**Gorlenko, M.V. et al.**	**2012**	**Functional Biodiversity of Soil Microbe Colonies Affected by**	**Moscow University Soil Science Bulletin,**

			Organic Substrates of Different Kinds	**2012, Vol. 67, No. 2, pp. 71–78**
Reliability				
Purpose of the study Description of endpoints	The influence of different organic substrates (litter manure, diesel fuel, and glyphosate) on characteristics of functional diversity in sodpodzol soils and land incubated in a sample experiment. Endpoints: multisubstrate testing, CO2 emission evaluation, and nitrogen fixation by gas chromatography			
Test compound, application procedure, exposure period, protocol	Roundup: applied to 0.5, 5 and 50 mg a.i./kg soil			
Experimental approach Statistical design, test environment	Refer to paper, no statistical analysis			
Test organisms	- Sod podzol soil (Moscow) - ML soil: china clay (20%), intermediate peat (10%) and mason's sand (70%)			
Biological effects	- The effects of humate and glyphosate on CO2 emissions and intensity of nitrogen fixation was weaker compared to diesel fuel treatment. - Soil microbe community did not show any markedly negative response to Glyphosate, especially in "natural" soil (Sod Podzol). Effects were more pronounced in ML soil.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				No statistical approach
3 Is the ecotoxicological manifestation level appropriate for the assessment?				Not applicable
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				Commercially formulated product, possibly with POEA
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?				-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?				no
Concluding weight of evidence	Differences reponses of microbe community were observed between natural microbe community (Sod podzol soil) and "modell soil" (ML soil).			
Type of info. (Critical, supporting, low weight)	Roundup as a glyphosate-based formulation has been utilized as one of the most commonly applied herbicides. The study could not be taken into account as critical information on the ERA of the active substance glyphosate itself. **Low weight**			
Consideration/concluding score	**UBA3**			

Bai, S.H. et al. (2012)

2716726	**Bai et al.**	**2012**	**Effects of pre-planting site management on soil organic matter and microbial community functional diversity in subtropical Australia**	**Applied Soil Ecology 62 (2012) 31– 36**
Reliability				
Purpose of the study Description of endpoints	Identify effects of 3 methods of weed control on microbial communitiies Methods investigated: (i) glyphosat ii) glyphosate + MCPA iii) top soil removal			

	Endpoints: soil organic matter, microbial biomass, soil respiration, NH4+-N availability, potentially mineralisable N (PMN) soil microbial community and functional diversity (Biolog GN2 plates)
Test compound, application procedure, exposure period, protocol	Touchdown™ applied at a rate of 4.3 kg / ha MCPA (2-methyl-4-chlorophenoxyacetic acid) : 2 kg / ha
Experimental approach Statistical design, test environment	Refer to paper
Test organisms	Refer to paper
Biological effects	- No significant difference between the herbicide and control plots, in water soluble organic carbon, hot water extractable organic C, hot water extractable total N and microbial biomass C and N (after 20 weeks) -NH4+-N availability and potentially mineralisable N (PMN) values were lower at week 20 than week 1 in the herbicide and scalping treatments. - SOM was significantly affected by scalping
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	_
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	_
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercially formulated product
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Single herbicide application at field rates did not impact soil organisms but it did not effectively control weeds either. Touchdown™ is a glyphosate-based formulation. It has been utilized as one of the most commonly applied herbicides. The study could not be taken into account as critical information on the ERA of the active substance glyphosate itself
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA 2**

Partoazar, M. et al. (2011)

2716740 2716804	**Partoazar, M. et al.**	**2011**	**The effect of glyphosate application on soil microbial activities in agricultural land**	**African Journal of Biotechnology Vol. 10(83), pp. 19419-19424, 21**
Reliability				
Purpose of the study Description of endpoints	assess the effects of glyphosate application on soil microbial activities			
Test compound, application procedure, exposure period, protocol	Soils from a citrus garden located in the city of Darab (Iran) with at least a “slow rate” application of glyphosate in the last 12 years. Refer to paper			
Experimental approach Statistical design,	Refer to paper			

test environment	
Test organisms	Refer to paper
Biological effects	In soil with a long historical use of glyphosate: - heterotrophic bacterial population was significantly increased - dehydrogenase activity was significantly increased - respiration rates increased up to 50 nM - inhibitory effects on respiration at 500 mM
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercially formulated product
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Not available
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	Regularly applications of commercially formulation of glyphosate over years may alter (increase) soil microbial activity and population. Increased microbial activity may be beneficial or detrimental toward plant growth, soil microbial ecology, and soil quality
Type of info. (Critical, supporting, low weight)	Supporting information
Consideration/concluding score	**UBA2**

Stachowski- Haberkorn, S. et al. (2008)

	Stachowski-Haberkorn, S. et al.	**2008**	**Impact of Roundup on the marine microbial community, as shown by an in situ microcosm experiment**	**Aquatic Toxicology 89 (2008) 232–241**
Reliability				
Purpose of the study Description of endpoints	Assess the impact of Roundup on the marine microbial community Endpoints: Temperature Gradient Gel Electrophoresis (TGGE) based on 16S and 18S rDNA, Flowcytometry			
Test compound, application procedure, exposure period, protocol	Roundup® tested at concentrations in the range of run-off events (1 and 10gL^{-1}), for more details refer to the paper			
Experimental approach Statistical design, test environment	7-day field experiment using microcosms; for more details refer to the paper			
Test organisms	natural marine microbial communities			
Biological effects	- differences between control and treatment fingerprint - significant increase in the prasinophyte-like population when Roundup concentration was increased to 10gL^{-1}.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				yes

3 Is the ecotoxicological manifestation level appropriate for the assessment?	-/-
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercially formulated product, possibly with POEA
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	No, study performed at concentrations of run-off events
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	Roundup as a glyphosate-based formulation has been utilized as one of the most commonly applied herbicides. The study could not be taken into account as critical information on the ERA of the active substance glyphosate itself.
Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Udawatta, R.P. et al. (2010)

2311941	**Udawatta, R.P. et al.**	**2010**	**Microbial resilience as influenced by an herbicide in soils from native prairie, CRP, and row crop management**	**19th World Congress of Soil Science, Soil Solutions for a Changing World 1 – 6 August 2010, Brisbane, Australia.**

Reliability	
Purpose of the study Description of endpoints	Examination of differences in soil microbial resilience as impacted by herbicide application on soils from prairie and row cop management. Endpoints: soil enzyme activites: fluorescein diacetate (FDA) and dehydrogenase
Test compound, application procedure, exposure period, protocol	Roundup Original Max 11 glyphosate concentrations from 0 to 2000 ppm
Experimental approach Statistical design, test environment	Refer to paper
Test organisms	4 soils types: i) native prairie ii) restored prarie iii) CRP and iv) corn-soybean management
Biological effects	- Small effects on FDA hydrolase activity - Effects highly variable for dehydrogenase activity - Soils from native prairie have greater enzyme resilience - Cropped soils have the smallest enzyme resilience
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Commercially formulated product,
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	-
2 Do the tested concentrations relate to measured or predicted environmental concentrations	-

(if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	Soils from native prairie have greater enzyme resilience while the corn-soybean cropped soils have the smallest enzyme resilience. This might suggest that continuous soil disturbance and application of other agri-chemicals may have negatively afffected microbial resilience for soils under crop management. Observed enzyme activities could be due to direct and/or indirect effects of pesticides which were not evaluated in the current study.
Type of info. (Critical, supporting, low weight)	No data for glyphosate technical or glyphosate acid deducible from the tested formulation. **Supporting information / Low weight**
Consideration/concluding score	**UBA2**

Zabaloy, M.C. et al. (2012)

2312004	**Zabaloy, M.C. et al.**	**2012**	**Assessment of microbial community function and structure in soil microcosms exposed to glyphosate**	**Applied Soil Ecology 61 (2012) 333– 339**

Reliability	
Purpose of the study Description of endpoints	Evaluation both the potential effects of glyphosate treatments on microbial community structure and function in laboratory incubation of soils. Endpoints: community respiration, microbial community structure (qPCR and t-RFLP)
Test compound, application procedure, exposure period, protocol	Solution of pure glyphosate (Nidera, 95% technical grade) was added to soil microcosms to final concentrations of 0.15 and 150 mg kg^{-1} More details in the paper
Experimental approach Statistical design, test environment	Refer to paper
Test organisms	- Soil from two sites in the Pampa region of Argentina (Vertic Argiudoll from Zavalla, ZAV; Petrocalcic Paleustoll from Coronel Dorrego, DOR), with long exposure to glyphosate - Soil from a natural grassland for control
Biological effects	- Minimal effects on both structural and functional measures of the microbial community. - Immediate respiratory response of the reference and chronically exposed soils to glyphosate was distinctive: - Glyphosate increased respiration in the reference grassland soil, potentially due to a stress response of glyphosate sensitive species - Glyphosate depressed respiration in the chronically exposed soil, probably as a result of selection for organisms acclimated for rapid assimilation of substrates from the cometabolic decay of the molecule.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	_
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light	-

conditions)?	
Concluding weight of evidence	- Respiratory response of the reference and chronically exposed soils to glyphosate was distinctive - Shifts in community metabolism were observed. => longer term studies involving repeated addition of glyphosate to previously unexposed soils are needed
Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Benslama, O. & Boulahrouf, A. (2013)

2716763	**Benslama, O. & Boulahrouf, A.**	**2011**	**Impact of glyphosate application on the microbial activity of two Algerian soils**	**Int.J.Curr.Microbiol.App.Sci (2013) 2(12): 628-635**

Reliability	
Purpose of the study Description of endpoints	Evaluation of the impact of glyphosate treatments on microbial community structure and function in vitro of two Algerian soils Endpoints: soil basal respiration and microbial enumeration.
Test compound, application procedure, exposure period, protocol	Roundup® (containing 450 g active ingredient/L, Monsanto) Glyphosate was applied at a rate of 2.16 mg a.i. kg^{-1} soil
Experimental approach Statistical design, test environment	Refer to paper
Test organisms	Forest soil & Saharan soil without past exposure of glyphosate
Biological effects	- Glyphosate addition to the forest soil had no effects on culturable microbial community but increased the basal respiration from the 4th to 16th days, than respiration decreased. - Saharan soil had a strong response in microbial activity and a marked increase in total culturable microorganisms after 30 days of incubation.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Commercially formulated product, possibly with POEA
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	-
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	Glyphosate has no negative effects on microbial activity, shift in microbial communitiy and function were observed. Further investigation is required to evaluate possible long-term ecological risks of using glyphosate in these regions.
Type of info. (Critical, supporting, low weight)	Roundup as a glyphosate-based formulation has been utilized as one of the most commonly applied herbicides. The study could not be taken into account as critical information on the ERA of the active substance glyphosate itself. **Supporting information**

Consideration/concluding score	**UBA2**

Pasaribu, A. et al. (2013).

2716805	**Pasaribu, A. et al.**	**2013**	**Effect of herbicide on sporulation and infectivity of vesicular arbuscular mycorrhizal (*Glomus mosseae*) symbiosis with peanut plant.**	**The Journal of Animal & Plant Sciences, 23(6): 1671-1678**

Reliability	
Purpose of the study Description of endpoints	Effect of three application rates of two widely used herbicides (alachlor and glyphosate) on sporulation and infection of peanut plant by *G. mosseae* under greenhouse conditions Endpoints: hyphal length, succinate dehydrogenase activity (SDH), spore number, infection intensity (i.e. internal hyphae)
Test compound, application procedure, exposure period, protocol	Roundup® applied at 1.08, 2.16 and 3.24 µg a.i. g^{-1} dry soil These application rates represented 0.5, 1 and 1.5 times the recommended field application rate (glyphosate: 800 g a.i./ha)
Experimental approach Statistical design, test environment	-
Test organisms	*Sorghum bicolor* (innoculum) and *G. mosseae* (VA mycorrhizal fungus)
Biological effects	- none of the herbicide treatments affected the external hyphal length and (SDH) - glyphosate had no significant effects at all application rates. - Phosphorus inflow through mycorrhizal hyphae was significantly increased with the application rates of glyphosate at the highest value
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	not applicable
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercially formulated product, possibly with POEA
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	symbiotic functions of *G. mosseae* with host plant could be affected by the depressive effects of herbicides that are apparently related to the types of herbicide and their rates of application.
Type of info. (Critical, supporting, low weight)	Roundup as a glyphosate-based formulation has been utilized as one of the most commonly applied herbicides. The study could not be taken into account as critical information on the ERA of the active substance glyphosate itself. **Supporting information**
Consideration/concluding score	**UBA2**

Yousaf, S. et al. (2013)

2716820	**Yousaf, S. et al.**	**2013**	**Effect of Pesticides on the Soil Microbial Activity**	**Pakistan J. Zool., vol. 45(4), pp. 1063-1067, 2013**
Reliability				
Purpose of the study Description of endpoints	Evaluation of the effect of Pendimethaline, Trifluralin, Glyphosphate, 2, 4-D, and MCPA (Chwastox) on microbial activities in soil. Endpoints: Microbial activities (CO2 production)			
Test compound, application procedure, exposure period, protocol	Glyphosate, 41EC *i.v.* 41% W/W applied at 1900 ml/ha Microbial activities (CO2 production) measured during incubation at 1, 2, 3, 4, 5, 7, 9, 11, and 15 day intervals			
Experimental approach Statistical design, test environment	No statistical approach			
Test organisms	Soil sampes without past exposure with glyphosate			
Biological effects	- CO2 production was not affected substantially - the total amount of CO2 produced during 15 days was suppressed			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?			yes	
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?			yes	
3 Is the ecotoxicological manifestation level appropriate for the assessment?			yes	
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?			-	
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?			-	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?			no	
Concluding weight of evidence	- Highly toxic effects on soil microbial activity.			
Type of info. (Critical, supporting, low weight)	Although the study has been performed with the active substance alone (no formulation), the study could not be taken into account as critical information on the ERA because of the lack of statistical analysis. The effects describes have not statistical basis.			
Consideration/concluding score	**UBA3**			

Druille, M. et al. (2013)

2716848	**Druille, M. et al.**	**2013**	**Glyphosate reduces spore viability and root colonization of arbuscular mycorrhizal fungi**	**Applied Soil Ecology 64 (2013) 99– 103**
Reliability				
Purpose of the study Description of endpoints	Evaluate the effect of glyphosate on arbuscular mycorrhizal fungi (AMF) spore viability and roots colonization. Endpoints: spore viability, total root colonization and percentage of arbuscules and vesicles			
Test compound, application procedure, exposure period, protocol	Glacoxan ® (48 g isopropylamine salt of glyphosate) 0, 0.26 and 1× recommended field rates, corresponding to 0, 0.8 and 3 L/ha of glyphosate			
Experimental approach	Refer to paper			

Statistical design, test environment	
Test organisms	- AMF from a grassland soil without past exposure of glyphosate - *Lolium multiflorum* (substrate)
Biological effects	- Reduction of spore viability by a factor 5.8- to 7.7 in treated soils even at the lower application rate. - Root colonization was significantly lower in plants grown in glyphosate treated soil than in untreated ones. - A decrease in arbuscular colonization (but not in vesicles) was found in plants grown in soils treated with the highest herbicide rate.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercially formulated product
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no

Concluding weight of evidence	- Symbiosis was functionality affected. - Soil residence time of glyphosate and/or its degradation products was enough to reduce AMF spore viability and their ability to colonize roots. This decrease in propagules viability may affect plant diversity, taking into account the different degrees of mycorrhizal dependency between plant species that may coexist in grassland communities.
Type of info. (Critical, supporting, low weight)	Glacoxan® a glyphosate-based formulation has been utilized. Thus, the study could not be taken into account as critical information on the ERA of the active substance glyphosate itself. However it contains relevant scientific information about the impact of glyphosate-formualted product on AMF. **Supporting information**
Consideration/concluding score	**UBA2**

Druille, M. et al. (2013)

2716849	**Druille, M. et al.**	**2013**	**Arbuscular mycorrhizal fungi are directly and indirectly affected by glyphosate application**	**Applied Soil Ecology 72 (2013) 143– 149**

Reliability	
Purpose of the study Description of endpoints	Investigation of the possible pathways through which glyphosate application affects AMF spores viability and root colonization in grassland communities. Endpoints: spore viability, total root colonization and percentage of arbuscules and vesicles
Test compound, application procedure, exposure period, protocol	Glacoxan ® (48 g isopropylamine salt of glyphosate) 0, 0.26 and 1× recommended field rates, corresponding to 0, 0.8 and 3 L/ha of glyphosate Exposure: soil (direct pathway) and plant foliage (indirect pathway).
Experimental approach Statistical design, test environment	Refer to paper

Test organisms	- AMF from a grassland soil without past exposure of glyphosate - *Paspalum dilatatum* & *Lotus tenuis* (plant substrates for AMF)
Biological effects	- Spore viability was reduced even under the lowest glyphosate rate, but only when it was applied on the soil. - Total root colonization for both species was similarly decreased when glyphosate was applied to plant foliage or on soil, with no difference between 0.8 and 3 l ha^{-1}. - The number of arbuscules was 20% lower when glyphosate was applied on plant foliage, than when it was applied on the soil.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercially formulated product
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no

Concluding weight of evidence	The findings illustrate that glyphosate application negatively affects AMF functionality in grasslands, due to different causes depending on the herbicide application "site" (soil *vs* foliage). While, under field conditions, the occurrence of direct and/or indirect pathways will depend on the plant cover at the time of glyphosate application, the consequences of this practice on the plant community structure will vary with the mycorrhizal dependence of the species composition regardless of the pathway involved.
Type of info. **(Critical, supporting, low weight)**	Glacoxan® a glyphosate-based formulation has been utilized. Thus, the study could not be taken into account as critical information on the ERA of the active substance glyphosate itself. However it contains relevant scientific information about the impact of glyphosate-formualted product on AMF. **Supporting information**
Consideration/concluding score	**UBA2**

Zaller, J.G. et al. (2014)

2716858	**Zaller, J.G. et al.**	**2014**	**Glyphosate herbicide affects belowground interactions between earthworms and symbiotic mycorrhizal fungi in a model ecosystem**	**Nature, Scientific Reports, 4 : 5634**

Reliability	
Purpose of the study Description of endpoints	Investigatation to what extent a globally-used glyphosate herbicide affects interactions between earthworms and arbuscular mycorrhizal fungi (AMF). Endpoints: root mycorrhization, soil AMF, spore biomass, AMF vesicles and propagules, soil hyphal biomass
Test compound, application procedure, exposure period, protocol	- Roundup Speed (7.2 g/l Glyphosat + 9.55 g/L Pelargonsäure) - Application directly onto the plants - application rate: 177.48 ml m^2 (5-fold higer than the recommended application rate of 1 x 33 ml/m^2)
Experimental approach, Statistical design, test	Mesocosms: greenhouse experiment with white clover refer to paper

environment	
Test organisms	- sterilised "field soil" - AMF: *Glomus mosseae* - *Trifolium repens* (host plant)
Biological effects	- Herbicides significantly decreased root mycorrhization, soil AMF spore biomass, vesicles and propagules. - Herbicide application and earthworms increased soil hyphal biomass and tended to reduce soil water infiltration after a simulated heavy rainfall. - Herbicide application in interaction with AMF led to slightly heavier but less active earthworms. - Leaching of glyphosate after a simulated rainfall was substantial and altered by earthworms and AMF.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercially formulated product,
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	
Concluding weight of evidence	Evidence for sizeable changes, which provide impetus for more general attention to side-effects of glyphosate-based herbicides on key soil organisms and their associated ecosystem services.
Type of info. (Critical, supporting, low weight)	Roundup Speed a glyphosate-based formulation has been utilized. Thus, the study could not be taken into account as critical information on the ERA of the active substance glyphosate itself. However it contains relevant scientific information about the impact of glyphosate-formualted product on AMF. **Supporting information**
Consideration/concluding score	**UBA2**

Mbanaso, F.U. et al. (2014) PDF available

2716705	**Mbanaso, F.U. et al.**	**2014**	**Potential microbial toxicity and non-target impact of different concentrations of glyphosate-containing herbicide (GCH) in a model Pervious Paving System.**	**Chemosphere (2014) 100:34-41.**
Reliability				
Purpose of the study Description of endpoints		Determination of the impact of different concentrations of glyphosate-containing herbicides on non-target microorganisms and on the pollutant retention performance of Pervious Pavement Systems (PPS). Endpoints: nutrients released, fungal and bacterial growth (Carbon dioxide evolution)		
Test compound, application procedure, exposure period, protocol		Refer to paper 7200, 720 and 72 mg glyphosate /L		
Experimental approach Statistical design, test environment		Refer to paper		
Test organisms		Fungi, bacteria, protists (*Colpoda cucullus* and *Colpoda steinii*)		

Biological effects	- Glyphosate-containing herbicides affected the retention capability (15%, 9% and 5% of added hydrocarbons were released by high (7200 mg L(-1)), medium (720 mg L(-1)) and low (72 mg L(-1)) glyphosate-containing herbicides concentrations respectively). - The concentrations of nutrients released also indicate a potential for eutrophication - highest fungal growth at 7200 mg L(-1) glyphosate-containing herbicides - highest bacterial growth at concentration of 720 mg L(-1) glyphosate-containing herbicides - Protists did not survive at doses of glyphosate-containing herbicides above 72 mg L(-1), but they survived at the lower concentration
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	-
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercially formulated product
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	no
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	- At the lowest concentration of glyphosate-containing herbicides (72 mg L(-1)), biodegradation processes may not be affected as all trophic levels required for optimum biodegradation of contaminants were present - The ciliates *Colpoda cucullus* and *Colpoda steinii* present a potential for use as biomarkers of herbicide-polluted environments.
Type of info. (Critical, supporting, low weight)	Glyphosate-based formulation has been utilized. Thus, the study could not be taken into account as critical information on the ERA of the active substance glyphosate itself. However it contains relevant scientific information about the impact of glyphosate-formualted product on AMF. **Low weight**
Consideration/ concluding score	**UBA3**

Krüger, M. et al. (2013)

2716438	**Krüger, M. et al. (2013)**	**2013**	**Glyphosate suppresses the antagonistic effect of *Enterococcus* spp. on *Clostridium botulinum*.**	**Anaerobe; 20:74-8**
Reliability				
Purpose of the study Description of endpoints	Assess the toxicity of glyphosate to the most prevalent *Enterococcus* spp. in the gastro-intestinal tract GIT.			
Test compound, application procedure, exposure period, protocol	- Roundup UltraMax (Monsanto, USA), 450 mg/ ml of glyphosate - N-(Phosphonomethyle)glycine (Sigma Aldrich, Germany). - Refer to paper			
Experimental approach Statistical design, test environment	Refer to paper			
Test organisms	Intestinal microflora (lactic acid producing bacteria)			
Biological effects	- The inhibitory effect of Roundup on the growth and toxin production of *C. botulinum* type B was more than 1 mg/m - The inhibitory concentrations of glyphosate to *C. botulinum* type B were 10-			

	100 fold higher than those that suppressed growth (0.1 mg/ml and 1 mg/ml) of *E. faecalis* - Supplementation of the medium with 1 or 10 mg/ml glyphosate Roundup and glyphosate reduced the cell numbers of *C. botulinum* type B about 100 fold after 5 days of cultivation, respectively.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	not applicable
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	not applicable
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercially formulated product,
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	no
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	not applicable
Concluding weight of evidence	A reduction of lactic acid producing bacteria in the gastro-intestinal tract microbiota by ingestion of strong biocides like glyphosate could be an explanation for the observed increase in levels of *C. botulinum* associated diseases
Type of info. **(Critical, supporting, low weight)**	Roundup UltraMax is a glyphosate-based formulation has been utilized as one of the most commonly applied herbicides. Thus, the study could not be taken into account as critical information on the ERA of the active substance glyphosate itself. Moreover, gastro-intestinal microbes are not the focus of this part of the risk assessment. However it contains relevant scientific information about the impact of glyphosate-formulated product on microbial communities. **Supporting information**
Consideration/concluding score	**UBA2**

Shehata, A.A. et al. (2013)

	Shehata, A.A. et al.	**2013**	**The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro.**	**Curr Microbiol. 66(4):350-8**
Reliability				
Purpose of the study Description of endpoints	Determination the real impact of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro.			
Test compound, application procedure, exposure period, protocol	- Roundup UltraMax - Concentrations of glyphosate tested: 5, 2.4, 1.2, 0.6, 0.3, 0.15 and 0.075 mg/ml			
Experimental approach Statistical design, test environment	Refer to paper			
Test organisms	Poultry microbiota (gut bacterial community)			
Biological effects	- highly pathogenic bacteria as *Salmonella Entritidis, Salmonella Gallinarum, Salmonella Typhimurium, Clostridium perfringens* and *Clostridium botulinum* are highly resistant to glyphosate - most of beneficial bacteria as *Enterococcus faecalis, Enterococcus faecium, Bacillus badius, Bifidobacterium adolescentis* and *Lactobacillus* spp. were found to be moderate to highly susceptible.			

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	not applicable
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	not applicable
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercially formulated product,
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	no
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	not applicable
Concluding weight of evidence	The toxicity of glyphosate to the most prevalent *Enterococcus* spp. could be a significant predisposing factor that is associated with the increase in *C. botulinum*-mediated diseases by suppressing the antagonistic effect of these bacteria on clostridia.
Type of info. (Critical, supporting, low weight)	Roundup UltraMax is a glyphosate-based formulation has been utilized as one of the most commonly applied herbicides. Thus, the study could not be taken into account as critical information on the ERA of the active substance glyphosate itself. Moreover, gastro-intestinal microbes are not the focus of this opart of the risk assessment. However it contains relevant scientific information about the impact of glyphosate-formualted product on microbial communities. **Supporting information**
Consideration/concluding score	**UBA2**

Pesce, S. et al. (2009)

2311702	**Pesce, S. et al.**	**2009**	**Response of spring and summer riverine microbial communities following glyphosate exposure.**	**Ecotoxicol Environ Saf. 72(7):1905-12**
Reliability				
Purpose of the study Description of endpoints			(i) evaluate the the impact of glyphosate on natural riverine microbial communities, and (ii) assess weather microbial response to glyphosate herbicide is seasonally dependent. Endpoints: algal biomass (chlorophyll a concentrations), bacterial activity ([3H]thymidine incorporation), bacterial community diversity (16S PCR-TTGE), eukaryotic community diversity (18S PCR- DGGE)	
Test compound, application procedure, exposure period, protocol			- Glyphosate at 10 µg/L - Spring and summer 14-day microcosm studies	
Experimental approach Statistical design, test environment			Refer to paper	
Test organisms			Algae, bacterial and eukaryotic community diversity	
Biological effects			- no major effect of glyphosate on algal biomass (chlorophyll a concentrations), bacterial activity ([3H]thymidine incorporation), or bacterial community diversity (16S PCR-TTGE detection). - Effects were observed only on summer algal community structure and eukaryotic diversity.	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	No information on the substance tested (i.e. active ingredient or formulated product)
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	no
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Even if the effects of a short pulse of glyphosate (10 µg/1) on riverine microorganisms seem to be limited, the responses of natural microbial communities to glyphosate exposure (and probably to other pesticide exposures) can clearly vary between the experiments, and can be seasonally dependent
Type of info. (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA3**

Krzysko-Lupicka T, Sudol T (2008)

2311484	**Krzysko-Lupicka T, Sudol T**	**2008**	**Interactions between glyphosate and autochthonous soil fungi surviving in aqueous solution of glyphosate.**	**Chemosphere 71(7):1386-91**
Reliability				
Purpose of the study Description of endpoints	Investigate i) survival of autochthonous fungi in soil treated with 1 mM aqueous solution of glyphosate and ii) interactions between these fungal strains and glyphosate iii) degradation of glyphosate by studied fungi was determined by means of TLC Endpoints: fungal species (qualitative and quantitative)			
Test compound, application procedure, exposure period, protocol	- Glyphosate (obtained from commercial formulation by precipitation from its aqueous solution with concentrated hydrochloric acid). - Glyphosate was used as the sole source of phosphorus or nitrogen or carbon in the concentrations of: 0.5, 1.0, 1.5 and 2.0 mM.			
Experimental approach Statistical design, test environment	Refer to paper			
Test organisms	Fungi from soil samples			
Biological effects	- fungal strains possessed high tolerance against the applied doses of glyphosate (0.5–2.0 mM). - significant increase of fungal dry mass in the presence of glyphosate (as a sole source of phosphorus) applied in concentrations of 1.0–1.5 mM - glyphosate induces phenotypic changes. - degradation of gylphosate by fungi lead to the formation of 2 types of compounds none of them was glycine nor AMPA. -Survival of *Fusarium* in soil environment is potentially dangerous.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes

2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	-
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	- Existence of interactions between microorganisms and herbicide, leading to qualitative changes in fungi population in the soil that was exposed to long-term action of this xenobiotic (glyphosate) - Survival of phytopathogenic, polyphagic fungi of Fusarium kind, capable to adapt to low concentration of glyphosate is unrest, because created the potential danger to agricultural environment.
Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Bonnet, J.L. et. al. (2007)

	Bonnet, J.L. et. Al.	**2007**	**Assessment of the potential toxicity of herbicides and their degradation products to nontarget cells using two microorganisms, the bacteria *Vibrio fischeri* and the ciliate *Tetrahymena pyriformis*.**	**Environ Toxicol. 22(1):78-91.**

Reliability	
Purpose of the study Description of endpoints	The potential toxicity of several herbicides-alachlor, diuron and its photo and biotransformation products, glyphosate and its metabolite aminomethyl phosphonic acid (AMPA)-to nontarget cells was assessed using two microorganisms frequently used in ecotoxicology, *Vibrio fischeri* and *Tetrahymena pyriformis*. Endpoints: IC(50) or EC(50), population growth, esterase activities
Test compound, application procedure, exposure period, protocol	- Glyphosate (glyphosate acid,45521, Pestanal) - AMPA (08385, purity > 99%) Glyphosate and AMPA were dissolved in pure water
Experimental approach Statistical design, test environment	- Flasks, tubes and microplates - refer to paper
Test organisms	Glyphosate : - Microtox (*V. fischeri*): 18.23 mg/L ±1.39 - Non specific esterase activities (*T. pyriformis*): - Population growth (*T. pyriformis*) : > 500 mg/L AMPA: - Microtox: 50.48 mg/L ±12.68 - Non specific esterase activities: 166.5 ± 3.28 - Population growth (*T. pyriformis*): > 5000 mg/L -Glyphosate and AMPA had a less negative effect on *T. pyriformis* than on *V. fischeri*. - Tests using the eukaryotic cell (*T. pyriformis*) were more sensitive than test using the prokaryotic cell (*V. fischeri*) - Glyphosate was found to be more toxic than AMPA.
Biological effects	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	-
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	no
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	This study demonstrates that it is essential to assess the toxicity of the metabolites formed to complete a more comprehensive study of the environmental impact of a polluting agent.
Type of info. (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA3**

Laatikainen T & Heinonen-Tanski H. (2002)

	Laatikainen T & Heinonen-Tanski H	**2002**	**Mycorrhizal growth in pure cultures in the presence of pesticides.**	**J Agric Food Chem. 19;54(8):3163-72.**
Reliability				
Purpose of the study Description of endpoints	Study of the effects of pesticides on 64 ectomycorrhizal fungi of boreal forest trees. Endpoints: fungal growth on petri dishes			
Test compound, application procedure, exposure period, protocol	- Rodeo (Monsanto): 480 g glyphosate / L - Dissolute in acetone and add to medium to final concentration of 1ppm (1mg a.i./L)			
Experimental approach Statistical design, test environment	Refer to paper			
Test organisms	64 ectomycorrhizal fungi of boreal forest trees (refer to paper)			
Biological effects	- strains from genus *Leccinum*, *P. involutus* and *C. geophilium* were sensitive to glyphosate - maneb, glyphosate and terbuthylazine stimulated the growth of some mycorrhizal fungi - growth stimulation of some ectomycorrhizal fungal strains was caused mainly by herbicides glyphosate, terbuthylazine and hexazinone (*Suillus* species)			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				not applicable
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?				not applicable
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				Commercially formulated product
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?				-
3 Have parameters influencing the endpoints been considered				no

(e.g. pH, temperature, light conditions)?	
Concluding weight of evidence	Knowledge on the capabilities of ectomycorrhizal fungi to tolerate pesticides might be useful in deciding which pesticides would be less harmful to mycorrhizal fungi when used in forest nurseries and in afforested fields.
Type of info. **(Critical, supporting, low weight)**	Rodeo is a glyphosate-based formulation. Thus, the study could not be taken into account as critical information on the ERA of the active substance glyphosate itself. However it contains relevant scientific information about the impact of glyphosate-formualted product on AMF. **Supporting Information**
Consideration/concluding score	**UBA2**

Estok, D. et al. (2002)

	Estok, D. et al.	**1989**	**Effects of the herbicides 2,4-D, glyphosate, hexazinone, and triclopyr on the growth of three species of ectomycorrhizal fungi.**	**Bull Environ Contam Toxicol. (6):835-9**

Reliability	
Purpose of the study Description of endpoints	Study the effect of the herbicides hexazinone, 2,4-D, triclopyr, and glyphosate on the growth of three ectomycorrhizal fungi species. Endpoints: fungal growth on petri dishes (colony area)
Test compound, application procedure, exposure period, protocol	- Roundup (336 g/L) - concentration range: 1, 10, 100, 1000, 5000, and 10.000 ppm
Experimental approach Statistical design, test environment	
Test organisms	-3 ectomycorrhizal fungi species *Cenococcum geophilum*, *Pisolithus tinctorius*, and *Hebeloma longicaudum*
Biological effects	- Neither species showed enhanced growth in the presence of herbicide - Inhibition of fungal growth at herbicide concentrations < 100 ppm
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercially formulated product
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	Herbicides reduced the growth of the ectomycorrhizal fungi species at concentrations < 100 ppm
Type of info. **(Critical, supporting, low weight)**	Roundup is a glyphosate-based formulation. Thus, the study could not be taken into account as critical information on the ERA of the active substance glyphosate itself. However it contains relevant scientific information about the impact of glyphosate-formualted product on AMF. **Supporting information**
Consideration/concluding score	**UBA2**

Kremer, R.J. & Means, N.E (2009)

2311470	**Kremer & Means**	**2009**	**Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms**	**Europ.J. Agronomy 31: 153–161**
Reliability				
Purpose of the study Description of endpoints	Review of the impacts of glyphosate on rhizosphere microorganisms and microbial activities based on published and new data from long-term field projects documenting effects of glyphosate applied to Glyphosate Resistant (GR) soybean and maize. Endpoints: root colonization and soil populations of *Fusarium* and selected rhizosphere bacteria, Nb of fungal species, bacterial cell density			
Test compound, application procedure, exposure period, protocol	Not applicable, because it is a review			
Experimental approach Statistical design, test environment	Review mostly based on field studies			
Test organisms	Host plants: glyphosate resistant soybean and maize Fungi: *Fusarium* spp. Rhizosphere bacteria: *Pseudomonas ssp*.			
Biological effects	- Glyphosate-induced Fusarium activity in the rhizosphere , but has negative impacts on soybean nodulation - Glyphosate: - increases the proportion of Mn-oxidizing bacteria - decreases pseudomonad component that antagonizes fungal pathogens - increases agrobacteria that may be involved in Mn oxidation - shifts microbial community due to apparent selection by glyphosate exudation.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	Not applicable			
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Not applicable			
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Not applicable			
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?	Review based on commercially formulated products			
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Not applicable			
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	Not applicable			
Concluding weight of evidence	- The role of glyphosate in biofilm formation and associated Mn transformation by rhizosphere bacteria remains to be clarified - Microbial groups and functions affected by glyphosate included Mn transformation and plant availability; phytopathogen–antagonistic bacterial interactions reduction in nodulation - A more informative approach to assess the impact of GR crops on the environment is to target sensitive indicator microbial groups and/or processes in addition to the more general tests targeting microbial community diversity. - Research on the impacts of glyphosate on mycorrhizal fungi is needed			

Type of info. (Critical, supporting, low weight)	Review based on commercially formulated products. However it contains relevant scientific information about the impact of glyphosate-formualted product on **Supporting information**
Consideration/concluding score	**UBA2**

Fernandez et al. (2009)

2311322	**Fernandez et al.**	**2009**	**Glyphosate associations with cereal diseases caused by Fusarium spp. in the Canadian Prairies**	**Europ. J. Agronomy 31: 133–143**

Reliability	
Purpose of the study Description of endpoints	Review on the effects of tillage systems and glyphosate use on the development of Fusarium head blight (FHB) and Common root rot (CRR) in wheat and barley in eastern Saskatchewan.
Test compound, application procedure, exposure period, protocol	Not applicable, because it is a review
Experimental approach Statistical design, test environment	Not applicable, because it is a review
Test organisms	Fungi responsible for Fusarium head blight (FHB) and Common root rot (CRR)
Biological effects	- Previous glyphosate use was consistently associated with higher FHB levels caused by the most important FHB pathogens, *Fusarium avenaceum* and *Fusarium graminearum*. - Relationship between previous glyphosate use and increased Fusarium infection of spikes and subcrown internodes of wheat and barley, or Fusarium colonization of crop residues. - *Cochliobolus sativus*, the most common CRR pathogen, was negatively associated with previous glyphosate use, while F. avenaceum, F. graminearum, and other fungi were positively associated, suggesting that glyphosate might cause changes in fungal communities.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Not applicable
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Not applicable
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Yes, but because of the close association between noncereal crops, reduced tillage and glyphosate use, it was not possible to completely separate the effects of these factors on *Fusarium* infections.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Not applicable
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Not applicable
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	Not applicable
Concluding weight of evidence	Consistent association between previous glyphosate use and *Fusarium* infections also warrants further research to elucidate the nature of this association and the underlying mechanisms determining these effects

Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Gimsing et al. (2004)

2310505	**Gimsing et al.**	**2004**	**Chemical and microbiological soil characteristics controlling glyphosate mineralisation in Danish surface soils**	**Appl. Soil Ecol. 27, 233–242**

Reliability	
Purpose of the study Description of endpoints	A comparison was made to several chemical and microbiological soil characteristics to identify their role in regulating the fate of glyphosate in these soils. Endpoint: mineralization of glyphosate (complete degradation to CO_2) , soil respiration rate, total bacterial and *Pseudomonas spp.*populations (plate counts)
Test compound, application procedure, exposure period, protocol	Glyphosate: 40wt.%, density 1.218, Aldrich Chemical Company
Experimental approach Statistical design, test environment	- 10 g of moist soil into a serum-flask and adding 100 ml 2.0 mM glyphosate solution. Corresponding to an initial concentration of 3.4 mg glyphosate g^{-1} soil (refer to paper) - In a parallel experiment the effect of inorganic phosphate on glyphosate mineralization was studied (refer to paper)
Test organisms	- soils used are from the A-horizon of five Danish agricultural soils representing the majority of soil types found in Denmark
Biological effects	- Glyphosate was rapidly adsorbed to iron and aluminium oxides, but were later released from these pools during mineralization. - In soils with high mineralisation rates the metabolite AMPA was formed and adsorbed. - Of all the soil factors tested, the rate of mineralisation was best correlated with the population size of *Pseudomonas* spp. bacteria in the soils. - Glyphosate mineralization did not appear to be correlated neither with the overall soil respiration activity nor with the abundance of heterotrophic bacteria (CFU count). - a good correlation (R^2 = 0.87) between glyphosate mineralisation and the specific abundance of *Pseudomonas* spp was observed - Phosphate addition had a stimulating effect on glyphosate degradation in soils with low mineralisation rates, but no effect or a negative effect on mineralisation in soils with high mineralisation rates. - Finally, mineralisation rates were higher in soils from organically managed soils than in soils from conventional farming.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	-
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	-
3 Is the ecotoxicological manifestation level appropriate for the assessment?	-
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	-
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no

Concluding weight of evidence	The results indicate that the activity of glyphosate mineralising bacteria (e.g. *Pseudomonas* spp.) was a major factor controlling the fate of glyphosate in the soils.
Type of info. **(Critical, supporting, low weight)**	**Low weight**
Consideration/concluding score	**UBA3**

Giovannetti et al. (2006)

2716477	**Giovannetti et al.**	**2006**	**Mycorrhizal fungi in ecotoxicological studies: soil impact of fungicides, insecticidesand herbicides**	**Prevent. Today 2, 47–62**

Reliability	
Purpose of the study Description of endpoints	Validating a model system for the use of the arbuscular mycorrhizal fungus *Glomus mosseae* as biological indicator of chemical substances (i.e. model organism for ecotoxicological studies) Endpoints: spore germination and fungal growth
Test compound, application procedure, exposure period, protocol	Risolutiv (30.4% of Glyphosate) tested at the following doses: 73, 36, 18, 9 and 5 mg a.i./l
Experimental approach Statistical design, test environment	pot cultivation of the mycorrhizal fungus with a host plant Refer to paper
Test organisms	- *Glomus mosseae* (AMF)
Biological effects	- No effect on the spore germination even at the highest dose (hormeosis) - Considerable inhibitory action on the growth of *G. mosseae*
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Not applicable
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercially formulated product
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no

Concluding weight of evidence	- The experimental system presented in the paper could turn out to be adequate for developing dose-effect curves with G. mosseae as model organism. - Glyphosate showed hormeosis effect on the spore germination of the AMF but considerably inhibited its growth
Type of info. **(Critical, supporting, low weight)**	Risolutiv is a glyphosate-based formulation. Thus, the study could not be taken into account as critical information on the ERA of the active substance glyphosate itself. However it contains relevant scientific information about the impact of glyphosate-formualted product on AMF. **Supporting information**
Consideration/concluding score	**UBA2**

Singh et al. (2014)

	Singh et al.	**2014**	**Microbial degradation of herbicides**	**Critical Reviews in Microbiology 1-17**
Reliability				
Purpose of the study Description of endpoints	Review all the available literature on the biodegradation of key herbicides, clodinafop propargyl, 2,4-dichlorophenoxyacetic acid, atrazine, metolachlor, diuron, glyphosate, imazapyr, pendimethalin and paraquat under the following objectives			
Test compound, application procedure, exposure period, protocol	Not assessed (review)			
Experimental approach Statistical design, test environment	Not relevant (review)			
Test organisms	Not relevant (review)			
Biological effects	Microorganisms known to degrade GP include *Pseudomonas* sp., *Arthrobacter atrocyaneus* and *Flavobacterium sp*			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	Not assessed in the review			
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Not assessed in the review			
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Not mentionned in the review			
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?	yes			
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Not mentionned in the review			
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	Not mentionned in the review			
Concluding weight of evidence	- Microbes are the main vehicle for remediation of herbicides, and new discoveries, such as novel biodegradation pathways, multispecies interactions and community-level responses to herbicides addition, are helping us to understand, predict and monitor the fate of herbicides - Phytoremediation in conjunction with rhizospheric microbes may provide a cheap, fast, eco-friendly and efficient rhizoremediation processes for the removal of explosive waste from the upper layers of the soil			
Type of info. (Critical, supporting, low weight)	**Low weight**			
Consideration/concluding score	**UBA3**			

Sheng et al. (2012)

	Sheng *et al.*	**2012**	**Cropping practices modulate the impact of glyphosate on arbuscular mycorrhizal fungi and rhizosphere bacteria in agroecosystems of the semiarid prairie**	**Can J Microbiol 58(8):990-1001**
Reliability				
Purpose of the study Description of endpoints	Investigate the influence of glyphosate use on the diversity of soil rhizosphere microorganisms (especially on arbuscular mycorrhizal fungi, AMF) under field conditions.			

	The paper focuses on the examination of 4 wheat-based production systems typical of the Canadian prairie over 2 years. Endpoints: AMF colonization rate (in-situ), 18S rDNA based DGGE + sequence analysis of the DNA fragments, PFLAs for fungi, AMF, gram- and gram+ bacteria and whole microbial community
Test compound, application procedure, exposure period, protocol	Roundup Weathermax applied at 450g a.i./ha at the 3- to 4-leaf stage of wheat (equivalent to recommended application rate of glyphosate).
Experimental approach Statistical design, test environment	Field study (refer to paper) of 4 wheat-based production systems were: continuous durum wheat and durum wheat – pea rotation plantations under no tillage and minimum tillage over 2 years
Test organisms	
Biological effects	- Glyphosate caused a shift in the species dominating the AMF community in the rhizosphere, possibly through the modification of host plant physiology but without any impact on the root colonization level. - Glyphosate stimulated rhizobacterial growth, but have no influence on saprotrophic fungi - Glyphosate stimulated rhizosphere bacteria only under no tillage pea (but not in urea-fertilized durum wheat). - Mitigation of the effects of glyphosate on rhizosphere bacteria through tillage suggests a reduction in residual glyphosate activity through increased adsorption to soil binding sites upon soil mixing.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	-
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercially formulated product
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes

Concluding weight of evidence	- The authors suggest that interactions between soil fertility, tillage, and cropping practices shape the influence of glyphosate use on rhizosphere microorganisms, and not glyphosate alone. - The authors heighted the fact that small effects of glyphosate on functionally important components of the agroecosystem can have a strong impact when these effects influence large areas continuously, as with the repeated use of herbicides in most cultivated fields and therefore recommend more large scale field studies to address this problem
Type of info. (Critical, supporting, low weight)	Roundup Weathermax is a glyphosate-based formulation. Thus, the study could not be taken into account as critical information on the ERA of the active substance glyphosate itself. However it contains relevant scientific information about the impact of glyphosate-formualted product on AMF **Supporting information**
Consideration/concluding score	**UBA2**

Sihtmäe et al. (2013)

BVL Nr.: 2716810	M. Sihtmäe, I. Blinova, K. Künnis-Beres, L. Kanarbik, M. Heinlaan, A. Kahru	2013	Ecotoxicological effects of different glyphosate formulations	Applied Soil Ecology 72 (2013) 215– 224

Reliability	
Purpose of the study Description of endpoints	The aim ofthis study was to evaluate the effects of isopropylamine salt of glyphosate and two glyphosate-based herbicides, Roundup MaxTM (containing POEA) and RoundupQuickTM(without POEA), on non-target species.
Test compound, application procedure, exposure period, protocol	Isopropylamine (IPA) salt of glyphosate (CAS no 38641-94-0),40% (w/v) solution in water (29.6% acid equivalents, AE, by weight),was purchased from Sigma–Aldrich. Herbicide formulations: Roundup QuickTM (spray, 0.72% AE by weight) with the composition of IPA salt of glyphosate (CAS no 38641-94-0)1%, water 94%, other additives 5% (not specified by the manu-facturer) Roundup MaxTM(granulated, 68% AE by weight)consisting of ammonium salt of glyphosate (CAS no 114370-14-8) 75%, surfactant POEA (CAS no 61791-26-2) 21%, sodium sulphite0.5%, other additives 3.5% (not specified by the manufacturer), were produced by Monsato Europe S.A. (Antwerpen, Belgium).
Experimental approach Statistical design, test environment	Laboratory ecotoxicity testing; Acute immobilization assay with crustacean *Daphnia magna*; Acute luminescence inhibition assay with bacterium *Vibriofischeri*; Bacterial growth inhibition assay; Bacterial viability assay (a 'spot'-test); Analysis of the bacterial numbers from the Roundup-spiked soils; Long-term outdoor experiments
Test organisms	Laboratory ecotoxicity testing was conducted with (i) two aquatic organisms – crustaceans *Daphnia magna* and marine bacteria *Vibrio fischeri*, (ii) five bacterial strains (*Escherichia coli* MG1655, *Pseudomonas putida* KT2440 and three bacterial isolates from the soil) and (iii) terrestrial plants *Raphanus sativus* and *Hordeum vulgare*.
Biological effects	In case of *D. magna*, IPA salt of glyphosate(48-h EC50 = 4.2 mg AE L−1) was about 10-fold more toxic than the tested Roundup formulations and no statistically significant differences between toxicities of Roundup MaxTM and RoundupQuickTM were found. In case of the recommended field rate of Roundup formulations the number of heterotrophic bacteria in soils 10 days after the treatment was only slightly higher (2–4times) than in the control soil. Remarkable increase in the numberof heterotrophic bacteria (45 to 48-fold) was observed at the 100-fold and 300-fold rec-ommended field rate of Roundup QuickTMat 10 and 21 days afterthe treatment, Results of the plant tests demonstrated that the recommendeddose of the Roundup formulations did not affect the plant growth of either test species from already 24 days after the soil treatment.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Yes, LC50 values were determined.
1 Is the substance tested representative and relevant for the substance being assessed?	Isopropylamine (IPA) salt of glyphosate
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	-/-

Concluding weight of evidence	Authors conclude that when reporting results of toxicity testing of glyphosate formulation it is veryimportant to provide the complete name of the tested product andall the possible information on its chemical composition, as toxicity ofthe glyphosate-based herbicides to non-target aquatic (crustaceansand bacteria) and terrestrial organisms (soil bacteria and plants)vary within a wide range.LC50 for Daphna is considered in B.9.13 1.Summary of the relevant literature on aquatic organisms.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Lane, M. et al. (2012)

BVL Nr.: 2716731	**M. Lanea, N.Lorenza, J. Saxenaa, C.Ramsierb, R. P. Dicka**	**2012**	**The effect of glyphosate on soil microbial activity, microbial community structure, and soil potassium**	**Pedobiologia 55 (2012) 335– 342**

Reliability	
Purpose of the study Description of endpoints	The objective of this study was to determine the effect of single or repeated glyphosate applications on microbial and K properties of soils
Test compound, application procedure, exposure period, protocol	The form of glyphosate used in this treatment was a K salt, branded Roundup PowerMax (Monsanto, St. Louis, MO, 540 g ae/ L). The application rate of glyphosate was calculated based on the maximum recommended field application rate of 1.74 kg ae ha−1 and a 2 mm interaction depth in the soil
Experimental approach Statistical design, test environment	A 54 day incubation study (Exp I) had a 3 × 5 factorial design with 3 soils (silt loam: fine, illitic, mesic Aeric Epiaqualf) of similar physical and chemical characteristics, that varied in long-term glyphosate applications (no, low, and high glyphosate field treatments) and five glyphosate rates (0, 0.5×, 1×, 2×, and 3× recommended field rates applied once at time zero). A second 6 month incubation study (Exp II) had a 3 ×3 factorial design with three soils (as described above) and three rates of glyphosate (0, 1×, and 2× recommended field application rates applied monthly). For each study microbial properties [respiration; community structure measured by ester linked fatty acid methyl ester (EL-FAME) analysis and microbial biomass K] and K fractions (exchangeable and non-exchangeable) were measured periodically.
Test organisms	For each study microbial properties [respiration; community structure measured by ester linked fatty acid methyl ester (EL-FAME) analysis and microbial biomass K] and K fractions (exchangeable and non-exchangeable) were measured periodically.
Biological effects	For Exp I, glyphosate significantly increased microbial respiration that was closely related to glyphosate application rate, most notably in soils with a history of receiving glyphosate. For Exp II, there was no significant effect of repeated glyphosate application on soil microbial structure (EL-FAME) or biomass K.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
1 Is the substance tested representative and relevant for the substance being assessed?	Roundup PowerMax

2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Authors conclude that glyphosate: (1) stimulates microbial respiration particularly on soils with a history of glyphosate application; (2) hasm no significant effect on functional diversity (EL-FAME) or microbial biomass K; and (3) does not reduce the exchangeable K (putatively available to plants) or affect non-exchangeable K.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

B.9.13 14.1 Summary of the relevant literature on soil non-target micro-organisms

Soil microorganisms play a very important role in soil fertility by assuming key ecological functions like matter decomposition and nutrient cycling. Additionally to these key ecological functions, plant biodiversity, productivity and variability are strongly depending on the association of the vascualr plants with symbiotic and generally mutualist fungi (Van der Heijden *et al*., 1998). Microorganisms involved in this close relationship can be for e.g. ectomycorrhizal or arbuscular mycorrhizal fungi (AMF).Therefore, information about how agricultural practices and especially pesticides significantly affect soil microorganisms in general and ectomycorrhizal or AM fungi in particular, is highly required in risk assessment. However, since soil microbial diversity is extremely difficult to measure because of is high complexity (Tiedje et al. 1999), the ERA of plant protection products on soil non-target micro-organisms is in practice restricted to the measurement of impact of pesticides on soil functional diversity (i.e. carbon and nitrogen mineralization rates, respiration rate, enzyme activities...) or bacterial and fungal biomass.

Additionnaly to the effects of glyphosate and glyphosat formulations on microbial function (i.e. respiration and nitrification), this section will also review the literature addressing the impact of the pespicide on microbial diversity (i.e. eukaryotes and procaryotes) without excluding literature on riverine or marine microbes as well as literature on mycorrhiza fungi.

Glyphosate is an organophosphonate herbicide that can be easily used as a source of P, C or N by either by gram-positive or gram-negative bacteria (van Eerd et al., 2003). Therefore, in most studies, the application of glyphosate at expected or higher field concentration rates is correlated with an immediate and significant increase in soil respiration (Accinelli et al., 2002; Partoazar, M. et al., 2011), microbial biomass (Lupwayi, N.Z., et al., 2004), C- and N-mineralizations (Lancaster et al., 2006; Haney et al., 2000a, 2002b). This stimulation of soil principal functional parameters is assumed to be linked to a rapid use of glyphosate as source of nutrients (Mijangos et al., 2009) usually correlated with a metabolisation of the pesticide. Araujo et al. (2003) demonstrated in two Brazilian soils a rapid biodegradation of glyphosate by soil microorganisms with the formation the metabolite AMPA, resulting in short- and long-term positive effect of the herbicide on the soil microbial activity (increase of 10–15% in the CO2 evolved and a 9–19% increase in FDA hydrolyses in the presence of glyphosate). However, in their paper Zalaboy et al. (2012) observed that effects which might appear as beneficial (i.e. stimulation of the soil respiration) in previously untreated soils are inversed in

chronically exposed soils (in this case a depression of soil respiration was observed over time).

This potential use of glyphosate as a source of P, C or N by soil non-target micro-organisms is likely to induce a shift in their community structures. Only few studies failed to detect significant effect on soil functional diversity after application of the herbicide (e.g. Liphadzi, et al. 2005; Bay et al., 2012). Zabaloy, et al. (2008) reported that "the addition of glyphosate at a dose 10 times higher than the normal field application rates caused minor changes to soil microbial activity, bacterial density and functional richness".

In rare cases, inhibitory effects have also been reported. In a land set-aside in the western part of Prague (Czech Republic), Ruzkova et al. (2011) found that repeated application of Roundup® desiccation caused a significant increase of microbial biomass (+69%), but also strongly decreased the immobilization of nitrates by the plants (nitrate-nitrogen ratio +86%) as well as the arylsulfatase activity (–28%).

In some studies, differences in microbial parameters are more a function of time and site quality than pesticides doses. For example, Gomez et al. (2009) detected significant differences in microbial respiration over the time but not between doses of applied glyphosate. In Hart et al. (2009), seasonality was a significant determinant of denitrifier and fungal abundance. Parallelly, Busse et al. (2001) found that variation in microbial community size, activity and metabolic diversity was more a function of time of year and land-use that herbicide treatment. Similar seasonnal effects were also reported in riverine microorganisms communities (Pesce et al., 2009).

Ratcliff et al. (2006) detected a community shift from fungal dominance to equal ratio with an enrichment of opportunistic cobiotrophic bacteria that use glyphosate as a nutrient and/or C source. Community shifts from bacterial to fungal dominance were also observed (Araujo et al., 2003). Lupwayi, et al. (2004) observed herbicide-induced shifts in microbial composition even when diversity indices among treatments did not differ. This study points out the importance to assess microbial diversity and composition when looking at the effects of pesticides on non-target micro-organisms. Community shift was also reported for microbial marine communities in in-situ microcosm experiment performed at run-off event concentrations (Stachowski- Haberkorn, S. et al., 2008).
In microcosm experiments performed with sediment microbes, Widenfalk et al. (2008) focused their monitoring on various levels of microbial community organization. Community-level endpoints like bacterial activity, fungal and total microbial biomass were not affected by pesticide exposure, whereas endpoints recorded at the "sub-community level" (e.g. Phospholipid Fatty-acid Analysis, 16S rRNA genotyping, T-RFLP) demonstrated significant shifts in bacterial community composition even at environmentally relevant concentrations. The same authors concluded that "Any shifts in community structure will, however, only have consequences on ecosystem function if the tolerant microorganisms cannot compensate for biogeochemical functions normally carried out by inhibited or eliminated microbial groups". Such community shifts coupled with a loss of function are clearly illustrated in Lancaster et al. (2006). The authors looked at how the combinations of pesticides may affect soil microbial activity differently than pesticides applied alone. They found that after 30 days, soils treated with glyphosate alone (applied as Roundup® WeatherMAX, Monsanto Co., St. Louis, MO) exhibited greater microbial biomass, cumulative C and N mineralization than all other

treatments. However, the addition of "Roundup® WeatherMax" reduced C mineralization in soils treated with the pesticides fluometuron, aldicarb, or mefenoxam + pentachloronitrobenzene formulations. The authors concluded that glyphosate based herbicides might alter the soil microbial response to other pesticides. A decrease of the microbial resilience (in term of enzymatic activity) of soils under pesticide managment was also reported in Udawatta, R.P. et al. (2010).

In the microbial rhizosphere, some studies reported a growth stimulation of the ectomycorrhizal fungi tested (e.g. Laatikainen & Heinonen-Tanski , 2002), but other studies cleary demonstrated a growth inhibition of the 3 ectomycorrhizal fungi they tested (e.g. Estok et al, 2002). Because of their key function in the symbiotic relationship with the vascular plant (especially on crop productivity), most of the studies on microbial rhizosphere deal with AM fungi. Some authors have already developed and validated a model system for the use of such AMF as biological indicators of chemical substances (Giovannetti et al., 2006). The authors demonstrated strong inhibitory effect of glyphosate (tested as formulated product) on the hyphal growth of the AM fungus *Glomus mosseae*. Based on these results, they proposed a test system for developing dose-effect curves with G. mosseae as model organism.

However, studies with "dose-effect curves" are rare, and most of the literature on microbial rhizosphere look at spore viability, colonisation of the host-plant, quality of the symbiosis or infection intensity in case of pathogenic fungi. In their paper, Zaller et al. (2013) demonstrated that glyphosate (tested as formulated product) significantly affected the quality of the symbiosis between the AM fungus investigated and its host-plant (decrease of the root colonisation , the number of vesicules and propagules). In a study performed with Roundup®, even if glyphosate had no significant effects on hyphal length, spore number, or infection intensity (Pasaribu, A. et al. (2013). However, the authors could demonstrated that the quality of the symbiosis could be strongly affected even reversed (i.e. phosphorus inflow through mycorrhizal hyphae was significantly increased with the application rates of glyphosate at the highest value). Druille et al. (2012a) reported strong negative effects of glyphosate (tested as formulated product) on spore viability and host-plant colonization of AMF from natural grassland communities. Furthermore in another study (Druille et al. , 2013b), they showed that (i) soil application of glyphosate might decrease the spore bank of AMF over time, and that (ii) foliage application might decrease the quality of the symbiosis. In the view of these results, the authors stressed the impact that such effects might have on plant biodiversity.
Krzysko-Lupicka & Sudol (2008) reported on qualitative changes in fungal populations in soils with chronic exposure of glyphosate. They observed an increased of the pathogenic fungus Fusarium at low concentration of glyphosate and highlight the potential danger of this funding for agricultural environment. Fremandez et al (2009) also reported on a consistant association between Fungi from the genus Fusarium and glyphosate exposure in soils.
These results on fungal community shift leadind to an increase infestationof pathogenic fungus, are also observed in gastro-intestinal microbial communities (Krüger at al., 2013, Shehata et al., 2013).
Although the application of glyphosate seems to have no strong negative effects on microbial functions as they are defined at the moment in the risk assessment of soil non-target micro-organisms (C- and N-mineralisation), important communitiy shifts are observed.

As stated in Lupwayi et al. or in Sheng et al. (2004 and 2012, respectively), these community shifts could have long-term effects on soil biological processes or impact other essential eco-

system services. These long-term effects might be specially pronounced in the case they influence large areas in a iterative form, like it is common for the repeated use of herbicides in most cultivated field. Therefore, the relevance of both microbial diversity and composition is of main importance and should be included in future risk assessment schemes looking at the impact pesticides on soil non-target micro-organisms.

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B.9.13.15 Effects on other non-target organisms (flora and fauna)

For the group of terrestrial non-target plants (NTA), a comprehensive database of 87 peer-reviewed papers was collected by the notifier. The notifier considered one publication (Boutin et al., 2010) to be rated in category “Klimisch2” and annotated with minimal remarks, whereas the remaining were considered not acceptable for risk assessment. The submitted publications were also evaluated by RMS and have been assigned according to an UBA screening. 27 studies were recognized as supporting information (category UBA2). Moreover, after the date of submission, during the administrative process and the public launching period of the evaluation a few more peer-reviewed relevant articles were newly published (mainly period of (2012-2014) and needed to be considered in a revised DAR.Within these references, 15 additional studies focusing on non target plants have been considered as supporting information for risk assessmen (Duke et al. 2012;Dalton, R.L. 2012; de Jong et al. 2001, Collin W. 2012, Collin W. 2012, Kilbride, et al. 2001, Marshall et al. 2003, Johal and Huber et al. 2009, Helander et al. 2009; Pfleeger et al. 2014, Damgaard et al. 2013, Tesfamariam, T.

et al. 2009, Akamatsu, F. et al. 2014, Allison, J.E. et al. 2013; Tuffi Santos et al. 2004; Olszyk, D. et al. 2013).

The following sections summarise the results and discussion as found in the published literature. View of the RMS will be given in the final summariesing chapter after the results of the study are presented and discussed.

B.9.13 15.1 General

Boutin et al. (2004)

glyphecotox_175	**Boutin, C., Elmegaard, N., Kjaer, C.**	**2004**	**Toxicity testing of fifteen non-crop plant species with six herbicides in a greenhouse experiment: Implications for risk assessment**	**Ecotoxicology Volume: 13 Issue: 4 Pages: 349-369**

Reliability	
Purpose of the study Description of endpoints	The objectives of this study were (1) to investigate the pattern of sensitivity of several types of plant species to six herbicides with different modes of action, and (2) to explore the feasibility of using non-crop plants commonly found in field boundaries as test species for herbicide risk assessment
Test compound, application procedure, exposure period, protocol	Roundup® Bio (360 g/l glyphosate with 480 g glyphosate-isopropylaminsalt), Monsanto; Four dosages plus control were sprayed, 0.01, 0.1, 1 and 5 or 10 times recommended label rates for agricultural use in Canada and Denmark.
Experimental approach, Statistical design, test environment	Greenhouse test, calculation of the EC50 the linear interpolation method for sublethal toxicity, also called the inhibition concentration approach (ICp) was used (as described in US EPA report EPA/600/4/-89-001 and 001A).
Test organisms	Fifteen species were selected, 5 species from the Asteraceae family (daisy family), four from the Lamiaceae family (mint family), two from the Polygonaceae family (buckwheat family) and the rest from four other families.
Biological effects	This paper presents the result of a greenhouse experiment where testing was performed with 15 non-crop plant species sprayed with 6 herbicides. EC50 values for non crop species range between 14 and 63 g a.s./ha.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Non crop species were tested
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	Authors states that the current suite of species prescribed in current guidelines will not be adequate for the protection of habitats, e.g., field margin species, in agricultural areas.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Dry weight of aerial parts were determined as endpoint.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	All herbicides were used as formulated products thereby containing a number of surfactant compounds.
2 Do the tested concentrations relate to predicted environmental concentrations?	See above
3 Have parameters influencing the endpoints been considered adequately?	yes

Concluding weight of evidence/proposed action	Study describes that field margin species may be not adequate protected and risk may be underestimated when non crop species are not tested for risk assessment.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

White, A.L., Boutin, C. (2007)

glyphecotox_646	**White, A.L., Boutin, C.**	**2007**	**Herbicidal effects on nontarget vegetation: Investigating the limitations of current pesticide registration guidelines**	**Environmental Toxicology and Chemistry 26 (12):2634-2643**

Reliability	
Purpose of the study Description of endpoints	Several crops and wild plant species were grown under greenhouse conditions following standard protocol for phytotoxicity testing. Plants were sprayed with five different herbicides at the four- to six-leaf stage, and biomass was recorded at 28 d after spray.
Test compound, application procedure, exposure period, protocol	Round-Up Original (Monsanto Canada, Mississauga, ON) containing 356 g ai/L glyphosate was applied. A nonionic surfactant (Agral 90; Norac Concepts, Ottawa, ON, Canada) containing nonylphenoxy polyethyoxyethanol was added, Label rates (defined as grams of active ingredient applied per hectare) selected.
Experimental approach, Statistical design, test environment	All species were exposed to a one-time herbicide application at the two- to six-leaf stage. At 28 d, visual observations were recorded, ANOVA.
Test organisms	Ten different crop species were paired with closely related wild plant relatives found in field margin habitats in Eastern Ontario.
Biological effects	Results showed that current regulatory protocol will likely underestimate herbicide phytotoxicity if testing does not include data for the complete tank-mix formulation. The present study also showed that the range in herbicide sensitivity among cultivars of the same crop can be quite extensive and that, depending on the cultivar included in a risk assessment, conclusions regarding the phytotoxicity of any given herbicide may differ.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	two- to six-leaf stage
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	IC25 for *solanum lycopersicon* was determined 51 g a.s. /ha.
3 Is the ecotoxicological manifestation level appropriate for the assessment, e.g. gene induction vs. apical endpoints like growth or reproduction?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	For all species for which it could be calculated, the IC25 was much lower for the formulated product than it was for the active ingredient alone, indicating that glyphosate is much less toxic to the species tested than the formulated product Round-Up Original.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Drift values would probably less than the label rate of 2,136 g ai/ha for Round-Up Original
3 Have parameters influencing the	yes

endpoints been considered adequately (e.g. pH, temperature, light conditions)?	
Concluding weight of evidence/proposed action	This study extends the current interest by presenting three experiments highlighting some of the limitations to current phytotoxicity testing guidelines.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Boutin et al, (2010)

glyphecotox_173	**Boutin, C., White, A.L., Carpenter, D.**	**2010**	**Measuring variability in phytotoxicity testing using crop and wild plant species**	**Environmental Toxicology and Chemistry 29 (2):327-337. DOI: 10.1002/etc.30.**

Reliability	
Purpose of the study Description of endpoints	The study was conducted in greenhouse or growth chamber environments with plants growing individually in pots and harvested 28 d after spraying with two herbicides, glyphosate and atrazine, as formulated products.
Test compound, application procedure, exposure period, protocol	Round-Up Original or Vision (Monsanto Canada), both formulations containing 356 g/L glyphosate [N- (phosphonomethyl) glycine], 2,136 g a.i./ ha for glyphosate additionally Agral 901 (Norac Concepts)
Experimental approach, Statistical design, test environment	At 28 d after herbicide exposure, all above-ground green plant material was harvested and placed in a forced-air dryer for a minimum of 72 h at approximately 708C for dry biomass determination, ANOVA.
Test organisms	Eight different herbaceous broad-leaf species from four families and with different life spans were included in the ecotype variability experiment
Biological effects	It was shown that test conditions induced a large variability in a given species' response to herbicides. Both crops and wild plant species responded quite variably when they were tested in different seasons as well as when tested in a greenhouse or in growth chambers.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	IC25 values are stated, no EC 50 values.
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	The HD calculated using species sensitivity distributions with the ecotype experiment data revealed that a factor of two generally separated the least sensitive and the most sensitive ecotypes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?	Application rate is supposed to be above the predicted drift exposure
3 Have parameters influencing the endpoints been considered ?	Different prevailing conditions were discussed.
Concluding weight of evidence/proposed action	The present study supports the inclusion of an uncertainty factor in risk assessments to account for the intrinsic variability in plant

	sensitivity to herbicides.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Martin, M.L., Ronco, A.E. (2006)

glyphecotox_489	**Martin, M.L., Ronco, A.E.**	**2006**	**Effect of mixtures of pesticides used in the direct seeding technique on nontarget plant seeds**	**Bull Environ Contam Toxicol 77 (2):228-36. DOI: 10.1007/s00128-006-1054-3.**

Reliability	
Purpose of the study Description of endpoints	Assessment of effects on germination and root elongation of seeds exposed to Roundup® Max formulation of glyphosate hernbicide.
Test compound, application procedure, exposure period, protocol	Roundup® Max (74.4% glyphosate)
Experimental approach Statistical design,test environment	Germination test with 2,5 to 2500 mg/L, assessment points were seed germination and seedling root elongation , regression analysis
Test organisms	*Lactuca sativa, Brassica napus, allium cepa, medicago sativa, Lolium perenne*
Biological effects	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance	Yes, considering that the first days of seedling groth are often the most sensitive stage of plant development.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	IC50 values are given : *L.sativa* 9.89 as mg/l, *L.perenne* 15.31 mg/L, *M.sativa*: 56.31 mg/L, *A.cepa*: 131.8 mg/L, *B.napus* 1164.31 mg/L
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	No effect on seed germination were observed with any concentration for any tested species.
Concluding weight of evidence	
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Olszyk et al. (2004)

glyphecotox_529	**Olszyk, D.M., Burdick, C.A., Pfleeger, T.G.,**	**2004**	**Assessing the risk to non-target plants from herbicides**	**J Agric Meteorol 60 (4):221-242**

	Lee, E.H., Watrud, L.S.			

Reliability	
Purpose of the study Description of endpoints	Paper addresses current trends in general ERA of plants, herbicide use in general, problems of formulations etc. in US.
Test compound, application procedure, exposure period, protocol	no endpoints, 10 years old
Experimental approach, Statistical design, test environment	no
Test organisms	no
Biological effects	nd
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	no
2 Is the magnitude of effects of biological significance?	no
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Review describes uncertainties s of phytotoxicity testing and gives recommendations for improvement.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	no
2 Do the tested concentrations relate to predicted environmental?	no
3 Have parameters influencing the endpoints been?	no
Concluding weight of evidence/proposed action	Not relevant in terms of risk assessment, but review indicates limitations of ERA in general.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Wagner et al. (2003)

glyphecotox_642	**Wagner, R., Kogan, M., Parada, A.M.**	**2003**	**Phytotoxic activity of root absorbed glyphosate in corn seedlings (Zea mays L.)**	**Weed Biology and Management 3:228-232**

Reliability	
Purpose of the study Description of endpoints	The purpose of the present study was to determine the relationship between the amount of glyphosate absorbed from roots, avoiding interaction of the herbicide with any substrate and its effect on plant growth. Also, the effects of glyphosate concentration and plant transpiration on herbicide's uptake were assessed.The treated plants presented a normal pattern of glyphosate allocation, with the apex the principal sink, accumulating more than 38% of mobilized glyphosate. When corn plants absorbed more than 0.6 mg they showed a decrease in growth. The relatively high glyphosate quantities allocated in the new leaves showed the relevance of the symplastic pathway in the translocation process for root absorbed glyphosate.
Test compound, application procedure, exposure period, protocol	Commercial herbicide solution of glyphosate isopropylamine salt (0.36 kg ae/L) (specific activity 1.0 GBq mmol, determined to be98.5% pure by HPLC, International Isotope München) to obtain 2% of the radiolabeled glyphosate in a100 mg /kg total glyphosate solution.
Experimental approach, Statistics, test environment	Growth chamber experiments were conducted in order to study the absorption, translocationand activity of glyphosate when applied to roots with aqueous solution avoiding any glyphosate–substrate interaction.
Test organisms	*Zea mays*
Biological effects	A linear relationship was found between glyphosatesolution concentration and glyphosate uptake over the range of 2–30 mg/L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance?	Small amounts of glyphosate absorbed by corn root stimulates its growth; however, a very low increase in these amounts starts to produce phytotoxic effects.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	no
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered?	nd
Concluding weight of evidence	Authors expect that if there is glyphosate available in the soil solution it could be absorbed from and root crop damagecould occur. Non target plant might therefore be exposed not only vial drift, but also via the soil.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Wibawa et al. (2009)

glyphecotox_647	**Wibawa, W., Bin Mohamad, R., Bin Puteh, A., Omar, D.,**	**2009**	**Residual Phytotoxicity Effects of Paraquat, Glyphosate and Glufosinate-Ammonium**	**International Journal of Agriculture and Biology 11 (2):214-216**

	Juraimi, A.S., Abdullah, S.A.		**Herbicides in Soils from Field-Treated Plots**	
Reliability				
Purpose of the study Description of endpoints			Soil residual phytotoxicity of commonly used herbicides in plantation crops in Malaysia were investigated through bioassay.	
Test compound, application procedure, exposure period, protocol			Roundup®R (360 g/ Lglyphosate Monsanto)	
Experimental approach, Statistics, test environment			400, 800, 1200 and 1600 g a.i. /ha were applied to field plots of 5 x 20 m2.	
Test organisms			cucumber and corn	
Biological effects			Glyphosate, when applied to the field in Malaysia at rates with ranges inclusive of their field recommended rates did not leave residues in the soil, which may cause phytotoxic effect to the indicator plants, corn and cucumber	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				nd
2 Is the magnitude of effects of biological significance?				nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?				nd
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				nd
2 Do the tested concentrations relate to predicted environmental concentrations?				nd
3 Have parameters influencing the endpoints been considered?				nd
Concluding weight of evidence			Field experiment in Malaysia, environmental conditions not comparable.	
Type of info. (Critical, supporting, low weight)			**low weight**	
Consideration/concluding score			**UBA3**	

Dalton, R.L. (2010)

	R. L. DALTON, and C. BOUTIN	**2010**	**Comparison of the effect of glyphosate and atrazine herbicides on non target plants grown singly and in microcosms.**	**Environmental Toxicology and Chemistry, Vol. 29, No. 10, pp. 2304–2315, 2010**
Reliability				
Purpose of the study Description of endpoints	The objective of the present study was to compare the response of plants to the herbicides atrazine and glyphosate when they were grown singly in pots versus when grown in microcosm communities to determine if single-species tests correspond well with more realistic conditions.			
Test compound, application procedure, exposure period, protocol	Two commercially available herbicides, Atrex1 Liquid 480 (Syngenta Crop Protection) and Roundup Original® (Monsanto). Roundup Original contains 356 g/L isopropylamine salt. The surfactant Agral 90 (Syngenta Crop Protection), containing nonylphenoxy polyethoxy ethanol, was added to give a concentration of 0.5% (v/v) as recommended on the product label for annual weed control and control of *Elymus repens* (L.) Gould			
Experimental approach Statistical design, test environment	Single-species definitive tests: 5 doses following a geometric progression and a control with 6 replicates each. Doses were selected by estimating the 20, 50, and 80 inhibition concentration (IC20, IC50, and IC80) from day 28 . Greenhouse microcosm experiments (28-d test period and 70 d): Seedlings were transplanted according to a standardized planting arrangement where the invasive species (*A. petiolata* for terrestrial microcosms and *P. arundinacea* for wetland microcosms) were assigned to the middle of the pot. At the end of the test period all			

	aboveground biomass was harvested and dried separately for each species. Outdoor microcosm experiment: Once transplanted into the microcosm pots, plants were left for one week in the greenhouse and then moved to an outdoor experimental compound. Dose–response curves were fit individually using nonlinear regression with one of five models (linear, exponential, logistics, gompertz, or hormesis; SYSTAT 11) Data were square-root or log-transformed if necessary to meet the assumptions of normality of residuals and homogeneity of variance, ANCOVA
Test organisms	Nine terrestrial and seven wetland plant species common to agroecosystems of Eastern Ontario and Western Quebec.
Biological effects	Greenhouse microcosms were generally more sensitive than single-species tests. Plants grown for an extended test period or in seminatural field conditions were generally less sensitive to herbicides. Sensitivity was found to be dependent on interactions between species and test conditions. Changes in community structure were observed in herbicide-treated microcosms that would not be predicted from single-species testing.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Biomass for terrestrial and wetland target species
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	For A. *petiolata* and *P. arundinacea*, increased performance and ability to recover corresponded in higher IC25 values than would be predicted by single-species tests. These species would be overprotected by single-species testing. Some desirable species, such as *L. americanus and S. lateriflorum*, with higher single-species IC25s and lower microcosm IC25s, would be underprotected by single-species tests.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Single-species tests are useful because they are inexpensive, can demonstrate dose–response patterns uncomplicated by other factors influencing growth, and are able to provide a measure of the sensitivity.. However, they are unable to predict subtle changes in community structure that may have important long-term consequences.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Roundup Original plus addition of surfactant
2 Do the tested concentrations relate to predicted environmental concentrations	In the present study, a number of IC25s were quite low compared to herbicide label rates (i.e., under 10%) and may be representative of environmentally relevant concentrations. However, since the response at a given concentration is often species- or test-dependent, laboratory data may yield different results than field data.
3 Have parameters influencing the endpoints been considered	yes
Concluding weight of evidence	Single-species tests are useful because they are inexpensive, can demonstrate clear dose–response patterns uncomplicated by other factors influencing growth, and are able to provide a measure of the sensitivity. However, they are unable to predict subtle changes in community structure that may have important long-term consequences.
Type of info. (Critical, supporting, low weight)	***supporting***
Consideration/concluding score	***UBA2***

de Jong et al.(2001)

	Frank M. W. de Jong, Helias A.	**2001**	**Development of a Field Bioassay for the Side-Effects of Herbicides**	**Arch. Environ. Contam. Toxicol. 40,**

	Udo de Haes		**on Vascular Plants Using Brassica napus and Poa annua**	**179–183 (2001) DOI: 10.1007/s002440010161**

Reliability	
Purpose of the study Description of endpoints	A field bioassay method has been developed for assessing side-effects of herbicides on Brassica napus and Poa annua
Test compound, application procedure, exposure period, protocol	Glyphosate (Agrichem glyphosat 2, 2.16 kg a.i./ha)
Experimental approach Statistical design, test environment	
Test organisms	Brassica napus and Poa annua
Biological effects	The resulting method appeared to be very sensitive. Pesticide drift was measured using water-sensitive paper. Effects were sometimes found below the detection limit of this method (at 8 m from the treated parcel with moderate wind speeds of 3-5 m/s).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations	nd
3 Have parameters influencing the endpoints been considered	The sensitivity of the test and the test conditions are discussed and an optimized method is presented.
Concluding weight of evidence	The aim of this study was the development of a a test procedure.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	***UBA3***

B.9.13 15.2 Ecological side effects (KIIA 8.16)

Neumann et al. (2006)

	G. Neumann, S. KohlsE. Landsberg, K. Stock-Oliveria Souza, V. Römheld,	**2006**	**Relevance of glyphosate transfer to non-target plants via the rhizosphere**	**Zeitschrift für Pflanzenkrankheiten un d Pflanzenschutz, Sonderheft, ISSN 0938-9938**

Reliability	
Purpose of the study Description of endpoints	In nutrient solution, rhizobox and pot experiments authors show that foliar applied glyphosate to target plants is released into the rhizosphere after a fast translocation from shoots to roots.
Test compound, application procedure, exposure period, protocol	Roundup®-Ultra (Monsanto, St. Louis, USA) was diluted as recommended by the manufacturer (1 l/200 l-1 deionized water) to obtain a concentration of 28.4 mM. In the nutrient solution experiment, glyphosate was applied with 0 %, 5 %, 50 % and 100 % (v/v) of the recommended concentration. In the rhizobox experiment, 0 % and 100 % (v/v) were foliar applied.

Experimental approach Statistical design, test environment	Seedlings were cultivated nutrient solution or planted into rhizoboxes. Measurements of 54Mn uptake and intracellular shikimate accumulation
Test organisms	*Glycine max, Helianthus annuus*
Biological effects	In the rhizosphere glyphosate can obviously be stabilized long enough to achieve negative effects on non-target plants. Such a negative side-effect is for example inhibited acquisition of micronutrients such as Mn, but also Zn, Fe and B, which are involved in plant own disease resistance mechanisms
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	No , as effects are involved in plant own disease resistance mechanisms
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Authors predict an increase in disease problems, particularly on soils with low micronutrient availability as already reported in the US due to transfer from target to non-target plants (e.g. from weed to trees in orchards)
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered?	yes
Concluding weight of evidence	Not relevant for the traditional risk assessment, but important to improve management of glyphosate long-term applications in agricultural practice.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Fernandez et al. (2009)

glyphecotox_375	**Fernandez, M.R., Zentner, R.P., Basnyat, P., Gehl, D., Selles, F., Huber, D.**	**2009**	**Glyphosate associations with cereal diseases caused by *Fusarium spp*. in the Canadian Prairies**	**Crop Science 47 (4):1574-1584. DOI 10.2135/cropsci2006.09.0596**

Reliability	
Purpose of the study Description of endpoints	This review deals primarily with the effects of tillage systems and glyphosate use on the development of FHB and CRR in wheat and barley in eastern Saskatchewan.
Test compound, application procedure, exposure period, protocol	Test compounds not stated, experimental units were selected randomly within Crop Districts in south-east and east-central Saskatchewan to represent the most common cropping practices in the area.
Experimental approach Statistical design, test environment	nd
Test organisms	*Fusarium* spp.
Biological effects	Glyphosate use was consistently associated with higher FHB levels caused by the most important Fusarium head blight pathogens, *Fusarium avenaceum* and *Fusarium graminearum.*
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s)	nd

studied?	
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	Because of the close association between non cereal crops, reduced tillage and glyphosate use, it was not possible to completely separate the effects of these factors on *Fusarium* infections.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Glyphosate might cause changes in fungal communities, which are not assessed in current risk assessment
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations ?	nd
3 Have parameters influencing the endpoints been considered?	nd
Concluding weight of evidence	Study established a relationship between previous glyphosate use and increased *Fusarium* infection of spikes and subcrown internodes of wheat and barley, or *Fusarium* colonization of crop residues.
Type of info. (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Piotrowicz-Cieslak et al. (2010)

glyphecotox_180	**Piotrowicz-Cieslak, A.I., Adomas, B., Michalczyk, D.J.**	**2010**	**Different Glyphosate Phytotoxicity of Seeds and Seedlings of Selected Plant Species**	**Polish Journal of Environmental Studies Volume: 19 Issue: 1 Pages: 123-129 Url:**

Reliability	
Purpose of the study Description of endpoints	The aim of this study was to compare the physiological responses of six plant species (popular crops or plants recommended as indicators of soil pollution) Percent germination, root length, seedling dry mass and myo-inositol content, as well as seedling leachate electroconductivity were determined in *Lepidium sativum*, *Sinapis alba*, *Sorghum saccharatum*, *Brassica napus*, *Lupinus luteus* and *Avena sativa*.
Test compound, application procedure, exposure period, protocol	(Roundup® Ultra 360 SL containing 360 g/L) at final concentrations: 1, 3, 7, 10, 40, 80, 120, 180, 240, 400, 750, 1000, 1500, 1700 or 2000 µM.
Experimental approach Statistical design, test environment	PHYTOTOXKIT™ (MicroBio Test Inc., Belgium), variance (F test) for two factor experiments (split-plot). The mean values of the plots were compared using q SNK test (Student-Newman-Keuls).
Test organisms	Seeds of oilseed rape (*Brassica napus*), white mustard (*Sinapis alba*), yellow lupin (*Lupinus luteus*), cress (*Lepidium sativum*), oats (*Avena sativa*) and sorghum (*Sorghum saccharatum*)
Biological effects	Even the dose 7- fold lower than that recommended in agronomical practice (7 µM, i.e. 3.0 L /ha Roundup® Ultra 360 SL inhibited root growth in *B. napus* and *A. sativa*, while it did not suppress root elongation in *Lepidium sativum* and it even increased root length in *Sinapis alba*. For glyphosate concentrations within the range 1-40 µM the sharpest drop in root length occurred in *Sorghum saccharatum*, which confirms the value of this plant as a herbicide sensor plant in biotests.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Germination test
2 Is the magnitude of effects of biological significance	nd

3 Is the ecotoxicological manifestation level appropriate for the assessment?	No, endpoints were stated at day 6: EC50 of root growth after six days for *Sinapis alba*, *Sorghum saccharatum*, *Brassica napus* and *Avena sativa* was 25, 22, 35 and 110 µM, respectively. In OECD 208 effects are usually determined between14 to 21 days.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial Product
2 Do the tested concentrations relate to predicted environmental concentrations	3.0 L /ha Roundup® Ultra
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	Not all indicator plants are equally suitable for analysis of biological activity of glyphosate residues.
Concluding weight of evidence	Indication that glyphosate inhibits root growth.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Eker et al. (2006)

Non-target plants	**Eker, S., Ozturk, L., Yazici, A., Erenoglu, B., Romheld, V., Cakmak, I.**	**2006**	**Foliar applied glyphosate substantialla reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus L.*) plants**	**J. Agric. Food Chem. 54: 10019-10025**

Reliability	
Purpose of the study Description of endpoints	To study the effect of glyphosate on shoot dry matter production, chlorophyll concentration, and the uptake, translocation, and tissue accumulation of Fe, Mn, Zn, and Cu in sunflower plants.
Test compound, application procedure, exposure period	- Roundup Ultra (480 g/ as isopropylamine salt) - Application: "subherbicidal rates of glyphosate": 1.25, 2.5, and 6% of the recommended application rate provided on the product label (equivalent to 0.39, 0.79, and 1.89 mM a.i., respectively). - sprayed on foliage in a volume of nearly 1.5 mL per plant
Experimental approach, Statistics, test environment	- Each treatment consisted of four independent replications, and each replication (pot) had two plants. - Statistics: Least significant difference (LSD) calculations were performed according to Student's t-test using MSTAT-C software
Test organisms	*Helianthus annuus*
Biological effects	- Reduction of the uptake and transport of Fe and Mn in plants. -glyphosate is antagonistic to the uptake, transport, and accumulation (tissue concentration) of Fe and Mn in sunflower plants
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*Helianthus annuus*
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Effects might reduce fitness of plants or change sensitiveness towards pest organisms but will probably not relate to population effects towards non target plants
3 Is the ecotoxicological manifestation level appropriate for the assessment?	See above
Environmental Relevance	

1 Is the substance tested representative and relevant for the substance being assessed?	Roundup Ultra, commercial formulation containing probably surfactants which can not completely separate from the effects of the active substance .
2 Do the tested concentrations relate to predicted environmental concentrations?	Concentrations close to drift rates have been tested (up to 6% of the recommended application rate)
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	The results suggest that residues or drift may result in impairments in Fe and Mn nutrition of nontarget plants, possibly due to the formation of poorly soluble glyphosate-metal complexes in plant tissues and/or rhizosphere interactions.
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Tesfamariam et al. (2006)

Non-target plants	**Tesfamariam T, Bott S, Cakmak I, Römheld V & Neumann G.**	**2009**	**Glyphosate in the rhizosphere – role of waiting times and different glyphosate binding forms in soils and phytotoxicity to non-target plants.**	**. European Journal of Agronomy 31: 126-131.**

Reliability	
Purpose of the study Description of endpoints	Investigation whether plant residues of glyphosate-treated weeds or direct soil application of glyphosate bears an intoxication risk for subsequently cultivated sunflower.
Test compound, application procedure, exposure period	nd
Experimental approach, Statistics, test environment	- Greenhouse studies on two soils with contrasting properties (acidic, sandy Arenosol, calcareous loess subsoil) - treated weed: model plant *Lolium perenne* - Treatments: direct soil application and treated-weeds
Test organisms	*Helianthus annuus* seedlings
Biological effects	- detrimental effectswere more pronounced after glyphosate weed application (90% biomass reduction of seedling) compared with direct soil application (55–70% biomass reduction) at waiting time 0 d. - increase in shikimate accumulation in theseedling root tissue - impairment of the manganese-nutritional status, detectable after 21 d.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*Helianthus annuus* seedlings
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Indication for general fitness
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Probably would affect the population integrity in the end due to high biomass reduction.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	dn
Concluding weight of evidence/proposed action	Study indicates that glyphosate in plant residues might effects plant fitness.

Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Collin W. (2012)

	Collin W. f, Carol A. Auer*	**2012**	**Annual Glyphosate Treatments Alter Growth of Unaffected Bentgrass (*Agrostis*) Weeds and Plant Community Composition**	**www.plosone.org, December 2012 | Volume 7 | Issue 12**

Reliability	
Purpose of the study Description of endpoints	Examination the effects of glyphosate on weedy bentgrass plants in the context of natural and agricultural plant communities (co-occurrence suggests a potential for pollen-mediated gene flow).
Test compound, application procedure, exposure period, protocol	Glyphosate was applied according to label specifications, Soil samples were taken in each subplot prior to the study and 3 years later at the same time of year.
Experimental approach Statistical design, test environment	Five field plots were established. Plots were not fertilized, irrigated or mowed during the 3 years of the study, no known history of herbicide use. Dependent variables were examined using analyses of variance (SAS ver. 9). Meadow 1 and 2 were established in a natural meadow along the Fenton River. Hayfield 1 and 2 were placed on the upper and lower edges of an agricultural hayfield. The Wasteland plot was established in an area bordered by a road, cow pasture and wetlands. Soil nutrient levels were analyzed as possible covariates. Data from all subplotswere combined to analyze treatment, year (treated as a random variable) and bentgrass species interaction effects. Survivorship was analyzed using a probit model to determine the significance of glyphosate application.
Test organisms	Changes in plant community composition GR (glyphosate resistance) creeping bentgrass (*Agrostis stolonifera* L.,CB), Agrostis called redtop (*Agrostis gigantea* Roth., RT)
Biological effects	After three years, the treated subplots in Meadow 2 and Hayfield 1 had lower plant species richness (14.5 to 7.7 species per subplot, and 19.2 to 12.2 species, respectively) and altered plant community assemblages compared to control subplots. Treatment in Meadow 1, Hayfield 2, and Wasteland subplots did not alter the metric of plant species richness, changes in plant community assemblages were observed. Plant community composition shifted in four plot sites due to treatment. This change could largely be attributed to the increased occurrence of early and late annuals. At the end of the third year, soils in subplots receiving glyphosate had higher nitrate concentrations (Meadow plots 1 and 2). The nitrate levels in Hayfield 2 did not change. Other soil nutrients (P, K, Ca, Mg) were not affected.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Vegetative growth and reproductive potential of two weedy bentgrasses, creeping bentgrass (CB) and redtop (RT)
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Results supported the hypothesis that CB and RT plants with mimicked GR trait would have higher levels of survivorship, vegetative growth and reproductive potential with selection pressure.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	GR experienced less competition from neighboring vegetation for resources such as light, soil nutrients, or soil moisture. CB and RT plants had more tillers, above-ground biomass, and flowers in sprayed subplots. GR is not part of the AIRII environmental risk assessment.

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Glyphosate formulation not stated. This result is relevant to long-term risk assessment for HR crops that can escape cultivation because changes in plant community assemblages can produce negative impacts on other trophic levels.
2 Do the tested concentrations relate to predicted environmental concentrations	nd
3 Have parameters influencing the endpoints been considered	nd
Concluding weight of evidence	Study mimicks GR trait, which is not part of the AIRII environmental risk assessment. Nevertheless, measuring community composition over time is important for describing them response of complex agricultural or natural plant communities to annual glyphosate applications.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Panigo et al. (2011)

	Elisa S.Panigo IgnacioM.Dellaferrera, JuanM.Acosta Adria´n G.Bender JuanI.Garetto, Mariel G.Perreta	**2011**	**Glyphosate-induced structural variations in *Commelina erecta L.* (Commelinaceae)**	**Ecotoxicology and Environmental Safety 76 (2012) 135–142**

Reliability	
Purpose of the study Description of endpoints	The purpose of this study was to characterize the shoot systemof *C. erecta*, and to identify the structural changes with the aim of determining the morphological syndromes that enable its vegetative regrowth and persistence after glyphosate application.
Test compound, application procedure, exposure period, protocol	The treatments included two doses of glyphosate: 900 and 1800 ga.e./ha, and the untreated control. The doses were selected based on the recommended use of the product according to the Guide of Phytosanitary Products for the Argentine Republic (CASAFE, 2005); the former dose being for use in the cropland and the latter, for less disturbed environments near croplands.
Experimental approach Statistical design, test environment	Plants from different origins (seeds and regrowth) and increasing concentrations of glyphosate were analyzed. Regrowth shoots (vegetative cloning axes) and seedlings and two glyphosate concentrations: 900 and 1800 ga.e./ha were used.
Test organisms	*Commelina erecta*, a glyphosate- tolerant weed
Biological effects	Foliar shape and size changes,change sin the length of internodes,variations in the quantity of inflorescences,low seed production,and difference sin the number of branches. After treatment, different phenotypic responses were observed as a result of differential sensitivity to glyphosate. Most of the vegetatively-propagated plants treated with 900 ga.e./ha revealed the same morphological structureas that of controls (i.e.three clearly identifiable zones:a basal orbranching zone,a middle zone where branch production is inhibited, and an apical zone where only floral branches are produced). By contrast, the 900ga.e./ha treated seedlings and the1800ga.e./ha treated regrowth shoots and seedlings showed two phenotypic responses,exhibiting differences in the quantity and location of branches and in the survival time of axes.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Morphological paramaters are suitable for ERA, nevertheless, GR species are not included in the environmental risk

	assessment fo AIRII renewal.
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	The different phenotypic responses and morphological changes suggest that *C. erecta* shows a very high intraspecific variability, which allows it to survive even in environments with intensive glyphosate application.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Glyphosate salt (Zamba, Nidera S.A.), Glyphosate formulation not stated.
2 Do the tested concentrations relate to predicted environmental concentrations	Appropriate for in field application rates. Drift rate are expected to be lower.
3 Have parameters influencing the endpoints been considered	nd
Concluding weight of evidence	The usage of increasing concentrations for full control of plants has adverse effects on the agroecosystem:(i) it makes the population grow increasingly tolerant over time and (ii) it creates conditions for the onset of glyphosate resistance.
Type of info. (Critical, supporting, low weight)	**Low relevance**
Consideration/concluding score	**UBA3**

Kilbride, et al. (2001)

	K. M. Kilbride, F. L. Paveglio	**2001**	**Long-Term Fate of Glyphosate Associated with Repeated Rodeo Applications to Control Smooth Cordgrass (*Spartina alterniflora*) in Willapa Bay, Washington**	**Arch. Environ. Contam. Toxicol. 40, 179–183 (2001) DOI: 10.1007/s002440010161**
Reliability				
Purpose of the study Description of endpoints	3-year study assessed whether glyphosate accumulates to levels potentially hazardous to aquatic biota associated with repeated applications of Rodeo® to control Spartina.			
Test compound, application procedure, exposure period, protocol	Rodeo (5% solution v/v) and LI-700t (2% solution). This tank mix represents the maximum concentrations allowed for spray-to-wet application of Rodeo and a nonionic surfactant.			
Experimental approach Statistical design, test environment	Determination of the short- and long-term fate in marine substrate associated with repeated applications of Rodeo to control Spartina; and (2) compare concentrations of glyphosate in the substrate resulting from these multiple applications with values from laboratory toxicity tests (LC50) of marine biota to assess potential effects to the estuarine environment. Two-factor analyses of variance (ANOVAs) were used to compare substrate concentrations of glyphosate among years and locations for mudflat and Spartina plots.			
Test organisms	Invasive species Smooth Cordgrass Spartina alterniflora			
Biological effects	Glyphosate concentrations in sediment from mudflat plots declined 88% to 96% from 1 day posttreatment in 1997 to 1 year after the second Rodeot applications in 1999. In contrast, glyphosate concentrations in Spartina plots increased 231% to 591% from 1997 to 1999 because Spartina rhizomes likely did not readily metabolize or exude it.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	Short- and long-term fate and potential effects to marine biota. To assess whether glyphosate levels potentially represent an acute hazard to aquatic biota, the highest geometric mean (GM) concentrations of glyphosate from mudflat and Spartina plots annually were			

	compared with values from laboratory toxicity tests for marine biota.
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	The highest GM concentration of glyphosate from mudflat plots (16.3 mg/g DW) was found 1 DPT during 1997 that was 820 and 245 times less than LC_{50} values for *E. estuaries* and the Pacific oyster, respectively. The highest GM concentration of glyphosate for *Spartina* plots (2.30 mg/g DW) was found 1 DPT during 1998; however, it was 1,734 and 5,812 times less than LC_{50} values for the amphipod and oyster, respectively.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The glyphosate concentrations in substrates from mudflat and *Spartina* plots were less than values for toxicity tests associated with adverse effects from short- or long-term exposure.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Rodeo® tank mix
2 Do the tested concentrations relate to predicted environmental concentrations	yes
3 Have parameters influencing the endpoints been considered	3-year field study with complex envirmonmental factors influencing endpoints.
Concluding weight of evidence	Authors state that long-term detrimental effects to aquatic biota from repeated application of Rodeot for *Spartina* control would be highly unlikely.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	***UBA2***

Marshall et al. (2003)

	E J P MARSHALL, V K BROWN, N D BOATMAN, P J W LUTMAN, G R SQUIRE– & L K WARD	**2003**	**The role of weeds in supporting biological diversity within crop fields**	**European Weed Research Society Weed Research 2003 43, 77–89**

Reliability	
Purpose of the study Description of endpoints	In this paper, evidence is sought for changes in weed floras and for information on the importance of weeds for other taxa and biodiversity in general. As an example of intensive agricultural management data are examined for the UK. The hypothesis is proposed that (a) weeds are important for farmland diversity and (b) changes in agricultural management have reduced the diversity of weed flora, with subsequent impacts on other non-target taxa. A review of the available published information has been used to evaluate the roles of weeds, changes in weed flora, associated taxa and changes in weed management.
Test compound, application procedure, exposure period, protocol	Review on changes in weed communities, Changes in farming systems, Links between weeds and other taxa, Interactions between crop management, weeds and biodiversity, Balancing biodiversity and crop production.
Experimental approach Statistical design, test environment	Literature review
Test organisms	The role of weeds in arable systems for other above-ground trophic levels are examined.
Biological effects	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance, e.g. is a very small	nd

statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	*nd*
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations	nd
3 Have parameters influencing the endpoints been considered	nd
Concluding weight of evidence	The aim must be to strike a balance between adequate weed control, including the prevention of weed seed build-up, and the requirement for some plants to support biological diversity. For some, clean crops and zero tolerance of weeds is the approach, with noncrop areas supporting biodiversity. Approaches need to be based on integrated weed management, although modifications to crop management in selected areas of fields, such as conservation headlands and uncropped wildlife strips (Marshall & Moonen, 2002), may provide sufficient resources for some species of concern.
Type of info. (Critical, supporting, low weight)	***supporting***
Consideration/concluding score	***UBA2***

Johal and Huber et al. (2009)

	G.S. Johal∗ , D.M. Huber	**2009**	**Glyphosate effects on diseases of plants**	**Europ. J. Agronomy 31 (2009) 144–152**

Reliability	
Purpose of the study Description of endpoints	Review report illustrated indirect effects of glyphosate on disease predisposition.
Test compound, application procedure, exposure period, protocol	Various studies are described probably using different king of commercially available products.
Experimental approach Statistical design, test environment	Resultare descirbed for various observations related to immobilization of specific micronutrients involved in disease resistance, reduced growth and vigor of the plant from accumulation of glyphosate in meristematic root, shoot, and reproductive tissues, altered physiological efficiency, or modification of the soilmicroflora affecting the availability of nutrients involved in physiological disease resistance.
Test organisms	Please refer to comprehensive overwie in table 1 of the paper.
Biological effects	Diseases increased by glyphosate, Mechanisms of predisposition to disease, Strategies to ameliorate glyphosate predisposition to disease are presented.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations	nd
3 Have parameters influencing the endpoints been	nd

considered	
Concluding weight of evidence	Strategies that could be used to protect crops from glyphosate-induced disease are recommended: The reduction of glyphosate application and the application of critical micronutrients before glyphosate is sprayed is discussed.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	***UBA2***

Helander et al. (2009)

	Helander, M., I. Saloniemi and K. Saikkonen.	**2012**	**Glyphosate in northern ecosystems.**	Trends in Plant Science 17:569-574.

Reliability	
Purpose of the study Description of endpoints	In this opinion article, the potential ecological, environmental and agricultural risks of intensive glyphosate use in boreal regions is discussed.
Test compound, application procedure, exposure period	Glyphosate
Experimental approach, Statistics, test environment	The opinion article the following topics are presented: „Glyphosate is weeding the fields“, „Glyphosate and phosphorus competing in soil“, „Biological consequences“, „Environmental concerns“.
Test organisms	nd
Biological effects	nd
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	In the review most of the cited literature was addressed in the literature review submitted with the Annex I Renewal of glyphosate (please refer to table below). Other literature cited in this review was either regarding efficacy of the active substance, transgenic plants (not part of the GAP for review), or previous to the evaluation period (pre-2001).
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Author	**Year**	**Reference**	**Annex/ Category**

Andréa MMd, Peres TB, Luchini LC, Bazarin S, Papini S, Matallo MB, Savoy VLT	2003	Influence of repeated applications of glyphosate on its persistence and soil bioactivity. Pesquisa Agropecuaria Brasileira 38:1329-1335.	Annex B.8 (Appendix), Considered as supporting information
Benachour N, Sipahutar H, Moslerni S, Gasnier C, Travert C, Seralini GE	2007	Time- and dose-dependent effects of roundup on human embryonic and placental cells. Archives of Environmental Contamination and Toxicology 53 (1):126-133.	Please refer to Vol. 3 B.6
Borggaard OK, Gimsing AL	2008	Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. Pest Manag Sci 64 (4):441-56.	Annex B.8 (Appendix), Considered as additional information
Bott S, Tesfamariam T, Kania A, Eman B, Aslan N, Roemheld V, Neumann G	2011	Phytotoxicity of glyphosate soil residues re-mobilised by phosphate fertilisation. Plant and Soil 342 (1-2):249-263.	Annex B.8 (Appendix), Considered as additional information
Busse MD, Ratcliff AW, Shestak CJ, Powers RF	2001	Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. Soil Biology & Biochemistry 33 (12-13):1777-1789.	Annex B.9 (Appendix) Considered as supporting information
Cakmak I, Yazici A, Tutus Y, Ozturk L	2009	Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean. European Journal of Agronomy 31 (3):114-119.	Annex B.8 (Appendix), Low weight
Castilla AM, Dauwe T, Mora I, Malone J, Guitart R	2010	Nitrates and Herbicides Cause Higher Mortality than the Traditional Organic Fertilizers on the Grain Beetle, Tenebrio molitor. Bulletin of Environmental Contamination and Toxicology 84 (1):101-105.	Annex B.9 (Appendix) Low weight
Druart C, Millet M, Scheifler R, Delhomme O, Raeppel C, de Vaufleury A	2011	Snails as indicators of pesticide drift, deposit, transfer and effects in the vineyard. Science of the Total Environment 409 (20):4280-4288.	Annex B.9 (Appendix) Considered as supporting information
Duke SO	2011	Glyphosate Degradation in Glyphosate-Resistant and -Susceptible Crops and Weeds. J Agric Food Chem 59 (11):5835-5841.	Considered Supporting information
Duke SO, Powles SB	2008	Glyphosate: a once-in-a-century herbicide. Pest Manag Sci 64 (4):319-25.	Supporting information
Eker S, Ozturk L, Yazici A, Erenoglu B, Romheld V, Cakmak I	2006	Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (Helianthus annuus L.) plants. Journal of Agricultural and Food Chemistry 54 (26):10019-10025.	

Evans SC, Shaw EM, Rypstra AL	2010	Exposure to a glyphosate-based herbicide affects agrobiont predatory arthropod behaviour and long-term survival. Ecotoxicology 19 (7):1249-57.	Annex B.9 (Appendix) UBA2
Fernandez MP, Zentner KP, DePauw RM, Gehl D, Stevenson FC	2007	Impacts of crop production factors on common root rot of barley in eastern Saskatchewan. Crop Science 47 (4):1585-1595.	Pathogens/ Diseases
Fernandez MR, Zentner RP, Basnyat P, Gehl D, Selles F, Huber D	2009	Glyphosate associations with cereal diseases caused by Fusarium spp. in the Canadian Prairies. European Journal of Agronomy 31 (3):133-143.	Pathogens/ Diseases
Gimsing AL, Borggaard OK, Bang M	2004	Influence of soil composition on adsorption of glyphosate and phosphate by contrasting Danish surface soils. European Journal of Soil Science 55 (1):183-191.	Annex B.8 (Appendix), Considered as additional information
Hedberg D, Wallin M	2010	Effects of Roundup and glyphosate formulations on intracellular transport, microtubules and actin filaments in Xenopus laevis melanophores. Toxicol In Vitro 24 (3):795-802.	UBA3, Low weight for traditional ERA
Johal GS, Huber DM	2009	Glyphosate effects on diseases of plants. European Journal of Agronomy 31 (3):144-152.	Annex B.9 (Appendix) Considered as Supporting information
Kremer RJ, Means NE	2009	Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. European Journal of Agronomy 31 (3):153-161.	Annex B.9 (Appendix) Considered as Supporting information
Laitinen P, Rämö S, Nikunen U, Jauhiainen L, Siimes K, Turtola E	2009	Glyphosate and phosphorus leaching and residues in boreal sandy soil. Plant and Soil 323 (1):267-283.	Annex B.8 (Appendix), Considered as supporting information
Laitinen P, Ramo S, Siimes K	2007	Glyphosate translocation from plants to soil - does this constitute a significant proportion of residues in soil? Plant and Soil 300 (1-2):51-60.	Annex B.8 (Appendix), Considered as additional information
Laitinen P, Siimes K, Eronen L, Ramo S, Welling L, Oinonen S, Mattsoff L, Ruohonen-Lehto M	2006	Fate of the herbicides glyphosate, glufosinate-ammonium, phenmedipham, ethofumesate and metamitron in two Finnish arable soils. Pest Manag Sci 62 (6):473-91.	Annex B.8 (Appendix), Considered as supporting information
Neumann G, Kohls S, Landsberg E, Stock-Oliveira Souza K, Yamada T, Romheld V	2006	Relevance of glyphosate transfer to non-target plants via the rhizosphere. Journal of Plant Diseases and Protection (Special Issue 20):963-969.	Annex B.9 (Appendix) Considered as Supporting information

Relyea RA	2005	The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. Ecological Applications 15 (2):618-627.	Annex B.9 (Appendix) Considered as Supporting information
Relyea RA	2005	The lethal impact of roundup on aquatic and terrestrial amphibians. Ecological Applications 15 (4):1118-1124.	Annex B.9 (Appendix) Considered as Supporting information
Richard S, Moslemi S, Sipahutar H, Benachour N, Seralini GE	2005	Differential effects of glyphosate and roundup on human placental cells and aromatase. Environmental Health Perspectives 113 (6):716-720.	Please refer to Vol. 3 B.6
Shushkova T, Ermakova I, Leontievsky A	2010	Glyphosate bioavailability in soil. Biodegradation 21 (3):403-10.	Annex B.8 (Appendix), Low weight
Stenrod M, Eklo OM, Charnay MP, Benoit P	2005	Effect of freezing and thawing on microbial activity and glyphosate degradation in two Norwegian soils. Pest Management Science 61 (9):887-898.	Annex B.8 (Appendix), Considered as additional information
Tanney JB, Hutchison LJ	2010	The effects of glyphosate on the in vitro linear growth of selected microfungi from a boreal forest soil. Can J Microbiol 56 (2):138-44.	UBA3, Low weight for traditional ERA
Tesfamariam T, Bott S, Cakmak I, Römheld V, Neumann G	2009	Glyphosate in the rhizosphere--Role of waiting times and different glyphosate binding forms in soils for phytotoxicity to non-target plants. European Journal of Agronomy 31 (3):126-132.	Annex B.9 (Appendix) Considered as Supporting information
Tsui MT, Chu LM	2003	Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. Chemosphere 52 (7):1189-97.	Annex B.9, (Annex), Considered as Supporting information
Tuffi Santos LD, Graca RN, Alfenas AC, Ferreira FA, Melo CAD, Machado MS	2011	Glyphosate reduces urediniospore development and Puccinia psidii disease severity on Eucalyptus grandis. Pest management science 67 (7):876-880.	UBA3, Low weight for traditional ERA
Vera MS, Lagomarsino L, Sylvester M, Perez GL, Rodriguez P, Mugni H, Sinistro R, Ferraro M, Bonetto C, Zagarese H, Pizarro H	2010	New evidences of Roundup (glyphosate formulation) impact on the periphyton community and the water quality of freshwater ecosystems. Ecotoxicology 19 (4):710-21.	Annex B.9, (Annex), Considered as Supporting information

Vereecken H	2005	Mobility and leaching of glyphosate: a review. Pest Management Science 61 (12):1139-1151.	Annex B.8 (Appendix), Considered as additional information
Weidenhamer JD, Callaway RM	2010	Direct and Indirect Effects of Invasive Plants on Soil Chemistry and Ecosystem Function. Journal of Chemical Ecology 36 (1):59-69.	Annex B.9, (Annex), Considered as Supporting information

Pfleeger et al. (2014)

	Pfleeger T1, Blakeley-Smith M, Lee EH, King G, Plocher M, Olszyk D.	**2014**	**Effects of single and multiple applications of glyphosate or aminopyralid on simple constructed plant communities.**	**Environ Toxicol Chem. 2014 Oct;33(10):2368-78. doi: 10.1002/etc.2686. Epub 2014 Aug 29.**

Reliability	
Purpose of the study Description of endpoints	Determination of effects due to multiple applications of herbicides on small constructed plant communities.
Test compound, application procedure, exposure period, protocol	Roundup Original, Plants were treated with glyphosate at target concentrations of 0 × , 0.01 × , 0.1 × , and 0.2× a field application rate (FAR) of 1122 g ha(-1) active ingredient (a.i.) for 3 yr in 1 location, and for 2 yr in a second location.
Experimental approach Statistical design, test environment	*Prunella vulgaris* L.var. lanceolata Fern, *Festuca roemeri* (Pavlick) Alexeev, *Clarkia amoena* (Lehm.) Nels., and *Cynosurus echinatus* L. were grown together in small field plots
Test organisms	See above
Biological effects	With glyphosate, plant volume tended to decrease for all species (especially *C. echinatus*), and the decreases generally became larger with more applications. Plant communities exposed to the 2 greatest concentrations initially differed from controls but then appeared to recover.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to predicted environmental concentrations	yes
3 Have parameters influencing the endpoints been considered	yes
Concluding weight of evidence	There was no simple conclusion in terms of potential response to multiple herbicide applications in the present study, because the results varied by species, herbicide, and herbicid concentration. Studies provided further evidence that low levels of glyphosate and aminopyralid can affect native vegetation and that there were differences in species sensitivity to these herbicides.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Damgaard et al. (2013)

2716473	**Damgaard, C., Strandberg, B., Mathiassen, S., Kudsk, P.**	**2013**	**The Effect of Nitrogen and Glyphosate on Survival and Colonisation of Perennial Grass Species in an Agro-Ecosystem: Does the Relative Importance of Survival Decrease with Competitive Ability?**	**Plose one; Published: April 11, 2013 DOI: 10.1371/journal.pone.0060992**

Reliability	
Purpose of the study Description of endpoints	The combined effect of nitrogen and glyphosate on the survival and colonization probability of two perennial grass species, *Festuca ovina* and *Agrostis capillaris*, which are known to differ in their responses to both glyphosate and nitrogen treatments, is calculated using pin-point cover data in permanent frames.
Test compound, application procedure, exposure period, protocol	The treatments included 4 glyphosate treatments (0; 14.4; 72 and 360 g a.i./ha RoundupBioH, Monsanto Crop Science, Denmark A/S, 360 g/L glyphosate as a isopropylamine salt) and 3 nitrogen treatments (0, 25 and 100 kg N/ha) applied in a full factorial design.
Experimental approach Statistical design, test environment	Field experiment
Test organisms	Two perennial grass species, *Festuca ovina* and *Agrostis capillaris*
Biological effects	Authors found that the relative importance of survival increased with the level of glyphosate for the glyphosate sensitive *A. capillaris* and decreased for the glyphosate tolerant *F. ovina*.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The elasticity of survival has proven useful for quantifying the relative importance of survival for regulating plant cover along an environmental gradient.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered?	nd
Concluding weight of evidence	The proposed method can enable predictions of the effects of agricultural practices on community dynamics in a relatively simple setup.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Tesfamariam, T. et al. (2009)

2716698	**Tesfamariam, T. et al.**	**2009**	**Fate of glyphosate stored in weed residues and the potential of phytotoxicity for following crops**	**The Proceedings of the International Plant Nutrition Colloquium XVI**

				UC Davis
Reliability				
Purpose of the study Description of endpoints	This study investigates the potential risk of glyphosate phytotoxicity to non-target plants in reduced tillage systems, where glyphosate is employed as a means of weed control and minimal tillage is done during sowing without removing the glyphosate desiccated weed residues.			
Test compound, application procedure, exposure period, protocol	Plants were sprayed with 15.60 ml of 28.4mM spray solution using a hand sprayer. Twelve hours after treatment, rye grass was harvested and shoot material was chopped into 1 cm length using scissors to be applied to the soil. Sunflower was directly sown.			
Experimental approach Statistical design, test environment	Shikimate analysis in the roots by HPLC. Mineral analysis.			
Test organisms	sunflower (*Helianthus annuus* L. cv Frankasol) as non-target and rye grass (*Lolium perenne* L. cv. Kelvin)			
Biological effects	Sunflower plants showed intracellular shikimate accumulation in roots on the Arenosol, but not in the Luvisol with a soil incorporation of glyphosate-treated rye grass.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	Potential risk of phytotoxicity to non-target plants in reduced tillage systems was studied			
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	Accumulation of high levels of shikimate in plant tissues is not considered to display a population effect.			
3 Is the ecotoxicological manifestation level appropriate for the assessment?	no			
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				nd
2 Do the tested concentrations relate to predicted environmental concentrations?				nd
3 Have parameters influencing the endpoints been considered?				nd
Concluding weight of evidence	Soil type dependent residual phytotoxicity is related to the detoxification capacity difference between the two soils.			
Type of info. (Critical, supporting, low weight)	**Low weight**			
Consideration/concluding score	**UBA 3**			

Akamatsu, F. et al. (2014)

BVL.Nr.	**Akamatsu, F. et al.**	**2014**	**Evaluation of glyphosate application in regulating the reproduction of riparian black locust (*Robinia pseudoacacia* L.) after clear-cutting, and the possibility of leaching into soil**	**Landscape and Ecological Engineering January 2014, Volume 10, Issue 1, pp 47-54**
Reliability				
Purpose of the study Description of endpoints	Examination of the regrowth-inhibiting effects of glyphosate (a widely used agricultural herbicide) application to stumps of black locust after cutting on the riverbank of the Tenryu River in accordance with current river management protocol.			
Test compound, application procedure, exposure period, protocol	Roundup Maxload, Nissan Chemical Industries Ltd., Tokyo, Japan), the active ingredient of which is the potassium salt of glyphosate			
Experimental approach Statistical design, test environment	Using a paintbrush amounts of undiluted solution of a herbicide were applied on stumps. The numbers ofstump sprouts, root suckers, and seedlings in the treatment and control quadrats were counted, Analysis of glyphosate in soil			

Test organisms	*Robinia pseudoacacia*, an invasive alien species in riparian forests
Biological effects	Glyphosate application completely inhibited stump sprouting. There were no significant differences in seedling densities between treatment and control. A max. concentration of glyphosate leaching into the soil.2.6 ± 0.7 mg/kg on day 1 after application was detected.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	In this study, the application of glyphosate to black locust stumps completely inhibited sprouting
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	no
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation tested.
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered?	nd
Concluding weight of evidence	Clear cuts and glyphosate application are not considered to lead to high exposition to the environment. Study is considerd to be relevant for efficacy assessment.
Type of info. (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA3**

Egan, J.F. et al. (2013)

BVL.Nr. 2716771	**Egan, J.F. et al.**	**2013**	**A comparison of the herbicide tolerances of rare and common plants in an agricultural landscape**	
Reliability				
Purpose of the study Description of endpoints	Authors combined a floristic plant survey with a series of greenhouse bioassay experiments to test the hypothesis that common plant species are more tolerant to herbicides than rare species.			
Test compound, application procedure, exposure period, protocol	Test cpompound is not further described, probably commercial product, 5 doses with field application rate and drift rate.			
Experimental approach Statistical design, test environment	Abundances of plant species across a 100-km2 region of farmland in northwest Lancaster County, Pennsylvania, USA; 4 habitat types: arable fields, grasslands, pastures, and forests. Collection of presence or absence data in the 10-m2, 100-m2, and 1000-m2 plots and percentage of cover of all species in the 1-m2 quadrats, Greenhouse bioassay experiments.			
Test organisms	*Asclepias* spp, *Bidens* spp, *Elymus* spp., *Polygonum* spp., *Vebena* spp.			
Biological effects	Large variation in size of individuals within a species before treatment and large variation in response to herbicide exposure.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	nd			
2 Is the magnitude of effects of biological significance to cause a (population) relevant	Large variation in size of individuals within a species before treatment and large variation in response to herbicide			

effect?	exposure was observed.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	A large variability in our dose–response curves and ED estimates constrains confidence in these results was observed.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Test cpompound is not further described, probably commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered?	nd
Concluding weight of evidence	Reproduction could be more sensitive to herbicide pollution than biomass or growth response. The data provided suggest that herbicide pollution may be a factor structuring plant communities.
Type of info. (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA3**

Boutin, C. et al. (2014)

BVL.Nr. 2716765	**Boutin, C. et al.**	**2014**	**Herbicide impact on non-target plant reproduction: What are the toxicological and ecological implications?**	**Environmental Pollution.Volume 185, February 2014, Pages 295–306**

Reliability	
Purpose of the study Description of endpoints	The objectives of this work were to quantify the phenological stages of non-target plants in in-situ field situations during herbicide spray and to compare plant susceptibility at different phenological stages
Test compound, application procedure, exposure period, protocol	Test compound not stated.
Experimental approach Statistical design, test environment	Vegetation/Phenology surveys, Greenhouse experiment for effects on reproduction, Comparing phenological stage and endpoints at time of spray.
Test organisms	Woodlots with non-target plants in in-situ field situations
Biological effects	Results demonstrated that a large number of non-target plants had reached reproductive stages during herbicide spray events in wood lots and hedgerows, both in Canada and Denmark where vegetation varies considerably.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Ecological risk assessments need to include reproductive endpoints.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered?	nd
Concluding weight of evidence	Plants should be tested for longer periods (to assess seed output) than currently recommended in guidelines.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Allison, J.E. et al. (2013)

BVL Nr. 2716757	Allison, J.E. et al.	2013	Influence of soil organic matter on the sensitivity of selected wild and crop species to common herbicides	Ecotoxicology October 2013, Volume 22, Issue 8, pp 1289-1302

Reliability	
Purpose of the study Description of endpoints	The purpose of this study was to assess the impact of high and low soil Organic matter content on the sensitivity of plants of differing nitrogen affinity to glyphosate, chlorimuron ethyl and dicamba. The goal is to understand if test conditions calling for low levels of OM (3 % as specified by OECD and USEPA guidelines) truly represent the worst-case scenario for toxicity assessments; and to understand if nitrogen affinity affects species response to herbicides under guideline test conditions.
Test compound, application procedure, exposure period, protocol	Vision (Monsanto Canada Inc.), containing 356 g acid equivalent (a.e.)/ L glyphosate isopropylamine salt; Seedlings were sprayed at the 2- to 4-true leaf stage.
Experimental approach Statistical design, test environment	Doses, including controls, consisted of five replicates for a total of 35 plants for each of the low and highOMsoil treatments; non-linear regression models
Test organisms	Species of low affinity and high affinity towards nitrogen
Biological effects	Glyphosate treated plants were more sensitive in high OM soil for eight species: *Urtica dioica*, *Poa palustris*, C. vulg*are*, *P. recta*, *L. sativa*, *R. sativus*, *Solanum lycopersicum*, and *Lolium perenne*.

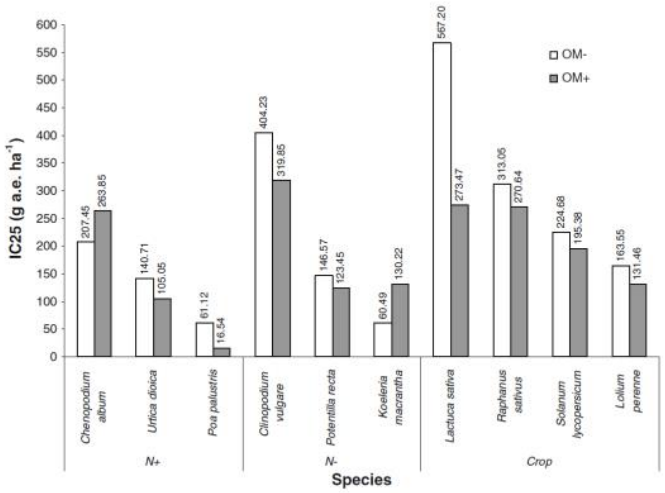

Fig. 1 Calculated IC25 values for ten plant species exposed to six doses of glyphosate. Plants were grown in two soil treatments, low organic matter (OM−) containing approximately 3 % OM, and high organic matter soil (OM+) containing approximately 9 % OM. Species are sorted either as crop species or according to the species affinity for high nitrogen (N+) environments or low nitrogen (N−) environments

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Yes IC25 were calculated.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Vision is a glyphosate-based formulation. Thus, the study could not be taken into account as critical

	information on the ERA of the active substance glyphosate itself.
2 Do the tested concentrations relate to predicted environmental concentrations?	A few IC25 values are below the estimated drift levels.
3 Have parameters influencing the endpoints been considered?	nd
Concluding weight of evidence	In most cases, plants growing in high OM soil were more sensitive to glyphosate than plants grown in low OM soil. IC25 values for the formulation ranged from 17 g a.i /ha to 567 g a.ai /ha.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Saunders et al. (2013)

BVL Nr. 2716492	**Lyndsay E. Saunders *, Melissa B. Koontz and Reza Pezeshki**	**2013**	**Root-Zone Glyphosate Exposure Adversely Affects Two Ditch Species**	**Biology 2013, 2, 1488-1496; doi:10.3390/biology2041488**

Reliability	
Purpose of the study Description of endpoints	The present laboratory experiment investigated whether two species commonly found in agricultural ditches in southcentral US were affected by root zone glyphosate in a dose-dependent manner, with the objective of identifying a sublethal concentration threshold.
Test compound, application procedure, exposure period, protocol	commercial product Roundup ProDry (EPA Registration No. 524-505) which contains 71.4% glyphosate in the form of an ammonium salt and 28% other ingredients (Monsanto Company, St. Louis, MO, USA). (0, 10, 1000, 10000 mg/L)
Experimental approach Statistical design, test environment	Root zone of individuals of *Polygonum hydropiperoides* and *Panicum hemitomon* were exposed to four concentrations of glyphosate. Leaf chlorophyll content was measured, and the ratio of aboveground biomass to belowground biomass and survival were quantified
Test organisms	*Polygonum hydropiperoides* and *Panicum hemitomon*
Biological effects	Glyphosate induced significant decreases in CCI after seven days. Root-to-shoot biomass ratios were not affected by glyphosate exposure treatments. Total mortality seen in the 1000 and 10000 mg/L treatments for *P. hydropiperoide*s and in 10,000 mg/L treatment for *P. hemitomon*.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Non-target terrestrial plants inhabiting semi-aquatic low-lying areas.
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	LC100 values stated. No derivation of LC50 values.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered?	yes
Concluding weight of evidence	Study shows taht high concentrations of comercial glyphosate concentrations relsutling from run off events can effect Non-target

	terrestrial plants inhabiting semi-aquatic low-lying areas Authors highligt that grass buffer and plant cover zones might contribute to remove herbicidal entry into the environment .
Type of info. (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

B.9.13 15.3Drift simulation

Ellis et al. (2003)

glyphecotox_362	**Ellis, J.M., Griffin, J.L., Linscombe, S.D., Webster, E.R.**	**2003**	**Rice (Oryza sativa) and corn (Zea mays) response to simulated drift of glyphosate and glufosinat**	**Weed Technology 17 (3):452-460**

Reliability	
Purpose of the study Description of endpoints	Field research was conducted during 3 yr to evaluate response of rice and corn to simulated drift rates representing 12.5, 6.3, 3.2, 1.6, and 0.8% of the usage rates of 1,120 g ai/ha glyphosate (140, 70, 35, 18, and 9 g/ha, respectively)
Test compound, application procedure, exposure period, protocol	Drift rates represented 12.5, 6.3, 3.2, 1.6, and 0.8% of the usage rate of 1,120 g ai/ha (140, 70, 35, 18, and 9 g/ha, respectively)
Experimental approach, Statistics,	Early-postemergence applications were made to two- to three-leaf rice and six-leaf corn, and late-postemergence applications to rice at panicle differentiation and to corn at nine-leaf stage (1 wk before tasseling). ANOVA
Test organisms	Rice and corn
Biological effects	Glyphosate consistently reduced rice plant height when the two highest rates were applied early, and heading was delayed 2 to 5 d. In 2 of 3 yr, the highest rate of glyphosate reduced rice yield 99 and 67% when applied early and 54 and 29% when applied late. Germination of rice seeds from glyphosate-treated plants was reduced in 1 of 2 yr and for only the highest rate. Early application of glyphosate reduced corn yield an average of 22 to 78% for the three highest rates, but only for the highest rate at the late timing (33%).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Six-leaf growth stage was assessed after 28d similar to OECD227.
2 Is the magnitude of effects of biological significance,	Injury greater 50 % was observed at drift simulation (70 g a.s. /ha) for corn height, corn injury after 14 DAT. Recovery was observed for this parameter after 28d.
3 Is the ecotoxicological manifestation level appropriate for the assessment	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Probably commercial product, but not stated.
2 Do the tested concentrations relate to predicted environmental concentrations ?	Yes, drift rates are simulated
3 Have parameters influencing the endpoints been considered?	nd, Field study.
Concluding weight of evidence/proposed action	Visual injury to both rice and corn associated with the lower herbicide, rates in some cases was minimal, but the negative effect on yield was significant. Visual injury alone, therefore, would not be a good indicator of potential

	yield loss from sublethal rates of glyphosate.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Blackburn, L.G., Boutin, C. (2003)

glyphecotox_172	**Blackburn, L.G., Boutin, C.**	**2003**	**Subtle effects of herbicide use in the context of genetically modified crops: A case study with glyphosate (Roundup® (R))**	**Ecotoxicology 12 (1-4):271-285**
Reliability				
Purpose of the study Description of endpoints			Paper presents results of literature review and results of a experiment performed with emphasis on non crop species	
Test compound, application procedure, exposure period, protocol			Roundup® Liquid	
Experimental approach, Statistics,			nd	
Test organisms			nd	
Biological effects			nd	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?			yes	
2 Is the magnitude of effects of biological significance,			nd	
3 Is the ecotoxicological manifestation level appropriate for the assessment			Application affectd F1 generation os species from Poaceae family, Brassicae family members were affectd in root and shoot development, Fabaceae members were affectes serious at alls doses tested	
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?			Commercial product	
2 Do the tested concentrations relate to predicted environmental concentrations ?			89o ga.s /ha, this application rate is supposed to be above the exposure anticipated to result from drift at typical glyphosate use rates	
3 Have parameters influencing the endpoints been considered?			Lack of uniform solution, lack of green leaves, yes	
Concluding weight of evidence/proposed action			More powerful experiment with non crop species were conducted later by the author, which might overwrite the present .	
Type of information (Critical, supporting, low weight)			**low weight**	
Consideration/concluding score			**UBA3**	

Al-Khatib et al. (2003)

glyphecotox_276	**Al-Khatib, K., Claassen, M.M., Stahlman, P.W., Geier, P.W., Regehr, D.L., Duncan, S.R., Heer, W.F.**	**2003**	**Grain sorghum response to simulated drift from glufosinate, glyphosate, imazethapyr, and sethoxydim**	**Weed Technology 17 (2):261-265**

Reliability	
Purpose of the study Description of endpoints	Field experiments were conducted at four locations in Kansas in 1999 and 2000 to evaluate grain sorghum response to simulated drift rates of four herbicides. Imazethapyr, glufosinate, glyphosate, and sethoxydim were applied at 1/3, 1/10, 1/33, and 1/100 of the use rate when plants were 10 to 20 cm tall.
Test compound, application procedure, exposure period, protocol	Use rates were 1/100, 1/33, 1/10, and 1/3 of the recommended use
Experimental approach, Statistics,	
Test organisms	sorghum
Biological effects	Visible crop injury increased as rates of each herbicide increased. The highest rate of glyphosate resulted in injury at all sites in both years. Injury ranged from 64 to 99% 8 WAT.not stetd if active ingriedien tor or commercial product,
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance,	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations ?	Yes, but at 1/ 100 and 1/33 drift rates, no significant effects observed.
3 Have parameters influencing the endpoints been considered?	nd
Concluding weight of evidence/proposed action	No information about product, no endpoints, field experiment in Canada not reliable for RA
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Felix et al. (2011)

glyphecotox_369	**Felix, J., Boydston, R., Burke, I.C.**	**2011**	**Potato Response to Simulated Glyphosate Drift**	**Weed Technology 25 (4):637-644. DOI: 10.1614/wt-d-11-00001.1.**

Reliability	
Purpose of the study Description of endpoints	Field studies were conducted in 2008 in Ontario, OR and Paterson, WA to determine the effect of simulated glyphosate drift on 'Ranger Russet' potato, including visual injury, shikimic acid accumulation, and tuber yield.
Test compound, application procedure, exposure period, protocol	Roundup® Original Max ®Glyphosate was applied at 8.5, 54, 107, 215, and 423 g ae ha21; which corresponds to 0.01, 0.064, 0.126, 0.254, and 0.5 of the lowestrecommended (846 g ha21) single application dose for glyphosate-resistant corn and sugar beet.
Experimental approach,	Glyphosate was applied when potato plants were at 10-cm height, stolon

Statistics,	hooking, tuber initiation, or bulking stage; ANOVA
Test organisms	Corn an sugar beet
Biological effects	The greatest visual foliar injury was observed when glyphosate was applied at a dose of 54 g ha21 or greater and potato plants were at the hooking stage.the lowest foliar injury was observed when glyphosate was applied to potato plants at the bulking stage. The I50 glyphosate dose at 42 d after treatment (DAT) was estimated to be 167 g ha21 for potatoes sprayed at the hooking stage
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	visual foliar injury data at 21 DAT
2 Is the magnitude of effects of biological significance,	The estimated I50 glyphosate doseat 21 DAT was lowest at hooking stage (80.3 g/ ha) followedby tuber initiation (156.4 g/ ha)
3 Is the ecotoxicological manifestation level appropriate for the assessment	80g/ha is the amount of glyphosate which can be predicted with an application rate of 2880 ga.s./ha.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	commercial product
2 Do the tested concentrations relate to predicted environmental concentrations ?	yes
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	Field study
Concluding weight of evidence/proposed action	Significant effects at concentrations related to predicted driftl concentrations, EC50 values stated for 21DAT.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Deeds et al. (2006)

glyphecotox_341	**Deeds, Z.A., Al-Khatib, K., Peterson, D.E., Stahlman, P.W.**	**2006**	**Wheat Response to Simulated Drift of Glyphosate and Imazamox Applied at Two Growth Stages**	**Weed Technology 20:23-31**

Reliability	
Purpose of the study Description of endpoints	The objectives of this research were to determine the.effects of simulated drift of glyphosate and imazamox.applied at the jointing and flowering stages of winter wheat on growth, yield, and seed germination, and to determine the correlation between early injury symptoms and grain yield.
Test compound, application procedure, exposure period, protocol	Roundup® Ultra Max ®Glyphosate at 1/1003, 1/333, 1/103, and 1/33 of usage rates of 840 g ae/ha glyphosate and 35 g/ha imzamox were applied individually to wheat in the early jointing or the early flower stages of growth. All glyphosate5 treatments included 2% ammonium sulfate by weight.
Experimental approach, Statistics,	Wheat plants were observed for injury symptoms and recovery throughout the growing season, and visible injury ratings were determined 1, 2, and 4 wk after treatment (WAT) using a scale of 0 to 100, with 0 equal to no wheat injury and 100 equal to plant mortality; regression analysis.
Test organisms	Wheat varieties
Biological effects	Glyphosate injury symptoms were noticeable on wheat plants within 4 to 7 d after treatment and peaked at 3 to 4 WAT. Symptom intensity differed depending on glyphosate rate and environmental conditions. Wheat injury ratings. Wheat injury ratings were highly correlated with yield reduction, and

	the correlation was more apparent between yield reduction and injury rating at 4 WAT than injury ratings at 1 and 2 WAT
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Jointing an flowering stage
2 Is the magnitude of effects of biological significance,	no
3 Is the ecotoxicological manifestation level appropriate for the assessment	no
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations ?	yes
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	Field experiment
Concluding weight of evidence/proposed action	No EC50 values calculated, but obviously ranging for visual injury between 0,05 and 0.25 of use rate (approx 40 to 210 g/ha).
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Gilreath et al. (2001)

glyphecotox_385	**Gilreath, J.P., Chase, C.A., Locascio, S.J.**	**2001**	**Crop injury from sublethal rates of herbicide. I. Tomato**	**Hortscience 36 (4):669-673**

Reliability	
Purpose of the study Description of endpoints	The objectives were to evaluate the extent of phytotoxic injury and the effect on yield of fresh market tomato exposed at three stages of development to levels of glyphosate known to be sublethal.
Test compound, application procedure, exposure period, protocol	Roundup® 4EC®; Monsanto Agricultural Products, St. Louis) were applied at three reproductive growth stages of 'Sunny' tomato applied at 0,1, 10, and 100 g/ha in a volume of 234 L
Experimental approach, Statistics,	
Test organisms	tomato
Biological effects	Exposure to 60 to 100 g/ha during the period 4 to 5.5 weeks after transplanting, just prior to bloom of the first cluster and during bloom, caused foliar injury and flower abscission, and reduced fruit set. Plants treated later were larger and more mature. They were less susceptibleto foliar injury.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance,	Yield reductions
3 Is the ecotoxicological manifestation level appropriate for the assessment	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations ?	yes
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	Field study
Concluding weight of evidence/proposed action	Field study in Florida with commercial formulation.

Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Gove et al. (2007)

glyphecotox_394	**Gove, B., Power, S.A., Buckley, G.P., Ghazoul, J.**	**2007**	**Effects of herbicide spray drift and fertilizer overspread on selected species of woodland ground flora: comparison between short-term and long-term impact assessments and field surveys**	**Journal of Applied Ecology 44 (2):374-384. DOI 10.1111/j.1365-2664.2007.01261.x.**

Reliability	
Purpose of the study Description of endpoints	Six species of woodland plants were exposed to the herbicide glyphosate at concentrations equivalent to those measured in spray drift trials (1–25% of the full application rate) in short-term greenhouse and long-term field experiments.
Test compound, application procedure, exposure period, protocol	Glyphosate (Egret, Cardel, France) in liquid form at a concentration of 360 g glyphosate/ L plus a polyoxyethylene amine surfactant; application rates vary between 2 and 10 L active ingredient (a.i.) /ha. The median application rate of 6 L (2160 g) a.i. /ha was chosen as the maximum dose rate (100%).
Experimental approach, Statistics, test environment	A glyphosate dosing regime of 0, 0·06, 0·3, 0·6 and 1·5 L a.i./ ha (0%, 1%, 5%, 10% and 25% of the median field application rate) was followed, covering the range of doses measured in spray drift situations. Non-parametric Kruskal–Wallis test.
Test organisms	Fourteen native woodland plant species
Biological effects	This study has shown that herbicide concentrations as low as 1–5% of the median field application rate can have biologically significant effects on several woodland species, among them species of conservation value.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance?	The vigour and fitness of understorey plants in woodland margins may be affected by herbicide applications to adjacent agricultural land.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered?	nd

Concluding weight of evidence	Relevant for general risk assessment. Authors recommend the adoption of no-spray buffer zones of at least 5 m to protect the majority of woodland species from the impacts of agrichemicals applied to adjacent land.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Pfleeger et al. (2008)

glyphecotox_544	**Pfleeger, T., Olszyk, D., Plocher, M., Yilma, S.**	**2008**	**Effects of low concentrations of herbicides on full-season, field-grown potatoes**	**Environmental Toxicology and Chemistry 30 (2):455-468. Doi 10.1002/Etc.394.**

Reliability	
Purpose of the study Description of endpoints	Field trials were conducted to determine if potato (*Solanum tubersum* L.) vegetative growth and tuber yield and quality were affected by herbicides at below recommended field rates.
Test compound, application procedure, exposure period, protocol	Commercial products, brand names were not stated. Herbicide characteristics are listed in Table 1. Herbicides were applied at 14 or 28 days after emergence (DAE) at 0.00056, 0.0032, 0.018, or 0.1 times the field application rate (FAR)
Experimental approach Statistical design, test environment	Potatoes were grown in fields at the Oregon State University Horticulture Farmwith herbicides applied at below recommended field applicationrates 14 d after emergence (DAE) or at 28 DAE. ANOVA
Test organisms	*Solanum tubersum*
Biological effects	Tuber yield and quality parameters were more affected by lower herbicide rates than were plant height or injury.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Mean plant height at time of spraying for 14 DAE plants in 2003, 2004, and 2005 was 0.213, 0.193, and 0.295 m, respectively. Mean plant height at time of spraying for 28 DAE plants in 2003, 2004, and 2005 was 0.393, 0.578, and 0.581 m, respectively.
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Glyphosate affected tuber production more in 2004 than in 2003.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Vegetative responses did not accurately predict yield and quality responses of tubers; therefore, reproductive responses should be considered in phytotoxicity test protocols for pesticide registration in the USA.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Unknown commercial product
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	Field study
Concluding weight of evidence	Reproductive responses should be considered in phytotoxicity test protocols for pesticide registration
Type of info. (Critical, supporting, low weight)	**low weight for ERA of glyphosate, critical for ERAin general**
Consideration/concluding score	**UBA2**

Pfleeger et al. (2011)

glyphecotox_179	**Pfleeger, T., Olszyk, D., Lee, E.H., Plocher, M.**	**2011**	**Comparing Effects of Low Levels of Herbicides on Greenhouse- and Field-Grown Potatoes (*Solanum Tuberosum* L.), Soybeans**	**Environmental Toxicology and Chemistry. Volume: 30 Issue: 2 Pages: 455-468 DOI: 10.1002/etc.394**

			(*Glycine Max* L.), and Peas (*Pisum Sativum* L.)	**ISSN: 1552-8618 (online)**

Reliability	
Purpose of the study Description of endpoints	Toxicology tests were conducted on potatoes, peas, and soybeans grown in a native soil in pots in the greenhouse and were compared to plants grown outside under natural environmental conditions to determine toxicological differences between environments, whether different plant developmental stages were more sensitive to herbicides, and whether these species were good candidates for plant reproductive tests.
Test compound, application procedure, exposure period, protocol	Roundup®, field application rate 832 g ha_1 a.i.; concentrations of 0, 0.00056, 0.00320, 0.01800 and 0.10000the FAR for each herbicide.
Experimental approach Statistical design,,test environment	For potatoes, herbicide treatments were applied each year at tuber initiation and bulking (generally 14 or 28 d after emergence.ANOVA,
Test organisms	*Pisum sativum, (Solanum tuberosum, Glycine max)*
Biological effects	The results indicate that potatoes were not more sensitive in either environment for the chemicals tested. potatoes exposed to glyphosate at different developmental stages had nonsignificant effects on tuber measures. However, vegetative measures were as sensitive or more sensitive when potatoes were exposed to glyphosate.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Authors found visual injury not to be necessarily the most sensitive endpoint.
2 Is the magnitude of effects of biological significance	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	Method was discussed including possible deficiencies
Concluding weight of evidence	General consideration for RA that ratio between greenhouse- and field-grown plants to be around 1.8. Results may be more or less sensitive than reality, and more restrictive regulations (safety factors) should be imposed to account for this variability.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Nandula et al. (2007)

glyphecotox_522	**Nandula, V.K., Reddy, K.N., Rimando, A.M., Duke, S.O., Poston, D.H.**	**2007**	**Glyphosate-resistant and -susceptible soybean (*Glycine max*) and canola (*Brassica napus*) dose response and metabolism relationships with glyphosate**	**Journal of Agricultural and Food Chemistry 55 (9):3540-3545. doi: 10.1021/jf0635681**

Reliability	
Purpose of the study Description of endpoints	Experiments were conducted to determine (1) dose response of glyphosate-resistant (GR) and -susceptible (non-GR) soybean [*Glycine max* (L.) Merr.]

	and canola (*Brassica napus* L.)
Test compound, application procedure, exposure period, protocol	Glyphosate-K was applied at 0.87, 1.73, 3.47, 6.93, 13.86, 27.72, 55.44, and 110.88 kg ae ha-1 to Asgrow 4603RR GR soybean and at 0.007, 0.015, 0.03, 0.06, 0.11, 0.22, 0.44, and 0.87 kg ha-1 to HBKC 5025 non-GR soybean.
Experimental approach Statistical design, test environment	Data were subjected to analysis of variance, and means were separated using Fisher's protected least significant difference (GR50 (glyphosate dose required to cause a 50% reduction in plant dry wt accumulation) values for GR and non-GR soybean and canola were calculated fitting nonlinear regression equations
Test organisms	Soybean and canola
Biological effects	GR50 non-GR soybean = 0.47 kg/ha, canola= 0.3 kg/ha
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Plants were subirrigated with water and fertilized as needed. Soybean plants at oneto two-trifoliate leaf (22 days old, 45 cm tall) growth stage and canola plants at four- to five-leaf (29 days old, 14 cm tall) growth stage were used for treatment.
2 Is the magnitude of effects of biological significance	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	No details about formulated product
2 Do the tested concentrations relate to predicted environmental concentrations	nd
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	The greenhouse was maintained at 25/20 ?C ((3 ?C) day/night temperature with natural light supplemented
Concluding weight of evidence	Additional information about endpoints for herbicidal product (GR50 non-GR soybean = 0.47 kg/ha, canola= 0.3 kg/ha)
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Koger et al. (2005)

glyphecotox_428	**Koger, C.H., Shaner, D.L., Krutz, L.J., Walker, T.W., Buehring, N., Henry, W.B., Thomas, W.E., Wilcut, J.W.**	**2005**	**Rice (*Oryza sativa*) response to drift rates of glyphosate**	**Pest Management Science 61 (12):1161-1167. Doi 10.1002/Pt.1113.**

Reliability	
Purpose of the study Description of endpoints	Greenhouse and field studies were conducted to investigate response of two rice varieties, Priscilla and Cocodrie, to sub-lethal rates of glyphosate in terms of injury, shikimate accumulation and yield.
Test compound, application procedure, exposure period, protocol	An isopropylamine salt of glyphosate (Roundup® Custom) was applied at 0, 26, 105 and 420 gAE/ ha to 31- to 37-cm-tall plants in the three-leaf growth stage. A nonionic surfactant (Induce□, a mixture of alkylarylpolyoxyalkane ethers and free fatty acids) was added at 2.5ml/l to each glyphosate solution
Experimental approach Statistic, test	In the greenhouse, more shikimate accumulated in Cocodrie than Priscilla at comparable glyphosate rates applied to plants at the three-leaf stage. In field

environment	studies, glyphosate was applied to both varieties when they were 74-cm tall and in the internode separation growth stage.
Test organisms	*Oryza sativa*
Biological effects	The highest rate of glyphosate reduced yield in Cocodrie by 92% whereas there was only a 60% yield reduction in Priscilla. The estimated IC50 of glyphosate on Cocodrie was 60 g ha−1 compared with 339 g ha−1 for Priscilla. The differences in the sensitivity of these two varieties to glyphosate may be related to the physiological state of the plants at the time of treatment. Both varieties were sprayed at internode elongation when the plants were 74cm tall.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	31- to 37-cm-tall plants in the three-leaf growth stage.
2 Is the magnitude of effects of biological significance	yes
3 Is the ecotoxicological effect appropriate for the assessment?	This research demonstrates that a drift event can be detected and any subsequent effect on rice yield can be measured, especially if the rice is exposed to sub-lethal rates of glyphosate at the beginning of the reproductive growth stage.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations	Yes,
3 Have parameters influencing the endpoints been considered?	nd
Concluding weight of evidence	Visual injury was apparent by 7 DAT and was a better parameter than height reduction for confirming glyphosate exposure.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Brown et al. (2009)

glyphecotox_303	**Brown, L.R., Robinson, D.E., Young, B.G., Loux, M.M., Johnson, W.G., Nurse, R.E., Swanton, C.J., Sikkema, P.H.**	**2009**	**Response of Corn to Simulated Glyphosate Drift Followed by In-Crop Herbicides**	**Weed Technology 23 (1):11-16. Doi 10.1614/Wt-08-067.1**

Reliability	
Purpose of the study Description of endpoints	Thirteen field experiments were conducted in Illinois, Indiana, Ohio, and Ontario from 2005 to 2007 to determine the effects of simulated glyphosate drift followed by in-crop applications of nicosulfuron/rimsulfuron plus dicamba/ diflufenzopyr or foramsulfuron plus bromoxynil plus atrazine on nontransgenic corn injury, height, stand count, shoot dry weight, and yield.
Test compound, application procedure, exposure period, protocol	Glyphosate, Roundup® WeathermaxH, Monsanto Canada Inc., Glyphosate1 was applied to corn at the 4- to 5-leaf stage at 0, 10, 50, 100, and 200 g/ha, representing approximately 0, 1, 5, 10, and 20% of the recommended rate (1,000 g/ha), respectively, to simulate herbicide drift. Conventional cornherbicides consisting of nicosulfuron/rimsulfuron (25 g/ha) plus dicamba/diflufenzopyr2 (200 g/ha), or foramsulfuron3 (35 g/ha) plus bromoxynil3 (280 g/ha) plus atrazine4 (1,000 g/ha) were applied 2 to 5 d after the simulated glyphosate drift application.
Experimental approach Statistic, test environment	randomized complete block design with four replications. Corn was planted
Test organisms	*corn*
Biological effects	Simulated glyphosate drift at 100 and 200 g/ha, resulted in 11 to 61% visual crop injury and a 19 to 45% decrease in corn height. Simulated glyphosate drift at 200 g/ha caused a reduction in shoot dry weight by 46%, stand count by 28% and yield by 49 to 56%.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance	nd
3 Is the ecotoxicological effect appropriate for the assessment?	No, as after glyphosate treatment additional herbicides were used.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product , plus additional herbicides
2 Do the tested concentrations relate to predicted environmental concentrations	yes
3 Have parameters influencing the endpoints been considered?	nd
Concluding weight of evidence	Glyphosate drift can result in an additive increase in crop injury from the application of in-crop herbicides in adjacent fields.
Type of info. (Critical, supporting, low weight)	Low weight
Consideration/concluding score	**UBA2-3**

Webster et al. (2004)

	T. M Webster,W.W Hanna and B. G Mullinix Jr	**2004**	**Bermudagrass (*Cynodon* spp) dose–response relationships with clethodim, glufosinate and glyphosate**	**Pest Manag Sci 60:1237–1244**
Reliability				

Purpose of the study Description of endpoints	The objective was to characterize the dose–response of common bermudagrass, three commercial triploid hybrid cultivars and eight experimental triploid hybrid cultivars to three common herbicides. Evaluation of the relative herbicide sensitivities of these bermudagrass cultivars provides knowledge of these bermudagrass cultivars that can be used in the development of herbicide-resistant turf-bermudagrass cultivars.
Test compound, application procedure, exposure period, protocol	Each herbicide was applied at eight doses on a geometric scale (with a multiplier of two) and included: 0, 0.0625, 0.125, 0.25, 0.5, 1, 2, and 4 times the registered herbicide use rate. The registered use rates were 0.84 kgAE/ha for glyphosate.
Experimental approach Statistical design, test environment	Greenhouse studies were conducted to evaluate the sensitivity of three commercial cultivars, eight experimental cultivars and common bermudagrass to clethodim, glufosinate and glyphosate. Data were examined using analysis of variance.
Test organisms	Each treatment was applied to 12 bermudagrass cultivars
Biological effects	I_{50} (*Plant color rating 17 DAT*) for cultivar 97-4= 0.34 kg/ha. I_{50} (plant vigour) for 97-4 was 0.39 kg/ ha.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Plant color are not considered to substantially effect population. Plant vigour/ phytotoxcitiy is a commom parameter in OECD 227 testing.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Formulation not stated, but recalculated for acid equivalents
2 Do the tested concentrations relate to predicted environmental concentrations	Minimum concentration equivalent to 6% use rate for a unknown formulation.
3 Have parameters influencing the endpoints been considered	nd
Concluding weight of evidence	IC50 far above the relevant enpoint for ERA, unknown commercial formulations
Type of info. (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Tuffi Santos et al. (2004)

	Tuffi Santos, LD., Sant'Anna-Santos, BF., Meira, RMSA., Ferreira, FA., Tiburcio, RAS. and Machado, AFL.	**2004**	**Leaf anatomy and morphometry in three eucalypt clones treated with glyphosate**	**Braz. J. Biol., 69(1): 129-136, 2009**
Reliability				
Purpose of the study Description of endpoints	This work aimed to evaluate the effects of simulated drift of glyphosate on the morphoanatomy of three eucalypt clones and to correlate the intoxication symptoms on a microscopic scale with those observed in this visual analysis.			
Test compound, application procedure, exposure period, protocol	The five treatments consisted of the following doses of Isopropylamine salt of N-phosphonometly glycine (glyphosate: Commercial brand name Roundup SC, Monsanto of Brazil Ltd): 0, 43.2, 86.4, 172.8 and 345.6 g e.a./ ha corresponding to 0, 3, 6, 12 and 24% of the glyphosate dose of 1.440 g e.a./ha.			
Experimental approach Statistical design,	Three month old seedlings, 30 cm high were planted in pots. After herbicide application, morphological changes in the aerial part of the plants were daily			

test environment	evaluated.
Test organisms	three eucalypt genoptypes
Biological effects	*E. grandis* was most susceptible to glyphosate with 75% seedling death at 45 DAA in plants treated with 345.6 g.ha–1 of glyphosate. Anatomic injuries were observed in the leaves of the plants exposed to glyphosate doses of 86.4; 172.8 and 345.6 g./ha in the three clones evaluated.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	no
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The anatomical and morphological responses in eucalypt plants treated with glyphosate, under simulated drift, are evident and, in general, associated with plant protection and rehabilitation.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation
2 Do the tested concentrations relate to predicted environmental concentrations	Yes. Drift rate are in the same range with an application rate of 2800 g a.a.i /ha. Glyphosate doses of 43.6 and 86.4 g.ha–1 caused a slight intoxication in the plants of the three clones, with a total recovery of those treated with 43.6 g.ha–1 being observed with time.
3 Have parameters influencing the endpoints been considered	nd
Concluding weight of evidence	Glyphosate altered the thickness and proportion of the leaf blade tissues, with the greatest changes being observed in the palisade parenchyma. Nevertheless, at drift rate plants seem to recover.
Type of info. (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	***UBA3***

Olszyk, D. et al. (2013)

2716801	**Olszyk, D. et al.**	**2013**	**Effects of low levels of herbicides on prairie species of the Willamette Valley, Oregon.**	
Reliability				
Purpose of the study Description of endpoints	The relative sensitivity of 17 noncrop plant species from Oregon's Willamette Valley was determined in response to glyphosate, tribenuron methyl (tribenuron), and fluazifop-p-butyl (fluazifop) herbicides.			
Test compound, application procedure, exposure period, protocol	Glyphosate in the form of its isopropylamine salt) was used as the formulated product, Roundup Original (Monsanto).			
Experimental approach Statistical design, test environment	Plant culture of prairie grass and forb species in greenhouse			
Test organisms	prairie grass and forb species			
Biological effects	The NOEC values were similar for many of the species, at 0.1 or 0.01 the field application rate for both parameters, reflecting the nonspecific phytotoxicity of glyphosate. *Elymus trachycaulus, Festuca arundinacea, Madia elegans, Potentilla gracilis,* and *Ranunculus occidentalis* were the most sensitive species, based on a			

	concentration calculated to reduce shoot dry weight by 25% (IC25 values) of 0.02 to 0.04 x a field application rate of 1112 g active ingredient (a.i.) per hectare.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Noncrop plant species in phytotoxicity were testing and considered tobe feasibile.
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations	yes
3 Have parameters influencing the endpoints been considered	yes
Concluding weight of evidence	For the most sensitive species, IC25 values were 22 g a.a/ha to 44 g a.i /ha .
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Hatterman-Valenti, H.M. (2014)

BVL Nr. 2716781	**Hatterman-Valenti, H.M.**	**2014**	**Simulated Glyphosate Drift to Potato Mother Plants and Effect on Daughter Tubers Used for Seed Production**	**Weed Technology 28(1):253-258. 2014**

Reliability	
Purpose of the study Description of endpoints	The objective of this research was to evaluate the effect of simulated glyphosate applied to nonirrigated seed potatoes in the late tuber bulking stage, a period of time when glyphosate is commonly applied as a preharvest aid to small grains.
Test compound, application procedure, exposure period	Roundup Weather Maxt, Monsanto Company, 800 North Lindbergh Blvd., St. Louis, MO 63167) rate at 0, 18, 35, 71, 141, and 282 g ae ha_1; 0.021, 0.042, 0.083, 0.167, and 0.33 the recommended rate of 846 g /ha for a preharvest application in small grains.
Statistical design, test environment	Field trials were conducted to evaluate the effect of simulated glyphosate drift rates applied to two non-irrigated potato cultivars in the late tuber bulking stage grown for potato seed production.
Test organisms	potato
Biological effects	Plants treated with the highest glyphosate rate of 282 g/ ha had reduced yields.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	no
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	no
3 Is the ecotoxicological manifestation level appropriate for the assessment?	*no*
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation
2 Do the tested concentrations relate to predicted environmental concentrations	yes
3 Have parameters influencing the endpoints been considered	nd

Concluding weight of evidence	Potential adverse growth and production in the succeeding potato crop from the contamination of a potato seed crop late in the growing seasons described.
Type of info. (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Hensley, J.B. et al.(2013)

BVL Nr. 2716782	**Hensley, J.B. et al.**	**2013**	**Response of Rice to Drift Rates of Glyphosate Applied at Low Carrier Volumes**	**Weed Technology 27(2):257-262. 2013**

Reliability	
Purpose of the study Description of endpoints	The objectives of this research were to evaluate the effects of simulated glyphosate drift applied to rice during the primary rice crop on the crop response and yield of treated rice in the primary and ratoon rice crops.
Test compound, application procedure, exposure period	Roundup Weathermax ®, 540 g ae /L, Monsanto Co.,applied at simulated drift rates of 6.3 and 12.5% of the labeled usage rate of 863 g ae/ ha, or 54 and 108 g /ha. Field studies were conducted near Crowley, LA in 2005 through 2007 to evaluate the effects of simulated herbicide drifton 'Cocodrie' rice. Applications were made with the spray volume varying proportionally to herbicide dosage based on a constant spray volume of 234 L/ ha and a glyphosate rate of 863 g ae/ ha.
Statistical design, test environment	Please refer to paper.
Test organisms	Cocodrie' rice
Biological effects	Visually rated rice crop injury ranged from 34 to 51% when the formulation was applied to rice at the one-tiller stage, representing more injury than any other timing evaluated in this study. Formulation applied to mature rice had no effect on rice plant height.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	*yes*
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation
2 Do the tested concentrations relate to predicted environmental concentrations	yes
3 Have parameters influencing the endpoints been considered	nd
Concluding weight of evidence	Simulated glyphosate drift applications resulted in reduced plant height at harvest and primary and total crop yield losses, with the greatest reduction in primary crop yield resulting from a simulated glyphosate drift application at 108 g/ha.
Type of info. (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Kutman, B.Y. et al. (2013)

BVL Nr. 2716788	**Kutman, B.Y. et al.**	**2013**	**Foliar Nickel Application Alleviates Detrimental Effects of**	***J. Agric. Food Chem.*, 2013, *61***

			Glyphosate Drift on Yield and Seed Quality of Wheat	**(35), pp 8364–8372**
Reliability				
Purpose of the study Description of endpoints	The possibility of using nickel (Ni), an essential divalent metal, for alleviating glyphosate drift damage to wheat was investigated in this study.			
Test compound, application procedure, exposure period	Roundup STAR (Monsanto) containing 441 g/L (glyphosate) potassium Plants were treated with either at tillering (ZS: 21−24; 29 days after sowing) or at booting (ZS: 47−49; 50 days after sowing) stage Visual injury symptoms, various growth parameters, shikimic acid accumulation, grain yield, and seed germination were investigated to demonstrate the effects of Ni on glyphosate damage.			
Statistical design, test environment	Greenhouse; analysis of variance (ANOVA)			
Test organisms	wheat (*Triticum durum*)			
Biological effects	The results presented in this study indicate that glyphosate rates as low as 1% or even 0.5% of the recommended herbicidal rate can disrupt seed set and thus significantly reduce wheat yield under controlled conditions.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	yes			
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Shoot dry weight and main stem height were significantly affected by glyphosate treatments. Glyphosate halved the grain yield.			
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Plants vigour may be affected by herbicide applications to adjacent agricultural land.			
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation			
2 Do the tested concentrations relate to predicted environmental concentrations	Yes, drift rate were simulated			
3 Have parameters influencing the endpoints been considered	nd			
Concluding weight of evidence	Glyphosate in this commercial formulation has effects on shoot dry weight of young plants, stem elongation, and grain yield of wheat at drift rates.			
Type of info (Critical, supporting, low weight)	**supporting**			
Consideration/concluding score	UBA2			

B.9.13 15.4Biochemical studies

Cruz-Hipolito et al. (2001)

glyphecotox_329	**Cruz-Hipolito, H., Rojano-Delgado, A., Dominguez-Valenzuela, J.A., Heredia, A., de Castro, M.D.L., de Prado, R.**	**2001**	**Glyphosate tolerance by Clitoria ternatea and Neonotonia wightii plants involves differential absorption and translocation of the herbicide**	**Plant and Soil 347 (1-2):221-230. doi:10.1007/s11104-011-0840-9.**

Reliability	
Purpose of the study Description of endpoints	The purpose of this work was to investigate the glyphosate tolerance mechanism for C. ternatea, N. wightii and an Amaranthus hybridus population susceptible to this herbicide in order to establish the basis for nontarget site-based mechanisms.
Test compound, application procedure, exposure period, protocol	The herbicide used was [14C]glyphosate-N phosphonomethyl) glycine of 52 mCi mmol−1 specific activity from American Radiolabeled Chemicals, Inc. (St.Louis, MO). Dose–response tests were conducted using the commercially formulated isopropylamine salt of glyphosate 360 g a.e./ L (Roundup® plus®).
Experimental approach	nd
Test organisms	Plants of *C. ternatea*, *N. wightii* and *A. hybridus* were sprayed with commercially formulated glyphosate at 500 g ae ha−1 as described above.
Biological effects	significant correlation between glyphosate differential absorption and epicuticular wax coverage has been found: high wax coverage leads to reduced glyphosate uptake. This provides new, solid evidence of the protective role of wax covering the lipid cuticle of higher plants
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	nd
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	Physiological study not relevant in terms of risk assessment
Type of info. (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

McMullin et al. (2012)

glyphecotox_497	**McMullin, R.T., Bell, F.W., Newmaster, S.G.**	**2012**	**The effects of triclopyr and glyphosate on lichens**	**Forest Ecology and Management 264:90-97. doi: 10.1016/j.foreco.2011.09.039.**

Reliability	
Purpose of the study Description of endpoints	Two commonly used silvicultural herbicides (triclopyr and glyphosate) were examined for their effects on lichens in northeastern Ontario.
Test compound, application procedure, exposure period, protocol	Glyphosate was formulated as Vision_ at 368 g a.e. isopropylamine salt L_1 (1.0–3.0 kg a.e. ha_1).
Experimental approach, Statistical design,	ANOVA
Test organisms	Lichen cover was comprised primarily of Cladonia species in the ubgenus *Cladina* (reindeer lichens)
Biological effects	Of eighteen lichen species treated in the glyphosate plots, eight species showed no reduction in abundance and 10 (56%) were negatively affected (Fig. 2b and d). Species most affected by glyphosate were *Cladonia uncialis*, *Bryoria furcellata*, and *T. granulosa*; with the latter two showing 100% mortality.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Lichens are not part of the current ERA procedure
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Glyphosate reduced the abundance, respectively in 40% and 56% of the boreal forest lichen species treated in this study. For most species that decreased in abundance effects were minor, but three species where strongly affected.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	Study describes ecotoxicological side effects towards lichens. Given the important functions of these two lichen species, their sensitivity to herbicide applications is relevant to forest managers.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Miteva et al. (2010)

glyphecotox_510	**Miteva, L.P.E., Ivanov, S.V., Alexieva, V.S.**	**2010**	**Alterations in glutathione pool and some related enzymes in leaves and roots of pea plants treated with the herbicide glyphosate**	**Russian Journal of Plant Physiology 57 (1):131-136. doi: 10.1134/s1021443710010188.**
Reliability				
Purpose of the study	the changes in the endogenous level of glutathione (total and oxidized) and the			

Description of endpoints	activities of glutathione reductase (GR) and glutathione S-transferase (GST) after treatment with glyphosate were studied in pea plants (*Pisum sativum* L., cv. Skinado).
Test compound, application procedure, exposure period, protocol	10 mM glyphosate (Roundup®, produced by Monsanto, United States).
Experimental approach Statistical design, test environment	The plant were treated at the stage of the third leaf development. Root treatment was made with 0.01 mM solution of glyphosate.
Test organisms	*Pisum sativum* L., cv. Skinado
Biological effects	It was found that glyphosate application to leaves provoked strong enhancement in the GST activity in leaves, while its root application stimulated the enzyme activity in the roots. The general internal thiol–disulfide balance has a great influence on biochemical processes, including photo synthesis, photorespiration and gene expression in the plant cell
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Changes in the glutathione levels andGR activity observed with the progress of the oxidative stress in plants. Apparently, the inhibiting of the shikimic acid pathway by glyphosate induces nonspecifically the oxidative stress. Despite the activation of the antioxidant system, oxidative stress appears to be the major reason for the injuries of the plants.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	no
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The conclusion from this study is only valid for glyphosate formulations that contain POEA.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	The concentration used for the leaf treatment was calculated on the basis of the field rate of the herbicide.
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	Biochemical study defining the oxidative stress related to mode of action of glyphosate
Type of info. (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Moldes et al. (2008)

glyphecotox_515	**Moldes, C.A., Medici, L.O., Abrahao, O.S., Tsai, S.M., Tsai, S.M., Azevedo, R.A.**	**2008**	**Biochemical responses of glyphosate resistant and susceptible soybean plants exposed to glyphosate**	**Acta Physiologiae Plantarum 30 (4):469-479. doi: 10.1007/s11738-008-0144-8.**
Reliability				
Purpose of the study Description of endpoints	The effect of glyphosate application on chlorophyll level, lipid peroxidation, catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GOPX) and superoxide dismutase (SOD) activities, soluble amino acid levels and			

	protein profile, in leaves and roots, was examined in two conventional (non-GR) and two transgenic (GR) soybean.
Test compound, application procedure, exposure period, protocol	Glyphosate (Agrisato 480 CS manufactured by ALKAGRO)
Experimental approach Statistical design, test environment	5-week-old plants were sprayed in an application chamber. The herbicide was diluted in water at 2:100 proportion and applied on the foliar surface
Test organisms	soybean cultivars
Biological effects	An improved adaptive capacity of the antioxidant pathway for detoxification of oxidative stress appears to be generated during glyphosate action, since CAT activity increased in roots of non-GR soybean cultivars. The total soluble amino acid content increased after glyphosate application, which might be responsible for reducing oxidative damage.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	The slight oxidative stress generated by glyphosate has no relevance to plant mortality.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The slight oxidative stress generated by glyphosate has no relevance to plant mortality.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	nd
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	The objective of this work was to study biochemical parameters that may be affected in roots and leaves of soybean plants exposed to glyphosate, focusing on the antioxidant response and soluble amino acid content, thus evaluating possible,biochemical markers for differential characterization of glyphosate-resistant and conventional soybean lines
Type of info. (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Alvarez-Moya et al. (2011)

glyphecotox_277	**Alvarez-Moya, C., Silva, M.R., Arambula, A.R.V., Sandoval, A.I., Vasquez, H.C., Montes, R.M.G.**	**2011**	**Evaluation of genetic damage induced by glyphosate isopropylamine salt using *Tradescantia* bioassay**	**Genetics and Molecular Biology 34 (1):127-130**

Reliability	
Purpose of the study Description of endpoints	Various concentrations of a glyphosate isopropylamine salt were tested using two methods of genotoxicity assaying, viz., the pink mutation assay with Tradescantia (4430) and the comet assay with nuclei from staminal cells of the same plant.
Test compound, application procedure, exposure period, protocol	N-(phosphonomethyl)-glycine 96% (CAS No. 1071- 83-6, lot 09816 PE) was obtained from Aldrich. The evaluated concentrations were 0.7, 0.07, 0.007 and 0.0007 mM.
Experimental approach Statistical design, test environment	In this assay, color changes in cells from floral parts are used to determine mutational events, ANOVA
Test organisms	The *Tradescantia*, clone (4430) (hybrid T. *Subacaulis* X T. *hirsutiflora*), which is highly sensitive to environmental mutagens was used.
Biological effects	Isopropylamine possesses strong genotoxic activity, but its detection can vary depending on the test systems used.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Authors believe that isopropylamine used in commercial farming can induce genetic damage, depending on the dose used and the physiological characteristics of the plants exposed to it
3 Is the ecotoxicological manifestation level appropriate for the assessment?	No, as genetic damage is not assessed in the current risk assessment.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes: N-(phosphonomethyl)-glycine 96%
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	Authors believe that isopropylamine used in commercial farming can induce genetic damage, depending on the dose used and the physiological characteristics of the plants exposed to it.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Pline et al. (2002)

glyphecotox_181	**Pline, W.A., Wilcut, J.W., Edmisten, K.L., Wells, R.**	**2002**	**Physiological and morphological response of glyphosate-resistant and non-glyphosate-resistant cotton seedlings to root-absorbed glyphosate**	**Pesticide Biochemistry and Physiology Volume: 72 Issue: 1 Pages: 48-58**

Reliability				
Purpose of the study Description of endpoints	Studies were conducted to determine relative tissue sensitivity in glyphosate-resistant (GR) and non-GRcotton seedlings to the herbicide glyphosate.			
Test compound, application procedure, exposure period, protocol	100, 10, 1, 0.1, or 0lM technical grade glyphosate (N-(phosphonoethyl)glycine, 95% purity, Sigma, St. Louis, MO). These concentrations would correspond to 169.1, 16.9, 1.69, 0.169, or 0ppm in a hydroponic solution, whereas a glyphosate application of 1:12kgaiha_1 would produce a 3.39 ppm concentration if the herbicide remained evenly distributed in the top 2.54 cm of the soil profile.			
Experimental approach Statistical design, test environment	Cotton seedlings were grown in hydroponic solutions containing technical grade glyphosate to ensure constant exposure to glyphosate. non-linear regression analysis (Weibull model)			
Test organisms	Seeds of Delta Pine & Land varieties 'DP 5415' (non-glyphosate resistant) and 'DP 5415RR' (GR)			
Biological effects	Glyphosate inhibited the growth of non-GR cotton cotyledons, hypocotyls, and roots 50% at concentrations of 23, 69, and 27µM glyphosate, respectively. Additionally, glyphosate inhibited the development of lateral roots at concentrations of 0.01 or 0:1µM glyphosate, in GR and non-GR cotton, respectively. Lateral roots of GR and non-GR cotton inhibited by glyphosate appeared shorter and were surrounded by a thick layer of necrotic cells or root exudate which was not present in roots from plants grown in media not containing glyphosate.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	Cotyledon, hypocotyl, and root tissue from GR and non-GR plants			
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Because seedlings may come in contact with glyphosate, either applied foliarly or via root absorption from root exudates from neighboring sensitive species in a field situation, the potential for glyphosate to slow or inhibit seedling establishment may exist.			
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes			
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, technical grade glyphosate (N-(phosphonoethyl)glycine, with 95% purity was tested.			
2 Do the tested concentrations relate to predicted environmental concentrations	nd			
3 Have parameters influencing the endpoints been considered	Controlled environment			
Concluding weight of evidence	Even though no endpoints were stated , observed effects on root development are considered to have an effect on seedling emergence.			
Type of info. (Critical, supporting, low weight)	**supporting**			
Consideration/concluding score	**UBA2**			

Mateos-Naranjo et al.(2013)

	E. Mateos-Naranjo, A. Perez-Martin	**2013**	**Effects of sub-lethal glyphosate concentrations on growth and photosynthetic performance of non-target species**	**Chemosphere 93 (2013) 2631–2638**

			Bolboschoenus maritimus	
Reliability				
Purpose of the study Description of endpoints	The aim of this study was to investigate the effect of sub-lethal glyphosate concentrations present in the nutrient solution on the growth and photosynthetic apparatus of *B. maritimus*.			
Test compound, application procedure, exposure period, protocol	Glimur LS; 360 g a.s./L, Sarabia SA, Spain After 3 months of seedling cultures, the pots (each one with 4–6 live tillers) were randomly allocated still inside the glasshouse to five glyphosate treatments in shallow trays (eight pots per tray, with one tray per treatment): 0,5, 10, 20 and 30 mg/L. Plants were subjected to glyphosate for 20 d. The entire solution was changed every 2 d.			
Experimental approach Statistical design, test environment	Growth analysis, Measurement of chlorophyll fluorescence , Photosynthetic pigments, Gas exchange, Statistica v. 6.0.			
Test organisms	*Bolboschoenus maritimus*, a rhizomatous emergent macrophyte considered one of the main components the perennial vegetation in terms of primary production in the Doñana marshes.			
Biological effects	The lethal concentration of glyphosate (LC50, the concentration of glyphosate that kills 50% of plants) was ca. 30 mg/l L and all plants survived during the whole study period, the percentage of dead tillers increased at 30 mg /l glyphosate, with values ca. 50%.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	Although B. maritimus plants were able to grow and survive after 20 d of exposure to glyphosate, the presence of this herbicide affected their growth, through a direct interaction with the root system. Particularly, at 30 mg/ L glyphosate, B. maritimus showed ca. 30% of biomass decrease.			
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	All plants survived during the whole study period, the percentage of dead tillers increased markedly at 30 mg /L glyphosate, with values ca. 50%.			
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The greatest growth reduction was recorded at 30 mg/L glyphosate concentration, atreatment where B. maritimus showed ca. 30% of biomass reduction 20 d after glyphosate application.			
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?	Combination of 20% Hoagland's solution and glyphosate herbicide (Glimur LS; 360 g active ingredient)			
2 Do the tested concentrations relate to predicted environmental concentrations	Glyphosate concentrations were chosen considering the model for risk assessment in estuaries by Solomon and Thompson (2003), which indicated that at doses of 8 Kg/ ha the amount of glyphosate in the open water ranged from 0.7 mg/ L to 5.4 mg/ L,exceeding the predicted concentration by far.			
3 Have parameters influencing the endpoints been considered	Controlled environment.			
Concluding weight of evidence	In *B. maritimus*, the assessment of toxicity thresholds of experimental glyphosate concentrations presented in nutrient solution ranging between 0 and 30 mg/ L glyphosate indicates that this species has a high tolerance to glyphosate stress, since all plants survived for the full 20 d of treatments. Sublethal effects on root development by difuse pollutions might effect ecosystems.			
Type of info. (Critical, supporting, low weight)	***supporting***			

Consideration/concluding score	***UBA2***

Olesen and Cedergreen (2010)

	CF Olesen and N.Cedergreen	**2010**	**Glyphosate uncouples gas exchange and chlorophyll fluorescence**	**Pest Manag Sci 2010; 66: 536–542**
Reliability				
Purpose of the study Description of endpoints	The aim of this study was to investigate the time course of changes in conductance, net CO2 assimilation and chlorophyll fluorescence of a stressor known to affect photosynthesis indirectly by inhibiting biochemical pathways 'downstream' of the photosynthetic electron transport chain.			
Test compound, application procedure, exposure period, protocol	Plants were sprayed with glyphosate at 11, 22, 45, 90, 180, 360 and 720 g AE /ha (Glyphonova; Cheminova, Denmark).			
Experimental approach Statistical design, test environment	Three seeds per pot were sown, and the plants were placed in a growth cabinet at a day/night temperature of 25/20 ◦C and an irradiance of 400 µmolm−2 s−1 photosynthetic active radiation (PAR) provided by sodium lamps (HQI; Phillips, The Netherlands) for 16 h/ day. The plants were watered with tap water. Gas-exchangemeasurements, chlorophyll fluorescence, three-parameter log-logistic dose–response model:			
Test organisms	barley, *Hordeum vulgare* L			
Biological effects	The biomass data were best described with a dose–response model including hormesis. Both the doses 360 and 720 g AE/ha stopped biomass growth completely within the 7 day ED50 was 150 ± 21 and 153 ± 38 g AE/ha for the two experiments respectively.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	Biochemical parameters are currently not part of the traditionel ERA according to OECD 227. Observations are made for visual phytotoxicity and mortality.			
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	The results demonstrate that changes in gas-exchange parameters are more sensitive biomarkers for glyphosate toxicity compared with chlorophyll fluorescence. ED50 of 150 ± 21 and 153 ± 38 g AE/ha for the two experiments are higher than relevant endpoint used in ERA.			
3 Is the ecotoxicological manifestation level appropriate for the assessment?	See above.			
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?	Formulation Glyphonova;for gas-exchange experiment, the plants were sprayed with technical glyphosate (isopropylamine salt; Cheminova, Denmark) formulated with 1 g L−1 polyoxyethylene (20) sorbitan monolaurate (Tween 20; Sigma Aldrich).			
2 Do the tested concentrations relate to predicted environmental concentrations	Tested concentrations are according to herbicide label rates. Appropriate for in field application rates. Drift rate are expected to be lower.			
3 Have parameters influencing the endpoints been considered	nd			
Concluding weight of evidence	The biomass data were best described with a dose–response Model and are the same magnitude as the proposed endpoint for ERA.			

Type of info. (Critical, supporting, low weight)	***supporting***
Consideration/concluding score	***UBA2***

Riechers et al. (1994)

	D.E.Riechers, L. M. Wax, R. A. Lieb1, and D.R. Bush	**1994**	**Surfactant-Increased Glyphosate Uptake into Plasma Membrane Vesicles Isolated from Common Lambsquarters Leaves**	**Plant Physiol. (1994) 105: 1419-1425**

Reliability	
Purpose of the study Description of endpoints	In the research reported here, isolated plasma membrane vesicles to examine glyphosate uptake and to asses the impact of various surfactants on glyphosate accumulation
Test compound, application procedure, exposure period, protocol	Technical grade glyphosate [N-(phosphonomethyl)Gly] was provided by Monsanto. Radiolabeled [14C]glyphosate with a specific activity of 1.89 GBq mmol and radiochemical purity of 98% as determined by HPLC was purchased from Amersham. Both technical and radiolabeled glyphosate were in the free acid form.
Experimental approach Statistical design, test environment	Please refer to paper.
Test organisms	*Chenopodium album L.*
Biological effects	Glyphosate uptake was stimulated by 0.01% (v:v) MON 0818, the cationic surfacant used in the commercial formulation of this herbicide for foliar application. This concentration of surfadant did not disrupt the integrity of the plasma membrane vesicles, as evidenced by the stability of imposed pH gradients and adive amino acid transport.Glyphosate uptake was very low compared to the acidic amino acid glutamate, indicating that glyphosate is not utilizing an endogenous amino acid carrier to enter the leaf cells and that the plasma membrane appears to be a significant barrier to cellular uptake.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Biochemical, fundamental research investigating the mode of action is generally not suitable for an indication of intrinsic sensitivity for ERA.
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Experimental set up is not relevant for ecotoxicological risk assessment. Glyphosate uptake was stimulated by 0.01% (v:v) MON 0818, the cationic surfadant used in the commercial formulation of this herbicide for foliar application. This concentration of surfactant did not disrupt the integrity of the plasma membrane vesicles, as evidenced by the stability of imposed pH gradients and adive amino acid transport.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	MON 0818 is the cationic surfadant used in the commercial formulation of this herbicide for foliar application.
2 Do the tested concentrations relate to predicted environmental concentrations	nd
3 Have parameters influencing the endpoints been considered	nd
Concluding weight of evidence	Experimental set up is currently not relevant for ecotoxicological risk assessment.

Type of info. (Critical, supporting, low weight)	**Low weight for ERA**
Consideration/concluding score	**UBA2-3**

Nafziger et al. (1983)

	E. D. NAFZIGER, J. M. WIDHOLM, AND F. W. SLIFE	**1983**	**Effects of Aspartate and Other Compounds on Glyphosate Uptake and Growth Inhibition in Cultured Carrot Cells'**	**Plant Physiol. (1983) 71, 623-626**

Reliability	
Purpose of the study Description of endpoints	In this paper, we report the effects of aspartate and other reversing agents on growth and glyphosate uptake by carrot cells incubated in herbicide. The effects of glyphosate and aspartate and on intracellular ammonia levels were examined as a test of the hypothesis that the herbicide may induce ammonia toxicity.
Test compound, application procedure, exposure period, protocol	79,4, The effect of aspartate on glyphosate uptake in carrot cells was tested by incubating cells in all combinations of 0.1, 0.2, 0.3, 0.4, and 0.5 mm glyphosate and 0, 1, and 2 mM aspartate. The40 cpm/ml of [methyl-'4CJglyphosate or 794.4 cpm/4mol.
Experimental approach Statistical design, test environment	The reversal of glyphosate inhibition of cell growth by aspartate was tested using all combinations of glyphosate at 0, 0.1, 0.2, 0.3, 0.4, and 0.5 mM and aspartate at 0, 1, and 2 mM.
Test organisms	Garden carrot (*Daucus carota* L. cv Danvers)
Biological effects	The 50% growth-inhibitory concentration ofglyphosate increased from 0.1 1 mm in the absence of aspartate to 0.14 and 0.19 in the presence of I and 2 mM aspartate, respectively. At glyphosate concentrations above 0.3 mm, aspartate was ineffective in reversing growth inhibition.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	no
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	no
3 Is the ecotoxicological manifestation level appropriate for the assessment?	no
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to predicted environmental concentrations	nd
3 Have parameters influencing the endpoints been considered	nd
Concluding weight of evidence	Mode of action study
Type of info. (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Dimitrov (2006)

	Boyan D.Dimitrov, Polina G.Gadeva1, Donka K.Benova and Maria V.Bineva	**2006**	**Comparative genotoxicity of the herbicides Roundup, Stomp and Reglone in plant and mammalian test systems**	**Mutagenesis vol. 21 no. 6 pp. 375–382, 2006**

Reliability	
Purpose of the study Description of endpoints	Evaluation the genotoxicity of three herbicides, Roundup, Stomp and Reglone, in plant (*Crepis capillaris*) and mammalian (mouse)

	test systems that measure the induction of structural chromosomal aberrations and micronuclei.
Test compound, application procedure, exposure period, protocol	Roundup is a liquid water-soluble organophosphorus herbicide, containing glyphosate [N-(phosphonomethyl) glycine, C3H8NO5P] as its active ingredient (a.i.) (CAS No. 1071-83-6; >90% purity). The experiments with the three herbicides were carried out at concentrations bracketing those used in agricultural practice: 0.05, 0.1, 0.5 and 1.0% a.i. for Roundup.
Experimental approach Statistical design, test environment	The experiments with plant assay were conducted using *C. capillaris* root meristems.
Test organisms	*Crepis capillaris L*
Biological effects	None of the three compounds produced a significant increase in the frequency of structural chromosomal aberrations in plant cells (P > 0.05) at concentrations tested. Roundup did not significantly increase micronucleus frequency in plant cells; a slight increase was observed with some treatments, e.g. 0.05% with the 24 h sample time, but these increases were not statistically significant (P > 0.05).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	no
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	no
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial Roundup formulation
2 Do the tested concentrations relate to predicted environmental concentrations	Concentrations bracketing those used in agricultural practice: 0.05, 0.1, 0.5 and 1.0% a.i. for Roundup.
3 Have parameters influencing the endpoints been considered	nd
Concluding weight of evidence	Roundup did not induce chromosomal aberrations or micronuclei in either test system.
Type of info. (Critical, supporting, low weight)	***supporting***
Consideration/concluding score	***UBA2***

Gomes et al. (2014)

2716479	**Gomes, M.P., Smeldbol, E., Chalifour, A., Henault-Ethier, L., Labrecque, M., Lepage, L., Lucotte, M., Juneau, P.**	**2014**	**Alteration of plant physiology by glyphosate and its by-productaminomethylphosphonic acid: an overview**	**Journal of Experimental Botany, Vol. 65, No. 17, pp. 4691 134703, 2014**

Reliability	
Purpose of the study Description of endpoints	The present review provides an overall picture of alterations of plant physiology caused by environmental exposure to glyphosate and its metabolite AMPA, and summarizes their effects on several physiological processes.
Test compound, application procedure, exposure period, protocol	nd
Experimental approach Statistical design, test environment	Review on uptake and translocation of glyphosate and AMPA in plants and on alteration of plant physiology by glyphosate and AMPA.

Test organisms	*nd*
Biological effects	nd
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations	nd
3 Have parameters influencing the endpoints been considered	nd
Concluding weight of evidence	Review articel focuses on photosynthesis, from photochemical events to C assimilation and translocation, as well as oxidative stress and shows that glyphosate has several secondary or indirect effects on plant physiology which may also explain its herbicidal effects.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	***UBA2***

Chauhan, B.S., Abugho, S.B. (2012)

BVL NR: 2716717	**Chauhan, B.S., Abugho, S.B.**	**2012**	**Threelobe Morningglory (*Ipomoea triloba*) Germination and Response to Herbicides**	**Weed Science 60(2):199-204. 2012**

Reliability	
Purpose of the study Description of endpoints	The objectives of this study were to determine the effects of scarification, temperature and light, salt and water stress, seed burial depth, and rice residue on germination and emergence of threelobe morningglory and to evaluate the response of this weed to commonly available POST herbicides in the Philippines.
Test compound, application procedure, exposure period, protocol	glyphosate at 250 and 500 g ai ha21 (Monsanto Phis. Inc., Muntinlupa City, Philippines)
Experimental approach Statistical design, test environment	Effect of Herbicides on Survival, Shoot Biomass, and Control of Threelobe Morningglory
Test organisms	Morningglory (*Ipomoea triloba*)
Biological effects	Glyphosate gave around 70% control when applied at the four- and six-leaf stages at 250 g ai ha, and to achieve greater than 90% control of threelobe morningglory, a dose of 500 g ai ha was required.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes fout to eight leaf stage
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation tested, not further specification

2 Do the tested concentrations relate to predicted environmental concentrations	Significant effects at concentrations related to drift concentrations can be most likely excluded.
3 Have parameters influencing the endpoints been considered	nd
Concluding weight of evidence	Control of Ipomoea species by glyphosate requires high applicationrates.
Type of info. (Critical, supporting, low weight)	low weight
Consideration/concluding score	UBA3

Banaszkiewicz, T., Wysocki, K. (2012)

BVL NR: 2716761	**Banaszkiewicz, T., Wysocki, K.**	**2012**	**Application of white mustard (Sinapis alba) biotest in the assessment of environmental contamination by glyphosate**	

Reliability	
Purpose of the study Description of endpoints	Research into this issue was carried out with the use of seeds (Phytotestkit test procedure) and young seedlings of white mustard (*Sinapis alba*) to examine the influence of glyphosate on photosynthesis in plants and to check usefulness of *Sinapis alba* for monitoring water contamination by glyphosate.
Test compound, application procedure, exposure period, protocol	Commercial formulation Glyfos 360 SL
Experimental approach Statistical design, test environment	Experiments in a phytotron chambe; effects on chlorophyll a fluorescence in white mustard seedlings, effects on root growth.
Test organisms	*Sinapis alba*
Biological effects	An IC50 dose inhibiting the growth of the roots after 3 days was 9.5 µl/L and 3.75 µl/L after 7 days. A drop in chlorophyll "a" photosynthetic activity was observed at the herbicide concentration of 1.8 rnl/l (equal to a 21/h a in 400 I of water).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	no
3 Is the ecotoxicological manifestation level appropriate for the assessment?	no
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation
2 Do the tested concentrations relate to predicted environmental concentrations	nd
3 Have parameters influencing the endpoints been considered	nd
Concluding weight of evidence	Water contamination did not result in inhibition of germination of white mus tard and chlorophyll "a" fluorescence can provide a good indicator of plant contamination by glyphosate.
Type of info. (Critical, supporting, low weight)	low weight
Consideration/concluding score	UBA3

Truta, E. et al. (2010)

BVL NR: 2716816	**Truta, E. et al.**	**2010**	**Evaluation of Roundup-induced toxicity on genetic material and on length growth of barley seedlings**	

Reliability	
Purpose of the study Description of endpoints	The objective of this study is to evaluate and to provide new data on the genotoxic potential by quantification of cytogenetic parameters in root tip meristems of barley seedlings obtained by germination of Roundup-treated seeds. Also, its influence on behaviour of length growth of barley seedlings was analyzed.
Test compound, application procedure, exposure period, protocol	Commercial formulation of Roundup, containing 360 g/L glyphosate, water solutions of Roundup were prepared (0.1%, 0.5%, 1.0% and 2.0%, v/v), to the following concentrations of active ingredient: 0.36, 1.80, 3.60, respectively, 7.20 mg/ml. The barley grains have been maintained for 3 h and 6 h in Roundup solutions. After treatment, the seeds have been washed with running tap water, and then transferred in glass Petri dishes for germination. Petri dishes were covered and maintained at 20 °C.
Experimental approach Statistical design, test environment	Cytogenetic , Length growthanalysis, mitotic index and chromosome aberrations
Test organisms	*Hordeum vulgare* L.
Biological effects	All tested concentrations have an inhibitory effect on plantlet growth, independently of exposure time (Fig. 5); the inhibitory rate is 86.8% in 2.0% – 3 h variant, and 89.2% in 2.0% – 6 h variant. Roundup induced damage on genetic level.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Seed germination test on petri dihes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Chromosome damage might have influence on subsequent plant growth and development.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Seed germination test on petri dihes probably overestimate effects, due to the lack of soil environment.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation, it is therfor possible that the toxic effects of Roundup are constituted by surfactant system.
2 Do the tested concentrations relate to predicted environmental concentrations	yes
3 Have parameters influencing the endpoints been considered	no
Concluding weight of evidence	Influence on genetic material are not part oft he traditional ERA, but can lead to undesired effects of herbicides in general towards plant health.
Type of info. (Critical, supporting, low weight)	supporting
Consideration/concluding score	UBA3

B.9.13 15.5 Summary of other non-target organisms (flora and fauna)

For the group of terrestrial non-target plants (NTA), a comprehensive database of 87 peer-reviewed papers was collected by the notifier. The notifier considered one publication (Boutin et al., 2010) to be rated in category “Klimisch2” and annotated with minimal remarks,

whereas the remaining were considered not acceptable for risk assessment. The submitted publications were also evaluated by RMS and have been assigned according to an UBA screening. 27 studies were recognized as supporting information (category UBA2).
Moreover, after the date of submission, during the administrative process and the public launching period of the evaluation a few more peer-reviewed relevant articles were newly published (mainly period of 2012-2014) and needed to be considered in a revised DAR. Within these references, additional studies focusing on non target plants have been considered as supporting information for risk assessment (Dalton, R.L. 2010; de Jong et al. 2001, Collin W. 2012, Kilbride, et al. 2001, Marshall et al. 2003, Johal and Huber et al. 2009, Helander et al. 2009; Pfleeger et al. 2014, Damgaard et al. 2013, Tesfamariam, T. et al. 2009, Akamatsu, F. et al. 2014, Allison, J.E. et al. 2013; Tuffi Santos et al. 2004; Olszyk, D. et al. 2013). The review of Gomes et al. (2014) provides an overall picture of the effects of glyphosate and AMPA on plant primary physiological processes.

Most of the cited studies were performed with plant protection products than for the active ingredient itself. It is known that surfactants or additives form a significant amount of plant protection products. The function of these compounds is supposed to enhance the herbicidal activity of the active ingredients by e.g. improving the dispersal and retention on the leaf surface or the uptake. Therefore, it is essential for regulatory ERA to take into account toxicity data considering the synergistic effects of these ingredients in formulation in order to avoid underestimation of glyphosate containing products.

The use of herbicides to control weeds in target areas may affect non-target terrestrial plants (NTP) in off- field situation. Potentially at risk are -besides NTP-, non-target arthropods or birds and mammals that are dependent on these plants for food and shelter. The objective of the risk assessment towards NTP, especially for herbicides, is to ensure that these organisms will not be harmed by unintended exposure due to drifting into the off- field area outside the intended spray zones. Under optimal spraying conditions and appropriate application techniques, total spray drift (the portion of herbicide achieving off-field area) was considered to range from (2.77% to 29.2% of the volume applied) depending on the crop to be sprayed (Ganzelmeier & Rautmann, 2000).

Several publications were evaluated that simulate drift towards different test organisms (Deeds et al., 2006; Ellis et al., 2003; Felix et al., 2011; Gove et al., 2007; Koger et al., 2005; Nandula et al., 2007; Pfleeger et al., 2011). In general it was observed that concentrations corresponding to predicted environmental exposure caused injuries to test plants depending on concentration, time of treatment, crop variety and experimental approach. The following conclusions were drawn:

- Gove et al. (2007) recommend the adoption of a buffer zones of at least 5 m to protect woodland species from the impacts of agrichemicals.
- Pfleeger et al. (2011) conducted toxicity test in greenhouse and under natural conditions and found that visual injury is not to be necessarily the most sensitive endpoint, but reproductive endpoints were more sensitive that vegetative ones in many cases.
- Pfleeger et al. (2011) propose more restrictive regulations (safety factors) should be imposed to account for the variability in sensitivity observed between greenhouse- and field-grown plants.

- Boutin et al. (2010) supports the inclusion of an uncertainty factor in risk assessments to account for the intrinsic variability in plant sensitivity to herbicides.
- Boutin et al. (2014) states that plants should be tested for longer periods in order to assess the seed output.
- Boutin et al.(2010) showed that crops and wild plant species can responded quite variably when tested in different seasons as well as when tested under different environmental conditions. These findings are in line with uncertainties of phytotoxicity testing described by Olzyk et al. (2004), who addresses current trends in general risk assessment of plants in US.

More limitations to current phytotoxicty testing were described, taking into account that herbicides can influence plant communities in terms of species composition and diversity:

- Though single-species tests are useful as they can demonstrate clear dose–response patterns independently from other factors influencing growth, these test are o flimited value to predict changes in community structure that may have long-term consequences. Greenhouse microcosms were more sensitive than single-species tests and changes in community structure were observed in herbicide-treated microcosms that would not be predicted from single-species testing (Dalton and Boutin, 2010).
- Pfleeger et al. (2014) showed effects of multiple herbicide applications on small plant communities. RMS agrees that it is of high relevance that system-wide effects of any extensive herbicide application should be evaluated.
- Additionally, it was shown that foliar applied glyphosate to target plants is released into the rhizosphere and might negatively affect non-target plants, disease problems and nutritional status (Neumann et al., 2006; Johal and Huber, 2009).

The extensive usage of herbicides in agricultural landscapes can reduce the diversity of plant species, with subsequent impacts on other non-target taxa (Marshall et al. 2003). A decrease of certain plant species might associate with impacts on birds and mammals, as well as arthropods by influencing food resources or plant cover to reproduce or to hide from predators (Norris and Kogan 2005). RMS agrees that is necessary to strike a balance between adequate weed control, and the requirement to support biological diversity. Moreover, RMS also favours modifications to crop management in selected areas of fields, and supports the extensiveness of uncropped strips to provide sufficient resources for biodiversity of non target (plant) organisms. Therefore, traditional ERA might be complemented taking into account a regional agroecosystem level - with exposure estimations considering the usage of herbicides in various indications and areas. Please refer also to chapter B 9.1.7.7 " Indirect effects via trophic interactions".

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B.9.13.16 Surface active substances in glyphosate-based formulations

Paganelli et al. (2010)

glyphnosubm_244	**PAGANELLI, A., GNAZZO, V., ACOSTA H., LOPEZ, S.L., CARRASCO, A.E.**	**2010**	**GLYPHOSATE-BASED HERBICIDES PRODUCE TERATOGENIC EFFECTS ON VERTEBRATES BY IMPAIRING RETINOIC ACID**	**CHEMICAL RESEARCH IN TOXICOLOGY (23): 1586-1595**
Reliability				
Purpose of the study Description of endpoints	To conduct an embryological approach to explore the effects of low doses of glyphosate in development - EP: neural crest markers			
Test compound, application procedure, exposure period, protocol	- Roundup Classic ® (48% (w/v) glyphosate salt); Glyphosate - 1/3000, 1/4000, and 1/5000-dilutions of Roundup Classic® prepared in 0.1× MBS (modified Barth's saline) - Treatments were performed from the 2-cell stage - 0.5 or 1 µM Ro-415253 was added at the 9-cell stage - Embryos were incubated in 0.1 x MBS. Cyclopamine was used at 100 µM concentration in 0.1 x MBS and was applied from the 2-cell stage until fixation. Embryos were fixed in MEMFA when sibling controls reached the desired stage.			
Experimental approach Statistical design, test environment	Refer to the study			
Test organisms	*Xenopus laevis* Chicken embryos			
Biological effects	Relevant experimental set up for ecotoxicological assessment: Effects on eggs Effects were detected in glyphosate-based formulations. Please refer also to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?			partly	
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?			partly	
3 Is the ecotoxicological manifestation level appropriate for the assessment?			partly	
Environmental Relevance				
1 Is the substance tested representative and		yes		

relevant for the substance being assessed?	
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	-/-
Concluding weight of evidence	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Romano et al. (2010)

glyphnosubm_249	**ROMANO, R.M. ROMANO, M.A. BERNARDI, M.M. FURTADO, P.V. OLIVEIRA, C.A.**	**2010**	**PREPUBERTAL EXPOSURE TO COMMERCIAL FORMULATION OF THE HERBICIDE GLYPHOSATE ALTERS TESTOSTERONE LEVELS**	**ARCHIVES OF TOXICOLOGY (84): 309-317**

Reliability	
Purpose of the study Description of endpoints	To evaluation the endocrine disruption potential of glyphosate formulation by assessment of rats prepubertal reproductive development. EP: progression of puberty, body development, hormonal production of testosterone, estradiol and corticosterone, and morphology of the testis
Test compound, application procedure, exposure period, protocol	Roundup Transorb purity: 480 g/L of glyphosate (648 g/L as isopropylamine salt) Duration of study: From postnatal day (PND) 23 until PND53 Dose levels: Control group – deionized water; 5, 50 or 250 mg/kg of body weight of glyphosate-Roundup Transorb Administration by gavage Dosing volume: 0.25 mL/100 g of body weight, Application time: between 7 and 8 a.m. each day
Experimental approach Statistical design, test environment	Animals per dose group: 4 treatment groups, 17 animals per group Animal selection No mention of avoiding selection of siblings within the same group to control for possible litter effects Administration: The glyphosate-Roundup Transorb was diluted in a watery suspension and administered once a day, by gavage; Dosing volume: 0.25 mL/100 g of body weight, Application time: between 7 and 8 a.m. each day
Test organisms	Wistar rats
Biological effects	Results showed that the herbicide (1) significantly changed the progression of puberty in a dose-dependent manner; (2) reduced the testosterone production, in semineferous tubules' morphology, decreased significantly the epithelium height ($P < 0.001$; control = 85.8 ± 2.8 µm; 5 mg/kg = 71.9 ± 5.3 µm; 50 mg/kg = 69.1 ± 1.7 µm; 250 mg/kg = 65.2 ± 1.3 µm) and increased the luminal diameter ($P < 0.01$; control = 94.0 ± 5.7 µm; 5 mg/kg = 116.6 ± 6.6 µm; 50 mg/kg = 114.3 ± 3.1 µm; 250 mg kg = 130.3 ± 4.8 µm); (4) no difference in tubular diameter was observed; and (5) relative to the controls, no differences in serum corticosterone or estradiol levels were detected, but the concentrations of testosterone serum were lower in all treated groups ($P <0.001$; control = 154.5 ± 12.9 ng/dL; 5 mg/kg = 108.6 ± 19.6 ng/dL; 50mg/dL = 84.5 ± 12.2 ng/dL; 250mg/kg = 76.9 ± 14.2 ng/dL).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-

3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA1 for assessment of surfactand effects (POEA)**

Romano et al. (2012)

glyphnosubm_250	**ROMANO, M.A., ROMANO, R.M., SANTOS, L.D., WISNIEWSKI, P., CAMPOS, D.A., DE SOUZA, P.B., VIAU, P., BERNARDI, M.M., NUNES, M.T., DE OLIVIERA, C.A.**	**2012**	**GLYPHOSATE IMPAIRS MALE OFFSPRING REPRODUCTIVE DEVELOPMENT BY DISRUPTING GONADOTROPIN EXPRESSION**	**ARCHIVES OF TOXICOLOGY (86) 4: 663-673**

Reliability	
Purpose of the study Description of endpoints	To investigate the effect of gestational maternal glyphosate exposure (50 mg/kg, NOAEL for reproductive toxicity) on the reproductive development of male offspring. EP: sexual behavior, partner preference; serum testosterone concentrations, estradiol, FSH and LH; mRNA and protein content of LH and FSH; sperm production and morphology of the seminiferous epithelium; weight of the testes, epididymis and seminal vesicles. The growth, the weight and age at puberty of the animals were also recorded.
Test compound, application procedure, exposure period, protocol	Roundup Transorb purity: 480 g/L of glyphosate (648 g/L as isopropylamine salt) Duration of exposure: From gestational day 18 to postnatal day (PND) 5 Dose levels: Control group – deionised water; 50 mg/kg bw of glyphosate
Experimental approach Statistical design, test environment	2 treatment groups Administration: Roundup Transorb was diluted in a watery suspension and administered once a day by gavage from Gestation Day 18 to Post Natal day 5; Dosing volume: 0.25 mL/100 g bw, Application time: between 7 and 8 a.m. each day Statistics: First the Kolmogorov–Smirnov tests for normality and the Bartlett test for homoscedasticity. For analysis of body growth the multi-way analysis of variance for repeated measures MANOVA) by a general linear model (GLM) was used. Weights were compared between different groups and ages, considering the expected changes with age. The sexual behavior and day of PPS were compared among the groups using the Mann–Whitney *U* test. Weights of seminal vesicle (drained and undrained) were compared by paired Student's *ttest.* All other parameters were analyzed by Student's *t-test*. Statistical differences were considered significant when the value of P was < 0.05. Values were expressed as means and the standard error of the mean (±SEM) for parametric and interquartile ranges of nonparametric analysis.
Test organisms	Wistar rats
Biological effects	Increases in sexual partner preference scores and latency time to the first mount; testosterone and estradiol serum concentrations; mRNA expression and protein content in the pituitary gland and the serum concentration of LH; sperm production

	and reserves; and height of the germinal epithelium of seminiferous tubules. Early onset of puberty but no effect on the body growth of the animals
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Benachour et al. (2007)

glyphnosubm_237	**BENACHOUR, N. SIPAHUTAR, H. MOSLERNI, S. GASNIER, C. TRAVERT, C. SERALINI, G. E.**	**2007**	**TIME- AND DOSE-DEPENDENT EFFECTS OF ROUNDUP ON HUMAN EMBRYONIC AND PLACENTAL CELLS.**	**ARCHIVES OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY (53) 126-133**

Reliability	
Purpose of the study Description of endpoints	To test the toxicity and endocrine disruption potential of Roundup (Bioforce®) on human embryonic 293 and placental-derived JEG3 cells, but also on normal human placenta and equine testis. EP: cell viability; aromatase activity inhibition
Test compound, application procedure, exposure period, protocol	Test item: Roundup Bioforce® and glyphosate Active substance(s): Glyphosate Purity: Glyphosate: not reported Roundup Bioforce® : 360 g/L acid glyphosate (equivalent to 480 g/L of isopropylamine salt of glyphosate)
Experimental approach, Statistical design, test environment	*Please refer to the study*
Test organisms	Human: Human embryonic kidney (HEK) 293 cell line (ECACC 85120602), choriocarcinoma-derived placental JEG3 cell line (ECACC 92120308) Horse: Equine testis (aromatase activity inhibition)
Biological effects	The median lethal dose (LD_{50}) of Roundup with embryonic cells is 0.3% within 1 h in serum-free medium, and it decreases to reach 0.06% (containing among other compounds 1.27 mM glyphosate) after 72 h in the presence of serum. In these conditions, the embryonic cells appear to be 2-4 times more sensitive than the placental ones. In all instances, Roundup (generally used in agriculture at 1-2%, i.e., with 21-

	42 mM glyphosate) is more efficient than its active ingredient, glyphosate, suggesting a synergistic effect provoked by the adjuvants present in Roundup
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Benachour et al. (2009)

glyphnosubm _238	**BENACHOUR, N. SERALINI, G. E.**	**2009**	**GLYPHOSATE FORMULATIONS INDUCE APOPTOSIS AND NECROSIS IN HUMAN UMBILICAL, EMBRYONIC, AND PLACENTAL CELLS**	**CHEMICAL RESEARCH IN TOXICOLOGY (22) 97-105**

Reliability	
Purpose of the study Description of endpoints	To evaluate the toxicity of four glyphosate (G)-based herbicides in Roundup formulations, from 10(5) times dilutions, on three different human cell types. The formulations have been compared to glyphosate alone and with its main metabolite AMPA or with one known adjuvant of R formulations, POEA (Polyethoxylated tallowamine) EP: Cell viability, membrane damage, apoptosis induction, cell morphology
Test compound, application procedure, exposure period, protocol	Test item: Glyphosate, Roundup Express®, Bioforce® or Extra 360, Grands Travaux®, Grands Travaux plus®; AMPA Active substance(s): Glyphosate Purity: Glyphosate and AMPA: not reported Roundup Express®: 7.2 g/L (R7.2) Bioforce® or Extra 360: 360 g/L (R360) Grands Travaux®: 400 g/L (R400) Grands Travaux plus®: 450 g/L (R450) Dose levels: Roundup formulations, glyphosate, AMPA and POEA: 14 concentrations ranging from 10 ppm to 2 % Additional AMPA concentrations: 4, 6, 8 and 10% POEA concentrations. 1 and 5 ppm Combined exposures of G, AMPA and POEA mixtures: For the two cell lines, the first mixture was the combination of glyphosate

	(0.4999%) with POEA (0.0001%); the second was the combination of glyphosate (0.4%) with AMPA (0.1%), and the third was AMPA (0.4999%) plus POEA (0.0001%). Combined exposures of G, AMPA and POEA mixtures: For the primary HUVEC cells, the first mixture was glyphosate (0.04999%) with POEA (0.0001%); the second was glyphosate (0.04%) with AMPA (0.01%), and the third was AMPA (0.04999%) plus POEA (0.0001%).
Experimental approach, Statistical design, test environment	MTT assay: Assessment of cell viability ToxiLight® assay: Bioluminescent assay for quantitative measurement of cell membrane damage Caspase-Glo® 3/7 assay Assessment of caspase activity or apoptosis induction Microscopy: Assessment of cell viability due to cell morphology Statistics: All data were reported as mean ± standard error. Statistical differences were determined by Student t-test using significant levels of 0.01.
Test organisms	Human embryonic kidney 293 cell line (ECACC 85120602) Human choriocarcinoma-derived placental JEG3 cell line (ECACC 92120308)
Biological effects	All R formulations: - cause total cell death within 24 h, through an inhibition of the mitochondrial succinate dehydrogenase activity, and necrosis, by release of cytosolic adenylate kinase measuring membrane damage - induce apoptosis via activation of enzymatic caspases 3/7 activity. This is confirmed by characteristic DNA fragmentation, nuclear shrinkage (pyknosis), and nuclear fragmentation (karyorrhexis), which is demonstrated by DAPI in apoptotic round cells Glyphosate provokes only apoptosis, and HUVEC are 100 times more sensitive overall at this level. The deleterious effects are not proportional to G concentrations but rather depend on the nature of the adjuvants AMPA and POEA separately and synergistically damage cell membranes like R but at different concentrations. Their mixtures are generally even more harmful with G. In conclusion, the R adjuvants like POEA change human cell permeability and possibly amplify toxicity induced by G, through apoptosis and necrosis. The real threshold of G toxicity must take into account the presence of adjuvants but also G metabolism and time-amplified effects or bioaccumulation.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Tested concentrations are far below agricultural recommendations and corresponds to low levels of residues in food or feed.
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes

Concluding weight of evidence	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Gasnier et al. (2009)

glyphnosub m_239	**GASNIER, C., DUMONT, C., BENACHOUR, N., CLAIR, E., CHAGNON, M. C., SERALINI, G. E**	**2009**	**GLYPHOSATE-BASED HERBICIDES ARE TOXIC AND ENDOCRINE DISRUPTORS IN HUMAN CELL LINES**	**TOXICOLOGY (262)3:184-191**

Reliability	
Purpose of the study Description of endpoints	To study xenobiotic toxicity, to four different formulations and to glyphosate EP: cytotoxicity(3 assays : Alamar Blue, MTT, ToxiLight), plus genotoxicity (comet assay), anti-estrogenic (on ERα, ERβ) and anti-androgenic effects (on AR) using gene reporter tests. Androgen to estrogen conversion by aromatase activity and mRNA
Test compound, application procedure, exposure period, protocol	Test item:Glyphosate, Roundup Express®, Bioforce® or Extra 360, Grands Travaux®, Grands Travaux plus® Purity: Glyphosate: not reported Roundup Express®: 7.2 g/L (R7.2) Bioforce® or Extra 360: 360 g/L (R360) Grands Travaux®: 400 g/L (R400) Grands Travaux plus®: 450 g/L (R450) Dose levels: Glyphosate: not reported Roundup Express®: 7.2 g/L Bioforce® or Extra 360: 360 g/L Grands Travaux®: 400 g/L Grands Travaux plus®: 450 g/L
Experimental approach, Statistical design, test environment	Replicates per dose level: 4 x 3 replicates Statistics: All data were reported as mean ± standard error. Statistical differences were determined by Student t-test using significant levels of 0.01 or 0.05.
Test organisms	Cell cultures: Hepatoma cell line HepG2, breast cancer cell line MDA-MB453-kb2
Biological effects	All parameters were disrupted at sub-agricultural doses with all formulations within 24h: - Human cell endocrine disruption from 0.5 ppm on the androgen receptor in MDA-MB453-kb2 cells for the most active formulation (R400), then from 2 ppm the transcriptional activities on both estrogen receptors were also inhibited on HepG2. - Aromatase transcription and activity were disrupted from 10 ppm. Cytotoxic effects started at 10 ppm with Alamar Blue assay (the most sensitive), and DNA damages at 5 ppm.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, partly
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH,	yes

temperature, light conditions)?		
Concluding weight of evidence	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report	
Type of info. (Critical, supporting, low weight)		
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**	

Clair et al. (2012)

glyphnosub m_242	**CLAIR, E., MESNAGE, R., TRAVERT, C., SERALINI, G.E.**	**2012**	**A GLYPHOSATE-BASED HERBICIDE INDUCES NECROSIS AND APOPTOSIS IN MATURE RAT TESTICULAR CELLS *IN VITRO*, AND TESTOSTERONE DECREASE AT LOWER LEVELS**	**TOXICOLOGY IN VITRO (26)2:269-279**

Reliability	
Purpose of the study Description of endpoints	To test glyphosate and its formulation on mature rat fresh testicular cells from 1 to 10000 ppm EP: Citotoxicity (adenylate kinase activities); measurements of caspases 3 and 7 (key-caspases of apoptosis) in cell cultures by means of bioluminescence-based method; study of chromatin condensation by DAPI-labelling; measurement of 3β-HSD activity; changes in testosterone production secreted from Leydig cells in medium
Test compound, application procedure, exposure period, protocol	Test item: Roundup Bioforce® and glyphosate Purity: Glyphosate: not reported; Roundup Bioforce®: 360 g/L acid glyphosate (corresponding to 100%)
Experimental approach, Statistical design, test environment	Experimental approach: please refer to the study Statistics: All data are present as means ± SEM. Statistically significant differences from controls were determined by an ANOVA test followed by Bonferroni post-test with p<0.001 (****), p<0.005 (***), p <0.01 (**) and p <0.05 (*).
Test organisms	Rat: Cell Culture: Leydig, Sertoli and germ cells
Biological effects	- From 1 to 48 h of Roundup exposure Leydig cells are damaged. - Within 24–48 h this formulation is also toxic on the other cells, mainly by necrosis, by contrast to glyphosate alone which is essentially toxic on Sertoli cells - Later it induces apoptosis at higher doses in germ cells and in Sertoli/germ cells co-cultures. - At lower non toxic concentrations of Roundup and glyphosate (1 ppm), the main endocrine disruption is a testosterone decrease by 35%.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Concentrations from 1 to 10000 ppm (from the range in some human urine and in environment to agricultural levels)

3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Daruich et al. (2001)

glyphnosub m_245	**DARUICH, J. ZIRULNIK, F. GIMENEZ, M. S.**	**2001**	**EFFECT OF THE HERBICIDE GLYPHOSATE ON ENZYMATIC ACTIVITY IN PREGNANT RATS AND THEIR FOETUSES**	**ENVIRONMENTAL RESEARCH (85)226-231**

Reliability	
Purpose of the study Description of endpoints	To study the effects of the herbicide glyphosate on several enzymes of pregnant rats EP: Enzymatic activity of three cytosolic enzymes : isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase, malic dehydrogenase in liver, heart, and brain of pregnant Wistar rats. Organ weights: Liver, hearts and brains of maternal females
Test compound, application procedure, exposure period, protocol	Test item: Herbycigon Active substance(s): Glyphosate
Experimental approach, Statistical design, test environment	Dose levels: 0 (tap water), glyphosate solution 0.5% w/v in tap water (0.2 ml glyphosate/ml water) glyphosate solution 1% w/v in tap water (4 ml glyphosate/ml water) Animals per test substance group: 8 Animals per control group: Tap water control group: 8 Low water and low food control group: 6 Administration: The test substance was prepared as solution in tap water. 35 mL of the test substance preparations were provided in water bottles per day and animal The treatment was administered during the 21 days of pregnancy
Test organisms	Wistar rats
Biological effects	Please refer to the study
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report

Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Dallegrave et al. (2003)

glyphnosubm_247	**DALLEGRAVE, E. MANTESE, F. D. COELHO, R. S. PEREIRA, J. D. DALSENTER, P. R. LANGELOH, A..**	**2003**	**THE TERATOGENIC POTENTIAL OF THE HERBICIDE GLYPHOSATE-ROUNDUP® IN WISTAR RATS**	**TOXICOLOGY LETTERS (142)45-52**

Reliability	
Purpose of the study Description of endpoints	To assess the teratogenicity of the herbicide glyphosate-Roundup(R) (as commercialized in Brazil) to Wistar rats.
Test compound, application procedure, exposure period, protocol	Test item: Roundup ® Active substance: Glyphosate Concentration: 360 g/L Surfactant Class: Polyoxyethyleneamine (POEA) Concentration: 18% (w/v) (POEA)
Experimental approach, Statistical design, test environment	Study type: Developmental toxicity study Guideline: Refers to the EPA (Environmental Protection Agency), 1996.Guidelines for Reproductive Toxicity Risk Assessment- EPA/630/R-96/009, Washington, USA, pp. 1-163.(reproductive toxicity protocols; segment II).
Test organisms	Wistar rats
Biological effects	Results showed: - a 50% mortality rate for dams treated with 1000 mg/kg glyphosate - Skeletal alterations in 15.4, 33.1, 42.0 and 57.3% of fetuses from the control, 500, 750 and 1000 mg/kg glyphosate groups, respectively. The authors conclude that glyphosate-Roundup(R) may toxic to the dams and induces developmental retardation of the fetal skeleton.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity in the Vol.1 of the BfR report
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Dallegrave et al. (2007)

glyphnosubm_248	**DALLEGRAVE, E. MANTESE, F. D. OLIVEIRA, R. T. ANDRADE, A. J. M. DALSENTER, P. R. LANGELOH, A.**	**2007**	**PRE- AND POSTNATAL TOXICITY OF THE COMMERCIAL GLYPHOSATE FORMULATION IN WISTAR RATS**	**ARCHIVES OF TOXICOLOGY (81):665-673**

Reliability	
Purpose of the study Description of endpoints	To elucidate whether glyphosate-Roundup® (commercial formulation) poses reproductive hazards to male and female offspring of rats exposed during pregnancy and lactation
Test compound, application procedure, exposure period, protocol	Test item: Roundup ® Active substance(s): Glyphosate Concentration: 360 g/L Surfactant: Polyoxyethyleneamine (POEA) Concentration: 18% (w/v) POEA
Experimental approach, Statistical design, test environment	Duration of study: 21-23 days during pregnancy; 21 days during lactation Dose levels: 0 (water), 50, 150, 450 mg/kg glyphosate-Roundup® Administration: Test substance preparations were prepared by diluting the Roundup-formulation with appropriate volumes of distilled water. Applications were done once daily by oral gavage Dosing volume: 10 mL/kg bw Statistics: Parametric data, expressed as mean ± standard error (SEM), were analyzed by repeated measure ANOVA or one-way ANOVA, followed by the Bonferroni test when appropriate. The nonparametric data, expressed as proportion or percentage, were analyzed by the chi-square test. Differences were considered statistically significant when $P < 0.05$.
Test organisms	Wistar rats
Biological effects	Glyphosate-Roundup (R) did not induce maternal toxicity but induced adverse reproductive effects on male offspring rats: a decrease in sperm number per epididymis tail and in daily sperm production during adulthood, an increase in the percentage of abnormal sperms and a dose-related decrease in the serum testosterone level at puberty, and signs of individual spermatid degeneration during both periods. There was only a vaginal canal-opening delay in the exposed female offspring.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes

Concluding weight of evidence	These findings suggest that in utero and lactational exposure to glyphosate-Roundup (R) may induce significant adverse effects on the reproductive system of male Wistar rats at puberty and during adulthood.
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Hokanson et al. (2007)

glyphnosub m_263	**HOKANSON, R., FUDGE, R., CHOWDHARY, R., BUSBEE, D.**	**2007**	**ALTERATION OF ESTROGEN-REGULATED GENE EXPRESSION IN HUMAN CELLS INDUCED BY THE AGRICULTURAL AND HORTICULTURAL HERBICIDE GLYPHOSATE**	**HUMAN & EXPERIMENTAL TOXICOLOGY (26) 747-752**

Reliability	
Purpose of the study Description of endpoints	To examine the toxicity of glyphosate as a function of its capacity to alter gene expression in the presence or absence of E2 (17ß-estradiol). The authors present data resulting from an investigation of the potential endocrine disruptive activities of a commercially available, unregulated, glyphosate herbicide. EP: In vitro DNA microarray analysis, quantitative real-time PCR (qrtPCR)
Test compound, application procedure, exposure period, protocol	Test item: Glyphosate formulation Source: Unknown retail supplier Purity: Not reported Concentration: 15% home use preparation Dose levels: 0.1, 0.01, 0.001 or 0.0001% dilutions of the glyphosate stock solution containing 15% glyphosate. Duration of exposure: 18 h
Experimental approach, Statistical design, test environment	Please refer to the study
Test organisms	MCF-7
Biological effects	DNA microarray analysis indicated that a large number of genes, 680 out of 1550 on the chip, were dysregulated by in vitro exposure to the commercial glyphosate herbicide For example: three genes – HIF1, CXCL12 and EGR1 –determined by DNA microarray analysis and quantitative real-time PCR to be dysregulated by exposure to glyphosate, combine to give a bewildering array of potential altered gene regulation effects. These include initiation of apoptosis in cells of cerebral and myocardial tissues, increased angiogenesis in tumors, retinal ischemia, hypertension, pre-eclampsia, fetal growth retardation and inactivation of tumor repressor genes. Altered EGR1 levels in response to glyphosate salts are less clear than for HIF1 and CXCL12, but appear to potentially impact rates of apoptosis initiation and alter the levels of vascularization associated with tumor formation.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	

1 Is an appropriate test species/ life-stage(s) studied?	
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	partly
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	There remains an unclear pattern of very complex events following exposure of human cells to low levels of glyphosate, but events surrounding the altered levels of expression of only three genes – EGR1, CXCL12 and HIF1 – out of the entire battery tested, are both complicated and potentially damaging to adult and fetal cells.
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA3**

Mesnage R. et al. (2012)

glyphnosub m_243	**MESNAGE R., BERNAY B., SERALINI G.-E.**	**2012**	**ETHOXYLATED ADJUVANTS OF GLYPHOSATE-BASED HERBICIDES ARE ACTIVE PRINCIPLES OF HUMAN CELL TOXICITY**	**TOXICOLOGY,** IN PRESS, CORRECTED PROOF, AVAILABLE ONLINE 20 SEPTEMBER 2012

Reliability	
Purpose of the study Description of endpoints	To study potential active principles for toxicity on human cells for 9 glyphosate-based formulations. As controls a major adjuvant (the polyethoxylated tallowamine POE-15), glyphosate alone, and a total formulation without glyphosate were used. EP: mitochondrial activities, membrane degradations, and caspases 3/7 activities
Test compound, application procedure, exposure period, protocol	Glyphosate (CAS: 1071-83-6; Sigma–Aldrich) POE-15 (CAS: 61791-26-2; ChemService) Formulating agents without Glyphosate: Genamin T200 (60–80% of POE-15) 9 Formulating agents with Glyphosate: Bayer GC (12.5% of G, 1–5% of POE-15) Clinic EV (42% of G, 11% of POE-15) Glyphogan (39–43% of G, 13–18% of POE-15) Roundup Grand Travaux (400 g/L of G, R GT) Roundup Grand Travaux plus (450 g/L of G, 90 g/L of EtO-EA, R GT+) Roundup Ultra (41.5% of G, 16% surfactant) Roundup Bioforce (360 g/L of G) Roundup 3plus (170 g/L of G, 8% surfactant) Topglypho 360 (360 g/L of G)
Experimental	Experiments were repeated at least 3 times in different weeks on 3 independent cultures

approach Statistical design, test environment	(n = 9). LC_{50} values were calculated by a nonlinear regression using sigmoid (5-parameters) equation with the GraphPad software Statistical differences were determined by Student's t-test using significant levels with $p < 0.01$ (**) and $p < 0.05$ (*).
Test organisms	hepatic (HepG2), embryonic (HEK293) and placental (JEG3) cell lines
Biological effects	**Mitochondrial respiration (SD activity):** All chemicals are cytotoxic, inducing similar dose-dependent patterns on HEK293, HepG2, and JEG3 in 24 h. 3 groups of differentially toxic formulations: The most toxic : adjuvants alone POE-15 (LC_{50} ~ 1–2 ppm; agricultural dilutions: 1–2% of the herbicide formulation containing adjuvants) and Genamin, themselves around 100-fold more toxic than a middle group. Middle group: the majority of formulations (6, with among them R GT and GT+). This middle group is again 100-fold more toxic than the third one which includes R Ultra, R Bioforce, R 3plus and finally G alone POE-15 diluted to the concentration at which it is present in Clinic E.V. (a formulation from the middle group) presented a similar toxicity than this GBH and to the middle group in general. It thus appears to be the toxic principle in human cells. Two formulations claiming a similar concentration of G (360 g/L) and different adjuvants (16% of POEA or other adjuvants), Glyphogan and R Ultra respectively, exhibited very different toxicities, 150- fold stronger on average for Glyphogan on the 3 cell lines **Cytotoxicity**: results obtained with all cell lines: The cytotoxicity induced by GBH is not linear to G concentrations The cytotoxicity induced by GBH is only linear to the 3 ethoxylated adjuvants. a The cytotoxicity induced by GBH is not linear to the non-ethoxylated formulations => Ethoxylated adjuvants can thus be considered as the active principle of the toxicity of GBH in human cells **Critical micelle concentration (CMC) of POE-15** Disruptions of the cellular membranes by micellization were observed **Membrane disruption / caspases activation**: POE-15 and R GT+ (containing also an ethoxylated adjuvant) induced more necrosis by membrane alterations rather than apoptosis G induced only apoptosis at higher levels. Ethoxylated adjuvants are thus not inert at all but cell membrane disruptors, and then induce severe mitochondrial alterations.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes: tested concentrations between 1 and 3 ppm and at environmental/occupational doses.
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes

Concluding weight of evidence	All formulations appeared more toxic than glyphosate, and 3 groups of differentially toxic formulations were experimentally separated according to their concentrations in ethoxylated adjuvants. Ethoxylated adjuvants alone and in formulations appeared as active principles for human cell toxicity
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA1 for assessment of surfactand effects (POEA)**

Walsh et al (2000)

glyphnosubm_067	**WALSH, L.P. MCCORMICK, C. MARTIN, C. STOCCO, D.M.**	**2000**	**ROUNDUP INHIBITS STEROIDOGENESIS BY DISRUPTING STEROIDOGENIC ACUTE REGULATORY (StAR) PROTEIN EXPRESSION.**	**ENVIRONMENTAL HEALTH PERSPECTIVES (108)769-776**

Reliability	
Purpose of the study Description of endpoints	To screen 8 currently used pesticide formulations for their ability to disrupt steroid hormone biosynthesis. EP: steroidogenic acute regulatory (StAR) protein expression in MA-10 cells; levels and activities of the P450scc and the 3p-hydroxysteroid dehydrogenase (3P-HSD) enzymes (conversion of cholesterol to pregnenolone and pregnenolone to progesterone; respectively)
Test compound, application procedure, exposure period, protocol	Roundup (180 g/L glyphosate): N-(phosphonomethyl) glycine
Experimental approach Statistical design, test environment	Please refer to the study: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1638308/pdf/envhper00309-0125.pdf
Test organisms	Mouse MA-10 Leydig tumor cell line
Biological effects	**Progesterone production and total cellular protein synthesis:** - Roundup decreased progesterone production in a dosage-dependent manner without inducing a parallel decrease in total protein synthesis (*indicating that this herbicide did not cause acute cellular toxicity or a general disruption in translation*). - Roundup also significantly disrupted steroidogenesis over time without inducing a parallel decrease in total protein synthesis. - The active ingredient in Roundup, glyphosate, did not alter steroidogenesis or total protein synthesis at any dose tested (0-100 gg/mL) **P450scc and -30-HSD enzyme activity, expression, and steroidogenesis** - Although Roundup significantly reduced (Bu)2cAMPstimulated steroidogenesis by 84%, effects were completely reversible. - Roundup also significantly reduced 22R-HC-driven steroidogenesis by 71%, indicating that it inhibited P450scc and/or 3,B-HSD enzyme activity. - Although Roundup did not alter 30-HSD enzyme activity, indicating that the herbicide was not acutely toxic to cells or mitochondria, it significantly reduced P450scc activity by 61%. **StAR protein and mRNA levels** Northern blot analysis revealed that Roundup did not alter StAR mRNA levels, indicating that Roundup disrupted StAR protein expression post-transcriptionally.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints	yes

been considered (e.g. pH, temperature, light conditions)?	
Concluding weight of evidence	The results indicate that the commercial formulation of the herbicide glyphosate Roundup® might affect reproductive function in animals
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

McDaniel at al. (2008)

glyphecotox_496	**MCDANIEL, T.V., MARTIN, P.A., STRUGER, J., SHERRY, J., MARVIN, C.H., MCMASTER, M.E., CLARENCE, S., TETREAULT, G.**	**2008**	**POTENTIAL ENDOCRINE DISRUPTION OF SEXUAL DEVELOPMENT IN FREE RANGING MALE NORTHERN LEOPARD FROGS (*RANA PIPIENS*) AND GREEN FROGS (*RANA CLAMITANS*) FROM AREAS OF INTENSIVE ROW CROP AGRICULTURE**	**AQUATIC TOXICOLOGY 88 (4):230-42**

Reliability	
Purpose of the study Description of endpoints	To assess whether amphibians that inhabit wetlands in areas of IRCA in southern Ontario show evidence of exposure to endocrine disrupting substances EP: altered gonad histology, altered or abnormal plasma steroid levels, or Vtg expression in male To test for possible associations with any observed health effects or biomarker responses in the amphibians EP: measurements of concentrations of a suite of pesticides and nutrients in farm ponds and agricultural drains in the area of the frog collection sites and from the surrounding watersheds
Test compound, application procedure, exposure period, protocol	In-situ measurements: Glyphosate concentrations were analyzed using gas chromatography with nitrogen/phosphorus detection. Glyphosate was detected in trace amounts (>5,000 ng/L) at several agricultural sites
Experimental approach Statistical design, test environment	Study sites: Agricultural sites were located in two regions of southwestern Ontario within the Thames River watershed; an area north of the city of London in Middlesex county and an area west of the city of Chatham in Chatham/Kent county Statistical analysis Data were log transformed, where necessary, to meet normality and homogeneity of variance requirements for parametric tests. If those criteria could not be met then non-parametric tests were used. Biological endpoints (circulating sex steroids, gonadosomatic index, diameter of TOFS) were compared amongst regions and between males with and without TOFS using one-way analysis of variance (ANOVA). The particular associations between concentrations of circulating sex steroids or the proportion of males with TOFS and atrazine concentrations were assessed using Pearson product moment. Goodness of fit tests were used to test the hypothesis that the frequency of TOFS were equal amongst regions, for this test, the sites within regions were pooled in order to increase sample sizes. In order to look at variation within region, individual sites with sample sizes greater than 20 animals in Chatham Region were compared for frequency of TOFS (Testicular Ovarian Follicles).
Test organisms	*Rana pipiens* and *Rana clamitans*
Biological effects	- Glyphosate was detected in several agricultural sites. - Occurrence of testicular ovarian follicles (TOFS) in male *R. pipiens* was

	significantly higher (42%; $p < 0.05$) at agricultural sites - There was no difference in circulating sex steroid levels between frogs from agricultural and reference sites and sex steroid levels did not correlate with pesticide concentrations in the environment - No differences were detected in the gonadosomatic indices or stage of spermatogenesis between frogs from agricultural and non-agricultural regions ($p > 0.05$). - Plasma Vtg-lpwas detected in only one male *R. pipiens* from an agricultural site. - Neither gonad size, gonad maturity nor sex steroid levels differed between normal males and those with testicular oocytes. - Proportion of testicular oocytes correlate with a mixture of pesticides and nutrients
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Monitoring study
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	-/-s
Concluding weight of evidence	No relevant for the risk assessment of glyphosate, however the study showed that mixtures of pesticides potentially have endocrine effects in amphibians inhabiting farm ponds and agricultural drains in intensive row crop agriculture
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA3**

Quassinti et al. (2009)

glyphecotox_235	**Quassinti, L., Maccari, E., Murri, O., Bramucci, M.**	**2009**	**EFFECTS OF PARAQUAT AND GLYPHOSATE ON STEROIDOGENESIS IN GONADS OF THE FROG RANA ESCULENTA IN VITRO**	**A Pesticide Biochemistry and Physiology 93 (2):91-95.**
Reliability				
Purpose of the study Description of endpoints	To assess how paraquat and glyphosat affect reproduction in amphibians EP: 17b-estradiol and testosterone levels			
Test compound, application procedure, exposure period, protocol	Glyphosate (Sigma–Aldrich) was solubilized at 100 mM concentration in Krebs Ringer Bicarbonate buffer Diluted solutions of herbicides were added to each cells culture well to reach the final concentrations of $10^{-}3$, $10^{-}4$, $10^{-}5$, and $10^{-}6$ M.			
Experimental approach Statistical design,	Experimental approach: ovarian tissue and testis of the water frog *Rana esculenta* were incubated in vitro			

test environment	in presence of different concentrations of the two herbicides Statistics: Data represent the mean ± S.D. of 4 determinations. Data were subjected to Levene's test for assay homogeneity of variance. Significant differences between groups were established by use Mann–Whitney U nonparametric test. The minimum level of significance considered was P < 0.05. All statistical analysis used SPSS version 13.0 for Windows.
Test organisms	*Rana esculenta* (water frog)
Biological effects	Glyphosate showed no effect on gonadal steroidogenesis even at high concentrations. Glyphosate does not exert a significant inhibition on testosterone production at the highest tested concentrations. Treatment with glyphosate showed no evidence of specific activity on 17b-estradiol production by frog ovary.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Glyphosate showed no effect on gonadal steroidogenesis
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA1 for assessment of surfactand effects (POEA)**

Moore et al . (2012)

BVL-Nr. 2716841	**Moore LJ, Fuentes L, Rodgers JH Jr, Bowerman WW, Yarrow GK, Chao WY, Bridges WC Jr.**	**2012**	**Relative toxicity of the components of the original formulation of Roundup to five North American anurans.**	**Ecotoxicology and Environmental Safety78, 128-33**

Reliability	
Purpose of the study Description of endpoints	The responses of five North American frog species that were exposed in an aqueous system to the original formulation of Roundup were compared. The formulated mixture of this herbicide as well as its components, isopropylamine (IPA) salt of glyphosate and the surfactant MON 0818 (containing polyethoxylated tallowamine (POEA)) were separately tested in 96h acute toxicity tests with Gosnerstage 25 larval anurans. Endpoint : mortality; LC50 values are expressed as glyphosate acid equivalents (ae) or as mg/L for MON0818 concentrations for

	comparison between the formulation and components.
Test compound, application procedure, exposure period	11 concentrations of theoriginal formulation of Roundup herbicide, nine concentrations of MON0818 and three concentrations of IPA salt of glyphosate
Experimental approach, Statistics, test environment	static (non-renewal) aqueous 96 h laboratory tests . Bioassays performed according to EPA- 821-R-02–012 (U.S. EPA, 2002). Chemical and physical measurements of testing conditions, dilution water, and test solutions were conducted according to the ASTM methods (ASTM, 2003).
Test organisms	*Rana pipiens*, *R. clamitans*, *R. catesbeiana*, *Bufo fowleri*, and *Hyla chrysoscelis* were reared from egg masses
Biological effects	R.pipiens was the most sensitive of five species. 96h-LC50 values for formulation tests for the five species ranged from 1.80 to 4.22 mg ae/L, and with 96h-LC50 values after exposure to MON 0818 ranging from 0.68 to 1.32 mg/L. No significant mortality was observed during exposures of 96h for any of the five species exposed to glyphosate IPA salt at concentrations up to 100 times the predicted environmental concentration (PEC).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, tests were performed separately with formulated products containing POEA, glyphosate alone and the surfactant
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	The results fully supports previous RMS evaluation. Surfactant MON 0818 containing POEA drives the toxicity of the herbicide formulations containign glyphosatge for amphibians.
Type of information (Critical, supporting, low weight)	**Critical**
Consideration/concluding score	**UBA1, also for assessment of surfactand effects (POEA)**

B.9.13 16.1 Summary of the relevant literature on surface active substances in glyphosate-based formulations

In glyphosate-based formulations –as in almost all plant protection products (PPP) –, a varing amount of co-formulants are added to improve the handling and efficacy of the product. A great amount of the co-formulants might consist of water to which substances with antifoaming or surface active properties are added.
Surfactant do have a mode of action that attack membranes, so to permit the active substance to enter cells and reach the target. A class of non-ionic surfactants, the so called alkylamine ethoxylates (ANEO), exert an (eco)toxicological effect that can be detected in glyphosate-

based formulations. Polyethoxylated alkylamine (POEA) are non-ionic surfactants belonging to the alkylamine ethoxylates.
The lead formulation for the assessment of glyphosate as active substance for PPP in the European Union does not contain alkylamine ethoxylates as surfactant. Nevertheless, since several glyphosate-based products are formulated with alkylamine ethoxylates, RMS considers it adequate to provide general background informations to other Member States in the European Union to facilitate the assessment of the risk arising from glyphosate-based PPP other than the lead formulation.
The toxicity of glyphosate-based products is greatly enhanced if the active substance is formulated with alklyamine ethoxylates (e.g. Figure B.0-1).

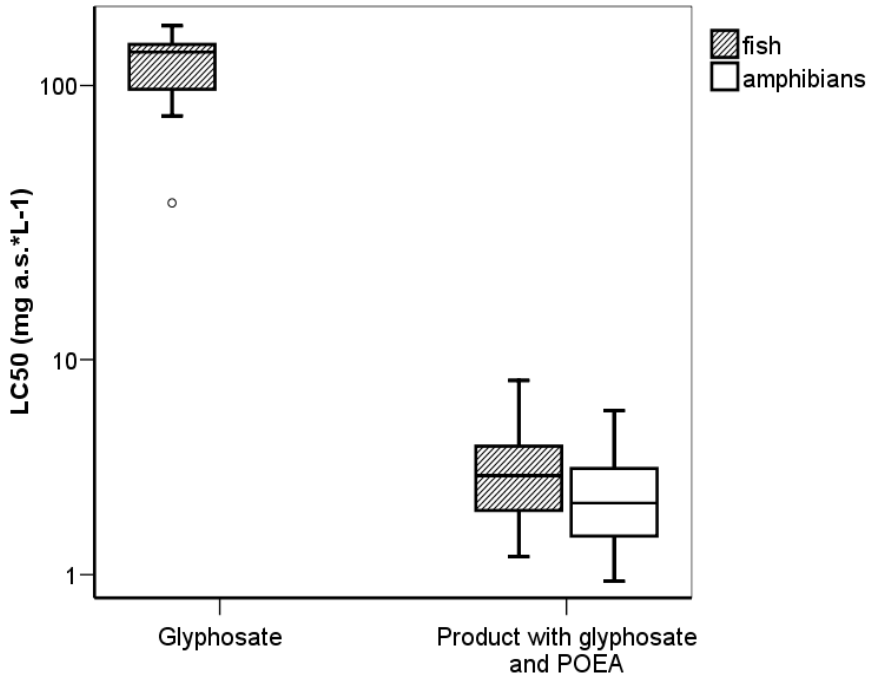

Figure B.0-1: LC_{50} values determined for fish and amphibians: exposed to glyphosate or to glyphosate-based products containing polyethoxylated alkylamines. Data submitted for authorization of different products. Box gives median and 50 %, whiskers 75 % values

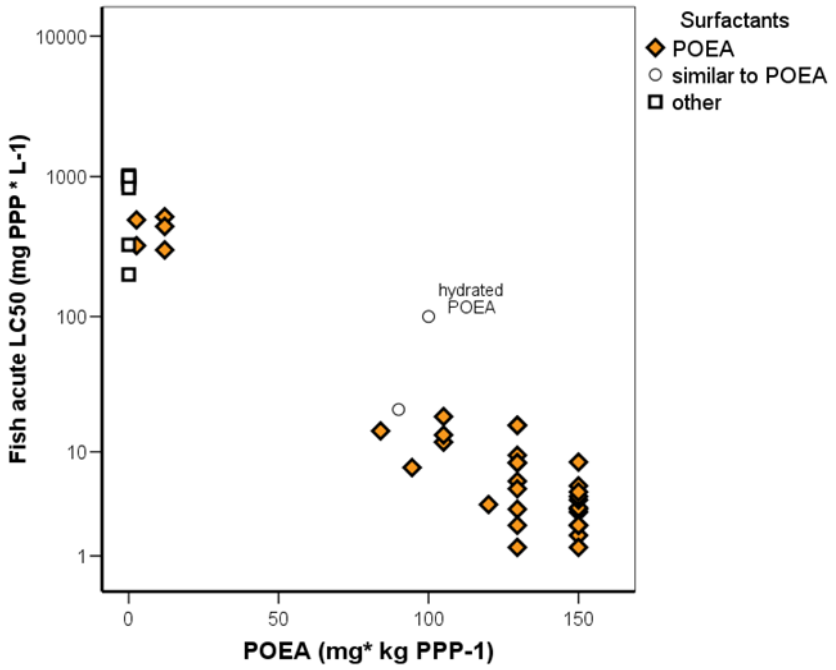

Figure B. 0-2: **LC_{50} for fish exposed to several glyphosate-based PPPs with different surfactants as a function of product surfactant content. POEA: polyethoxylated alkylamines; similar to POEA: other alkylamine ethoxylates. Other: other surfactant classes.**

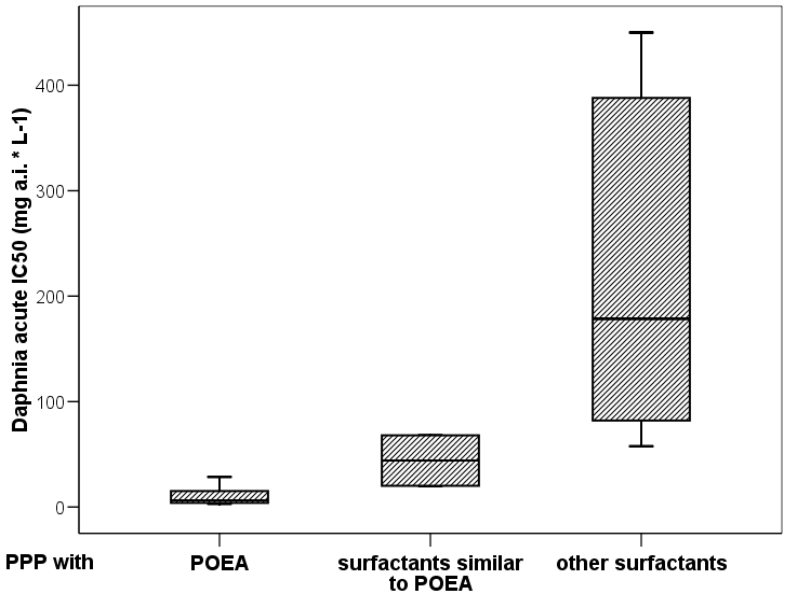

Figure B. 0-3: **LC_{50} for Daphnia exposed to several glyphosate-based PPPs with different surfactants. POEA: polyethoxylated alkylamines; similar to POEA: other alkylamine ethoxylates. Other: other classes. Data submitted for authorization of different products. Box: median/ 50 %, whiskers 75 % values**

As can be seen in Figure B.0-1, the acute toxicity of PPP with glyphosate and POEA for fish and amphibians (stage Gosner 25, see Gosner 1960) is comparable, as was discussed in the respective chapter.

The clearly higher toxicity of some PPP with glyphosate as active substances can be predicted from the surfactant 'class' in the formulation and can be depicted as relative to the content of the surfactant and not of the amount of active substance in the product (Figure B. 0-2).

Similar data showing enhanced toxicity of glyphosate-based PPP when formulated with POEA are available for Daphnia (Figure B. 0-3) and algae (data not shown).

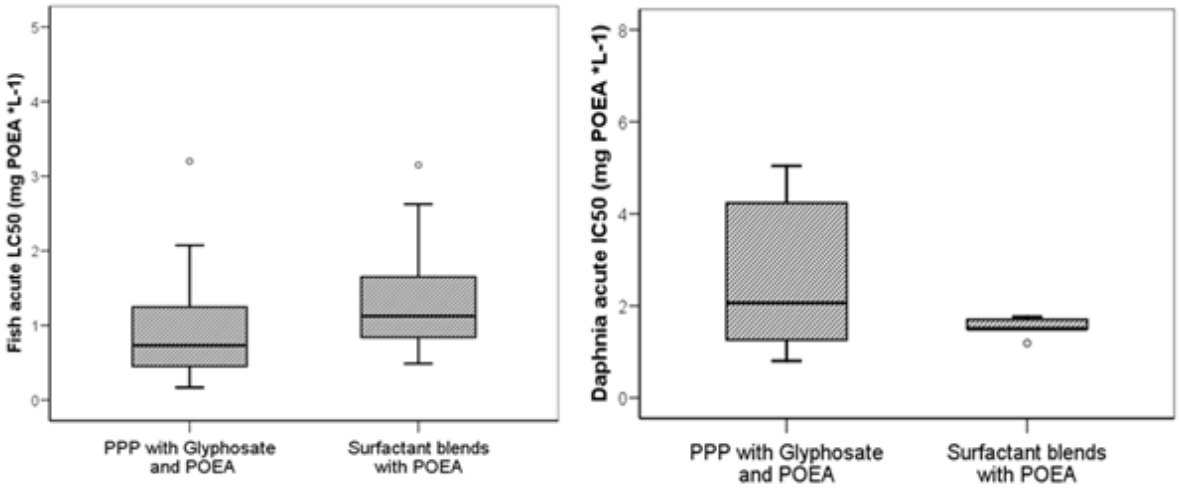

Figure B. 0-4: LC_{50} data for fish and daphnids exposed either to glyphosate-based products containing alkylamine ethoxylates or to the surfactants alone. Data submitted for authorization of different products. Box gives median and 50 %, whiskers 75 % values

During authorization processes for different glyphosate-based products, several toxicity data were generated with fish, daphnid and algae exposed to the surfactant blend alone (Figure B. 0-4). All data show that the toxicity of the surfactant does drive the toxicity of the product, in part because glyphosate itself is only moderately acutely toxic to the tested organisms.

Summarizing the data available, the acute risk for non-target organisms exposed to glyphosate-based products containing alkylamine ethoxylates can be assessed in the opinion of RMS based on the product tests submitted with the registration dossiers.

Hovewer, several studies published and peer reviewed that were submitted with this dossier and allocated to different themes (e.g. fish, aquatic invertebrates, algae, amphibians) do rise concerns on the effects of glyphosate and glyphosate-based formulation on endpoints regarding genotoxicity, mutagenicity, development or reproduction of non target organisms (Paganelli et al., 2010; Romano et al., 2010 and 2012; Gasnier et al., 2009; Dallegrave et al., 2003 and 2007; Hockanson et al., 2007; Walsh et al., 2000, McDaniel et al., 2008).
For the evaluation of studies with glyphosate and glyphosate-based products in in-vitro and in-vivo experimental set-ups with the aim to detect possible endocrine, genotoxic, carcenogenetic effect please refer also to the specific chapters assessing human toxicity (Vol.1, chapter 2.6.7.2, developmental toxicity and teratogenicityand respective Vol. 3 of this report).

RMS considers several of the observed effects to be mediated by the surfactants included in the formulatios. In different studies, the effects were clearly more pronounced in treatments where the tested products contained alkylamine exthoxylated surfactants (e.g. Benachour et al., 2007 and 2009); glyphosate acid treatment (when tested alone) did extremely seldom show any effects on biomarkers or higher endpoints (e.g. Quassinti, 2009) . In the paper of

Mesnage et al. (2012) and Moore et al. (2012), the direct actue toxic effects of co-formulants belonging to the alkylamine ethoxylates class has been identified and very well characterized. In chapter Vol 3 of this report, chapter B.6.13, an evaluation of the potential chronic toxicity, carcinogenicity reproduction and developmental toxicity of POEA is performed.
All (eco)toxicological data available give strong evidence that the toxicity of glyphosate-base formulation with POEA arisises from the surfactant. Nevetheless, even if this evidence relieves for the time being the active substance glyphosate from the suspect of being potentially carcenogenetic, endocrine disruptive and mutagenic, it does not tell the same for the surfactant class of the alkylamine ethoxylates.

Walsh et al. (2000) report that glyphosate based formulations, but not glyphosate alone, might affected the steroidogenesis pathway by inhibiting the progesterone production. Levine et al. (2007) determined that Roundup® branded formulation and a Roundup blank formulation without glyphosate decreased the hCG-stimulated increase in progesterone production. These findings indicate that the effect on progesterone is largely attributable to the surfactant, insofar as it decreases progesterone production upon mitochondrial membrane disruption.

Other finding (e.g. Dallegrave et al., 2007, Knapp, 2007 and 2008) give indication on reproductive toxicity of a commercial Roundup® formulation and the surfactant formulation MON 0818 (POEA) on reproduction. As stated in Vol 3, chapter B.6.13.3 "(...) Nonetheless, the published findings suggest that offspring development was in fact a particularly sensitive target of Roundup and the POE-tallowamine. The findings in young male rats might indicate impairment of spermatogenesis (...)".

More indications exisits that glyphosate-based products with ethoxylated alkylamines might interfere with the endocrine system of vertebrates (e.g. estrogen synthesis, aromatase activity Soso et al., 2007; Richard et al., 2005). Moreover, it is not clear how glyphosate-based PPP with alkylamine exthoxylated affect the process of amphibian methamorphosis (please refer to the respective chapter B.9.13 10.1).

Therefore, the authorization of glyphosate-based products with alkylamine exthoxylated surfactants might require the generation of further data. The requests should cover the clarification of the effect of POEA on endocrine enpoints (e.g by a fish screening assay) and thyroid mediated processes (e.g. by extended amphibian metamorphosis tests) with representative POEA.

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B.9.13.17 Overview of the concluding weight of evidence and assessment

The following sections summarise the results and discussion as found in the published literature, as well as a concluding weight of evidence. Further RMS assessment is given in the final summarising chapter after the results of the studies are presented and discussed.

Author	Year	Title	Organism	Concluding weight of evidence	Type of info	Score
Santillo, et al.	1989	Response of songbirds to glyphosate-induced habitat changes on clearcuts	Verte-brates	Authors state that leaving untreated palches of vegetation and staggering herbicide treatments on large elearcuts will maintain bird populations similar to those on untreated clearcuts.	Critical	UBA1
Santillo, et al.	1989	Response of small mammals and habitat to glyphosate application on clearcuts	Verte-brates	RMS emphasiszes that herbicides cause indirect effects and high-lights the need for risk mitigation measures by the Member States, proposing compensation measures as a suitable tool.	Critical	UBA1
Oliveira, et al.	2007	Effects of the herbicide Roundup on the epididymal region of drakes *Anas platyrhynchos*.	Vert-ebrates	The unusual way of exposure for reproductive studies via gavage and not via dietary uptake is criticized by the notifier. For the mallard duck as an aquatic dabbling bird it is assumed acceptable as a worst case exposure scenario.	Supporting information	UBA2
Eason, et al.	2007	Effects of Atrazine and Glyphosate ingestion on body weight and nutritional well-being of *Coturnix quail*	Verte-brates	The poor quality of the paper suggests that the experimenters had no or minor experience with animal toxicological testing and should have never given the permission to kill in sum 72 vertebrate organisms. The study was not further considered relevant after checking the experimental design.	Low weight	UBA3
Stoleson, et al.	2011	Ten-year response of bird communities to an operational herbicide-shelterwood treatment in a northern hardwood forest	Verte-brates	The silvicultural practice of shelterwood systems and thus the experimental approach is quite specific for North American practices and not transferable to European agricultural practices. Nevertheless, the authors describe an impressive example of indirect effects of herbicides on 'ecosystem level' and the complexity of an assessment that is not covered by 'standard ERA' by far. The transferability is further restricted by the use of a tank mixture of two herbicides. The study is recommended to be considered as an example of indirect effects and profound statistical analysis of monitoring data.	Supporting information	UBA2
Sullivan, et al.	2003	Ecosystem disturbance: impact of glyphosate herbicide on plant and animal diversity in terrestrial systems	Verte-brates	The paper deals with the impact of the Anglo-Saxon practice of managing the vegetation for purposes of enhancing forest and other crop yields. This includes especially the control of roadside vegetation and intends the maintenance of ecological processes in terrestrial ecosystems.	Supporting information	UBA2
Filizadeh, et al.	2011	Toxicity determination of three sturgeon species exposed to glyphosate	Fish	The description of the study is deficient; however, the LC50's 96 hours after exposure (20-26 mg a.i./L) are below the acute studies provided by the notifier and are located rather near the chronic toxicity of a full life cycle test (25.7 mg/L). Because the content of POEA that is usually grossly determining the toxicity of Roundup formulations was not stated by the authors, the study could not be taken into account as additional or critical information on the ERA of the active substance glyphosate.	Low weight	UBA3
Guilherme, et al.	2010	European eel (*Anguilla anguilla*) genotoxic and pro-oxidant responses following short-term exposure to Roundup® a glyphosate-based herbicide	Fish	The study is well conducted, except for the statistical evaluation. Regardless, the results can be taken as an indicator of genotoxic but not of oxidative stress effects of realistically low water concentrations in fish. Study describes physiological parameters, no mortality endpoints are stated.	Supporting information	UBA2
Hued, et al..	2012	Exposure to a Commercial Glyphosate Formulation (Roundup) Alters Normal Gill and Liver Histology and Affects Male Sexual Activity of *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes)	Fish	There are some obscurities in the description of the statistics and the test substance. The study could be taken as a further source of information that realistic concentrations of Glyphosate in surface waters could have a pronounced long-term effect on the populations of fishes. It is not distinguishable if the effect on the endpoints was due to the active ingredient glyphosate or (more likely) to the surfactant that was contained at 15.3 % of the formulation.	Supporting information	UBA2
Kelly, et al.	2010	Synergistic effects of glyphosate formulation and parasite infection on fish malformations and survival	Fish	The experiment shows a general mechanism of the ecotoxicological theory: many multiple stressors act additively on the endpoints observed; It is of limited value for ERA because the factors are not considered separately and safety factors should cover uncertainties caused by synergisms or enhanced toxicity.	Low weight	UBA3
Salbego, et al.	2010	Herbicide formulation with glyphosate affects growth, acetylcholinesterase activity, and metabolic and hematological parameters in piava (*Leporinus obtusidens*)	Fish	Physiological endpoints measured do not contribute to an ERA and the species is not representative for common protection goals. There was no indication of the percentage content of tallow amine surfactants within the Roundup formulation tested.	Low weight	UBA3

Tierney, et al..	2006	Changes in juvenile coho salmon electro-olfactogram during and after short-term exposure to current-use pesticides	Fish	Very interesting and well conducted study with a comprehensive description of the experimental design and statistics. The endpoints are considered ecologically relevant, but the validity and relevance are lowered by the artificial design.	Supporting information	UBA2
Soso, et al., et al.	2007	Chronic exposure to sub-lethal concentration of a glyphosate-based herbicide alters hormone profiles and affects reproduction of female Jundia (*Rhamdia quelen*)	Fish	The design of the study as a limit-test makes the interpretation of the tendencies and results difficult. The study has shown slight effects at elevated concentrations. An extrapolation to lower doses would most probably not reveal significant effects, in the given statistical design. The POEA content of the formulation was not stated.	Supporting information	UBA2
De Menezes, et al.	2011	Roundup Effects on Oxidative Stress Parameters and Recovery Pattern of *Rhamdia quelen*	Fish	There was little indication of adverse effects of the tested formulation on biomarker concentrations in different tissues of *Rhamdia quelen* at elevated concentrations compared to predicted environmental concentrations after spray application of Glyphosate. The findings support the classification of Glyphosate even in a formulation containing the potentially more toxic POEA as non to moderately toxic towards fish. Nevertheless, it cannot be distinguished between the effect of POEA and glyphosate	Supporting information	UBA2
Kreutz, L.C., et al.	2011	Altered haematological and immunological parameters in silver catfish (*Rhamdia quelen*) following short term exposure to sublethal concentration of glyphosate	Fish	The study could be taken as supplementary information that environmentally relevant concentrations of glyphosate could induce subtle changes of the haematological status of fish. It could not be assessed if those changes have the potential to affect the health status of an individual and thus to cause effects relevant for a whole population.	Supporting information	UBA2
Kreutz, L.C. et al.	2008	Acute toxicity test of agricultural pesticides on silver catfish (Rhamdia quelen) fingerlings	Fish	The LC50 for the exposure of R. quelen reported here is far below toxicities reported from other acute studies with fish under laboratory conditions. This is most probably due to the composition of the tested formulation of glyphosate (Roundup), as discussed by the authors as well. The study can be seen as additional evidence of enhanced toxicity caused by the POEA in glyphosate formulations. However, the study is not suited to trigger the aquatic risk assessment of glyphosate.	Supporting information	UBA2
Folmar, LC, et al.	1979	Toxicity of the Herbicide Glyphosate and Several of Its Formulations to Fish and Aquatic Invertebrates.	Fish	The paper is considered one of the key publications on the enhancing effect of adding tallow amine surfactants to glyphosate-based herbicides and could not be ignored even in a recent risk assessment. Since the concentration series and the spacing factors were not described appropriately, the study has formally a low reliability. Nevertheless, it could be shown that most of the toxicity of the product was due to the POEA. Nowadays, products formulated by means of POEA are not expected to be neither notified nor registered in the future. The publication supports this practice.	Supporting information	UBA2
Evrard, E et al.	2010	Impacts of mixtures of herbicides on molecular and physiological responses of the European flounder *Platichthys flesus*	Fish	Main finding: Low concentrations of glyphosate are suited to alter the gene expression patterns of the liver of European flounders. There are many uncertainties in transferring the results of this study to populations of fish, which is the relevant organisational level for an environmental risk assessment. A simple and a complex mixture has been tested so far, that causes a limited use of the results.	Supporting information	UBA2
Cavalcante, et al.	2008	Genotoxic effects of Roundup® (R) on the fish *Prochilodus lineatus*	Fish	Physiological study with the commercial formulation . No distinction between the activie substance and surfactants.	Supporting information	UBA2
Langiano, V. et al.	2008	Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus*	Fish	Prochilodus lineatus is more sensitive to Roundup® than rainbow trout (Oncorhynchus mykiss).	Supporting information	UBA2
Ferreira, D et al.	2010	Assessment of oxidative stress in *Rhamdia quelen* exposed to agrichemicals	Fish	No visible histological changes, Indication for general fitness	Supporting information	UBA2
Haller, W.T et al.	2003	Toxicity of 19 adjuvants to juvenile *Lepomis macrochirus* (bluegill sunfish)	Fish	Monsanto's MON 0818 and Entry II are 68 to 73% and.35% ethoxylated tallow amine surfactants, respectively.The material safety.data sheet for MON 0818 lists 96-h toxicity to bluegill sunfish.at 1.3 ppm, similar to the 1.6-ppm LC50 obtained in this study.	Supporting information	UBA2
Zhidenko, A.A., et al.	2007	The influence of Roundup® on the dynamics of histological changes in organs of carps	Fish	Action of Roundup® and environmentally realistic concentrations leads to alterations in organs of carp which might lead to functional changes in organ function.	Supporting information	UBA2
Ortiz-Ordoñez, E et al.	2011	Effect of Yerbimat Herbicide on Lipid Peroxidation, Catalase Activity, and Histological Damage in Gills and Liver of the Freshwater Fish Goodea Atripinnis	Fish	Biochemical damage, as evidenced by high LPX and CAT inhibition in gill tissue, was apparent following chronic Yerbimat exposure, indicative of damage due to oxidative stress, might lead to cellular damage and death.	Supporting information	UBA2
Cattaneo, R., et al.	2011	Toxicological Responses of Cyprinus carpio Exposed to a Commercial Formulation Containing Glyphosate	Fish	Short-term exposure can affect their physiological conditions, nevertheless no discrimination between glyphosate and POEA possible.	Supporting information	UBA2
Modesto, K.A., et al.	2010	Effects of Roundup® Transorb on fish: Hematology, antioxidant defenses and acetylcholinesterase activity	Fish	Hematological parameters in fish can change in response towards chemical stressors; however, these alterations are non-specific to a wide range of substances. After 24 and 96 h the antioxidant defenses were apparently enough to combat ROS, preventing the occurrence of oxidative damage. The exposure to RDT for 96 h led to an inhibition of AChE in brain and muscle but at rates which may not be considered a life-threatening situation.	Supporting information	UBA2
Evrard, E., et al.	2010	Impacts of mixtures of herbicides on molecular and physiological responses of the European flounder *Platichthys flesus*	Fish	Significant alterations of liver gene expressions were detected for contaminated vs control fishes, particularly at the levels of methionine metabolism, lipid transport and metabolism, immunity and respiratory chain.	Supporting information	UBA2
Smith, B.C., et al.	2004	Toxicity of four surfactants to juvenile rainbow trout: Implications for use over water	Fish	Surfactant pose environmetal hazard, displaying non-specific narcosis .	Supporting information	UBA2
Kreutz, L.C., et al.	2008	Acute toxicity test of agricultural pesticides on silver catfish (*Rhamdia quelen*) fingerlings	Fish	The 96-h LC50 determined for the glyphosate-based herbicide Roundup® (7.3mg/L) was much lower than that for the active substance glyphosate itself.	Supporting information	UBA2
Xie, L., et al.	2005	Evaluation of estrogenic activities of aquatic herbicides and surfactants using an rainbow trout vitellogenin assay	Fish	Glyphosate did not induce elevated levels of vitellogenin in juvenile rainbow trout compared with control fish.	Supporting information	UBA2
Le Mer, C. et	2012	Effects of chronic exposures to	Fish	Authors could not observe induced production of vitllogenin or a	Supporting	UBA2

al.		the herbicides atrazine and glyphosate to larvae of the threespine stickleback (*Gasterosteus aculeatus*)		change in sex ratio in early life stages of stickleback.	information	
Fan, J. Y et al..	2013	Herbicide Roundup® and its main constituents cause oxidative stress and inhibit acetylcholinesterase in liver of *Carassius auratus*.	Fish	The present study showed that Roundup, glyphosate and POEA may cause changes in the metabolic and enzymatic parameters of fish.	Supporting information	UBA2
Nwani, C. D et al.	2013	Induction of micronuclei and nuclear lesions in *Channa punctatus* following exposure to carbosulfan, glyphosate and atrazine.	Fish	The study, indicated that the MN and NL assays are sensitive tools for evaluating the mutagenic and genotoxic effects of carbosulfan, glyphosate and atrazine. Nevertheless study investigated the commercial products and therfore, emphasizes the importance to assess commercial products for their ability to induce genotoxicity.	Supporting information	UBA2
Richard, S., et al..	2014	Effect of a Glyphosate-Based Herbicide on Gene Expressions of the Cytokines Interleukin-1β and Interleukin-10 and of Heme Oxygenase-1 in European Sea Bass, *Dicentrarchus labrax* L.	Fish	In vitro studies could be sensitive biomarkers to evaluate the effect of exposure to pollutants chronically present in the environment. Nevertheless. study investigates a formulated commercial product, probably containing POEA.	Supporting information	UBA2
Sandrini, J. Z., et al.	2013	Effects of glyphosate on cholinesterase activity of the mussel *Perna perna* and the fish *Danio rerio* and *Jenynsia multidentata*: In vitro studies.	Fish	Glyphosate was able to inhibit cholinesterase activity in vitro in a concentration-dependent manner for all species and fractions tested. Inhibition seems to occur at relative high concentrations of glyphosate.	Supporting information	UBA2
Syedkolaei, S. J. G et al.	2013	Toxicity evaluation of Malathion, Carbaryle and Glyphosate in common carp fingerlings (*Cyprinus carpio*, Linnaeus, 1758), translation from arabic	Fish	Biochemical parameters are sensitive biomarkers, showing a dose-response relationship between Roundup concentrations and AChE activities in the different tissues in *C. carpio*.	Supporting information	UBA2
Erhunmwunse, N. O., et al.	2013	Pathological alterations in the liver of post-juvenile African Catfish *(Clarias gariepinus*) exposed to sublethal concentrations of the herbicide Glyphosate.	Fish	The observed alterations in the liver of *C. gariepinus* provide evidence to use pathological change in fish as an indicator for monitoring the effect of exposure to low levels of toxicants, which are capable of altering the physiological profile of an organism.	Supporting information	UBA2
Moreno, N. C., et al. B.	2014	Genotoxic effects of the herbicide Roundup Transorb®and its active ingredient glyphosate on the fish *Prochilodus lineatus*.	Fish	Glyphosate and RT cause damage to the DNA molecule of *P. Lineatus* at concentrations higher than those predicted in the environment. It is evident that further studies are needed to determine if the changes on biochemical traits are translated to sublethal long-term effects.	Supporting information	UBA2
Uren Webster, T. M et al.	2013	Effects of glyphosate and its formulation, Roundup®, on reproduction in zebrafish (*Danio rerio*).	Fish	Results demonstrate that the tested chemicals cause reproductive toxicity in zebrafish, although only at concentrations unlikely to occur in the environment.	Supporting information	UBA2
Gholami-Seyedkolaei, S. J., et al.	2013	Effect of a glyphosate-based herbicide in *Cyprinus carpio*: Assessment of acetylcholinesterase activity, hematological responses and serum biochemical parameters	Fish	Biochemical parameters are sensitive biomarkers, showing a dose-response relationship between Roundup concentrations and AChE activities in the different tissues in *C. carpio*.	Supporting information	UBA2
Gholami-Seyedkolaei, S. J. et al.	2013	Optimization of recovery patterns in common carp exposed to roundup using response surface methodology: Evaluation of neurotoxicity and genotoxicity effects and biochemical parameters	Fish	Recovery trend of fishes exposed to Roundups. It is evident that further studies are needed to determine if the changes on biochemical traits are translated to sublethal long-term effects.	Low weight	UBA3
De Souza Filho, J., et al.	2013	Mutagenicity and Genotoxicity in Gill Erythrocyte Cells of *Poecilia reticulata* Exposed to a Glyphosate Formulation.	Fish	The commercial formulation containg POEA shows a gradual increase in the number of damaged cells, indicating a concentration-dependent effect and that this herbicide was mutagenic and genotoxic to *P. reticulata.*	Supporting information	UBA2
Akinsorotan, A., et al.	2013	Acute Toxicity And Behavioural Changes On African Catfish (*Clarias gariepinus)* Exposed To Dizensate (Glyphosate Herbicide).	Fish	The study was carried out to determine the toxicity of dizensate herbicide to the most cultivable fish species in Nigeria. The four days median lethal concentration (96h LC50) was determined as 43.65 mg/l.	Supporting information	UBA2
de Castilhos Ghisi, N., et al.	2012	Genotoxic effects of the herbicide Roundup® in the fish *Corydoras paleatus* (Jenyns 1842) after short-term, environmentally low concentration exposure.	Fish	Authors conclude that short-term exposure of the pesticide Roundup® is genotoxic to the bioindicator fish species *C. paleatus*, even at a relatively low concentration. However, this low concentration tested did not result in clastogenic and/or aneugenic effects. Moreover, the European Community limit (i.e., 0.10 µg/L of pesticide for surface waters) is below the concentration that was tested in this experiment	Supporting information	UBA2
Vera-Candioti, J., et al.	2013	Evaluation of the genotoxic and cytotoxic effects of glyphosate-based herbicides in the ten spotted live-bearer fish *Cnesterodon decemmaculatus* (Jenyns, 1842).	Fish	In terms of cytotoxic effects neither Panzer nor Credit were capable to induce alterations in erythrocyte:erythroblast ratios, at least within the concentration ranges used.	Supporting information	UBA2
Guilherme, S., et al.	2012	Differential genotoxicity of Roundup® formulation and its constituents in blood cells of fish (*Anguilla anguilla*): considerations on chemical interactions and DNA damaging mechanisms.	Fish	The analysis of oxidatively induced breaks suggested that oxidation of DNA bases was not a dominant mechanism of damage.	Supporting information	UBA2
Guilherme, S. et al.	2012	DNA damage in fish (*Anguilla anguilla*) exposed to a glyphosate-based herbicide - Elucidation of organ-specificity and the role of oxidative stress	Fish	The analysis of oxidatively induced breaks suggested that oxidation of DNA bases was not a dominant mechanism of damage..	Supporting information	UBA2
Guilherme, S., et al.	2014	DNA and chromosomal damage induced in fish (*Anguilla anguilla* L.) by aminomethylphosphonic acid (AMPA)—the major environmental breakdown	Fish	Effects on biochemical responses like changes on chromosomal damage are currently considered as supporting information on commercial products in the field of environmental risk assessment. Nevertheless, it is evident that further studies are needed to determine if the changes on biochemical traits are translated to sublethal long-	Supporting information	UBA2

		product of glyphosate.		term effects.		
Guilherme, S. et al.	2014	Are DNA-damaging effects induced by herbicide formulations (Roundup® and Garlon®) in fish transient and reversible uponcessation of exposure?	Fish	Roundup®: a recovery was observed when considering non-specific DNA damage onday 14 post-exposure. Effects on biochemical responses like changes on chromosomal damage are currently considered as supporting information on commercial products in the field of environmental risk assessment. Nevertheless, it is evident that further studies are needed to determine if the changes on biochemical traits are translated to sublethal long-term effects.	Supporting information	UBA2
Armiliato, N., Ammar, et al..	2014	Changes in Ultrastructure and Expression of Steroidogenic Factor-1 in Ovaries of Zebrafish *Danio rerio* Exposed to Glyphosate.	Fish	No assessment possible due to lack of information regarding the formulation tested.	Low weight	UAB3
Glusczak, L., Loro, et al..	2011	Acute exposure to glyphosate herbicide affects oxidative parameters in Piava (*Leporinus obtusidens*).	Fish	High concentrations of glyphosate-based herbicide used in agricultural fields can cause changes in oxidative stress parameters in Piava.	Supporting information	UBA2
Penders, E.J.M., et al.	2012	Genotoxic Effects in the Eastern Mudminnow (*Umbra pygmaea*) After Prolonged Exposure to River Rhine water, as Assessed by Use of the In Vivo SCE	Fish	The SCE assay points at increased DNA damage upon exposure of the fish to River Rhine water. The data corroborate that in vivo genotoxicity of River Rhine water is at present lower than in 1978. The paper deals with the River Rhine water impact of DNA damage . The test substances River Rhine was described in detail as a mixture of pollutants, but can not consultated for the risk assessment of the active ingredient'glyphosate'.	Low weight	UBA3
Fuzinatto, C.F., et al.	2013	Induction of micronucleus of Oreochromis niloticus exposed to waters from the Cubatão do Sul River, southern Brazil	Fish	Pollution of aquatic environments, especially surface water from rivers that have potentially genotoxic substances, is a critical problem. The paper deals with the genotoxic otetial of River water. The test substances displays a mixture of pollutants, but can not consultated for the risk assessment of the active ingreient'glyphosate'itself.	Low weight	UBA3
El-Shebly, A.A., et al.	2008	Effects of Glyphosate Herbicide on Serum Growth Hormone (GH) Levels and Muscle Protein Content in Nile Tilapia (*Oreochromis Niloticus* L.)	Fish	Authors observed that the Roundup even used at low-concentrations affected the growth of *O niloticus*.	Low weight	UBA3
Kaya, I. et al.	2012	Investigation of effects on plasma nitric oxide, malondialdehyde and total sialic acid levels of glyphosate in Kars Creek transcaucasian barb (*Capoeta capoeta* [guldenstaedt, 1773]) in Turkey.	Fish	Korfosat 48 SL, a commercial formualtion elevated biochemical parameters related to lipid peroxidation.	Low weight	UBA3
Shiogiri, N.S. et al.	2012	Acute exposure of a glyphosate-based herbicide affects the gills and liver of the Neotropical fish, *Piaractus mesopotamicus*	Fish	The overall toxicity of commercial formulations of glyphosate partly depends on the toxicity of its associated compounds, results provide evidence for the moderate toxicity of glyphosate in the RR formulation, but severe level of damage in the liver of fish.	Low weight	UBA3
Uchida, M. et al.	2012	Toxicity evaluation of glyphosate agrochemical components using Japanese medaka (*Oryzias Jatipes*) and DNA microarray gene expression analysis	Fish	No statistically significant gene expression change was detected in adult medaka liver tissues that were exposed to glyphosate alone, showing that the surfactants in formulations play a significant role.	Supporting information	UBA2
Bayir, M. et al.	2013	Alterations in Fatty Acids of Polar Lipids in Salmo trutta on Long-term Exposure to a Glyphosate-Based Herbicide (Roundup®)	Fish	Roundup effects fatty acid metabolism at sublethal concentrations.	Low weight	UBA3
Harayashiki, C.A.Y. et al.	2013	Toxic effects of the herbicide Roundup in the guppy *Poecilia vivipara* acclimated to fresh water	Fish	Exposure to Roundup did not alter the biochemical parameters analyzed, though differences between genders were observed and deserve further investigations. Findings from the present study suggest that exposure to environmentally relevant concentrations of Roundup may negatively affect at long-term the reproduction of *P. vivipara*, with consequent changes in fish populations inhabiting environments contaminated with the herbicide.	Supporting information	UBA2
Nwani, C.D. et al.	2013	Investigation on Acute Toxicity and Behavioral Changes in *Taliapia zillii* due to glyphosate-based herbicide, ForceUp	Fish	The commercial formualtion shows relatively low toxcity compared to other formulations.	Supporting information	UBA2
Okonkwo, F.O. et al.	2013	Toxicological Studies on the Short Term Exposure of *Clarias albopunctatus* (Lamonte and Nichole 1927) to Sub-lethal Concentrations of Roundup	Fish	Short term exposure of *Clarias albupunctatus* to sub-lethal concentrations of Roundup was found to be toxic to the fish.	Supporting information	UBA2
Yusof, S. et al.	2008	Effect of glyphosate-based herbicide on early life stages of Java medaka (*Oryzias javanicus*): A potential tropical test fish	Fish	Results show that high concentrations of Roundup and glyphosate induce developmental toxicity in *Java medaka*.	Supporting information	UBA2
Marques, A. et al.	2014	Progression of DNA damage induced by a glyphosate-based herbicide in fish (*Anguilla anguilla*) upon exposure and post-exposure periods Insights into the mechanisms of genotoxicity and DNA repair	Fish	Oxidative damage was implicit as an important mechanism of genetic damage, which showed to be transient, since DNA integrity returned to the control levels on the first day after cessation of exposure.	Low weight	UBA3
Prévot d'Alvise, N. et al.	2013	Acute toxicity of a commercial glyphosate formulation on European sea bass juveniles (*Dicentrarchus labrax* l.): gene expressions of heme oxygenase-1 (ho-1), acetylcholinesterase (ache) and aromatases (cyp19a and cyp19b)	Fish	Results showed complex tissue-specific transcriptional responses after 96h of exposure to a sublethal concentration.	Supporting information	UBA2
Schneider, S, et al.	2013	Glyphosate: Fish Short-Term Reproduction Assay (FSTRA) with the Fathead Minnow (*Pimephales promelas*).	Fish	There were no apparent effects on survival, growth, reproduction, secondary sex characteristics, GSI, VTG or gonad histopathology in male or female fish exposed to glyphosate for 21 days. Based on the endpoints evaluated, glyphosate does not appear to impact the function of the hypothalamuspituitary- gonadal (HPG) endocrine axis in fathead minnows.	Supporting information	UBA2

Dutra, B.K., et al.	2011	Effect of Roundup®(R) (glyphosate formulation) in the energy metabolism and reproductive traits of *Hyalella castroi* (Crustacea, Amphipoda, Dogielinotidae)	Invertebrates	Pyhsiological study, including survival EC50 approx. at 2.16 mg/l of glyphosate.	Supporting information	UBA2
Achiorno, C.L., et al.	2008	Toxicity of the herbicide glyphosate to *Chordodes nobilii* (Gordiida, Nematomorpha)	Invertebrates	The minimum concentration tested(0.1 mg a.e./L) decreased larval infectivity. Thisvalue is below the guidance level for glyphosate in freshwatersystems (0.24 mg/ l), established to protect the aquatic biota in Argentina.	Supporting information	UBA2
Brausch, J.M., et al.	2007	Toxicity of three polyethoxylated tallowamine surfactant formulations to laboratory and field collected fairy shrimp, *Thamnocephalus platyurus*	Invertebrates	POEA was very toxic to *T. platyurus* with average 48-h LC50s of 2.01, 2.70, and 5.17 µg/L for POEA surfactants having an oxide:tallowamine ratio of 15:1, 10:1, and 5:1,respectively.	Supporting information	UBA2
Brausch, J.M., et al.	2007	Acute and Sub-Lethal Toxicity of Three POEA Surfactant Formulations to *Daphnia magna.*	Invertebrates	POEA was very toxic to *Daphnia magna* POEA 15:1= 0.85 mg/L, POEA 10:1=0.097 mg/L POEA 5:1= 0.18mg/L	Supporting information	UBA2
Iummato, M.M. et al.	2013	Evaluation of biochemical markers in the golden mussel *Limnoperna fortune*i exposed to glyphosate acid in outdoor microcosms	Invertebrates	The increase in lipid peroxidation levels and the decrease in SODand CES activities observed in *L. fortunei* indicate that glyphosate had adverse effects on the metabolism the bivalve.	Supporting information	UBA2
Le, T.H., et al.	2010	Effects of glyphosate and methidathion on the expression of the Dhb, Vtg, Arnt, CYP4 and CYP314 in *Daphnia magna*	Invertebrates	Tested substance not specified	Low weight	UBA3
Magbanua, F.S., et al.	2013	Understanding the combined influence of fine sediment and glyphosate herbicide on stream periphyton communities	Invertebrates	Population, community and ecosystem variables responded differently to multiple stressors, highlighting the value of combining structural, functional taxonomic and trait data to assess the effects of anthropogenic stressors.	Supporting information	UBA2
Bringolf, R.B., et al.	2007	Acute and chronic toxicity of glyphosate compounds to glochidia and juveniles of *Lampsilis siliquoidea* (Unionidae)	Invertebrates	MON 0818 was most toxic of the compounds tested and the 48-h median effective concentration (0.5 mg/L) for *L. siliquoidea* EC50 values are taken into account.	Critical	UBA1
Tsui, M.T.K., et al.	2004	Comparative toxicity of glyphosate-based herbicides: aqueous and sediment porewater exposures	Invertebrates	EC50 values are taken into account. Please refer to Summary of relevant literature, Chapter 9.13.9.6	Supporting information	UBA2
Chen, C.Y., et al.	2004	Multiple stress effects of Vision (R) herbicide, pH, and food on zooplankton and larval amphibian species from forest wetlands	Invertebrates	Vision®Commercial formulation containing POEA. Commercial formulation. The conclusion from this study is only valid for glyphosate formulations that contain POEA. EC50 values are taken into account. Please refer to Summary of relevant literature, Chapter 9.13.9.6	Supporting information	UBA2
Mensah, P.K., et al.	2011	Acute toxicity of Roundup® herbicide to three life stages of the freshwater shrimp *Caridina nilotica* (Decapoda: Atyidae)	Invertebrates	The neonates of *C. nilotica* were found to be most sensitive with a mean 96h $LC_{50}$of 2.5 mg/L.	Supporting information	UBA2
Zeynep et al.	2009	Acute Toxicity of the Herbicide Glyphosate on *Daphniamagna**	Invertebrates	EC50 values taken in to account for the formulation. Please refer to Summary of relevant literature, Chapter 9.13.9.6	Supporting information	UBA2
Conners, D.E., et al.	2004	Evaluation of lethality and genotoxicity in the freshwater mussel *Utterbackia imbecillis* (Bivalvia : Unionidae) exposed singly and in combination to chemicals used in lawn care	Invertebrates	LC50 taken into account. Please refer to Summary of relevant literature, Chapter	Supporting information	UBA2
Frontera, J.L., et al.	2011	Effects of Glyphosate and Polyoxyethylenamine on Growth and Energetic Reserves in the Freshwater Crayfish *Cherax quadricarinatus* (Decapoda, Parastacidae)	Invertebrates	Physiollogical traits are affected, which might affect fitness. The decrease ofboth glycogen and lipid reserves, as observed in the mixture,could have lead to lower protein levels and decreasedsomatic growth in juvenile crayfish *C. quadricarinatus.*	Supporting information	UBA2
Mottiera, Bouchartb, et al.	2013	Effects pof glyphosate –based herbicides on embryo-larval development and metamorphosis in the Pacific oyster, *Crassostrea gigas*	Invertebrates	Predicted environmental concentrations might be lower (for one indication per area).	Supporting information	UBA2
Dominguez-Cortinas, G., et al.	2008	Analysis of the toxicity of glyphosate and Faena® using the freshwater invertebrates *Daphnia magna* and *Lecane quadridentata*	Invertebrates	The LC_{50} values show that this freshwater rotifer is 11-foldmore susceptible to the commercial formulation (Faena) than to the active ingredient (glyphosate). This effect might be due to the synergistic activity of other components of the industrial formulation that increase the toxicity of the compound. *Daphnia magna* is almost 20-fold moresusceptible to Faena than to glyphosate. LC_{50} taken into account. EC50 (esterases activity)of glyphosate is 1500-fold smaller than the LC50.	Supporting information	UBA2
Demetrio, P.M., et al.	2012	Effects of Pesticide Formulations and Active Ingredients on the Coelenterate *Hydra attenuata* (Pallas, 1766)	Invertebrates	With glyphosate, higher and significant effects were detected for the formulation at lower concentrations,with a reversal of the behavior at higher concentrations.	Supporting information	UBA2
Melnichuk, S.D., et al.	2007	Effects of Fakel herbicide on vital activity of *Ceriodaphnia affinis* in acute and chronic experiments	Invertebrates	Fakel herbicide exerted the greatest inhibitory influence on number of young per broodat concentration of 10 mg/dm3.	Supporting information	UBA2
Akcha, F., et al.	2012	Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos	Invertebrates	Out of the three bioassays conducted at the laboratory, only one assay revealed glyphosate to have an embryotoxic effect at concentrations of 2.5 µg L−1 ($p < 0.001$) upwards. Taking into account the data from the three bioassays, the main effects ANOVA revealed significant differences between the assays ($p < 0.001$), with assay 2 differing from assays 1 and 3. The potential embryotoxicity of glyphosate was studiesd but the study tested a highest concentration of 5.0 µg L did not reslut in a EC50values. Therefore, the estimated EC50 for one bioassay resulted in EC50 a.s > 5µg/L.	Supporting information	UBA2
Cuhra, M., et al.	2013	Clone- and age-dependent toxicity of a glyphosate	Invertebrates	Based on conservative modelling assumptions, the worst-case concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L. Putting these results in context results in a margin of	Critical	BA1

		commercial formulation and its active ingredient in *Daphnia magna*.		safety of around 5. No significant effects on fecundity or abortion rates were seen at concentrations 0.05–0.45 mg/l for glyphosate IPA.		
Avigliano, L., et al.	2014	Effects of Glyphosate on Growth Rate, Metabolic Rate and Energy Reserves of Early Juvenile Crayfish, *Cherax quadricarinatus M.*	Invertebrates	Glyphosate is able to reduce growth rates and protein and lipid.reserves in chronically exposed early juvenile crayfish at concentrations of at 40 mg/L. Based on conservative .modelling assumptions, the worst-case concentrations expected in surface waters exposed to drift lie around 0.1 mg a.i./L.	Supporting information	UBA2
Mona, M. H., et al.	2013	Evaluation of cytotoxic effects of atrazine and glyphosate herbicides on *Biomphalaria glabrata* snails.	Invertebrates	Biochemical parameter were analysed, no lethal or reproductive endpoint, Non-standard test system, no dose response.	low weight	UBA3
dos Santos, K. C et al.	2014	Genotoxic and biochemical effects of atrazine and Roundup®, alone and in combination, on the Asian clam *Corbicula fluminea*.	Invertebrates	*C. fluminea* revealed to be a good biomonitor of environments contaminated by the herbicides ATZ and RD; EROD and LPO appeared as potential biomarkers. Mixture effects are complex and variable, either intensifying or mitigating the effects promoted by compounds.	Supporting information	UBA2
Janssens, L., & et al.	2013	Synergistic effects between pesticide stress and predator cues: Conflicting results from life history and physiology in the damselfly *Enallagma cyathigerum*.	Invertebrates	Synergistic interactions were not present for growth rate and food intake, they were identified for antioxidant defence and oxidative damage. Effects on biochemical responses are currently considered as supporting information on commercial products in the field of environmental risk assessment. Nevertheless, it is evident that further studies are needed to determine if the changes on biochemical traits are translated to sublethal long-term effects.	Supporting information	UBA2
Kumar, A., et al.	2013	Toxic impacts of two organophosphorus pesticides on the acetylcholinesterase activity and biochemical composition of freshwater fairy shrimp *streptocephalus dichotomus*.	Invertebrates	Since the test compound was not described in detail and further effects in the same range were also observed in the control treatments the study has limited reliability.	low weight	UBA3
Omran, N.E., et al.	2013	The endocrine disruptor effect of the herbicides atrazine and glyphosate on *Biomphalaria alexandrina* snails	Invertebrates	The data obtained indicated that sublethal doses (LC10) oft he commercial formulation Herfosate for 3 weeks effects the endocrine systems of *B. alexandrina* snails.	Supporting information	UBA2
Avigliano, L. et al.	2014	Effects of glyphosate on egg incubation, larvae hatching, and ovarian rematuration in the estuarine crab *Neohelice granulata*	Invertebrates	A significantly lower number of hatched larvae per female was detected in the RoundupUltramax treatment, a clear embryonic mortality was associated with this formulation, which contained a glyphosate concentration of 2.5 mg/L. Also taking into account that pure glyphosate, at the same concentration, did not significantly reduce the number of hatched larvae, these results indicate that Roundup compounds other than glyphosate may be responsible for the embryonic mortality.	Supporting information	UBA2
F. Geret,T. et al.	2011	Effects of Low-Dose Exposure to Pesticide Mixture on Physiological Responses of the Pacific Oyster, *Crassostrea gigas*	Invertebrates	Low doses and the mixture of pesticides effect the metabolic responses under controlled conditions. Effects on enzyme performances are currently considered as supporting information on commercial products in the field of environmental risk assessment. Nevertheless, it is evident that further studies are needed to determine if the changes on biochemical traits are translated to sublethal long-term effects.	low weight	UBA3
Boonsoong & Buliangpoti	2012	Acute toxicity of Roundup® and carbosulfan to the Thai fairy shrimp, *Branchinella thailandensis*	Invertebrates	The commercial formulation Roundup shows a relatively high toxicity (LC50: 0.319 ppm) towards the *Branchinella thailandensis*.	low weight	UBA3
Ray, P et al.	2008	Deleterious effect of herbicides on waterhyacinth biocontrol agents *Neochetina bruchi* and *Alternaria alternata*	Algae/ aquatic plants	Waterhyacinth considered as target species	low weight	UBA3
Romero, D.M et al.	2011	Oxidative stress induced by a commercial glyphosate formulation in a tolerant strain of *Chlorella kessleri*	Algae/ aquatic plants	The EC50-96h obtained for *C. kessleri* was higher than those used in risk assessment.	Supporting information	UBA2
Debenest, T., et al.	2010	Effects of Pesticides on Freshwater Diatoms	Algae/ aquatic plants	No details about Glyphosate in particular.	low weight	UBA3
Inderjit, I et al.	2010	Effect of herbicides with different modes of action on physiological and cellular traits of *Anabaena fertilissima*	Algae/ aquatic plants	Comparative study designed to examine toxicity of propanil, pretilchlor and glyphosate on physiological and cellular characteristics of *A. fertilissima*.	low weight	UBA3
Romero, D.M et al.	2011	Oxidative stress induced by a commercial glyphosate formulation in a tolerant strain of *Chlorella kessleri*	Algae/ aquatic plants	The freshwater species *Chlorella* in Europe according to Algae Base((http://www.algaebase.org/search/species/detail/?species_id=40443) species distribution in South America, Asia, in Europe in Romania and Spain. Tolerant strain used .	low weight	UBA3
Ma, J., Xu, L et al., Y.	2002	Toxicity of 40 herbicides to the green alga *Chlorella vulgaris*	Algae/ aquatic plants	Presented EC_{50} values will be taken into account.	Supporting information	UBA2
Ma, J., Wang, S., et al.	2006	Toxicity assessment of 40 herbicides to the green alga *Raphidocelis subcapitata*	Algae/ aquatic plants	Presented EC50 values will be taken into account. Freshwater species , taxonomic synonym *Pseudokirchneriella subcapitata*	Supporting information	UBA2
Ma, J. et al.	2002	Differential sensitivity to 30 herbicides among populations of two green algae *Scenedesmus obliquus* and *Chlorella pyrenoidosa*	Algae/ aquatic plants	Presented EC50 values will be taken into account. EC_{50}= 56 mg/L	Supporting information	UBA2
Ma, J., et al.	2006	Toxicity of 21 herbicides to the green alga *Scenedesmus quadricauda*	Algae/ aquatic plants	Presented EC50 values will be taken into account. EC_{50}= 70.5 mg/L	Supporting information	UBA2
Ma. J., et al.	2001	Acute toxicity of 33 herbicides to the green alga *Chlorella pyrenoidosa*	Algae/ aquatic plants	Presented EC50 values will be taken into account. EC_{50}= 3.5 mg/L	Supporting information	UBA2
Tsui, M.T.K., Chu, L.M.	2003	Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors	Algae/ aquatic plants	Presented EC50 values will be taken into account.	Supporting information	UBA2
Perez, G.L.,	2011	Effects of Herbicide Glyphosate	Algae/	Review chapter in book.	Supporting	UBA2

et al.		and Glyphosate-Based Formulations on Aquatic Ecosystems	aquatic plants		information	
Cedergreen, N., Streibig, J.C.	2005	The toxicity of herbicides to non-target aquatic plants and algae: assessment of predictive factors and hazard	Algae/ aquatic plants	Presented EC50 values will be taken into account.	Supporting information	UBA2
Turgut, C., Fomin, A.	2002	Sensitivity of the rooted macrophyte *Myriophyllum aquaticum* (Vell.) Verdcourt to seventeen pesticides determined on the basis of EC_{50}	Algae/ aquatic plants	Presented EC50 values will be taken into account. Pigment content was more senstive endpoint than other parameters. The test item is not characterized in the study. In addition, the endpoint of 0.22 mg/L is based on chlorophyll a content, which is not a standard endpoint for aquatic plant risk assessment.	Supporting information	UBA2
Sobrero, M.C., et al.	2007	Effects of the glyphosate active ingredient and a formulation on *Lemna gibba* L. at different exposure levels and assessment end-points	Algae/ aquatic plants	Presented EC50 values will be taken into account	Supporting information	UBA2
Sura, S. et al.	2012	Effects of glyphosate and two herbicide mixtures on microbial communities in prairie wetland	Algae/ aquatic plants	The results from this study indicate that glyphosate at 225 µg/ Lcan have an effect on the productivity of aquatic microbial communities over relatively short time periods. Differences in the toxicity of co-formulants preclude making general conclusions across all formulations of glyphosate herbicides	Supporting information	UBA2
Annett, R et al.	2014	Impact of glyphosate and glyphosate-based herbicides on the freshwater environment	Algae/ aquatic plants	Comprehensive review article.	Supporting information	UBA2
Vera, M.S., et al.	2014	Comparative Effects of Technical-Grade and a Commercial Formulation of Glyphosate on the Pigment Content of Periphytic Algae	Algae/ aquatic plants	Results showthat both glyphosate and the commercial formulation Glifosato Atanor stimulate the growth of the auto- trophic fraction in the periphyton. Chlorophyll content is not a standard endpoint for aquatic plant risk assessment.	low weight	UBA3
Issa, A.A.E. et al.	2012	Alterations in some metabolic activities of *Scenedesmus quadricauda* and *Merismopedia glauca* in response to glyphosate herbicide	Algae/ aquatic plants	Glyphosate herbicide inhibited the growth and some metabolic activities in the tested algae, this effect depend on the algal species, the type and concentration of the herbicide.	low weight	UBA3
Qiu, H. et al.	2012	Physiological and biochemical responses of *Microcystis aeruginosa* to glyphosate and its Roundup® formulation	Algae/ aquatic plants	*M. aeruginosa* could utilize glyphosate as a sole P-resource, and glyphosate had a positive dosage-response on the cell number and Chl-a content of M. aeruginosa from 0 to 5 mg/L. Roundup® showed a hormesis effect on *M. aeruginosa* growth.	low weight	UBA3
Sun, K. et al.	2012	Ecological risks assessment of organophosphorus pesticides on bloom of *Microcystis wesenbergii*	Algae/ aquatic plants	Fluorescence of chlorophyll a and the photochemical efficiency were enhanced in the range of natural concentrations. Stimulatingeffect varied among tested parameters; hormetic enhancement on physiological processes might be a factor to induce a water bloom of *M. wesenbergii.*	low weight	UBA3
Contardo-Jara, V., et al.	2009	Bioaccumulation of glyphosate and its formulation Roundup Ultra in Lumbriculus variegatus and its effects on biotransfor-mation and antioxidant enzymes	Sediment-	The bioaccumulation factor (BCF) varied between 1.4 and 5.9 for the different concentrations, and was higher than estimated from log Pow. The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.	Supporting information	UBA2
Baker, L.F. et al.	2014	The direct and indirect effects of a glyphosate-based herbicide and nutrients on Chironomidae (Diptera) emerging from small wetlands	Sediment-	Although direct toxicity of Roundup WeatherMax was not apparent, the authors observed longer-term impacts, suggesting that the indirect effects of this herbicide deserve more consideration when assessing the ecological risk of using herbicides in proximity to wetlands.	Supporting information	UBA2
Vera, M.S., et al.	2010	New evidences of Roundup® (glyphosate formulation) impact on the periphyton community and the water quality of freshwa-ter ecosystems	Microcosm	Changes in community structure: Roundup®produced a clear delay in periphytic colonization in treated mesocosms and values of the periphytic mass variables (dry weight, ash-free dry weight and chlorophyll a) were always higher in control mesocosms. Despite the mortality of algae, mainly diatoms, cyanobacteria was favored in treated mesocosms. It was observed that glyphosate produced a long term shift in the typology of mesocosms, ''clear'' turning to ''turbid'', which is consistent with the regional trend in shallow lakes in the Pampa plain of Argentina.	Supporting information	UBA2
Perez, G.L., et al.	2007	Effects of the herbicide Round-up® on freshwater microbial communities: a mesocosm study	Microcosm	Presented EC50 values will be taken into account. 6mg/l elicitated a change in community structure.	Supporting information	UBA2
Marı´a Solange Vera et al.	2012	Direct and indirect effects of the glyphosate formulation Glifosato Atanor on freshwater microbial communities	Microcosm	A single application of Glifosato Atanor caused a fast stimulation of bacterioplankton and planktonic picocyanobacteria as well as of chlorophyll a concentration in the water column, accelerating the eutrophication of the system.	Low weight	UBA3
Howe, et al..	2004	Toxicity of glyphosate-based pesticides to four North American frog species	Amphibians	Important data are presented that prove the high toxic potential of polyethoxylated tallow amines to different species of amphibians. The POEA treatment showed the highest toxicity in the acute tests. Dependent on the formulation that will be assessed by an ERA, the enhanced toxicity by surfactant additives should be considered and could be referenced to this well conducted and informative publication. Can be used for aquatic assessments. The chronic studies have several weaknesses (statistics, control survival rates, dose-response-relationship). Nevertheless, the results indicate several open questions in the assessment of chronic exposure of amphibian to formulated glyphosate products. None of the effects was observed in the treatments with glyphosate alone	Critical	UBA1 , also for asses smen t of surfa ctand effect s (POE A
Thompson, D.G., et al.	2004	Chemical and biomonitoring to assess potential acute effects of Vision (R) herbicide on native amphibian larvae in forest wetlands	Amphibians	No data available on the variance of larval response allocated to site/year/block factors. Pooling of results on biological and chemical responses over sites and years may lead to misleading interpretation of results. Effects of glyphosate application at site level with direct comparison of sprayed/not sprayed wetland not reported.	Supporting information	UBA2
Edge, C.B et al.	2011	Exposure of juvenile green frogs (*Lithobates clamitans*) in littoral enclosures to a glyphosate-based herbicide	Amphibians	Coformulants not known. Glyphosate was not tested alone. Product was applied to soil and water. Exposure pattern not clear.	Low weight	UBA3

Wojtaszek, B.F., et al.	2004	Effects of Vision (R) herbicide on mortality, avoidance response, and growth of amphibian larvae in two forest wetlands	Amphibians	The study presents acute mortalities for two amphibian species. The data can be employed in the assessment of formulations containing POEA as surfactants. No data for glyphosate technical or glyphosate acid deducible from the tested formulation. Lead formulation for EU renewal of glyphosate approval contains no POEA	Critical	UBA1 for assessment of surfactand effects (POEA)
Brodman, R., et al.	2010	Interaction of an Aquatic Herbicide and Predatory Salamander Density on Wetland Communities	Amphibians	Formulated product not relevant for current assessment (glyphosae + NPE). Glyphosate was not tested per se.	Low weight	UBA3
Cauble, K., Wagner, R.S.	2005	Sublethal effects of the herbicide glyphosate on amphibian metamorphosis and development	Amphibians	Unfortunately, the precise formulation identification is not possible. Moreover, experimental details are missing (e.g. mortalities in controls). Supporting for fromulations containing POEA (LC50 same range as other publications with Glyphosate+POEA formulations).	Supporting information	UBA2 for assessment of surfactand effects (POEA)
Dinehart, S.K et al.	2010	Acute and chronic toxicity of Roundup WeatherMAX® and Ignite® 280 SL to larval *Spea multiplicata* and *S. bombifrons* from the Southern High Plains, USA	Amphibians	The study presents slightly differing acute mortalities between the species and land use type, which can be assessed togheter with other glyphosate formulations containing surfactants of relevant toxicity like the one tested in this study. No clear interaction between the species, origin from crop- or grassland and treatments on survival could be shown. The chronic data was insufficiently analysed, or it was ambiguously described.	Supporting	UBA1 for assessment of surfactand effects (POEA)
Edginton, A.N., et al.	2004	Comparative effects of pH and Vision (R) herbicide on two life stages of four anuran amphibian species	Amphibians	Results Vision® can be used for the assessment of glyphosate formulations with POEA	Supporting	UBA1 for assessment of surfactand effects (POEA)
Jayawardena UA, Navaratne AN, et al.	2011	Acute and chronic toxicity of four commonly used agricultural pesticides on the Asian common toad, *Bufo melanostictus* Schneider.	Amphibians	Many details of the experimental procedures, the use of statistics and the identity of the tested substances are lacking. The study is therefore considered not applicable for a specific use in ERA.	Low weight	UBA3
Jones, D.K., et al..	2010	Roundup® and amphibians: The importance of concentration, application time, and stratification	Amphibians	Formulation ingredient undisclosed. No separate test of glyphosate as acid or salt.	Supporting information	UBA2
Jones, D.K., et al.	2011	Competitive stress can make the herbicide Roundup® more deadly to larval amphibians	Amphibians	Formulation ingredient undisclosed. No separate test of glyphosate as acid or salt.	Supporting information	UBA2
Lajmanovich, R.C. et al.	2003	Induction of mortality and malformation in Scinax nasicus tadpoles exposed to glyphosate formulations	Amphibians	The study confirms the relatively high toxicity of glyphosate preparations possibly mediated by POEA surfactants.	Supporting	UBA1 for assessment of surfactand effects (POEA)
Lajmanovich, R.C., et al.	2011	Toxicity of Four Herbicide Formulations with Glyphosate on *Rhinella arenarum* (Anura: Bufonidae) Tadpoles: B-esterases and Glutathione S-transferase Inhibitors	Amphibians	Acute endpoints reliable, reporting of experimental details not exaustive, formulations partly unknown	Supporting	UBA1 for assessment of surfactand effects (POEA)
Relyea R.A.	2005	The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities	Amphibians	The study confirms the relatively high toxicity of glyphosate preparations possibly mediated by POEA surfactants. Not relevant for the risk assessment of glyphosate due to weakness in methodological accuracy.	Supporting	UBA2
Relyea R.A.	2005	The lethal impact of roundup on aquatic and terrestrial amphibians	Amphibians	The study confirms the relatively high toxicity of glyphosate preparations possibly mediated by POEA surfactants.	Supporting	UBA2
Relyea R.A.	2012	New effects of Roundup on amphibians: Predators reduce herbicide mortality; herbicides induce antipredator morphology.	Amphibians	The study confirms the relatively high toxicity of glyphosate preparations mediated by surfactants.	Supporting	UBA2
Lajmanovich, R.C., et al.	2010	Activity levels of B-esterases in the tadpoles of 11 species of frogs in the middle Paraná River	Amphibians	The study is not relevant for ERA of glyphosate because the substance was not applied nor monitored explicitly. Most likely, the notifier assigned erroneously a high relevance to the study.	Low weight	UBA3

		floodplain: Implication for ecological risk assessment of soybean crops				
McDaniel, T.V., et al.	2008	Potential endocrine disruption of sexual development in free ranging male northern leopard frogs (*Rana pipiens*) and green frogs (*Rana clamitans*) from areas of intensive row crop agriculture	Amphibians	Study is a field survey on the impact of an exposure to the sum of possible substances employed in agricultural management on amphibian biomarkers and development. The investigation of the effects of single substances was not part of the study. The impact of glyphosate cannot be determined.	Low weight	UBA3
Wagner, N., et al.	2013	Questions concerning the potential impact of glyphosate-based herbicides on amphibians.	Amphibians	Maximum Predicted Environmental Concentrations PEC in european surface waters (sw) are predicted excluding the direct application to the water bodies, since this use is not authorized in Europe Please refer to Vol. 3 B 8.6.2 for calualted PECsw values. Most conservative avalable FOCUS Step 1 PECsw value is 104.81µg glyphosate/L. Results support evaluation of published literature by RMS	Critical	UBA 1/ UBA 2
Dornelles, M. F., et al.	2013	Effect of Atrazine, Glyphosate and Quinclorac on Biochemical Parameters, Lipid Peroxidation and Survival in Bullfrog Tadpoles (*Lithobates catesbeianus*).	Amphibians	Supporting information on biochemical response of tadpoles to environmental relevant concentrations of commercial formulation containing glyphosate and POEA.	supporting	UBA 2 also for assessment of surfactand effects (POEA
Moore LJ, et al.	2012	Relative toxicity of the components of the original formulation of Roundup to five North American anurans.	Amphibians	The results fully supports previous RMS evaluation. Surfactant MON 0818 containing POEA drives the toxicity of the herbicide formulations containign glyphosatge for amphibians.	Critical	UBA 1
Lajmanovich, R. C. et al.	2013	Individual and Mixture Toxicity of Commercial Formulations Containing Glyphosate, Metsulfuron-Methyl, Bispyribac-Sodium, and Picloram on *Rhinella arenarum* Tadpoles.	Amphibians	Commercial formulation of glyphosate, not containing POEA. Similar tests supported the conclusions that the surfactant system in UltraMax © is of similar toxicity as POEA, but the present paper reports a much toxicity of the formulated product compared to other results.	Supporting	UBA2
Meza-Joya, F.L., et al.	2013	Toxic, cytotoxic, and genotoxic effects of a glyphosate formulation (roundup®SL–cosmoflux®411F) in the direct-developing frog *eleutherodactylus johnstonei.*	Amphibians	Tested formulation not comparable to lead formulation. No areal spray as intended use in Europe, no glyphosate-based product with Cosmoflux®411F authorized in Europe. However, tested application rates are equal to intended uses in South America.	Supporting / critical	UBA 2
Lanctôt, C., et al.	2013	Effects of the glyphosate-based herbicide Roundup WeatherMax® on metamorphosis of wood frogs *Lithobates sylvaticus* in natural wetlands.	Amphibians	Critical regarding the results for GLY. Supporting information regarding the effects of formulated products different from the lead formulation in Europe.	Critical	UBA1
Yadav, S. et al.	2013	Toxic and genotoxic effects of Roundup on tadpoles of the Indian skittering frog (*Euflictis cyanophlyctis*) in the presence and absence of predator stress.	Amphibians	Low weight because of the unclear identity of the tested compound. Possibly Roundup original with POEA.	Low weight	UBA3
Güngördü, A.	2013	Comparative toxicity of methidathion and glyphosate on early life stages of three amphibian species: *Pelophylax ridibundusPseudepidalea viridis*and*Xenopus laevis*	Amphibians	Supporting information for the assessment of effect of glyphosate-based formulated products containing POEA.	Supporting	UBA 2
Edge, C et al.	2014	Variation in amphibian response to two formulations of glyphosate-based herbicies	Amphibians	Supporting information for the assessment of the tested formulations.	Supporting	UBA 2
Wagner, N., Lötters, S.	2013	Possible correlation of the worldwide amphibian decline and the increasing use of glyphosate in the agrarian industry	Amphibians	Fertilisers and pesticides – can affect amphibian individuals, in agricultural areas, but do not necessarily effect the population level. Authors empahsise that population viability analysis based on sufficient data and/or long-term field monitoring is necessary to investigate potential impacts on amphibian populations. (Tallowamine) surfactants, apparently ranked among the most harmful pesticides for amphibians. Nevertheless, risks for amphibian populations due to Glyphosate use cannot be named per se because species, life-stage, formulation and application-specific reactions have been observed.	Supporting	UBA 2
Wagner, N., Lötters, S.	2013	Effects of Water Contamination on Site Selection by Amphibians: Experiences from an Arena Approach With European Frogs and Newts	Amphibians	Water contamination with the tested environmentally relevant concentrations did not seem to lead to avoidance of contaminated pools. Perception and avoidance (shown by residence time) of contaminated water bodies should be investigated by arena approaches; however, to study other (relevant) behavior, such as oviposition site selection, authors suggest to use more sophisticated designs.	Supporting	UBA 2
Plötner J. & Matschke J	2012	Akut-toxische, subletale und indirekte Wirkungen von Glyphosat und glyphosathaltigen Herbiziden auf Amphibien – eine Übersicht.	Amphibians	Authors state that the findings presented to the acute toxic, sublethal and indirect effects of glyphosate herbicides on amphibians illustrate an ecological hazard potential, especially if herbicides are applied in approprate in high concentrations and over extended periods of time.	Supporting	UBA 2
Brodeur, J. et al.	2014	Synergy between glyphosate- and cypermethrin-based pesticides during acute exposures in tadpoles of the common South American Toad *Rhinella arenarum.*	Amphibians	he study shows that glyphosate and cypermethrin-based pesticides exhibit synergic interactions during acute exposures in tadpoles of the common South American toad, *R. arenarum*.	Supporting	UBA 2

Bosch, B., et al.	2011	Micronucleus test in post metamorphic *Odontophrynus cordobae* and *Rhinella arenarum* (Amphibia: Anura) for environmental monitoring	Amphibians	High concentrations Roundup ® can lead to basal spontaneous occurrence of MNE in postmetamorphic stages.The data can be employed in the assessment of formulations containing POEA as surfactants. No data for glyphosate technical or glyphosate acid deducible from the tested formulation. Lead formulation for EU renewal of glyphosate approval contains no POEA.	Low weight	UBA3
Berger, G., et al.	2013	Glyphosate applications on arable fields considerably coincide with migrating amphibians	Amphibians	Depending on a) age- and species-specific main migration periods, b) crop species, c) Glyphosate application mode for crops, and d) the presumed DT50 value (12 days or 47 days) of Glyphosate up to 100% coincidence was calculated. The amphibians regularly co-occur with pre-sowing/ pre-emerging applications to maize in spring and with stubble management prior to crop sowing in late summer and autumn. Siccation treatment in summer coincides only with early pond-leaving juveniles. Authors suggest in-depth investigations of both acute and long-term effects of Glyphosate applications on amphibian populations not only focussed on exposure during aquatic periods but also terrestrial life stages.	Supporting	UBA 2
Edge, C et al.	2014	Variation in amphibian response to two formulations of glyphosate-based herbicies	Amphibians	The results of this experiment support prior studies that have found some formulations of glyphosate-based herbicides are toxic to amphibian larvae at concentrations at the upper end of those measured in the environment and that toxicity differs among formulations.	Supporting	UBA 2
Ruamthum, W. et al.	2011	Effect of glyphosate-based herbicide on acetylcholinesterase activity in tadpoles, *Hoplobatrachus rugulosus*	Amphibians	Supporting information on biochemical response of tadpoles to environmental relevant concentrations of commercial formulation containing glyphosate and probably POEA.	Low weight	UBA3
Junges, C.M. et al.	2013	Effectiveness evaluation of glyphosate oxidation employing the H2O2/UVC process: Toxicity assays with *Vibrio fischeri* and *Rhinella arenarum* tadpoles	Amphibians	Ecotoxicity tests were performded for evaluating the effectiveness of decontamination by AOPs. Results have limited value for ERA.	Low weight	UBA3
Schneider, S, et al.	2012	Glyphosate: Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances.	Amphibians	Authors observed no effects observed on normalized hind-limb length, stage, or thyroid histology. Glyphosate was not found to interfere with the normal function of the hypothalamus.	Supporting	UBA 2
Brodeur, J.C. et al.	2012	Evidence of Reduced Feeding and Oxidative Stress in Common Tree Frogs (*Hypsiboas pulchellus*) from an Agroecosystem Experiencing Severe Drought	Amphibians	Authors observded that adult *H. pulchellus* were not affected by glyphosate because no significant difference was observed when comparing animals sampled before) and after the application. As drought maybe influenced the results it is stated that aquatic and terrestrial anuran species may be more exposed or be more vulnerable than the arborescent species studied here, and more research should be conducted before generalizing results to other circumstances or anuran species.	Supporting	UBA 2
Fuentes, L. et al.	2014	Role of sediments in modifying the toxicity of two roundup formulations to six species of larval anurans.	Amphibians	If used according to label instructions, the original formulation of Roundup® and Roundup WeatherMAX® herbicides should pose minimal risk to Anuran amphibians in actual field applications.	Supporting	UBA 2
Thibodeau, J. et al.	2012	Oxidation of retinoic acids in hepatic microsomes of wild bullfrogs *Lithobates catesbeianus* environmentally-exposed to a gradient of agricultural contamination	Amphibians	Long-term objective of these investigations is the development of in vitro assays and non-destructive biomarkers associated with impacts on RA metabolism and signalling in an environmental context.	Low weight	UBA3
Paetow, L.J., McLaughlin, J.D.	2013	Mortality of American Bullfrog Tadpoles Lithobates catesbeianus Infected by *Gyrodactylus jennyae* and Experimentally Exposed to *Batrachochytrium dendrobatidis*	Amphibians	There was no significant difference in mortality between tadpoles exposed only to *Bd* and tadpoles additionally exposed to atrazine or glyphosate, which suggests that the pesticide exposures did not alter the immune system or enhance the virulence of the pathogens in this experiment.	Low weight	UBA3
Lanctot, C. et al.	2014	Effects of glyphosate-based herbicides on survival, development, growth and sex ratios of wood frog (*Lithobates sylvaticus*) tadpoles. II: Agriculturally relevant exposures to Roundup WeatherMax® and Vision® under laboratory conditions	Amphibians	Pulse exposures to Roundup WeatherMax® at a much lower and environmentally relevant concentration (0.21 mg a.e./L) did not affect the survival to metamorphic climax (Gs42) of wood frog tadpoles. Authors conclude that agriculturally relevant exposure to Roundup WeatherMax® and chronic exposure to POEA (1.43 mg/L) is highly toxic to L. sylvaticus tadpoles under laboratory conditions. Survival, growth and mRNA results also indicate that Roundup WeatherMax® has greater toxicity than Vision® formulation containing the POEA surfactant.	Supporting	UBA 2
Katzenberger, M. et al.	2014	Swimming with Predators and Pesticides: How Environmental Stressors Affect the Thermal Physiology of Tadpoles	Amphibians	Environmental contamination influence thermal performance. Roundup itself not beeing in mixture with other stressors did not have any effect on CTmax estimates of tadpoles.	Low weight	UBA3
Hanlon, S.M., Parris, M.J.	2013	The interactive effects of chytrid fungus, pesticides, and exposure timing on gray treefrog (*Hyla versicolor*) larvae	Amphibians	Roundup ameliorated the effects of Bd, illustrating the complexity of interactions of multiple perturbations on amphibians.Previous research has shown that exposure to multiple stressors usually results in combined effects that reduce fitness (e.g., growth, survival) more so than exposure to individual pressures.	Low weight	UBA3
Hanlon, S.M. et al.	2013	Mouthparts of Southern Leopard Frog, *Lithobates sphenocephalus*, Tadpoles not Affected by Exposure to a Formulation of Glyphosate	Amphibians	Authors conclude that more research is necessary to elucidate the mechanisms by which pesticides affect amphibians throughout larval development.	Low weight	UBA3
Figueiredo, J., Rodrigues, D.J.	2014	Effects of four types of pesticides on survival, time and size to metamorphosis of two species of tadpoles (*Rhinella marina* and *Physalaemus centralis*) from the southern Amazon, Brazil	Amphibians	LC50 values for the formulation is determinded. No separate test of glyphosate as acid or salt performed. Differences in the toxicity of co-formulants preclude making general conclusions across all formulations of glyphosate herbicides	Supporting	UBA 2
Edge, C. et al.	2012	Laboratory and field exposure of two species of juvenile amphibians to a glyphosate-based herbicide and *Batrachochytrium dendrobatidis*	Amphibians	In this experimnatl setup exposure to Roundup WeatherMax poses a negligible risk of acute mortality or sublethal effects on LSI, body condition or the incidence of chytridiomycosis.	Low weight	UBA3

Edge, C. et al.	2012	A silviculture application of the glyphosate-based herbicide Visionmax to wetlands has limited direct effects on amphibian larvae	Amphibians	As one wetland was assigned both as the control and the other side as the treatment the integrity of the impermeable barriers might be compromised. Nevertheless, leakage was demonstrated to be two orders of magnitude below the lowest concentrations. It seems that trend lower toxicity can being observed in field studies compared to laboratory and mesocosm studies.	Low weight	UBA3
Navarro-Martín, L. et al.	2014	Effects of glyphosate-based herbicides on survival, development, growth and sex ratios of wood frogs (Lithobates sylvaticus) tadpoles. I: Chronic laboratory exposures to VisionMax®	Amphibians	Results indicate that chronic exposures to VisionMax® in the laboratory have direct effects on the metamorphosis of *L.sylvaticus* tadpoles by decreasing development rates and meta-morphic success. Analysis of mRNA levels indicates that exposure to VisionMax® affects key genes linked to the hormonal cascade inducing metamorphosis in developing anuran tadpoles. Further studies are needed to determine if the changes of mRNA levelsof these key genes are translated to sublethal long-term effects as metamorphs reach adult stages.	Supporting	UBA 2
Rick A. Relyea	2009	A cocktail of contaminants: how mixtures of pesticides at low concentrations affect aquatic communities.	Amphibians	Author concludes that when pesticides were combined, the mix of herbicides had no negative effect on the survival and metamorphosis of amphibians, but the mix of insecticides and the mix of all ten pesticides eliminated 99% of leopard frogs.	Low weight	UBA3
M. H. Bernal a , K. R. Solomon b & G. Carrasquilla	2009	Toxicity of Formulated Glyphosate (Glyphos) and Cosmo-Flux to Larval and Juvenile Colombian Frogs 2. Field and Laboratory Microcosm Acute Toxicity	Amphibians	The study presents the mortality data for the terrestrial stages of eight species of frogs are as nominal applica-tion rate (kg ae glyphosate / ha) in order to allow a comparison with field application rates. Mortality at the rate of 3.69 kg ae /ha, which corresponds to the maxi-mum annual application rate was between 15% and 35% and the LC50 values 3-8-fold higher than the application rate . With a application rate of 1.85 kg ae / ha, which corresponds to the simple application rate of an application, the mortality rates were in were 3 of 8 experiments between 15% and 35% and the LC50 values 3-8 times higher than that application rate. The authorization procedure currently has no harmonized models and evaluation approaches, but would currently –with derivation of safety factors- not provide adequate protection of amphibians.	Supporting	UBA 2
Addison, P.J., Barker, G.M.	2006	Effect of various pesticides on the non-target species *Microctonus* hyperodae, a biological control agent of *Listronotus bonariensis*	Arthropod	Species are not relevant for central zone, no data presented for glyphosate	Low weight	UBA3
Albajes, R., et al.	2011	Albajes, R., Lumbierres, B., Pons, X.	Arthropod	no significant changes observed, GM corn tested	Low weight	UBA3
Bueno, A.F., et al.	2011	Effects of pesticides used in soybean crops to the egg parasitoid *Trichogramma pretiosum*	Arthropod	Glyphosate (Roundup® Ready) 972 grams ha-1 were classified as harmfull for T. pretiosum eggs and harmless to the other parasitoid stages . Glyphosate 960 (Roundup® Original) was classified as slightly harmful for eggs and harmless for pupae of the parasitoid .	Supporting	UBA 2
Lipok, J.	2009	Dual action of phosphonate herbicides in plants affected by herbivore-Model study on black bean aphid *Aphis fabae* rearing on broad bean *Vicia faba* plants	Arthropod	Number of aphids accompanied with treated plants cannot be used for risk assessment, no effects observed.	Low weight	UBA3
Evans, S.C et al..	2010	Exposure to a glyphosate-based herbicide affects agrobiont predatory arthropod behaviour and long-term survival	Arthropod	No endpoints on mortality. Tested concentrations higher thatn expected drift rates. But authors demonstrate that arthropod predators inhabiting agroecosystems around the world exhibit subtle shifts in behaviour and reproduction during or after exposure to herbicide.	Supporting	UBA 2
Griesinger, et al.	2010	Effects of a glyphosate-based herbicide on mate location in a wolf spider that inhabits agroecosystems	Arthropod	Behavioral study , with POEA containing product. Tested concentrations probably higher than drift rates.	Low weight	UBA3
Michalková, V., Pekár, S.	2009	How glyphosate altered the behaviour of agrobiont spiders (Araneae: Lycosidae) and beetles (Coleoptera: Carabidae)	Arthropod	*Pardosa* and *Poecilus* are standard test species in RA, predation rate, locomotion speed, avoidance, defence and mating behavior nor standard parameters. Biological control potential of the two species should not be directly reduced following herbicide application in the field.	Supporting	UBA 2
Schier, A.	2006	Field study on the occurrence of ground beetles and spiders in genetically modified, herbicide tolerant corn in conventional and conservation tillage systems	Arthropod	Field study with climatic extremes and uncertainties. The results of this multi year study indicate that total abundance of ground beetles and spiders were not affectd due to reduced soil tillage combined with glyphosate treatment.	Supporting	UBA 2
Ainsworth, N.	2003	Integration of herbicides with arthropod biocontrol agents for weed control	Arthropod	No information relevant for risk assessment.	Low weight	UBA3
Renaud, A., et al.	2004	Influence of four soil maintenance practices on Collembola communities in a Mediterranean vineyard	Arthropod	abundance was highest in natural flora practice and in the practice with a postemergence herbicide. Glyphosate treatment weed cover was preserved.	Supporting	UBA 2
Wrinn, K.M., et al.	2012	Predator cues and an herbicide affect activity and emigration in an agrobiont wolf spider	Arthropod	Authors conclude that predation risk andmherbicide application likely interact to affect the movement of a major arthropod predator.	Low weight	UBA3
Benamu, et al.	2010	Effects of the herbicide glyphosate on biological attributes of *Alpaida veniliae* (Araneae, Araneidae), in laboratory	Arthropod	Authors conclude that sublethal effects are relevant from an ecological point of view, since the reduction of the arthropod performance may create risks to arthropod biodiversity conservation in agroecosystems.	Supporting	UBA 2
Castilla, A.M. et al.	2010	Nitrates and Herbicides Cause Higher Mortality than the Traditional Organic Fertilizers on the Grain Beetle, *Tenebrio molitor*	Arthropod	Herbicide mixture, deficiencies in test design.	Low weight	UBA3

Bernard, et al.	2010	Reducing the Impact of Pesticides on Biological Control in Australian Vineyards: Pesticide Mortality and Fecundity Effects on an Indicator Species, the Predatory Mite *Euseius victoriensis (*Acari: Phytoseiidae)	Arthropod	No effects detected.	Low weight	UBA3
Santos, M.J.G., et al.	2010	Joint effects of three plant protection products to the terrestrial isopod *Porcellionides pruinosus* and the collembolan *Folsomia candida*	Arthropod	ER_{50} reproduction at approx.12xPEC AC_{50} for avoidance at approx 10xPEC	Supporting	UBA 2
El Sebai, O.A., et al.	2012	Side-Effect of Certain Herbicides on Egg Parasitoid *Trichogramma evanescens* (West.) (Hymenoptera: Trichogrammatidae)	Arthropod	Treated *S. cerealella* eggs exposed to low concentrations of glyphosate had a high parasitism viability (85%) and higher percent of *T. evanescens* adult emergence (91%), what was closely similar to the untreated 89% and 95%, respectively.	Supporting	UBA 2
Menezes, C.W.G. et al.	2012	Reproductive and toxicological impacts of herbicides used in Eucalyptus culture in Brazil on the parasitoid *Palmistichus elaeisis* (Hymenoptera: Eulophidae)	Arthropod	The commercial formulation did not reduce the parasitism and emergence of *P. elaeisis.*	Supporting	UBA 2
Rittman, S. et al.	2013	Glyphosate-Based Herbicide Has Contrasting Effects on Prey Capture by Two Co-Occurring Wolf Spider Species	Arthropod	The commercial formulation affects the predatory interactions of both *Tigrosa and Pardosa* differently. Pesticide treatment can have selective implications for animals living in agricultural systems.	Supporting	UBA 2
Kaneda, S., et al.	2009	Effects of herbicides, glyphosate, on density and casting activity of earthworm, Pheretima (Amynthas) carnosus	earthworms	Not considered because of deficiencies in translation.	Low weight	UBA3
Casabe, N., et al.	2007	Ecotoxicological Assessment of the Effects of Glyphosate and Chlorpyrifos in an Argentine Soya Field	earthworms	Detailed study on the toxicity of this special commercial formulation,nevertheles with limited value for other formulations, as toxicity of the glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant system in the product.	Supporting	UBA 2
Correia, F.V., Moreira, J.C.	2010	Effects of glyphosate and 2,4-D on earthworms (Eisenia foetida) in laboratory tests	earthworms	Study will be considered.	Supporting	UBA 2
Verrell, P., Van Buskirk, E.	2004	As the worm turns: *Eisenia fetida* avoids soil contaminated by Glyphosate-based herbicide	earthworms	Study will be considered. No GLP, no OECD, no standard method, but results and conclusion shown credibly. Detailed study on the toxicity of this special commercial formulation,nevertheles with limited value for other formulations, as toxicity of the glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant system in the product.	Supporting	UBA 2
Santos, M.J.G. et al.	2012	Pesticide application to agricultural fields: effects on the reproduction and avoidance behaviour of *Folsomia candida* and *Eisenia andrei*	earthworms	Glyphosate did not seem to affect either earthworms or collembolans in the recommended field dose. Detailed study on the toxicity of this special commercial formulation,nevertheles with limited value for other formulations, as toxicity of the glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant system in the product.	Supporting	UBA 2
Yasmin, S., D'Souza, D.	2003	Effect of Pesticides on the Reproductive Output of *Eisenia fetida*	earthworms	No GLP, no OECD, no standard method, but results and conclusion shown credibly. Detailed study on the toxicity of this special commercial formulation,nevertheles with limited value for other formulations, as toxicity of the glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant system in the product.	Supporting	UBA 2
Zhou, C.F. et al.	2013	Subacute toxicity of copper and glyphosate and their interaction to earthworm (*Eisenia fetida*)	earthworms	During the 28 d exposures, therewas no significant difference in weight or the cocoon production between the control group and the GPS group. Our results showed that GPS had very low toxicity to the earthworms, which is consistent with other studies.	Supporting	UBA 2
Zhou, C.F., et al.	2012	Does glyphosate impact on Cu uptake by, and toxicity to, the earthworm *Eisenia fetida*?	earthworms	Toxicity to earthworms was not observed in this study.	Supporting	UBA 2
Moreno et al.	2009	Rainfed olive farming in south-eastern Spain: Long-term effect of soil management on biological indicators of soil quality	earthworms	Field study design lasting over 40 years.Supporting evidence.	Supporting	UBA 2
Negga et al.	2011	Exposure to Mn/Zn ethylene-bis-dithiocarbamate and glyphosate pesticides leads to neurodegeneration in *Caenorhabditis elegans*	earthworms	Study indicates that *C. elegans* are vulnerable to glyphosate-containing herbicides at environmentally relevant concentrations in terms of neurotoxicity. Toxicity of glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant system in the product.	Low weight	UBA3
Piola, L., et al.	2013	Comparative toxicity of two glyphosate-based formulations to *Eisenia andrei* under laboratory conditions	earthworms	Results highlight the importance of ecotoxicological assessment not only of the active ingredients, but also of the different formulations.	Supporting	UBA 2
Jeffrey D. et al.	2010	Direct and Indirect Effects of Invasive Plants on Soil Chemistry and Ecosystem Function	earthworms	Herbicides used to control invasive species can impact plant chemistry and ecosystems in ways that have yet to be fully explored.	Supporting	UBA 2
Druart et al.	2010	Towards the development of an embryotoxicity bioassay with terrestrial snails: Screening approach for cadmium and pesticides	earthworms	Relevant information about formulations containing POEA.	Supporting	UBA 2
Muangphra, P., et al.	2012	Genotoxic Effects of Glyphosate or Paraquat on Earthworm Coelomocytes	earthworms	Glyphosate causes only aneugenic effects and therefore, does not pose a risk of gene mutation in this earthworm.	Supporting	UBA 2

Wang, Y. et al.	2012	Toxicity assessment of 45 pesticides to the epigeic earthworm *Eisenia fetida*	earthworms	Authors highlight that results from single-pesticide experiments did not actually reflect field situations in which multiple pesticides or pesticide mixtures are used.	Supporting	UBA 2
Negga, R., et al.	2011	Exposure to Glyphosate- and/or Mn/Zn-Ethylene-bis-Dithiocarbamate-Containing Pesticides Leads to Degeneration of ?-Aminobutyric Acid and Dopamine Neurons in *Caenorhabditis elegans*	earthworms	The commercial formulation induces changes in neuron morphology in the model organism *C. elegans*.	Supporting	UBA 2
Zhou, C.F., et al.	2014	Inhibition effect of glyphosate on the acute and subacute toxicity of cadmium to earthworm *Eisenia fetida*	earthworms	Glyphosate is a strong chelating agent for heavy metals. The results of the study indicated that the Cd availability and, consequently, toxicity were reduced by the presence of glyphosate.	Supporting	UBA 2
Buch, A.C. et al.	2012	Toxicity of three pesticides commonly used in Brazil to *Pontoscolex corethrurus* (Müller, 1857) and *Eisenia andrei* (Bouché, 1972)	earthworms	For the pesticides tested in the present experiment there were no important and very few significant differences between both species in their sensitivity to the three pesticides used. The commercial formualtion was found to be non-toxic for both species at the concentrations used.	Supporting	UBA 2
Fusilero, M.A. et al.	2013	Weed management systems and other factors affecting the earthworm population in a banana plantation	earthworms	The use of commercial preparation of herbicides paraquat and glyphosate under the tested field conditions did not affect the earthworm population. Other factors found to affect the population were ground cover, rainfall and soil organic matter.	Supporting	UBA 2
García-Torres, T. et al.	2014	Exposure Assessment to Glyphosate of Two Species of Annelids	earthworms	Results revealed that the species *O. tyrtaeum* was more sensitive to exposure to glyphosate than *E. fetida*, decreasing mortality and biomass. In addition, there were adverse effects in adult fecundity and viability of cocoons by exposure to high concentrations of glyphosate. This study demonstrated adverse effects at concentrations which exceed the predicted concentrations of 6 mg/kg.	Supporting	UBA 2
Accinelli, C. et al	2002	Short time effects of pute and formulated herbicides on soil microbial activity and biomass	micro-organisms	These results support the absence of adverse effects of glyphosate and glufosinate-ammonium on soil microbial population. The paper focuses on ecosystem function and does inform on ecosystem structure diversity.	Supporting	UBA 2
Araujo, A.S.F., et al.	2003	Effect of glyphosate on the microbial activity of two Brazilian soils	micro	Glyphosate was biodegraded by soil microorganisms with the formation AMPA, and that the herbicide had positive effect on the soil microbial activity in short- and long-term. Detection of a community shift, was not discussed.	Supporting	UBA 2
Cycon, M., Kaczynska, A.	2004	Effects of selected pesticides on soil microbial activity in nitrogen and carbon transformation	micro	Application of glyphosate at recommended field rates did not have negatively effect on soil microbial activity measured by SIR and nitrogen transformation 28 days treatment. However, it is impossible to draw a general conclusion regarding the effect of glyphosate on soil microorganisms because a number of factors influence on the activity of this agrochemical in soil ecosystem, therefore estimation of two parameters only may be not adequate in some situations	Critical	UBA1
Gomez, E., et al.	2009	Impact of glyphosate application on microbial biomass and metabolic activity in a Vertic Argiudoll from Argentina	micro	The results of this study demonstrate an initial inhibitory effect that affected the microbial cells, which showed to be temporary, indicating that no harmful effects should be expected in the short-term when glyphosate is applied at doses equivalent or higher than those usually applied in the field.	Supporting	UBA 2
Haney, R.L., Senseman, S et al.	2002	Effect of Roundup® ultra on microbial activity and biomass from selected soils	micro	Roundup® Ultra appeared to be rapidly degraded by soil microbes regardless of soil type or organic matter content, and increased their population and activity even at high application rates, without adversely affecting microbial activity	Supporting	UBA 2
Haney, R.L., et al.	2002	Soil carbon and nitrogen mineralization as affected by atrazine and glyphosate	micro	Atrazine plus glyphosate stimulated microbial activity more than atrazine alone.	Supporting	UBA 2
Hart et al.	2009	Separating the effect of crop from herbicide on soil microbial communities in glyphosate-resistant corn	micro	Neither GR corn nor glyphosate had significant impacts on the denitrifying bacteria and fungi in this study	Low weight	UBA 3
Kyaw, K.M., Toyota, K.	2007	Suppression of nitrous oxide production by the herbicides glyphosate and propanil in soils supplied with organic matter	micro	herbicides used in this study had no severely adverse effects on the overall soil microbial community.	Low weight	UBA 3
			micro			
Lupwayi et. al	2004	Soil microbial biomass and diversity after herbicide application	micro	Herbicides applied once at recommended rates did not have significant or consistent effects on microbial C or diversity indices. However, shifts in microbial community structures were sometimes evident where microbial C and diversity were not different..Such shifts can lead to successions in the microbial communities that could have longterm effects on soil biological processes. Therefore, it is important to incorporate measures of microbial diversity and composition in herbicide ERA studies.	Supporting	UBA 2
Malkomes, H.-P.	2007	Influence of differently formulated glyphosate herbicides and a herbicidal reference compound on microbial activities in soil	micro	Independently from the tested formulations the overall effects of field-related dosages of glyphosate induced only relatively small effects on the investigated microbial activities in the soil.	Supporting	UBA 2
Mijangos, et al.	2009	Effects of glyphosate on rhizosphere soil microbial communities under two different plant compositions by cultivation-dependent and -independent methodologies	micro	Glyphosate was quickly used by soil microorganisms as a source of nutrients which resulted in a stimulation of the activity and functional diversity of the cultivable portion of the heterotrophic soil microbial community.Effects on the rhizosphere soil microbial community which were, interestingly, more enhanced in triticale than in "triticale þ pea" pots. Biolog was more sensitive to detect changes in soil microbial communities induced by glyphosate and plant composition than PCR-DGGE.	Supporting	UBA 2

Ratcliff, A.W., et al.	2006	Changes in microbial community structure following herbicide (glyphosate) additions to forest soils	micro	No major changes in microbial community structure assessed by C utilization, PLFA, and standard cultural and microscope methods. In forest soils following the addition of the recommended field-rate concentration of glyphosate commercial formulation has a benign affect on community structure when applied at the recommended field rate, and produces a non-specific, short-term stimulation of bacteria at a high concentration.	Supporting	UBA 2
Zabaloy, M.C., et al.	2008	An integrated approach, to evaluate the impacts of the herbicides glyphosate, 2,4-D and metsulfuron-methyl on soil microbial communities in the Pampas region, Argentina	micro	The addition of these herbicides at a dose 10 times higher than the normal field application rates caused minor changes to soil microbial activity, bacterial density and functional richness. The specific changes varied among herbicides, with the effects of glyphosate most pronounced.	Supporting	UBA 2
Lancaster, S.H., et al.	2006	Soil microbial activity is affected by Roundup® WeatherMax and pesticides applied to cotton (Gossypium hirsutum)	micro	For all variables measured, pasture soil exhibited greater microbial activity and biomass than cultivated soil. Cumulative C mineralization after 30 days was greater in soils treated only with glyphosate as compared to all other treatments. .Nitrogen mineralization was greater in soils that had been treated with applications that included glyphosate as compared with soils that were not treated with glyphosate. .Soil microbial biomass C increased relative to non-treated soils when glyphosate was applied alone. Soil microbial biomass N was not affected.	Supporting	UBA 2
Bennicelli, R.P., et al.	2009	Influence of pesticide (glyphosate) on dehydrogenase activity, pH, Eh and gases production in soil (laboratory conditions)	micro	Glyphosate caused an inhibition of DHA activity in all investigated soils up to 21st day.CO2 formation increased in the case of Terric Histosols and Eutric Fluvisols, but decreased in the case of Mollic Gleysols.Glyphosate caused an increase of N2O concentration in all investigated soils. Eh, pH and CO2 concentration had high correlations with DHA activity.	Supporting	UBA 2
Lancaster, S.H., et al.	2010	Effects of repeated glyphosate applications on soil microbial community composition and the mineralization of glyphosate	micro	Changes in the dissipation or distribution of glyphosate following repeated applications of glyphosate may be related to shifts in the soil microbial community composition.	Supporting	UBA 2
Ruzkova, M., et al.	2011	Microbiological characterization of land set-aside before and after Roundup® desiccation	micro	Roundup® desiccation caused effects on enzyme activita, microbial biomass and and nitrate-nitrogen ratio.	Supporting	UBA 2
Widenfalk, A., et al.	2008	Effects of pesticides on community composition and activity of sediment microbes - responses at various levels of microbial community organization	micro	The study showed that community-level end points failed to detect these changes, underpinning the need for application of molecular techniques in aquatic ecotoxicology.	Supporting	UBA 2
Liphadzi, K.B., et al.	2005	Soil microbial and nematode communities as affected by glyphosate and tillage practices in a glyphosate-resistant cropping system	micro	Soil health in a glyphosate-resistant cropping system was similar to that of cropping systems that used conventional herbicides.	Supporting	UBA 2
Busse, M.D., et al.	2001	Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities.	micro	Long-term, repeated applications had minimal effects on seasonal microbial characteristics, which was more a function of time of year and site. Tests in artificial media are of limited relevance for glyphosate	Supporting	UBA 2
Gorlenko, M.V. et al.	2012	Functional Biodiversity of Soil Microbe Colonies Affected by Organic Substrates of Different Kinds	micro	Differences reponses of microbe community were observed between natural microbe community (Sod podzol soil) and “modell soil” (ML soil).	Low weight	UBA3
Bai et al.	2012	Effects of pre-planting site management on soil organic matter and microbial community functional diversity in subtropical Australia	micro	Single herbicide application at field rates did not impact soil organisms but it did not effectively control weeds either. Touchdown™ is a glyphosate-based formulation. It has been utilized as one of the most commonly applied herbicides. The study could not be taken into account as critical information on the ERA of the active substance glyphosate itself.	Supporting	UBA 2
Partoazar, M. et al.	2011	The effect of glyphosate application on soil microbial activities in agricultural land	micro	Regularly applications of commercially formulation of glyphosate over years may alter (increase) soil microbial activity and population. Increased microbial activity may be beneficial or detrimental toward plant growth, soil microbial ecology, and soil quality.	Supporting	UBA 2
Stachowski-Haberkorn, S. et al.	2008	Impact of Roundup on the marine microbial community, as shown by an in situ microcosm experiment	micro	Roundup as a glyphosate-based formulation has been utilized as one of the most commonly applied herbicides. The study could not be taken into account as critical information on the ERA of the active substance glyphosate itself.	Supporting	UBA 2
Udawatta, R.P. et al.	2010	Microbial resilience as influenced by an herbicide in soils from native prairie, CRP, and row crop management	micro	Soils from native prairie have greater enzyme resilience while the corn-soybean cropped soils have the smallest enzyme resilience. This might suggest that continuous soil disturbance and application of other agri-chemicals may have negatively afffected microbial resilience for soils under crop management. Observed enzyme activities could be due to direct and/or indirect effects of pesticides which were not evaluated in the current study.	low weight	UBA3
Zabaloy, M.C. et al.	2012	Assessment of microbial community function and structure in soil microcosms exposed to glyphosate	micro	Respiratory response of the reference and chronically exposed soils to glyphosate was distinctive. Shifts in community metabolism were observed. => longer term studies involving repeated addition of glyphosate to previously unexposed soils are needed	Supporting	UBA 2
Benslama, O. & Boulahrouf, A.	2011	Impact of glyphosate application on the microbial activity of two Algerian soils	micro	No negative effects on microbial activity, shift in microbial community and function were observed.	Supporting	UBA 2
Pasaribu, A. et al.	2013	Effect of herbicide on sporulation and infectivity of vesicular arbuscular mycorrhizal (*Glomus mosseae*) symbiosis with peanut plant.	micro	Symbiotic functions of *G. mosseae* with host plant could be affected by the depressive effects of herbicides that are apparently related to the types of herbicide and their rates of application. Roundup as a glyphosate-based formulation has been utilized as one of the most commonly applied herbicides. The study could not be taken into account as critical information on the ERA of the active substance glyphosate itself.	Supporting	UBA 2

Yousaf, S. et al.	2013	Effect of Pesticides on the Soil Microbial Activity	micro	Although the study has been performed with the active substance alone (no formulation), the study could not be taken into account as critical information on the ERA because of the lack of statistical analysis. The effects describes have not statistical basis.	Low weight	UBA3
Druille, M. et al.	2013	Glyphosate reduces spore viability and root colonization of arbuscular mycorrhizal fungi	micro	Symbiosis was functionality affected. Soil residence time of glyphosate and/or its degradation products was enough to reduce AMF spore viability and their ability to colonize roots. This decrease in propagules viability may affect plant diversity, taking into account the different degrees of mycorrhizal dependency between plant species that may coexist in grassland communities. Glacoxan® a glyphosate-based formulation has been utilized. Thus, the study could not be taken into account as critical information on the ERA of the active substance glyphosate itself. However it contains relevant scientific information about the impact of glyphosate-formualted product on AMF	Supporting	UBA 2
Druille, M. et al.	2013	Arbuscular mycorrhizal fungi are directly and indirectly affected by glyphosate application	micro	Findings illustrate that glyphosate application negatively affects AMF functionality in grasslands, due to different causes depending on the herbicide application "site" (soil *vs* foliage). While, under field conditions, the occurrence of direct and/or indirect pathways will depend on the plant cover at the time of glyphosate application, the consequences of this practice on the plant community structure will vary with the mycorrhizal dependence of the species composition regardless of the pathway involved.	Supporting	UBA 2
Zaller, J.G. et al.	2014	Glyphosate herbicide affects belowground interactions between earthworms and symbiotic mycorrhizal fungi in a model ecosystem	micro	Evidence for sizeable changes, which provide impetus for more general attention to side-effects of glyphosate-based herbicides on key soil organisms and their associated ecosystem services.	Supporting	UBA 2
Mbanaso, F.U. et al.	2014	Potential microbial toxicity and non-target impact of different concentrations of glyphosate-containing herbicide (GCH) in a model Pervious Paving System.	micro	At the lowest concentration of glyphosate-containing herbicides biodegradation processes may not be affected as all trophic levels required for optimum biodegradation of contaminants were present. The ciliates *Colpoda cucullus* and *Colpoda steinii* present a potential for use as biomarkers of herbicide-polluted environments.	Low weight	UBA3
Krüger, M. et al. (2013)	2013	Glyphosate suppresses the antagonistic effect of *Enterococcus* spp. on *Clostridium botulinum*.	micro	A reduction of lactic acid producing bacteria in the gastro-intestinal tract microbiota by ingestion of strong biocides like glyphosate could be an explanation for the observed increase in levels of *C. botulinum* associated diseases. Moreover, gastro-intestinal microbes are not the focus of this part of the risk assessment. However it contains relevant scientific information about the impact of glyphosate-formulated product on microbial communities.	Supporting	UBA 2
Shehata, A.A. et al.	2013	The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro.	micro	The toxicity of glyphosate to the most prevalent *Enterococcus* spp. could be a significant predisposing factor that is associated with the increase in *C. botulinum*-mediated diseases by suppressing the antagonistic effect of these bacteria on clostridia. Roundup UltraMax is a glyphosate-based formulation has been utilized as one of the most commonly applied herbicides. Thus, the study could not be taken into account as critical information on the ERA of the active substance glyphosate itself. Moreover, gastro-intestinal microbes are not the focus of this opart of the risk assessment. However it contains relevant scientific information about the impact of glyphosate-formualted product on microbial communities.	Supporting	UBA 2
Pesce, S. et al.	2009	Response of spring and summer riverine microbial communities following glyphosate exposure.	micro	Even if the effects of a short pulse of glyphosate (10 µg/ l) on riverine microorganisms seem to be limited, the responses of natural microbial communities to glyphosate exposure (and probably to other pesticide exposures) can clearly vary between the experiments, and can be seasonally dependent.	Low weight	UBA3
Krzysko-Lupicka T, Sudol T	2008	Interactions between glyphosate and autochthonous soil fungi surviving in aqueous solution of glyphosate.	micro	Existence of interactions between microorganisms and herbicide, leading to qualitative changes in fungi population in the soil that was exposed to long-term action of glyphosate. Survival of phytopathogenic, polyphagic fungi of Fusarium kind, capable to adapt to low concentration of glyphosate is unrest, because created the potential danger to agricultural environment.	Supporting	UBA 2
Bonnet, J.L. et. Al.	2007	Assessment of the potential toxicity of herbicides and their degradation products to nontarget cells using two microorganisms, the bacteria *Vibrio fischeri* and the ciliate *Tetrahymena pyriformis*.	micro	This study demonstrates that it is essential to assess the toxicity of the metabolites formed to complete a more comprehensive study of the environmental impact of a polluting agent.	Low weight	UBA3
Laatikainen T & Heinonen-Tanski H	2002	Mycorrhizal growth in pure cultures in the presence of pesticides.	micro	Knowledge on the capabilities of ectomycorrhizal fungi to tolerate pesticides might be useful in deciding which pesticides would be less harmful to mycorrhizal fungi when used in forest nurseries and in afforested fields.	Supporting	UBA 2
Estok, D. et al.	1989	Effects of the herbicides 2,4-D, glyphosate, hexazinone, and triclopyr on the growth of three species of ectomycorrhizal fungi.	micro	Herbicides reduced the growth of the ectomycorrhizal fungi species at concentrations < 100 ppm. Paper contains relevant scientific information about the impact of glyphosate-formualted product on AMF.	Supporting	UBA 2
Kremer & Means	2009	Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms	micro	Review based on commercially formulated products. However it contains relevant scientific information about the impact of glyphosate-formualted products: The role of glyphosate in biofilm formation and associated Mn transformation by rhizosphere bacteria remains to be clarified - Microbial groups and functions affected by glyphosate included Mn transformation and plant availability; phytopathogen–antagonistic bacterial interactions reduction in nodulation - A more informative approach to assess the impact of GR crops on the environment is to target sensitive indicator microbial groups and/or processes in addition to the more general tests targeting microbial community diversity. Research on the impacts of glyphosate on mycorrhizal fungi is needed.	Supporting	UBA 2
Fernandez et al.	2009	Glyphosate associations with cereal diseases caused by *Fusarium* spp. in the Canadian Prairies	micro	Consistent association between previous glyphosate use and *Fusarium* infections also warrants further research to elucidate the nature of this association and the underlying mechanisms determining these effects.	Supporting	UBA 2

Gimsing et al.	2004	Chemical and microbiological soil characteristics controlling glyphosate mineralisation in Danish surface soils	micro	The results indicate that the activity of glyphosate mineralising bacteria (e.g. *Pseudomonas* spp.) was a major factor controlling the fate of glyphosate in the soils.	Low weight	UBA3
Giovannetti et al.	2006	Mycorrhizal fungi in ecotoxicological studies: soil impact of fungicides, insecticidesand herbicides	micro	The experimental system presented in the paper could turn out to be adequate for developing dose-effect curves with G. mosseae as model organism.Glyphosate showed hormeosis effect on the spore germination of the AMF but considerably inhibited its growth	Supporting	UBA 2
Singh et al.	2014	Microbial degradation of herbicides	micro	Microbes are the main vehicle for remediation of herbicides, and new discoveries, such as novel biodegradation pathways, multispecies interactions and community-level responses to herbicides addition, are helping us to understand, predict and monitor the fate of herbicides - Phytoremediation in conjunction with rhizospheric microbes may provide a cheap, fast, eco-friendly and efficient rhizoremediation processes for the removal of explosive waste from the upper layers of the soil	Low weight	UBA3
Sheng *et al.*	2012	Cropping practices modulate the impact of glyphosate on arbuscular mycorrhizal fungi and rhizosphere bacteria in agroecosystems of the semiarid prairie	micro	The authors suggest that interactions between soil fertility, tillage, and cropping practices shape the influence of glyphosate use on rhizo-sphere microorganisms, and not glyphosate alone. The authors heighted the fact that small effects of glyphosate on functionally important components of the agroecosystem can have a strong impact when these effects influence large areas continuously, as with the repeated use of herbicides in most cultivated fields and therefore recommend more large scale field studies to address this problem.	Supporting	UBA 2
M. Sihtmäe, I. et al.	2013	Ecotoxicological effects of different glyphosate formulations	micro	Authors conclude that when reporting results of toxicity testing of glyphosate formulation it is veryimportant to provide the complete name of the tested product andall the possible information on its chemical composition, as toxicity ofthe glyphosate-based herbicides to non-target aquatic (crustaceansand bacteria) and terrestrial organisms (soil bacteria and plants)vary within a wide range.LC50 for *Daphna* is considered in B.9.13 1.Summary of the relevant literature on aquatic organisms.	Supporting	UBA 2
M. Lanea, et al.	2012	The effect of glyphosate on soil microbial activity, microbial community structure, and soil potassium	micro	Authors conclude that glyphosate: (1) stimulates microbial respiration particularly on soils with a history of glyphosate application; (2) has no significant effect on functional diversity or microbial biomass K; and (3) does not reduce the exchangeable K (putatively available to plants) or affect non-exchangeable K.	Supporting	UBA 2
Boutin, C., et al.C.	2004	Toxicity testing of fifteen non-crop plant species with six herbicides in a greenhouse experiment: Implications for risk assessment	Plants	Study describes that field margin species may be not adequate protected and risk may be underestimated when non crop species are not tested for risk assessment.	Supporting	UBA 2
White, A.L., Boutin, C.	2007	Herbicidal effects on nontarget vegetation: Investigating the limitations of current pesticide registration guidelines	Plants	This study extends the current interest by presenting three experiments highlighting some of the limitations to current phytotoxicity testing guidelines.	Supporting	UBA 2
Boutin, C., et al.	2010	Measuring variability in phytotoxicity testing using crop and wild plant species	Plants	The present study supports the inclusion of an uncertainty factor in risk assessments to account for the intrinsic variability in plant sensitivity to herbicides.	Supporting	UBA 2
Dalton, R.L., Boutin, C.	2010	Comparison of the effects of glyphosate and atrazine herbicides on nontarget plants grown singly and in microcosms	Plants	Authors state that Single-species tests are useful because they are inexpensive, can demonstrate clear dose–response patterns uncomplicated by other factors influencing growth, and are able to provide a measure of the sensitivity of a given species to glyphosate and atrazine. However, they are unable to predict subtle changes in community structure that may have important long-term consequences.	Supporting	UBA 2
Martin, M.L., Ronco, A.E.	2006	Effect of mixtures of pesticides used in the direct seeding technique on nontarget plant seeds	Plants	No effect on seed germination were observed with any concentration for any tested species.	Supporting	UBA 2
Olszyk, D.M., et al.	2004	Assessing the risk to non- target plants from herbicides	Plants	Not relevant in terms of risk assessment, but review indicates limitations of ERA in general.	Supporting	UBA 2
Wagner, R., et al.	2003	Phytotoxic activity of root absorbed glyphosate in corn seedlings (*Zea mays* L.)	Plants	Authors expect that if there is glyphosate available in the soil solution it could be absorbed from and root crop damage could occur. Non target plant might therefore be exposed not only vial drift, but also via the soil.	Supporting	UBA 2
Wibawa, W., et al.	2009	Residual Phytotoxicity Effects of Paraquat, Glyphosate and Glufosinate-Ammonium Herbicides in Soils from Field-Treated Plots	Plants	Field experiment in Malaysia.	Low weight	UBA3
R. L. DALTON, and C. BOUTIN	2012	Comparison of the effect of glyphosate and atrazine herbicides on non target plants grown singly and in microcosms.	Plants	Single-species tests are useful because they are inexpensive, can demonstrate clear dose–response patterns uncomplicated by other factors influencing growth, and are able to provide a measure of the sensitivity. However, they are unable to predict subtle changes in community structure that may have important long-term consequences.	Supporting	UBA 2
Frank M. W. de Jong, Helias A. Udo de Haes	2001	Development of a Field Bioassay for the Side-Effects of Herbicides on Vascular Plants Using *Brassica napus* and *Poa annua*	Plants			
G. Neumann, et al.	2006	Relevance of glyphosate transfer to non-target plants via the rhizosphere	Plants	Authors predict an increase in disease problems, particularly on soils with low micronutrient availability as already reported in the US due to transfer from target to non-target plants (e.g. from weed to trees in orchards). Not relevant for the traditional ecotoxicological risk assessment, but important to improve management of glyphosate long-term applications in agricultural practice.	Supporting	UBA 2

Fernandez, M.R., et al.	2009	Glyphosate associations with cereal diseases caused by *Fusarium spp*. in the Canadian Prairies	Plants	Study established a relationship between previous glyphosate use and increased *Fusarium* infection of spikes and subcrown internodes of wheat and barley, or *Fusarium* colonization of crop residues.	Low weight	UBA3
Piotrowicz-Cieslak, A.I., et al.	2010	Different Glyphosate Phytotoxicity of Seeds and Seedlings of Selected Plant Species	Plants	Indication that glyphosate inhibits root growth.	Supporting	UBA 2
Eker, S., et al.	2006	Foliar applied glyphosate substantialla reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus L.*) plants	Plants	The results suggest that residues or drift may result in impairments in Fe and Mn nutrition of nontarget plants, possibly due to the formation of poorly soluble glyphosate-metal complexes in plant tissues and/or rhizosphere interactions.	Supporting	UBA 2
Tesfamariam T, et al.	2009	Glyphosate in the rhizosphere – role of waiting times and different glyphosate binding forms in soils and phytotoxicity to non-target plants.	Plants	Study indicates that glyphosate in plant residues might effects plant fitness.	Supporting	UBA 2
Collin W. et al.	2012	Annual Glyphosate Treatments Alter Growth of Unaffected Bentgrass (*Agrostis*) Weeds and Plant Community Composition	Plants	Study mimicks GR trait, which is not part of the AIRII environmental risk assessment. Nevertheless, measuring community composition over time is important for describing them response of complex agricultural or natural plant communities to annual glyphosate applications.	Supporting	UBA 2
Elisa S.Panigo et al.	2011	Glyphosate-induced structural variations in *Commelina erecta L.* (Commelinaceae)	Plants	The usage of increasing concentrations for full control of plants can have adverse effects on the agroecosystem:(i) it makes the population grow increasingly tolerant over time and (ii) it creates conditions for the onset of glyphosate resistance. GR species are not included in the environmental risk assessment fo AIRII renewal	Low weight	UBA3
K. M. Kilbride, F. L. Paveglio	2001	Long-Term Fate of Glyphosate Associated with Repeated Rodeo Applications to Control Smooth Cordgrass (*Spartina alterniflora*) in Willapa Bay, Washington	Plants	Authors state that long-term detrimental effects to aquatic biota from repeated application of Rodeot for *Spartina* control would be highly unlikely.	Supporting	UBA 2
E J P MARSHALL, et al.	2003	The role of weeds in supporting biological diversity within crop fields	Plants	Authors state thatthe aim must be to strike a balance between adequate weed control, including the prevention of weed seed build-up, and the requirement for some plants to support biological diversity. For some, clean crops and zero tolerance of weeds is the approach, with noncrop areas supporting biodiversity. Approaches need to be based on integrated weed management, although modifications to crop management in selected areas of fields, such as conservation headlands and uncropped wildlife strips (Marshall & Moonen, 2002), may provide sufficient resources for some species of concern.	Critical	UBA1
G.S. Johal*, D.M. Huber	2009	Glyphosate effects on diseases of plants	Plants	Review report illustrated indirect effects of glyphosate on disease predisposition.	Supporting	UBA 2
Helander, M., et al.	2012	Glyphosate in northern ecosystems.	Plants	In the review most of the cited literature was addressed in the literature review submitted with the Annex I Renewal of glyphosate (please refer to table below). Other literature cited in this review was either regarding efficacy of the active substance, transgenic plants (not part of the GAP for review), or previous to the evaluation period (pre-2001).	Supporting	UBA 2
Pfleeger T, et al.	2014	Effects of single and multiple applications of glyphosate or aminopyralid on simple constructed plant communities.	Plants	There was no simple conclusion in terms of potential response to multiple herbicide applications in the present study, because the results varied by species, herbicide, and herbicid concentration.	Supporting	UBA 2
Damgaard, C., et al.	2013	The Effect of Nitrogen and Glyphosate on Survival and Colonisation of Perennial Grass Species in an Agro-Ecosystem: Does the Relative Importance of Survival Decrease with Competitive Ability?	Plants	The proposed method can enable predictions of the effects of agricultural practices on community dynamics in a relatively simple setup.	Supporting	UBA 2
Tesfamariam, T. et al.	2009	Fate of glyphosate stored in weed residues and the potential of phytotoxicity for following crops	Plants	Soil type dependent residual phytotoxicity is related to the detoxification capacity difference between the two soils.	Low weight	UBA3
Akamatsu, F. et al.	2014	Evaluation of glyphosate application in regulating the reproduction of riparian black locust (*Robinia pseudoacacia* L.) after clear-cutting, and the possibility of leaching into soil	Plants	Clear cuts and glyphosate application are not considered to lead to high exposition to the environment. Study is considerd to be relevant for efficacy assessment.	Low weight	UBA3
Egan, J.F. et al.	2013	A comparison of the herbicide tolerances of rare and common plants in an agricultural landscape	Plants	Reproduction could be more sensitive to herbicide pollution than biomass or growth response. The data provided suggest that herbicide pollution may be a factor structuring plant communities.	Low weight	UBA3
Boutin, C. et al.	2013	Herbicide impact on non-target plant reproduction: What are the toxicological and ecological implications?	Plants	Plants should be tested for longer periods (to assess seed output) than currently recommended in guidelines.	Supporting	UBA 2
Allison, J.E. et al.	2013	Influence of soil organic matter on the sensitivity of selected wild and crop species to common herbicides	Plants	In most cases, plants growing in high OM soil were more sensitive to glyphosate than plants grown in low OM soil. IC25 values for the formulation ranged from 17 g a.i /ha to 567 g a.ai /ha.	Supporting	UBA 2
Lyndsay E. Saunders, et al.	2013	Root-Zone Glyphosate Exposure Adversely Affects Two Ditch Species	Plants	Study shows taht high concentrations of comercial glyphosate concentrations relsutling from run off events can effect Non-target terrestrial plants inhabiting semi-aquatic low-lying areas Authors highligt that grass buffer and plant cover zones might contribute to remove herbicidal entry into the environment .	Low weight	UBA3

Ellis, J.M., et al.	2003	Rice (*Oryza sativa*) and corn (*Zea mays*) response to simulated drift of glyphosate and glufosinat	Plants	Visual injury to both rice and corn associated with the lower herbicide, rates in some cases was minimal, but the negative effect on yield was significant. Visual injury alone, therefore, would not be a good indicator of potential yield loss from sublethal rates of glyphosate.	Supporting	UBA 2
Blackburn, L.G., Boutin, C.	2003	Subtle effects of herbicide use in the context of genetically modified crops: A case study with glyphosate (Roundup® (R))	Plants	More powerful experiment with non crop species were conducted later by the author, which might overwrite the present .	Low weight	UBA3
Al-Khatib, K., et al.	2003	Grain sorghum response to simulated drift from glufosinate, glyphosate, imazethapyr, and sethoxydim	Plants	No information about product, no endpoints, field experiment in Canada not reliable for RA	Low weight	UBA3
Felix, J., et al.	2011	Potato Response to Simulated Glyphosate Drift	Plants	Significant effects at concentrations related to predicted driftl concentrations, EC50 values stated for 21DAT.	Supporting	UBA 2
Deeds, Z.A., et al.	2006	Wheat Response to Simulated Drift of Glyphosate and Imazamox Applied at Two Growth Stages	Plants	No EC50 values calculated, but obviously ranging for visual injury between 0,05 and 0.25 of use rate (approx 40 to 210 g/ha).	Supporting	UBA 2
Gilreath, J.P., et al.	2001	Crop injury from sublethal rates of herbicide. I. Tomato	Plants	Field study in Florida with commercial formulation. The objectives were to evaluate the extent of phytotoxic injury and the effect on yield of fresh market tomato exposed at three stages of development to levels of glyphosate known to be sublethal.	Low weight	UBA3
Gove, B., et al.	2007	Effects of herbicide spray drift and fertilizer overspread on selected species of woodland ground flora: comparison between short-term and long-term impact assessments and field surveys	Plants	Relevant for general risk assessment. Authors recommend the adoption of no-spray buffer zones of at least 5 m to protect the majority of woodland species from the impacts of agrichemicals applied to adjacent land.	Supporting	UBA 2
Pfleeger, T., et al.	2008	Effects of low concentrations of herbicides on full-season, field-grown potatoes	Plants	Reproductive responses should be considered in phytotoxicity test protocols for pesticide registration. Low weight for ERA of glyphosate, critical for ERA in general.	Supporting	UBA 2
Pfleeger, T., et al.	2011	Comparing Effects of Low Levels of Herbicides on Greenhouse- and Field-Grown Potatoes (*Solanum Tuberosum* L.), Soybeans (*Glycine Max* L.), and Peas (*Pisum Sativum* L.)	Plants	General consideration for RA that ratio between greenhouse- and field-grown plants to be around 1.8. Results may be more or less sensitive than reality, and more restrictive regulations (safety factors) should be imposed to account for this variability.	Supporting	UBA 2
Nandula, V.K., et al.	2007	Glyphosate-resistant and -susceptible soybean (*Glycine max*) and canola (*Brassica napus*) dose response and metabolism relationships with glyphosate	Plants	Additional information about endpoints for herbicidal product (GR50 non-GR soybean = 0.47 kg/ha, canola= 0.3 kg/ha)	Supporting	UBA 2
Koger, C.H., et al.	2005	Rice (*Oryza sativa*) response to drift rates of glyphosate	Plants	Visual injury was apparent by 7 DAT and was a better parameter than height reduction for confirming glyphosate exposure.	Supporting	UBA 2
Brown, L.R., et al.	2009	Response of Corn to Simulated Glyphosate Drift Followed by In-Crop Herbicides	Plants	Glyphosate drift can result in an additive increase in crop injury from the application of in-crop herbicides in adjacent fields. after glyphosate treatment additional herbicides were used.	Supporting	UBA 2
T. M Webster, et al.	2004	Bermudagrass (*Cynodon* spp) dose–response relationships with clethodim, glufosinate and glyphosate	Plants	IC50 far above the relevant enpoint for ERA, unknown commercial formulations	Low weight	UBA3
Tuffi Santos, LD., et al.	2004	Leaf anatomy and morphometry in three eucalypt clones treated with glyphosate	Plants	Glyphosate altered the thickness and proportion of the leaf blade tissues, with the greatest changes being observed in the palisade parenchyma. Nevertheless, at drift rate plants seem to recover.	Low weight	UBA3
Olszyk, D. et al.	2013	Effects of low levels of herbicides on prairie species of the Willamette Valley, Oregon.	Plants	For the most sensitive species, IC25 values were 22 g a.a/ha to 44 g a.i /ha .	Supporting	UBA 2
Hatterman-Valenti, H.M.	2014	Simulated Glyphosate Drift to Potato Mother Plants and Effect on Daughter Tubers Used for Seed Production	Plants	Potential adverse growth and production in the succeeding potato crop from the contamination of a potato seed crop late in the growing seasons described.	Low weight	UBA3
Hensley, J.B. et al.	2013	Response of Rice to Drift Rates of Glyphosate Applied at Low Carrier Volumes	Plants	Simulated glyphosate drift applications resulted in reduced plant height at harvest and primary and total crop yield losses, with the greatest reduction in primary crop yield resulting from a simulated glyphosate drift application at 108 g/ha.	Low weight	UBA3
Kutman, B.Y. et al.	2013	Foliar Nickel Application Alleviates Detrimental Effects of Glyphosate Drift on Yield and Seed Quality of Wheat	Plants	Glyphosate in this commercial formulation has effects on shoot dry weight of young plants, stem elongation, and grain yield of wheat at drift rates.	Supporting	UBA 2
Cruz-Hipolito, H., et al.	2001	Glyphosate tolerance by *Clitoria ternatea* and Neonotonia wightii plants involves differential absorption and translocation of the herbicide	Plants	Physiological study not relevant in terms of risk assessment.	Low weight	UBA3
McMullin, R.T., et al.	2012	The effects of triclopyr and glyphosate on lichens	Plants	Study describes ecotoxicological side effects towards lichens. Given the important functions of these two lichen species, their sensitivity to herbicide applications is relevant to forest managers.	Supporting	UBA 2

Miteva, L.P.E., et al.	2010	Alterations in glutathione pool and some related enzymes in leaves and roots of pea plants treated with the herbicide glyphosate	Plants	Biochemical study defining the oxidative stress related to mode of action of glyphosate.	Low weight	UBA3
Moldes, C.A., et al.	2008	Biochemical responses of glyphosate resistant and susceptible soybean plants exposed to glyphosate	Plants	The objective of this work was to study biochemical parameters that may be affected in roots and leaves of soybean plants exposed to glyphosate, focusing on the antioxidant response and soluble amino acid content, thus evaluating possible,biochemical markers for differential characterization of glyphosate-resistant and conventional soybean lines.	Low weight	UBA3
Alvarez-Moya, C., et al.	2011	Evaluation of genetic damage induced by glyphosate isopropylamine salt using *Tradescantia* bioassay	Plants	Authors believe that isopropylamine used in commercial farming can induce genetic damage, depending on the dose used and the physiological characteristics of the plants exposed to it.	Supporting	UBA 2
Pline, W.A., et al.	2002	Physiological and morphological response of glyphosate-resistant and non-glyphosate-resistant cotton seedlings to root-absorbed glyphosate	Plants	Even though no endpoints were stated, observed effects on root development are considered to have an effect on seedling emergence.	Supporting	UBA 2
E. Mateos-Naranjo, A. et al.	2013	Effects of sub-lethal glyphosate concentrations on growth and photosynthetic performance of non-target species *Bolboschoenus maritimus*	Plants	In *B. maritimus*, the assessment of toxicity thresholds of experimental glyphosate concentrations presented in nutrient solution ranging between 0 and 30 mg/ L glyphosate indicates that this species has a high tolerance to glyphosate stress, since all plants survived for the full 20 d of treatments. Sublethal effects on root development by difuse pollutions might effect ecosystems.	Supporting	UBA 2
CF Olesen and N.Cedergreen	2010	Glyphosate uncouples gas exchange and chlorophyll fluorescence	Plants	The biomass data were best described with a dose–response model and are the same magnitude as the proposed endpoint for ERA.	Supporting	UBA 2
D.E.Riechers, et al.	1994	Surfactant-Increased Glyphosate Uptake into Plasma Membrane Vesicles Isolated from Common Lambsquarters Leaves	Plants	Experimental set up is currently not relevant for ecotoxicological risk assessment. Glyphosate uptake was stimulated by 0.01% (v:v) MON 0818, the cationic surfacant used in the commercial formulation of this herbicide for foliar application.	Supporting	UBA 2
E. D. NAFZIGER, et al.	1983	Effects of Aspartate and Other Compounds on Glyphosate Uptake and Growth Inhibition in Cultured Carrot Cells'	Plants	Mode of action study	Low weight	UBA3
Boyan D.Dimitrov, et al.	2006	Comparative genotoxicity of the herbicides Roundup, Stomp and Reglone in plant and mammalian test systems	Plants	Roundup did not induce chromosomal aberrations or micronuclei in either test system.	Supporting	UBA 2
Gomes, M.P., et al.	2014	Alteration of plant physiology by glyphosate and its by-productaminomethylphosphonic acid: an overview	Plants	Review articel focuses on photosynthesis, from photochemical events to C assimilation and translocation, as well as oxidative stress and shows that glyphosate has several secondary or indirect effects on plant physiology which may also explain its herbicidal effects.	Supporting	UBA 2
Chauhan, B.S., Abugho, S.B.	2012	Threelobe Morningglory (*Ipomoea triloba)* Germination and Response to Herbicides	Plants	Control of Ipomoea species by glyphosate requires high application rates.	Low weight	UBA3
Banaszkiewicz, T., Wysocki, K.	2012	Application of white mustard (*Sinapis alba*) biotest in the assessment of environmental contamination by glyphosate	Plants	Water contamination did not result in inhibition of germination of white mus tard and chlorophyll "a" fluorescence can provide a good indicator of plant contamination by glyphosate.	Low weight	UBA3
Truta, e. Et al.	2010	Evaluation of roundup-induced toxicity on genetic material and on length growth of barley seedlings	Plants	Infuence on genetic material are not part of he traditional ERA, but can lead to undesired effects of herbicides in general towards plant health.	Low weight	UBA3
Paganelli, a., et al.	2010	Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid	surfactant	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report.	Supporting	UBA 2
Romano, r.m. Et al.	2010	Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels	surfactant	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report. UBA1 for assessment of surfactant effects (POEA	Supporting	UBA 1-2
Romano, m.a., et al.	2012	Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression	surfactant	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report UBA2 for assessment of surfactant effects (POEA)	Supporting	UBA 2
Benachour, n. et al.	2007	Time- and dose-dependent effects of roundup on human embryonic and placental cells.	surfactant	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report. UBA2 for assessment of surfactant effects (POEA)	Supporting	UBA 2
Benachour, n. et al.	2009	Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells	surfactant	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report. UBA2 for assessment of surfactant effects (POEA)	Supporting	UBA2
Gasnier, c., et al.	2009	Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines	surfactant	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report. UBA2 for assessment of surfactant effects (POEA)	Supporting	UBA2
Clair, e., mesnage, r., et al.	2012	A glyphosate-based herbicide induces necrosis and apoptosis in mature rat Testicular cells *in vitro*, and testosterone decrease at lower levels	surfactant	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report. UBA2 for assessment of surfactant effects (POEA)	Supporting	UBA2

Daruich, et al.	2001	Effect of the herbicide glyphosate on enzymatic activity in pregnant rats and their foetuses	surfactant	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report. UBA2 for assessment of surfactant effects (POEA)	Supporting	UBA2
Dallegrave, et al.	2003	The teratogenic potential of the herbicide glyphosate-roundup® in wistar rats	surfactant	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report. UBA2 for assessment of surfactant effects (POEA)	Supporting	UBA2
Dallegrave, et al.	2007	Pre- and postnatal toxicity of the commercial glyphosate formulation in wistar rats	surfactant	These findings suggest that in utero and lactational exposure to glyphosate-Roundup (R) may induce significant adverse effects on the reproductive system of male Wistar rats at puberty and during adulthood. UBA2 for assessment of surfactant effects (POEA)	Supporting	UBA2
Hokanson, r., et al.	2007	Alteration of estrogen-regulated gene expression in human cells induced by the agricultural and horticultural herbicide glyphosate	surfactant	There remains an unclear pattern of very complex events following exposure of human cells to low levels of glyphosate, but events surrounding the altered levels of expression of only three genes – EGR1, CXCL12 and HIF1 – out of the entire battery tested, are both complicated and potentially damaging to adult and fetal cells.	Low weight	UBA3
Mesnage r., et al.	2012	Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity	surfactant	All formulations appeared more toxic than glyphosate, and 3 groups of differentially toxic formulations were experimentally separated according to their concentrations in ethoxylated adjuvants. Ethoxylated adjuvants alone and in formulations appeared as active principles for human cell toxicity. UBA1 for assessment of surfactant effects (POEA)	critical	UBA1
Walsh, l.p. Et al.	2000	Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (star) protein expression.	surfactant	The results indicate that the commercial formulation of the herbicide glyphosate Roundup® might affect reproductive function in animals. UBA2 for assessment of surfactant effects (POEA).	Supporting	UBA2
Mcdaniel, t.v., et al.	2008	Potential endocrine disruption of sexual development in free ranging male northern leopard frogs (*rana pipiens*) and green frogs (*rana clamitans*) from areas of intensive row crop agriculture	surfactant	No relevant for the risk assessment of glyphosate, however the study showed that mixtures of pesticides potentially have endocrine effects in amphibians inhabiting farm ponds and agricultural drains in intensive row crop agriculture.	Low weight	Uba3
Quassinti, l., et al. .	2009	Effects of paraquat and glyphosate on steroidogenesis in gonads of the frog rana esculenta in vitro	surfactant	Glyphosate showed no effect on gonadal steroidogenesis. UBA1 for assessment of surfactant effects (POEA)	critical	UBA1
Moore LJ, et al.	2012	Relative toxicity of the components of the original formulation of Roundup to five North American anurans.	surfactant	The results fully supports previous RMS evaluation. Surfactant MON 0818 containing POEA drives the toxicity of the herbicide formulations containign glyphosatge for amphibians. UBA1, also for assessment of surfactand effects (POEA)	critical	UBA1

Does glyphosate cause cancer?

Preliminary assessment of the carcinogenic risk of glyphosate with regard to the recent IARC evaluation

BfR Communication, 1st April 2015

As ”Rapporteur Member State”, Germany is responsible for the scientific assessment of the active substance glyphosate in the framework of EU re-evaluation. Within Germany the Federal Institute for Risk Assessment (BfR) is the competent authority for the human health risk assessment and is therefore issuing comments on the classification of glyphosate by IARC as "probably carcinogenic to humans" (Group 2A) based on the short report as published in the 20 March 2015 issue of the "Lancet" journal. [1,2]

Preface

The International Agency for Research on Cancer (IARC) is the specialized cancer agency of the World Health Organization (WHO). The main objective of the IARC is to promote international collaboration in cancer research. The evaluations of carcinogenic risk are made by international Working Groups of independent scientists and are qualitative in nature. The *Monographs* Programme provides scientific evaluations based on a comprehensive review of the scientific literature, but it remains the responsibility of individual governments and other international organizations to recommend regulations, legislation, or public health intervention. [1] IARC communications are not understood as basis for regulatory purposes [3].

The established procedure for *Monographs* evaluations is described in the Programme’s Preamble. The *Monographs* represent the first step in carcinogen risk assessment, which involves examination of all relevant information in order to assess the strength of the available evidence that an agent could alter the age-specific incidence of cancer in humans. The *Monographs* may also indicate where additional research efforts are needed, specifically when data immediately relevant to an evaluation are not available. Evaluations are performed by panels of international experts, selected on the basis of their expertise. [1]
For Volume 112, a Working Group of 17 experts from 11 countries met at IARC on 3–10 March 2015 to assess the carcinogenicity of **tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate**. The in-person meeting followed nearly a year of review and preparation by the IARC secretariat and the Working Group, including a comprehensive review of the latest available scientific evidence. [1]

The Working Group classified the herbicide **glyphosate** as "probably carcinogenic to humans" (Group 2A). This category is used when there is *limited evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals. *Limited evidence of carcinogenicity* means that a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence. *Sufficient evidence of carcinogenicity* means, that the Working Group considers that a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. [1]

The classification of glyphosate as "probably carcinogenic to humans" (Group 2A) was published in the 20 March 2015 issue of the “Lancet” journal [2] and is in contrast to other evaluations performed by supranational bodies such as the WHO/FAO-Panel of the Joint Meeting of Pesticide Residues (JMPR, 2004, [4]), or by national regulatory agencies such as the United States Environmental Protection Agency (U.S. EPA, 2012, [5]) that had concluded that glyphosate was not carcinogenic. The database on which the IARC evaluation is based is at present not known, since a background monograph (Volume 112) that is usually produced by IARC following the evaluation meetings has not

yet been released. Therefore, a comprehensive and scientifically sound consideration of the data and arguments that led to the IARC - conclusion is not possible at the moment. However, the aim is to publish the monograph within six months of the Working Group meeting, including concise statements of the principal line(s) of arguments that emerged, the conclusions of the Working Group on the strength of the evidence for each group of studies, citations to indicate which studies were pivotal to these conclusions, and an explanation of the reasoning of the Working Group in weighing data and making evaluations. When there are significant differences of scientific interpretation among Working Group Members, a brief summary of the alternative interpretations is provided, together with their scientific rationale and an indication of the relative degree of support for each alternative.

In the absence of more reliable information from IARC, BfR has tried to allocate the findings that are mentioned in the brief "Lancet" publication [2] to certain studies in our database and, by doing so, to put them into perspective.

(1) First, the new IARC classification for glyphosate as a carcinogenic substance is based on *limited evidence* in humans (see above). This risk is derived from three epidemiological studies conducted in the USA, Canada and Sweden and based on a statistical correlation between the exposure to glyphosate and an increased risk of non-Hodgkin lymphoma (NHL) [2]. These three mentioned studies are not new studies and have been evaluated before. However, in other studies based on data of the very large cohort study, the also cited "Agricultural Health Study" (AHS), no correlation between glyphosate exposure and an increased risk of NHL was observed. The current assessment report (RAR) of the BfR to the EU based on the evaluation of over 30 epidemiological studies and came to the overall conclusion that there is no validated relationship between the exposure to glyphosate and an increased risk of NHL or other types of cancer.
(2) Second, IARC points to findings of studies based on animal experiments submitted by the producers of glyphosate as evidence for the carcinogenic effect of glyphosate. All these studies were also considered in the above mentioned assessment report (RAR) of the BfR, and thus support the conclusion of the Joint Meeting on Pesticide Residues (JMPR) of the WHO/FAO responsible for the assessment of active substances in pesticides: "*In view of the absence of a carcinogenic potential in animals and the lack of genotoxicity in standard tests, the Meeting concluded that glyphosate is unlikely to pose a carcinogenic risk to humans*" [4]. The BfR does not have any information as to how many of the 11 long-term studies on rats and mice that were assessed as valid were available to the IARC.
(3) Third, IARC concluded that a glyphosate formulation promoted skin tumours [2]. In general, testing of formulations should not be used for toxicological evaluation of active substances because co-formulants may alter the outcome to a large extent. This principle is especially important in the case of the assessment of glyphosate because a high number of studies demonstrated a clearly higher toxicity including genotoxicity of glyphosate formulations in comparison with the active substance glyphosate. Therefore, the claim, based on this 2-stage cancer model in mice, that a highly concentrated, skin-irritating formulation containing the active substance promotes skin tumours is not considered by the institutions in the EU to be evidence for the carcinogenic properties of glyphosate.

It is not possible to fully examine the postulated indications for the genotoxic potential of the active substance glyphosate based on the brief report published by IARC [2], in particular due to the fact that the assessment included studies using formulations that are not specified in any detail.

BfR comments on "limited evidence of carcinogenicity" in humans as mentioned by IARC

- **Short evaluation of the crucial studies reported in Volume 3 of the draft Renewal Assessment Report (RAR) of the BfR from December 2014**

De Roos *et al.* (2005, [6]) reported AHS data evaluating glyphosate use and multiple cancer endpoints. No significant association was noted for glyphosate with the studied cancers, including NHL. There is a suggested association mentioned in the abstract with further research required. In an earlier publication based on another data set, however, De Roos *et al.* (2003, [7]) had reported an association between NHL and glyphosate use.

McDuffie *et al.* (2001, [8]) mentioned a non-significant positive association between self-reported glyphosate exposure and NHL in a Canadian study.

Blair *et al.* (2009, [9]), in contrast, did not report an association between glyphosate use and NHL in the AHS.

Hardell and Eriksson (1999, [10]) investigated in a case-control study the incidence of NHL in relation to pesticide exposure in Sweden. 404 cases and 741 controls have been included. The authors discussed an increased risk for NHL especially for phenoxyacetic acids. Glyphosate was included, but only 7 of 1145 subjects in the study gave exposure histories. The authors reported an elevated odds ratio (OR) of 2.3 for glyphosate. This OR was not statistically significant and was based on only 4 "exposed" cases and 3 "exposed" controls.
A further study was submitted by Hardell *et al.* (2002, [11]). This study pools data from Hardell and Eriksson (1999, [10]) with data from Nordström *et al.* (1998, [12]). In multi-variate analyses, the only significantly increased risk was found with a heterogeneous category of "other herbicides" and not for glyphosate.

Eriksson *et al.* (2008, [13]) reported a case-control study which included 910 cases of NHL and 1016 controls living in Sweden. The highest risk was calculated for MCPA. Glyphosate exposure was reported by 29 cases and 18 controls, and the corresponding OR was 2.02.

Fritschi *et al.* (2005, [14]) submitted a case-control study with 694 cases of NHL and 694 controls in Australia. Substantial exposure to any pesticide was associated with an increase of NHL. However, no association between NHL and glyphosate was observed.

In a comprehensive review of the AHS publications, Weichenthal *et al.* (2010, [15]) noted that increased rates of cancers in the studies were not associated with glyphosate use.

Mink *et al.* (2012, [16]) submitted a comprehensive review of all above mentioned epidemiologic studies of glyphosate and cancer. To examine potential cancer risks in humans they reviewed the epidemiologic literature to evaluate whether exposure to glyphosate is associated causally with cancer risk in humans. They also reviewed relevant methodological and biomonitoring studies of glyphosate. The review found non consistent pattern of positive associations indicating a causal relationship between total cancer (in adults or in children) or any site-specific cancer and exposure to glyphosate. However, one of the authors of this paper is a consultant for Monsanto.

Schinasi and Leon (2014, [17]) published the results of epidemiologic research on the relationship between NHL and occupational exposure to pesticides. Phenoxy herbicides, carbamate insecticides, organophosphorus insecticides and lindane were positively associated with NHL. Here, in a meta-analysis based on the studies by Eriksson (2008, [13]) and Cocco *et al*. (2012, [18]), an association between a subtype of NHL (B-cell lymphoma) and glyphosate could be elicited.

18 epidemiological studies did not reveal an association between glyphosate and specific cancer types, which were discussed in detail in the BfR assessment report (RAR) to the EU.

- **Main BfR comment after IARC publication from April 2015 on "limited evidence of carcinogenicity" in humans**

For all epidemiologic studies, BfR has no information on the actual product used. Some of the studies mention "Roundup", but they do not state explicitly if also other glyphosate formulations have been used. Therefore, a possible influence of co-formulants in the formulations on the outcome of the studies cannot be assessed. Based on a comparison of the results of studies with glyphosate and studies with glyphosate formulations in the RAR it is probable that different co-formulants in glyphosate formulations may influence the toxic effects of the glyphosate formulations.
A "biological gradient" (that is higher exposure stronger effect) was only discussed in one paper (McDuffie, 2001, [8]). The OR between glyphosate exposure and occurrence of NHL in all studies is likely to be slightly increased, but not significant in most cases (based on studies with interviews and no measurements of the volume and of glyphosate used). There is certainly no "clear evidence".

Table 1: Summary of the BfR assessment of the epidemiological studies mentioned by IARC.

Short evaluation of the crucial studies in the draft of the Renewal Assessment Report (RAR) of the BfR from December 2014	**Main BfR comment after IARC publication on strength of evidence (none, low, medium, high) based on study type, internal and external validity and estimated effect size**	**Internal validity, such as quality aspects of the study, sample size, measurement biases, statistical uncertainty.**	**External validity & relevance for the BfR assessment: how close is the measured endpoint to the health endpoint of concern;**
De Roos *et al.* (2003, [7]) had reported an association between NHL and glyphosate use.	No unequivocal evidence for causation of NHL by glyphosate based on a pooled analysis of three case control studies in the Midwestern United States (NHL diagnosed between 1979-1986) and reported exposures with 47 pesticides. Logistic regression and hierarchical model provide significant effect (OR, 2.1 with 95% confidence interval (CI) 1.1 to 4.0) and non-significant effect (OR, 1.6 with 95% CI 0.9 to 2.8), respectively, the latter with adjustment for multiple exposures and using prior probability of 0.3 for glyphosate as being carcinogenic. Contrary to common standards, the authors consider the result from the hierarchical model as significant. The description of the study	The internal validity cannot be assessed fully due to limitations in the reporting of the study. The past exposure status for a wide range of pesticides has been assessed in interviews, which is inherently prone to recall and interviewer bias. The study showed four out of 47 pesticides with lower limits of 95% confidence intervals greater than 1.0, indicative for a significant effect. The 47 pesticides may constitute multiple testing so that 5% of effects	The relevance of the study for the current risk question is high. It is not known whether exclusion of females from the study population compromises the applicability of the findings to the general, European population.

	design, analysis and results do not allow assessing methodological quality.	may show up by chance alone. The approximation of the relative risk using the OR is justified for NHL being a rare disease.	
McDuffie *et al.* (2001, [8]) mentioned a non-significant positive association between self-reported glyphosate exposure and NHL in a Canadian study.	OR_{adj} = 1.20 (0.83/1.74): low effect size, not significant; no unequivocal evidence for causation of NHL by glyphosate. Well performed case control study on the male Canadian population from 6 provinces with one of four rare tumours (517 cases, 1506 controls). The study has some limitations typical of a case-control study (recall bias, misclassification of pesticide exposure) and without appropriate adjustment for multiple testing (multiple exposures and multiple endpoints).	Low/medium Multiplicity of pesticide exposure reported, but not the correlations. Tiered approach starting with pesticide classes, but no adjustment for multiple testing (many pesticides, four tumours). While in this publication only NHL is considered, the study was planned and evaluated for four tumours.	Low/medium Should be considered for assessment as it is a well performed study exploring the endpoint NHL, which however is a collection of diseases. The problem of multiple exposures is not easily overcome in reality; therefore it should not be over-stressed.
Eriksson *et al.* (2008, [13]) reported a case-control study which included 910 cases of NHL and 1016 controls living in Sweden. The highest risk was calculated for MCPA. Glyphosate exposure was reported by 29 cases and 18 controls, and the corresponding OR was 2.02.	OR = 2.02 (1.10-3.71) medium effect size, significant; a multivariate analysis gave no significant results. Case control study in 4/7 Swedish regions; all new cases during 29 months. 910 cases, 1016 controls from population registry. The study has some limitations typical of a case-control study (recall bias, misclassification of pesticide exposure) and without appropriate adjustment for multiple testing (multiple exposures).	Low/medium OR values and confidence intervals cannot be reproduced. The reported dependency from use intensity sounds logical but might as well be attributable to reporting bias.	Medium Study reported NHL diagnosis and subtypes according to WHO classification

The studies cited in the brief “Lancet” publication [2] were re-evaluated regarding strength of evidence and validity and there was no unequivocal evidence for a clear and strong association of NHL with glyphosate because of the limitations of these epidemiological studies such as being based on interviews with farmers or family members, the number of cases involved, and no knowledge of the actual amount of glyphosate or the type of glyphosate formula used. Even though the OR for an association between the exposure to glyphosate and NHL was slightly increased in all studies, it was not significant in the McDuffie study [8], significant in the Eriksson study (based on 29 cases) [13] and not unequivocal in De Roos [7] (a further study with data from the AHS in 2005 by De Roos [6] found no clear association between glyphosate and NHL, based on a large number of participating farmers), allowing no solid epidemiological statement on the basis of these three epidemiological studies. The studies need to be put in the context of the other epidemiological and experimental studies undertaken. Probably, further research needs to be carried out to study the usage and the impact of the formulation used in the field situation.

BfR comments on ”sufficient evidence of carcinogenicity” in animals as mentioned by IARC

- Renal tumours in male CD-1 mice

“In male CD-1 mice, glyphosate induced a positive trend of a rare tumour, renal tubule carcinoma. [2]*”*

The finding mentioned by IARC goes apparently back to the study of Knezevich and Hogan (1983, [19]) in which in fact an increase of this tumour was seen. This one was a 2-year feeding study in CD-1 mice (50 per sex and dose) with dietary dose levels of 0, 1000, 5000, and 30000 ppm (equal to a mean daily intake of 157, 814, and 4841 mg/kg bodyweight in males and 190, 955, or 5874 mg/kg bw by females, i.e., including extremely high doses of approximately 5-fold of the limit dose in carcinogenicity testing methods according to the OECD-Guidelines). It had been available for previous EU evaluation (1998–2002) yet and the outcome with regard to kidney tumours was reported as follows:

“In males, the incidence of renal tubule adenoma was marginally increased (0/49, 0/49, 1/50, 3/50 in the control, low, mid and high dose groups, respectively). According to an additional expert statement included in the study report, one tumour of that type was also seen in the male control group. In the absence of any pre-neoplastic kidney lesions suggesting an adverse effect of treatment, this finding was considered spurious.”

Looking at this study once more, the former evaluation can be, in principle, confirmed. The increase in renal tubular tumours at the top dose level was not statistically significant. The additional case in the control group was apparently found during re-examination of the kidneys on request of U.S. EPA in 1985 by an independent pathologist (Marvin Kuschner, M.D., Huntington, New York). According to his re-analysis, there was no evidence of cytotoxic or pre-neoplastic changes. Renal tumours were all of the cortical epithelial type and occurred in one male control animal, one male animal receiving the mid dose and three high dose males. The tumour at the mid and two of the three tumours occurring at the high dose level had been seen at gross pathological examination before yet because they were quite large. In the original study report, all of them had been described as adenoma. Upon re-examination, the pathologist was not able to clearly distinguish if these tumours were rather benign (adenoma) or malign (carcinoma) but for the tumour occurring at the mid dose level, “most evidence of atypicality” was reported. Thus, the statement in the previous EU report that these tumours were adenoma seems equivocal and this perhaps different histological view might explain the IARC opinion that they were rather carcinomas.

On the basis of these tumours in mice, the U.S. EPA originally classified glyphosate as *”possibly carcinogenic to humans”* (Group C) in 1985. After a re-evaluation of that mouse study, the U.S. EPA changed its classification to *”evidence of non-carcinogenicity in humans”* (Group E) in 1991. In 1993, 2-years after the re-examination, the U.S. EPA considered these renal tumours as spontaneous because their occurrence at the high dose level was not statistically significant *(*EPA, 1993 [20]). It was also stated that: *”An independent group of pathologists and biometricians also conducted extensive evaluations of these adenomas and reached the same conclusion.”*

Meanwhile, more long-term studies with glyphosate in mice have become available allowing a better comparison of tumour incidences.

Table 2: Incidences of renal tubule tumours in the four available glyphosate studies in male CD-1 mice.

Study	[redacted], **1983, [19]**	[redacted] **1993, [21]**	[redacted] **[22]**	[redacted] **2009, [23]**
Dose levels	0, 1000, 5000, 30000 ppm	0, 100, 300, 1000 mg/kg bw/d	0, 1600, 8000, 40000 ppm	0, 500, 1500, 5000 ppm
Control	1/49	1/50	0/50	0/51
Low dose	0/49	1/50	0/50	0/51

Mid dose	1/50	0/50	0/50	0/51
High dose	3/50	0/50	2/50	0/51

No renal tubule tumours were seen in female mice in any of these studies.

From a today's perspective, it may be added that this type of tumour was seen in very few animals only in two of three further studies in male CD-1 mice and, in one of them, again, also among the control animals. One might suspect also an increase in the study by [redacted] [22] employing an extremely high dose of 40000 ppm (corresponding to a mean daily dose of 4348 mg/kg bw, i.e., similar to that one in the study by [redacted] [19]) but, again, the incidence was low and the difference to the control group did not gain statistical significance. Thus, usually one would not expect classification and labelling of a substance for these findings because there is no convincing evidence of a relation to treatment. On the other hand, a high dose phenomenon cannot be completely excluded. The extremely high doses of 30000 or 40000 ppm had not been employed in the studies by [redacted] (1993, [21]) in which the highest dose of 1000 mg/kg bw/day can be expected to be equivalent to a dietary level of perhaps 10000 ppm or by [redacted] (2009, [23]) with even "only" 5000 ppm being the top dose level. It should be taken into consideration that the systemically absorbed amount of glyphosate (estimated 20%) is virtually completely excreted in the urine. Thus, if an animal is continuously administered an exaggerated dose of such a substance for 18 months or 2 years, the kidney has to deal with a permanent burden. Even if these tumours were somehow related to glyphosate administration, they would not be relevant for man who might be reasonably expected to receive much lower doses via their diet. The high doses in the studies by [redacted] (1983, [19]) and by [redacted] (1997 [22]) were more than 8000 times higher as the ADI of 0.5 mg/kg bw. Based on a recent analysis of glyphosate measurement in human urine samples (Niemann *et al*., 2015, [24]) a daily intake of more than 1% of the ADI by consumers is very unlikely resulting in an even higher **margin of safety of more than 800000**. Taking into account that tumours occurred in long-term studies in mice with continuous exposure to such high doses, any risk for humans can be reasonably excluded.

- Haemangiosarcoma in male mice

"A second study reported a positive trend for haemangiosarcoma in male mice. [2]*"*
Here, it seems clear that the 2-year feeding study by [redacted]. (1993, [21]) is the source of this information because reference to the 2004 WHO/FAO (JMPR, [4]) evaluation was made and this was the only long-term study in mice that had been taken into consideration by JMPR. In fact, in the JMPR report, a slightly higher incidence of haemangiosarcoma in male mice is mentioned but was considered not treatment-related. In the report, the following is stated: *"Owing to the lack of a dose-response relationship, the lack of statistical significance and the fact that the incidences recorded in this study fell within the historical ranges for controls, these changes are not considered to be caused by administration of glyphosate."*
This study had been also used in the previous EU evaluation of glyphosate (1998-2002) but its description was rather brief and tumours of the vascular system were apparently not discussed as a possibly treatment-related effect. The same is true for the current EU evaluation.

The actual incidences of haemangiosarcoma in three of the four available studies in CD-1 mice are given in Tables 3 and 4 for males and females, respectively. In the oldest study by [redacted] (1983, [19]), haemangiosarcoma was not listed as a particular histopathological entity. It is not known which of the studies have been available to IARC.

<u>Table 3:</u> Occurrence of haemangiosarcoma in three available glyphosate studies in male CD-1 mice.

Study	[redacted] **1993, [21]**	[redacted] **1997, [22]**	[redacted] **2009, [23]**
Dose levels	0, 100, 300, 1000 mg/kg bw/d	0, 1600, 8000, 40000 ppm	0, 500, 1500, 5000 ppm
Control	0/50	0/50	0/51

Low dose	0/50	0/50	0/51
Mid dose	0/50	0/50	0/51
High dose	4/50	2/50	0/51

<u>Table 4:</u> Occurrence of haemangiosarcoma in three available glyphosate studies in female CD-1 mice.

Study	**[redacted] 1993, [21]**	**[redacted] 1997, [22]**	**[redacted]., 2009, [23]**
Dose levels	0, 100, 300, 1000 mg/kg bw/d	0, 1600, 8000, 40000 ppm	0, 500, 1500, 5000 ppm
Control	0/50	0/50	1/51
Low dose	2/50	0/50	1/51
Mid dose	0/50	2/50	0/51
High dose	1/50	0/50	1/51

From the two Tables, it is obvious that this tumour of the vascular system may be found in both sexes and at all dose levels at a low incidence. At least for the studies by [redacted] (1997, [22]) and [redacted]. (2009, [23]), it was detected either in the skin or in spleen.
There was no evidence of a possibly treatment-related increase in females in any of the studies. In males, in contrast, haemangiosarcoma was seen in two studies at very high dose levels but not in the controls or at lower dose levels. This finding was also not reported by [redacted] (2009, [23]) but, in this study, the dose levels were not as high as in the studies by [redacted] (1993, [21]) or by [redacted] (1997, [22]). Thus, as for the renal tumours discussed above, a "high dose phenomenon" cannot be excluded. On the other hand, the increases were not statistically significant. According to the JMPR evaluation, they were within the historical control range [4].
Indeed, in the study report of [redacted] (1993, [21]), the following control incidences may be found:
Based on six long-term studies, the incidences in haemangiosarcoma in untreated males varied from 0/50 to 4/50. Thus, the actual study incidence at the top dose level was at the upper edge of this range. In females, control incidence was lower ranging from 0/50 to 2/50, also covering the findings in the study with glyphosate. However, the information given in that study is not sufficient to current standards because it is not reported when these studies were performed.
For the study by [redacted] (1997, [22]), no historical data for haemangiosarcoma have been submitted and were not requested by the RMS since the increase was not statistically significant.

Thus, most probably, classification and labelling of glyphosate for carcinogenicity would be unlikely if the CLP criteria were applied. It cannot be said with certainty if the haemangiosarcoma in mice were treatment-related or not. In any case, as outlined above for renal tumours, the margin of safety to expected human exposure would be so extremely wide that no risk is anticipated.

<u>Further considerations on carcinogenicity of glyphosate in the mouse:</u>

In the current re-evaluation of glyphosate in the EU, Germany, acting as the RMS, suggested for glyphosate "*very limited evidence of carcinogenicity*" in mice without a need for classification and labelling. The basis for this proposal was a higher incidence in malignant lymphoma in Swiss mice, i.e., a strain with a high background incidence ([redacted] 2001, [25]). Since this study was apparently not available to IARC (otherwise, it would have been certainly used as the first place argument for carcinogenicity of glyphosate), the incidences of this tumour in all five valid long-term studies in mice is given in Table 5 (copied from Volume 1 of the RAR [26] that had been available for Member States and public consultation in 2014 yet, with slight modifications).

<u>Table 5:</u> Total incidence of malignant lymphoma in long-term studies with glyphosate in different mouse strains.

Study, Strain		Males				Females			
[redacted] 2009, [23] Crl:CD-1 (ICR) BR	Dose (ppm)	0	500	1500	5000	0	500	1500	5000
	Affected	0/51	1/51	2/51	5/51	11/51	8/51	10/51	11/51
[redacted] 2001, [25] HsdOLA:MF1 (Swiss albino)	Dose (ppm)	0	100	1000	10000	0	100	1000	10000
	Affected	10/50	15/50	16/50	19/50*	18/50	20/50	19/50	25/50*
[redacted] 1997, [22] Crj:CD-1 (ICR)	Dose (ppm)	0	1600	8000	40000	0	1600	8000	40000
	Affected	2/50	2/50	0/50	6/50	6/50	4/50	8/50	7/50
[redacted] 1993, [21], CD-1 (not further specified)	Dose (mg/kg bw/d)	0	100	300	1000	0	100	300	1000
	Affected**	4/50	2/50	1/50	6/50	14/50	12/50	9/50	13/50

* increase statistically significant, for females based on percentage and not on total number of affected mice
** based on histological examination of lymph nodes with macroscopic changes

Apart from the statistically significant increase in Swiss mice, a higher number of affected top dose males was also seen in two other studies ([redacted] 1997 [22] and [redacted] 2009 [23]) but was contravened later by historical control data.
The resulting assessment by the RMS was as follows (copied from the revised RAR, Volume 1, April 2015):

"A treatment-related effect in the study by [redacted] (2001, [25]) in Swiss albino mice cannot be completely excluded. However, the weak increase in malignant lymphoma even over the historical control of the performing laboratory was clearly confined to this single study and strain since it was not reproducible in four other valid long-term studies. Thus, there is only very limited evidence of a carcinogenic potential of glyphosate as a high-dose phenomenon in mice of a susceptible strain. Perhaps, age-related neoplastic changes might be exacerbated by long-lasting administration of high doses. Swiss albino mice with high background prevalence of malignant lymphoma could be more vulnerable than other strains.

Since the more frequent occurrence of malignant lymphoma was confined to a very high dose level that was administered over a long period, glyphosate is considered unlikely to pose a carcinogenic risk in humans. Classification and labelling for carcinogenicity is not considered appropriate by the RMS because of the following considerations:

(1) The presumed effect was observed in only one of five long-term studies in mice in a strain with a rather high background incidence of malignant lymphoma. Taking into account the huge amount of information on historical control incidences, there was no evidence of a similar effect in any other study.
(2) Although the increase in lymphoma incidence in the study by [redacted] (2001, [25]) was statistically significant in both sexes, it was still within the (small) historical control range of the performing laboratory for females. No evidence of a similar effect in female mice was obtained in any other study.
(3) No evidence of carcinogenicity with a higher lymphoma incidence was obtained in a total of six valid 2-year studies in rats in which sufficiently high dose levels were employed.

(4) The high dose with a significantly higher lymphoma incidence (1460 mg/kg bw/day) is above the limit dose for carcinogenicity testing according to OECD-Guidelines and more than 2900 times higher than the proposed ADI and the margin to the expected consumer exposure is even wider."

The same arguments are applicable with regard to both renal tumours and haemangiosarcoma under the worse-case assumption that they were in fact treatment-related. Thus, to a certain extent, both tumour types in mice mentioned by IARC might support this assessment.

- Pancreatic islet-cell adenoma in male rats

"Glyphosate increased pancreatic islet-cell adenoma in male rats in two studies. [2]*"*
Evidence of pancreatic tumours was first obtained in a study by [redacted] in 1990 [27]. In this study, a large variation in the incidence of islet-cell adenoma without a clear dose-response was observed in both sexes. However, it was noted that the incidence in all treated male groups was markedly higher than in the concurrent control group (Table 6). In the low and high dose groups, the incidence was above the upper edge of the laboratory's historical control range (8.5%). In the literature, according to JMPR (2004, [4]) higher control incidences of up to 17% were mentioned. The JMPR (2004, [4]) considered these tumours spontaneous in nature because of the lacking dose response and, accordingly, missing statistical significance in the Peto trend test. Furthermore, the detection of the only carcinoma in the male control group was considered not to support the assumption of a substance-related effect because there was no neoplastic progression.
For similar reasons, U.S. EPA (1993, [20]) regarded the pancreatic tumours as not treatment-related. Also for the first EU evaluation (1998–2002), this study had been available yet. The pancreatic tumours were disregarded because of the lacking dose response and because the incidences were all within the historical control data from the literature. Furthermore, there was no evidence of pre-neoplastic changes such as hyperplasia. In the current re-evaluation of glyphosate in the EU, the pancreatic tumours had not been addressed again.

<u>Table 6:</u> Incidence of islet-cell tumours in the pancreas of male and female rats in the glyphosate study by [redacted] (1990, [27]).

Sex/Dose	Control	2000 ppm (89/113 mg/kg bw/day in m/f)	8000 ppm (363/457 mg/kg bw/day in m/f)	20000 ppm (940/1183 mg/kg bw/day in m/f)
Males	1*/58	8/57**	5/60	7/59
Females	5/60	1/60	4/60	0/59

*carcinoma; **p<0.05

It was first not clear which one might be the second long-term study in rats with a higher incidence of pancreatic tumours in males as mentioned by IARC [2]. For its identification, the incidences from all valid 2-year studies on rats (apart from that one by [redacted] 1990, [27]) and that one from the very early study by [redacted] (1981, [28]) have been tabulated in Table 7. The latter study is now considered in the EU not acceptable, due to reporting deficiencies and since the dose levels employed were much too low.

<u>Table 7:</u> Pancreatic islet-cell tumours in long-term studies with glyphosate in male rats.

Study	Control	Low dose	Mid dose	Second mid dose	High dose
[redacted] 2009, [23]	4/51	1/51 (1500 ppm)	2/51 (5000 ppm)	-	1/51 (15000 ppm)
[redacted] 2001, [29]	1/53	2/53 (2000 ppm)	0/53 (6000 ppm)	-	1/52 (20000 ppm)
[redacted] 1997, [30]	4/50	1/50 (3000 ppm)	2*/50 (10000 ppm)	-	1/50 (30000 ppm)
[redacted] 1996, [31]	3/48	0/30 (100 ppm)	0/32 (1000 ppm)	-	1/49 (10000 ppm)

[redacted]., 1993, [21]	7/50	1/24 (10 mg/kg bw/day)	2/17 (100 mg/kg bw/day)	2/21 (300 mg/kg bw/day)	1/49 (1000 mg/kg bw/day)
[redacted] 1981, [28]	0/50	5/49 (3 mg/kg bw/day)	4/50 (10.3 mg/kg bw/day)	-	3*/50 (31.5 mg/kg bw/day)

*including one carcinoma

Based on this comparison, it seems that the study by [redacted] (1981, [28]) may be the second one suggesting an effect on the frequency of pancreatic neoplasia in treated rats although considered by IARC, but there was no dose response relationship. Furthermore, it is very unlikely that such an effect would not have been observed in more recent studies that were performed according to modern standards at much higher dose levels.

When these five other valid studies are taken into consideration, a remarkable variability in the incidence of pancreatic islet cell tumours becomes obvious but no increase was observed. In addition, the pancreas was never mentioned as one of the target organs of glyphosate-related toxicity.

Thus, based on weight of evidence and taking into account the uncertainties with regard to lacking dose response in the studies by [redacted] (1981, [28]) and by [redacted] (1990, [27]) as well as the low reliability of the [redacted] study [28], an effect of glyphosate on islet cell tumours of the pancreas in rats is unlikely.

- Promotion of skin tumours in mice by a formulation

"A glyphosate formulation promoted skin tumours in an initiation-promotion study in mice. [2]*"*
In general, testing of formulations should not be used for toxicological evaluation of active substances because co-formulants may alter the outcome very much.
Most likely, IARC used a publication that is known also from the re-evaluation of glyphosate in the EU. In the following, the RMS evaluation of this study is given (copied from Volume 1 of the RAR):
"George et al. (2010, [32]) used a 2-stage cancer model in mice to evaluate a glyphosate formulation for tumour promotion. A known tumour promoter, 12-o-tetradecanoyl-phorbol-13-acetate (TPA) was used as a positive control and for comparison with glyphosate effects after exposure to a tumour initiator, 7,12-dimethylbenz[a]anthracene. Proteomics were later applied to extrapolate a basis for glyphosate formulation tumour promotion. The results are considered by the authors to indicate a tumour promoting potential of glyphosate. However, the formulation Roundup was used in the study and not the active substance glyphosate. Furthermore, the up- and down-regulation of protein expression is not sufficient to prove a carcinogenic effect."
It must be emphasised that formulations under the trade mark Roundup often contain strongly irritating surfactants that may have contributed to the outcome.

General comments on the methodology and the approach taken by RMS

The general principles for methodology and the approach taken by RMS are described in the BfR Guidance Document for Health Assessments which can be downloaded from the following address: http://www.bfr.bund.de/cm/364/guidance_document_for_health_assessments.pdf.

This health evaluation of glyphosate is based on the following sources:

- Toxicological and ADME studies that were submitted by the Glyphosate Task Force (GTF) for this re-evaluation.
- Toxicological studies and ADME studies that had been reported in the previous DAR (1998, [33]) already and, thus, were part of previous EU evaluation. However, they were subject to re-assessment by the RMS according to current quality standards and were used only when regarded as acceptable or at least supplementary. In very few cases, NOAELs/LOAELs were revised. Unacceptable (old or new) studies were usually deleted with justifications given in the respective sections of Volume 3. In exceptional cases, such studies are still mentioned, i.e., if they were formerly taken into consideration for, e.g., ADI setting.
- Scientific publications and other relevant information that were submitted either by the GTF or by third parties or of which the RMS was aware before. It must be emphasised that a large part of the publications was on formulations different from the representative one and, thus, is of limited value for the toxicological evaluation of the active ingredient. With rather few exceptions in the areas of genotoxicity and human data, mainly scientific literature published since 2000 was assessed.

Due to the large number of submitted toxicological studies, the RMS was not able to report the original studies in detail and an alternative approach was taken instead. The study descriptions and assessments as provided by the GTF were amended by deletion of redundant parts (such as the so-called ”executive summaries”) and new enumeration of Tables. Obvious errors were corrected. Each new study was commented by the RMS. These remarks are clearly distinguished from the original submission by a caption, are always written in italics and may be found on the bottom of the individual study summaries.

Furthermore, in Volume 3, an assessment was performed on the individual study level. Overall evaluation of the diverse toxicological endpoints was transferred into Volume 1 (section 2.6).

The technical databases that have been used for the literature search include: Web of ScienceSM, BIOSIS Previews®, CAB Abstracts® (CABI), MEDLINE®, and CA Plus (Chemical Abstracts Plus). The searches were made on glyphosate acid, glyphosate salts (including isopropyl amine, potassium, ammonium, and methylamine), and AMPA, and their related chemical names and CAS numbers. Searches based on these search terms were also found suitable to identify publications that consider glyphosate and surfactants (such as polyoxyethylenealkylamines, or POEA) in the context of glyphosate formulations.

Additional publications cited in a recent document prepared by the NGO ”Earth Open Source” (Antoniou, *et al*., 2011, [34]) have also been included in the literature review.

The peer-reviewed publications identified for inclusion during the literature search were reviewed and classified into one of the categories listed below:

- **Category 0 publications:** These are publications in which glyphosate is only mentioned as an example substance or is discussed/studied in a context that is not relevant or related to any of the regulatory sections or the exposure/hazard assessments within this submission; the publication is therefore outside of the scope of this submission.
- **Category 1 publications:** These are publications which discuss glyphosate in a context relevant or related to the regulatory dossier sections and the conclusions fall within the

conclusions of the exposure/hazard assessment. The publication is submitted with minimal or no comment or discussion.

- **Category 2 publications:** These are publications which discuss glyphosate in a context relevant or related to the regulatory dossier sections and have conclusions that call into question the endpoints/conclusions in the exposure/hazard assessment. Additionally, Category 2 also includes publications with conclusions that support the risk/hazard assessment, and may be included in discussion of other relevant publications. For selected Category 2 publications, an OECD Tier-II type summary is provided in addition to a reliability assessment (Klimisch rating, see Klimisch *et al*. 1997, [35]); limited comments and critical remarks are provided, as appropriate.
- **Category 3 publications:** These are publications that discuss glyphosate in a context relevant or related to (1) non-regulatory endpoints that need to be addressed as per new Regulation (EC) 1107/2009; or (2) in a context relevant to sensitive allegations that have emerged or could emerge in the media; or (3) in a context relevant to the regulatory dossier sections and have conclusions that are in disagreement with endpoints/conclusions in the exposure/hazard assessment (although the experimental design seems relevant at first glance). An OECD Tier-II type summary is provided and a Klimisch rating assigned, and supplemented with critical review and discussion.
- **Category 'E' publications:** These are peer-reviewed publications that were cited in the Earth Open Source document. This category includes publications that were already captured by the literature search and are addressed within the appropriate discipline, as well as publications that were out of scope of the search (primarily as a result of being published prior to 2001). Publications already captured in the literature search were assigned a Category 1, 2 or 3 rating (as appropriate) in addition to a Category 'E' rating. An OECD Tier-II type summary has been prepared and a Klimisch rating assigned for each of the Category 'E' publications. All Category 'E' publications are reviewed within the appropriate discipline, with most of the reviews provided within the toxicology dossier under Section IIA 5.10.

A full description of the literature search methodology was provided by the GTF in a separate document (Carr and Bleeke, 2012, [36]). Five separate publication subject areas are addressed in the literature review:

1. Developmental and Reproductive Toxicity (DART) and Endocrine Disruption (ED)
2. Neurotoxicity
3. Carcinogenicity
4. Genotoxicity
5. Category E and other publications

The publications on subject areas 1-4 are presented in the chapters on Genotoxicity, Long Term Toxicity and Carcinogenicity, Reproductive Toxicity and Neurotoxicity of the report. Furthermore, publications are presented in the chapters "Further toxicological studies" and "Medical data". Important publications are presented in summaries as quoted from the articles followed by Klimisch ratings and by RMS comments on the paper.

In the process of public consultation after the submission of the first draft of this RAR PAN-Europe, PAN-Germany and PAN-UK conducted a PubMed literature search on the keywords 'glyphosate' and 'toxicity' and stated they got significant differences in comparison with the literature search conducted by the notifiers. The GTF repeated the PubMed search on June 11, 2014, using the same keywords (Glyphosate Task Force, 2014, [37]).
Overall, a total of 504 articles were identified in the search. Of those, 349 were from the time period of 2001 to 2012, and thus were considered relevant to the glyphosate submission, and were further evaluated as to whether or not they were included in either the original literature search, included in the May 2012 submission, or as part of the ongoing update of the search, as of the time of June 11 PubMed search. There were 266 reviewed for the submission (222 were included), with an additional 34 reviewed after the submission (29 selected for submission). Of the 49 remaining articles, 43 were considered to be not relevant based on the subject of the article (the majority were either on genetically

modified crops, efficacy or weed resistance). The remaining 6 were added to the literature review, and of those 4 were considered to be relevant and were selected for submission in the update.

Thus, of the 349 articles identified in the search, only 4 were determined to be relevant and were not already identified in the GTF literature search process.

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Volume 4
General information

Annex C

Summary, Scientific Evaluation and Assessment

RMS: Germany **Co-RMS: Slovakia**

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Volume 4

Annex C

Summary, Scientific Evaluation and Assessment

RMS: Germany **Co-RMS: Slovakia**

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Volume 4 submitted by	Document
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Cheminova	Glyphosate_RAR_22_Volume4_Cheminova_2015-01-29.doc
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Helm AG	Glyphosate_RAR_22_Volume4_Helm_2015-01-29.doc
Monsanto Europe S.A.	Glyphosate_RAR_22_Volume4_Monsanto_2015-01-29.doc
Nufarm GmbH & Co. KG	Glyphosate_RAR_22_Volume4_Nufarm_2015-01-29.doc
Rotam Agrochemical Europe Ltd.	Glyphosate_RAR_22_Volume4_Rotam_2015-01-29.doc
Sabero Europe B.V.	Glyphosate_RAR_22_Volume4_Sabero_2015-01-29.doc
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United Phosphorus Ltd. / Cerexagri s.a.s	Glyphosate_RAR_22_Volume4_UPL_2015-01-29.doc
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Renewal Assessment Report

31 August 2015

Glyphosate
Addendum 1 to RAR

Assessment of
IARC Monographs
Volume 112 (2015):
Glyphosate

RMS: Germany

Version history (Addendum 1)

Date	**Reason for revision**
31 August 2015	First Draft of Addendum 1

Preface

In February 2015 a revised health risk assessment report on glyphosate prepared by the Federal Institute for Risk Assessment (BfR) was discussed at the expert meeting of the European Food Safety Authority (EFSA). Subsequently, the report was amended by the BfR. This revision comprised additional evaluation tables as well as additional amendments for more clarification on some factual matters. On 1 April 2015 BfR sent this supplemented and revised version of the report to the Federal Office of Consumer Protection and Food Safety (BVL) for forwarding to EFSA.

The International Agency for Cancer Research (IARC) of the World Health Organization (WHO) evaluated glyphosate as "probably carcinogenic to humans (Group 2A)", based on the available and evaluated studies by IARC. The full report on glyphosate from the IARC monograph (Volume 112) has been publicly available since 29 July 2015.

As Rapporteur Member State (RMS) for the European renewal of approval of glyphosate, Germany was commissioned by EFSA to evaluate the IARC Monographs Volume 112 on glyphosate by 31 August 2015, so that this scientific analysis could be included in the renewal process of the active substance glyphosate. Once this addendum has been subjected to a consultation process with the other Member States and a subsequent discussion in a separate Expert Meeting of EFSA at the end of September 2015, the results of this Addendum may be considered in the "EFSA Conclusion on the peer review of the pesticide risk assessment" of glyphosate.

Abstract

Based on the studies on cancer in humans IARC concluded: „*There is limited evidence in humans for the carcinogenicity of glyphosate*". The Rapporteur Member State (RMS) agrees with IARC that the other IARC categories are not suitable for the classification of the evidence from studies in humans. **The evaluation of the epidemiological studies by the RMS is comparable to IARC. However**, RMS adopts a more cautious view since **no consistent positive association was observed**, and the most powerful study showed no effect. The IARC interpretation is more precautionary. It was also noted that **in the epidemiological studies a differentiation between the effects of glyphosate and the co-formulants is not possible.**

Based on carcinogenicity studies in experimental animals IARC concluded that glyphosate induced a positive trend in the incidence of rare renal tumours; a positive trend for haemangiosarcoma in male mice and increased pancreatic islet-cell adenoma in male rats in two studies, and therefore: „*There is sufficient evidence in animals for the carcinogenicity of glyphosate*". A much larger number of animal studies have been performed to evaluate the carcinogenic potential of glyphosate than necessary by the legal requirements. In mice, a total of five long-term carcinogenicity studies using dietary administration of glyphosate were considered. In rats, seven chronic toxicity and carcinogenicity studies using dietary administration of glyphosate and two studies with application via drinking-water were reviewed.

- Renal tumours:

  In two studies in CD-1 mice and one study in Swiss albino mice, the statistical analysis with the Cochran-Armitage test for linear trend yielded a significant result, whereas the analysis by pair-wise comparisons indicated no statistically significant differences between the groups and the incidences were within the historical control range of up to 6% for adenoma and carcinoma combined. A confounding effect of excessive toxicity cannot be excluded at the highest doses of 1460 - 4841 mg/kg bw/d. In both studies in CD-1 mice, but not in Swiss albino mice, the body weight gain was decreased by more than 15% compared to controls, but mortality/survival was not affected.

- Haemangiosarcoma:

  In two studies in CD-1 mice, the incidences of haemangiosarcoma in male mice were reconsidered for statistical evaluation. For both studies, the statistical analysis with the Cochran-Armitage test for linear trend yielded a significant result, whereas the analysis by pair-wise comparisons indicated no statistically significant differences between the groups. The background incidences for haemangiosarcoma in male CD-1 mice were up to 12% if multiple organs were considered. Therefore, the observed incidences for haemangiosarcoma were spontaneous and unrelated to treatment.

- Pancreatic and other tumours:

  The statistically significant increase in pancreatic tumours incidences in the male rats of the low dose groups are considered incidental. With regard to the positive trend for liver cell adenoma in male rats and thyroid C-cell adenoma in female rats for the study of Stout and Ruecker, IARC also noted a lack of evidence for progression.

- Malignant lymphoma:

  IARC also considered a review article containing information on five long-term bioassay feeding studies in mice, in which a statistically significant increase in the incidence of malignant lymphoma was reported, but the Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information. In three studies in CD-1 mice, the incidences of malignant lymphoma in male mice were reconsidered for statistical evaluation by the RMS. For two studies, the statistical analysis with the Cochran-Armitage trend test yielded a significant result, whereas the analysis by pair-wise comparisons indicated no statistically significant differences between the groups for all three studies. The incidences observed in the above studies, with a maximum of 12%, were all within the historical control range. Therefore, the observed malignant lymphomas were spontaneous and unrelated to treatment.

For an overall conclusion, the large volume of animal data for glyphosate has been evaluated using a weight of evidence approach. It should be avoided to base any conclusion only on the statistical significance of an increased tumour incidence identified in a single study without consideration of the biological significance of the finding. In summary, based on the data from five carcinogenicity studies in mice and seven chronic toxicity and carcinogenicity studies in rats, **the weight of evidence suggests that there is no carcinogenic risk related to the intended herbicidal uses and, in addition no hazard classification for carcinogenicity is warranted for glyphosate according to the CLP criteria.**

Based on the mechanistic and other studies, IARC concluded: „*There is mechanistic evidence for genotoxicity, oxidative stress, inflammation, immunosuppression, receptor-mediated effects, and cell proliferation or death of glyphosate*". Glyphosate has been tested in a broad spectrum of mutagenicity and genotoxicity tests *in vitro* and *in vivo*. **Taking into account all available data and using a weight of evidence approach, it is concluded that glyphosate does not induce mutations *in vivo* and no hazard classification for mutagenicity is warranted according to the CLP criteria. In the absence of sufficient evidence for a carcinogenic risk related to the intended herbicidal uses the mechanistic and other studies do not provide further evidence for a carcinogenic mechanism.**

AMPA has been tested for mutagenicity and genotoxicity *in vitro* and *in vivo* in an adequate range of assays. Taking into account all available data and using a weight of evidence approach, it is concluded that AMPA does not induce mutations *in vivo* and no hazard classification for mutagenicity is warranted according to the CLP criteria.

Glyphosate-based formulations have been extensively tested for mutagenicity and genotoxicity *in vitro* and *in vivo* in a wide range of assays. However, since formulation compositions are considered proprietary, the specific composition of the formulations tested was not available for the published studies. Positive results from *in vitro* chromosomal damage assays and tests for DNA strand breakage and SCE induction were reported in published studies. For specific glyphosate-based formulations, *in vivo* mammalian chromosomal aberration or micronucleus assays as well as tests for DNA adducts, DNA strand breakage and SCE induction gave positive results in some published studies. However, no

regulatory studies for these endpoints were provided. Thus, **for the different glyphosate-based formulations, no firm conclusions can be drawn with regard to a need for classification according to the CLP criteria.**

Considering the low level of metabolism and the chemical structure of glyphosate, glyphosate radical formation initiating oxidative stress appears unlikely. However, uncoupling or inhibition of mitochondrial oxidative phosphorylation also represents an established mechanism for ROS generation. Notably, uncoupling of oxidative phosphorylation by glyphosate has been reported in rat liver microsomes and a glyphosate formulation. Induction of oxidative stress can provide a mechanistic explanation for any observed cytotoxic/degenerative and indirectly genotoxic effects of substances. However, from the sole observation of oxidative stress and the existence of a plausible mechanism for induction of oxidative stress through uncoupling of mitochondrial oxidative phosphorylation alone, genotoxic or carcinogenic activity in humans cannot be deduced for glyphosate and glyphosate based formulations. Furthermore, the RMS concludes that the evidence from available data does not allow the conclusion that glyphosate caused immunosuppression. However it is to note that due to the small number of studies assessed and the fact that all studies show limitations, no robust information is available to conclude on the immunomodulatory action of glyphosate.

Glyphosate was included into the U.S. EPA Endocrine Disruptor Screening Program's (EDSP). It was concluded that, based on the Tier 1 assays that had been performed at different independent laboratories and taking into account the 'higher tier' regulatory safety studies, glyphosate should not be considered an endocrine disrupter or to have other receptor-mediated effects. Information on apoptosis and proliferation in cell systems from humans and mice was reported, but this was not considered as additional mechanistic evidence for carcinogenicity of glyphosate.

Results of four occupational and two para-occupational studies using various glyphosate-containing plant protection products have been evaluated in the International Agency for Cancer Research (IARC) monograph, which were carried out between 1988 and 2007 in different countries of North America and Europe. The recorded exposure values in these studies were below or in the same order of magnitude as those predicted in the Renewal Assessment Report (RAR). For resources on dietary exposure and for results on biological markers IARC refers to several selected reports from national food- and bio-monitoring programmes as well as to some studies in the public literature. With respect to exposure, no relevant deviating conclusions between the RAR and IARC were identified.

In addition, the RMS strongly recommends further genotoxicity studies in compliance with OECD test guidelines in general and for the representative formulation as confirmatory information for the authorisation of plant protection products.

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1 Exposure Data

1.1 Identification of the agent

The information reported in the sections 1.1.1 - 1.1.4 of the IARC Monographs Volume 112 (2015, ASB2015-8421) is generally summarized in line with the information in the cited references and with the information given in the RAR (2015, ASB2015-1194). Regarding section 1.1.4 it is noted that a different specification was derived by the RMS than by FAO (2000, ASB2015-8587). In summary, these sections appear to be an appropriate summary of the available knowledge on glyphosate.

1.2 Production and use

1.2.1 Production

1.2.1.1 Manufacturing process

In the IARC Monographs Volume 112 (2015, ASB2015-8421) it is stated that: "*To increase the solubility of technical-grade glyphosate acid in water, it is formulated as its isopropylamine, monoammonium, potassium, sodium, or trimesium salts*".

The manufacture and use of different active substance variants is not a glyphosate-specific feature; it is a common issue for many active substances. This circumstance has to be considered in the zonal/national authorisation procedure of the plant protection product. Thus, for the evaluation and assessment of the toxicological properties of active substance variants differently from the representative variant in the Annex I renewal, further studies may therefore be required for a bridging between the different variants of active substances on Member State level.

1.2.2 Uses

1.2.2.1 Agriculture

In the IARC Monographs Volume 112 (2015, ASB2015-8421) it is stated that: "*Common application methods include broadcast, aerial, spot and directed applications (EPA, 1993a).*" It should be noted that within the European Union, applications of plant protection products by aircraft are generally prohibited according to Directive 2009/128/EC (2009, ASB2015-8588). Only very few exceptions, for which it has to be applied particularly, can be granted, if no other effective method of pest control is available, e.g. for applications in the forest or on steep slopes in viticulture in Germany. However, no herbicidal applications by aircraft have ever been authorized. Thus, there is no aerial application of glyphosate-containing plant protection products, at least in Germany.

Within the scope of the European authorization procedure for glyphosate, only downwards directed applications have been intended and have been taken into account for risk assessment.

1.3 Measurement and analysis

Not one of the about 40 studies evaluated in Volume 3 sections B.5.2 - B.5.4 (2015, ASB2015-1194) are mentioned in the IARC Monographs Volume 112 (2015, ASB2015-8421). In section 1.3 of the IARC monograph in total 16 analytical reports from the open literature are cited. Two of them are merely

mentioned in the general introduction. Details of the remaining 14 studies are described in Table 1.1 of the IARC monograph. All details listed in Table 1.1 of the IARC monograph correspond exactly to the data of the cited studies. However, the limit of detection reported in these studies is estimated only and not statistically validated. A revised version of Table 1.1, listed in the Annex as Table A-5.5-1, additionally contains for that reason the limit of quantification, which is the only parameter that allows the evaluation of numerical data in other studies. In addition, Table A-5.5-1 contains the derivatisation agent (if used), a statement on the extent of validation data presented in cited studies and those sections of the IARC monograph, which refer to studies reported in section 1.3.

Due to the fact that quantitative analytical results will be more reliable if stable isotope labelled glyphosate is used as internal standard, it should be mentioned that the methods by Lee et al. (2001, ASB2015-8239) and Botero-Coy et al. (2013, ASB2015-7882) use such special internal standards.

Three of the studies reported in section 1.3 of the IARC monograph are cited in other sections. These are the studies by Acquavella et al. (2004, ASB2012-11528), Chang et al. (2011, ASB2015-7895) and Curwin et al. (2007, ASB2012-11597), which are mentioned in sections 1.4.1, 4.1.2, and 4.1.5. Due to missing analytical validation data in these studies, it is not possible to assess the reliability of results presented in these three studies. All other reports are not cited outside of section 1.3 of the IARC monograph.

In summary, the IARC Monographs Volume 112 (2015, ASB2015-8421) provides an overview on several studies published in scientific journals. About 50% of the methods reported in these studies are considered as sufficiently validated, even if the extent of validation data does not fully correspond to requirements of Regulation (EC) No 1107/2009 (2009, ASB2015-8589) as detailed in SANCO/825/00 rev. 8.1 (2010, ASB2015-8438).

1.4 Occurrence and exposure

1.4.1 Exposure

1.4.1.1 Occupational exposure

In section 1.4.1 of the IARC Monographs Volume 112 (2015, ASB2015-8421) results of four occupational and two para-occupational studies using various glyphosate-containing plant protection products are cited and summarized in Table 1.2. The studies were carried out between 1988 and 2007 in different countries of North America and Europe. Four of these studies (Centre de Toxicology du Québec, 1988, ASB2015-7889; Lavy et al., 1992, TOX9650912; Johnson et al., 2005, ASB2012-11859, and Curwin et al., 2007, ASB2012-11597) have not yet been included in the RAR (2015, ASB2015-1194). Nevertheless, all six exposure studies have been roughly evaluated now (see Table A-5.5-2). A short summary of the evaluation of these studies is given in section 5.1.

1.4.1.2 Community exposure

For residues in food and feed references were made to several food monitoring reports and data from the EU, Denmark, United Kingdom and Brazil. The information are freely available, however, not included in the RAR due to the “safe-use” approach for the assessment of active substances under Regulation (EC) No 1107/2009 (2009, ASB2015-8589). The “safe-use” concept relies on supervised field trial data treated at the maximum application rates for the active substance, resulting in a more conservative exposure scenario compared to food monitoring results.

All studies reported by IARC on biological markers for glyphosate are also included in the RAR (2015, ASB2015-1194).

1.4.2 Exposure assessment

The methodology for the exposure assessment of glyphosate will be described in IARC Monographs Volume 112 for Malathion, which has not yet been published.

2 Studies of Cancer in Humans

In the section on cancer in humans (epidemiological studies) the IARC describes in Tables 2.1. and 2.2 the primary cohort and control studies with the reference, study location, study design, population size, exposure assessment methods, organ site, exposure category, exposed cases, risk estimate (95% confidence intervals) and covariates controlled and comments. Overall, these descriptions reflect the information in the articles (Instead of the cases and the response rates, it would have been helpful to detail the actual cases analysed.) The general discussion of the epidemiological studies was not available since it will appear in the IARC Monographs Volume 112 on Malathion which as of today has not been published. There are small differences in the way the strength of evidence may be judged and the limitations of the studies according to the descriptions in either report (RAR and IARC monograph). For example, the RMS considers it problematic that Hardell et al. (2002, ASB2012-11839) put two studies one on NHL and the other only on HCL together – different types of cancer without inclusion of the other respective cancer group – and analysed them together. Even though IARC does weighting and uses quality criteria it is not always detailed. It is not described in detail how the literature search and the selection of literature were done for the IARC report. Overall, BfR agrees that the relevant studies on NHL-lymphoma are included in the IARC monograph.

The epidemiological studies face several problems: only a small number of cancer cases are observed in all the individual studies, making it difficult to obtain clear results. Also the number of adequate epidemiological studies is limited. There are a lot of problems with confounders: in most studies glyphosate is analysed together with several other pesticides/insecticides so that the effects of each individual substance are difficult if not impossible to disentangle. Farmers who use one chemical substance may also use another. It is not clearly stated in which formulation glyphosate is used that is, it could be different brands with slightly different chemical mixtures and co-formulants, which may have carcinogenic effects. The exposure cannot be easily measured. For example no measures from biomarkers from the blood are used. Exposure is measured through interviews or questionnaires. Here, there is a big recall problem to judge the amount of exposure to the chemicals. Furthermore, there may be a recall biases since individuals with cancer are more likely to think about possible reasons for their cancer than healthy individuals. Moreover, in these studies we find a problem with the classification of the cancers. NHLs are not consistently defined over time. The definition has changed over time due to the use of different diagnostic methods: first morphological methods, than modern immunological methods were applied. Therefore, the NHLs reported do not always comprise the same cancers. For instance, some include, others exclude hairy cell leukaemia. Multiple myelomas may also be considered presently as NHL but not previously. Some studies are thus not comparable and some comparisons are difficult because of the in- and exclusion of certain subtypes which are not the same. This may bias the picture. The same applies to the combination in meta-analyses. IARC notes in quite a number of studies that there is limited power for glyphosate exposure. On the other hand, evidence from epidemiological studies has to be considered with all necessary care since at least uncertainties due to extrapolating from animal to human toxicology is avoided in this approach.

2.1 Cohort studies

12 publications have been reported by IARC in section 2.1. These publications are summarized in Table 2.1-1). The conclusion of most of these studies is that glyphosate did not cause different types of cancer or did not increase the risk of all cancers.

Glyphosate did not significantly increase the risk of prostate cancer, pancreatic cancer, melanoma, lung cancer, colon cancer, rectum cancer, kidney cancer, urinary bladder cancer, breast cancer, childhood cancer and all types of cancer. Cohort studies reported also no increased risk of all lymphohaematopoietic cancers, non-Hodgkin lymphoma (NHL), multiple myeloma, and of monoclonal gammopathy which is considered to be a premalignant disorder that often precedes multiple myeloma. The results on NHL and multiple myeloma are discussed together with the results of case-control studies below (see section 2.2).

Table 2.1-1: Discussion of studies in section 2.1 Cohort studies of the IARC monograph

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Alavanja et al., 1996, ASB2015-7849	The Agricultural Health Study (AHS), large prospective cohort study	The only cohort study to date to have published findings on exposure and the risk of cancer at many different sites.	The data of this study were used in further studies. Conclusions are described there.	The AHS study was described in the RAR as basis for a number of publications.	Data of this publication were used for further studies. Conclusions on glyphosate are presented with these studies.
Alavanja et al., 2003, ASB2012-11535	Use of pesticides and prostate cancer risk (based on AHS)	No significant exposure-response association of glyphosate with cancer of prostate was found.	Agreement	Yes, page 531	No significantly increased risk of prostate cancer.
Andreotti et al., 2009, ASB2012-11544	Pesticide use and risk of pancreatic cancer (based on AHS)	The odds ratio for ever- versus never-exposure to glyphosate was 1.1 (0.6-1.7) while the odds ratio for the highest category of level of intensity-weighted lifetime days was 1.2 (0.6-2.6)	Agreement	Yes, Page 531	No significantly increased risk of pancreatic cancer.
Blair et al., 2011, ASB2015-7868	Impact of pesticide exposure misclassification on estimates of relative risks in the AHS	Nondifferential exposure misclassification biases relative risk estimates towards the null in the AHS and tends to decrease the study power.	Glyphosate was not assessed in this study.	No, no assessment of glyphosate in this study	No assessment of glyphosate in this study
Dennis et al., 2010, ASB2015-8439	Pesticide use and risk of melanoma (based on data of AHS)	Exposure to glyphosate was not associated with cutaneous melanoma within the AHS.	Agreement	No	No increased risk of melanoma.
De Roos et al., 2005a, ASB2012-11605	Cancer incidence among glyphosate-exposed pesticide applicators (based on data of the AHS)	No increased risk of all cancers and of cancers in lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate and of melanoma, all lympho-haematopoietic cancers, NHL and leukaemia. For multiple myeloma the relative	Agreement with the reported results and the conclusion on limited power of the study. Further discussion of multiple	Yes, page 539	No increased risk of all cancers and of cancers in lung, oral cavity, colon, rectum, pancreas,

		risk was 1.1 (0.5-2.4) when adjusted for age, but was 2.6 (0.7-9.4), when adjusted for multiple confounders. The study had limited power for the analysis of multiple myeloma. Missing data limit the interpretation of the findings.	myeloma in this study see also re-evaluation by Sorahan (2015, ASB2015-2284), below		kidney, bladder, prostate and of melanoma, all lympho-haematopoietic cancers, NHL and leukaemia. Interpretation of multiple myeloma is limited.
De Roos et al., 2005b, ASB2015-8437	Response in the discussion on the study of De Roos et al., 2005a, ASB2012-11605 (see above)	The study had limited power for the analysis of multiple myeloma. Missing data limit the interpretation of the findings.	Agreement	No, the paper is no study but only a response in the discussion on study of De Roos et al., 2005a, ASB2012-11605 (see above).	See De Roos et al., 2005a, ASB2012-11605
Engel et al., 2005, ASB2012-11613	Pesticide use and breast cancer risk	No difference in incidence of breast cancer for women who reported ever applying glyphosate (odds ratio 0.9 (0.7-1.1); Women who never used glyphosate but whose husband had used (no information on duration of use): odds ratio 1.3 (0.8-1.9)	Agreement	Yes, page 531	No significantly increased risk of breast cancer.
Flower et al., 2004, ASB2012-11620	Parental pesticide application and cancer risk in children; (based on data of AHS)	"For all the children of the pesticide applicators, risk was increased for all childhood cancers combined, for all lymphomas combined, and for Hodgkin lymphoma, compared with the general population." Limited power of the study for glyphosate exposure.	The cited IARC conclusion considers the risk for children of all pesticide applicators. However, this statement is not relevant for the assessment of glyphosate. There was an increased odds ratio in result of application of pesticides aldrin, dichlorvos and ethyl dipropylthiocarbamate. However, the results for glyphosate did not demonstrate any risk for childhood cancer. The odds ratios for maternal use and paternal use of glyphosate are	Yes, page 531	No increased risk of childhood cancer.

			even clearly below 1. Agreement with the limited power of the study.		
Landgren et al., 2009, ASB2012-11875	Pesticide exposure and risk of monoclonal gammopathy (based on data of AHS)	No association between exposure to glyphosate and risk of monoclonal gammopathy of undetermined significance, a premalignant plasma disorder that often precedes multiple myeloma; odds ratio 0.5 (0.2-1.0)	The study authors conclude a nonsignificant decrease of monoclonal gammopathy of undetermined significance (MGUS), on the large data base of the AHS.	Yes, page 531	Nonsignificant decrease of risk of MGUS which usually precedes multiple myeloma
Lee et al., 2007, ASB2015-8228	Pesticide use and risk of colorectal cancer (based on data of AHS)	Most of the 50 pesticides studied were not associated with risk of cancer of the colorectum, and the relative risks with exposure to glyphosate were 1.2 (0.9-1.6), 1.0 (0.7-1.5) and 1.6 (= 0.9-2.9) for cancers of the colorectum, colon and rectum respectively.	Agreement	No	No significantly increased risk of colorectal cancers.
Sorahan, 2015, ASB2015-2284	Glyphosate and multiple myeloma, re-analysis of AHS data; data of the study of De Roos et al., 2005a, ASB2012-11605 (see above) are reanalysed	Sorahan confirmed that the excess risk of multiple myeloma was present only in the subset with no missing information.	The author concluded that "*this secondary analysis of AHS data does not support the hypothesis that glyphosate use is a risk factor for multiple myeloma*".	No, study was published after completion of the RAR.	No significantly increased risk of multiple myeloma based on the AHS data

2.2 Case–control studies on non-Hodgkin lymphoma, multiple myeloma, and leukaemia

16 studies have been reported in section 2.2 of the IARC monograph and are summarized including comments of the RMS in Table 2.2-1.

Two of these 16 studies did not mention glyphosate (Waddell et al., 2001, ASB2015-8037 and Zahm et al., 1990, ASB2013-11501).

Five studies reported no increased risk of non-Hodgkin lymphoma and/or leukaemia or multiple myeloma. (Brown et al., 1990, TOX2003-999; Cantor et al., 1992, ASB2015-7885; Karunanayake et al., 2012, ASB2012-11865; Lee et al., 2004a, ASB2015-8238, and Orsi et al., 2009, ASB2012-11985).

Some of the reported studies had according to the IARC assessment in agreement with the RMS assessment a limited or even very limited power to assess effects of glyphosate. In three studies only 4 exposed cases have been compared with 2, 3 or 5 control subjects (Cocco et al., 2013, ASB2014-7523; Hardell and Eriksson, 1999, ASB2012-11838; and Nordström et al., 1998, TOX1999-687).

Further studies reported different, contradictory results. Depending from the used method of statistical analysis the risk was increased in some cases or not increased in other cases.

The relevant studies on non-Hodgkin lymphoma have been selected by Schinasi and Leon (2014, ASB2014-4819) to perform a meta-analysis. For the analysis of an association between glyphosate and non-Hodgkin lymphoma the following studies have been used: De Roos et al., 2003, ASB2012-11606; De Roos et al., 2005a, ASB2012-11605; Eriksson et al., 2008, ASB2012-11614; Hardell et al., 2002, ASB2012-11839; McDuffie et al., 2001, ASB2011-364, and Orsi et al., 2009, ASB2012-11985.

Furthermore, for the analysis of an association between glyphosate and B cell lymphoma 2 studies have been used: Eriksson et al., 2008, ASB2012-11614 and Cocco et al., 2013, ASB2014-7523.

2 of the 6 studies used for the analysis of non-Hodgkin lymphoma reported no increased risk of non-Hodgkin lymphoma (De Roos et al., 2005a, ASB2012-11605 and Orsi et al., 2009, ASB2012-11985).

3 of the above cited 7 studies were considered by IARC to have limited or even very limited power (Hardell et al., 2002, ASB2012-11839 and Cocco at al., 2013, ASB2014-7523) or a low participation rate (McDuffie et al., 2001, ASB2011-364).

Finally, IARC referred in a publication in Lancet (Guyton et al., 2015, ASB2015-7076) to 3 studies (De Roos et al., 2003, ASB2012-11606; McDuffie et al., 2001, ASB2011-364, and Eriksson et al., 2008, ASB2012-11614) in context with the conclusion that there was limited evidence in humans for carcinogenicity of glyphosate. These 3 studies are discussed by RMS in Table 2.2-2.

Table 2.2-1: Discussion of studies in section 2.2 Case-control studies on non-Hodgkin lymphoma (NHL), multiple myeloma and leukaemia of the IARC monograph

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Brown et al., 1990, TOX2003-999	Pesticide exposure and other agricultural risk for leukaemia	The odds ratio for glyphosate was 0.9 (0.5-1.6). The study had limited power to assess effects of glyphosate.	Agreement	No, because released before 2000	No increased risk of leukaemia, limited power of the study.
Brown et al., 1993, TOX2002-1000	Pesticide exposure and multiple myeloma	The odds ratio for glyphosate was 1.7 (0.8-3.6). The study had limited power to assess effects of glyphosate.	Agreement	No, because released before 2000	Limited power of the study to assess effects of glyphosate.
Cantor et al., 1992, ASB2015-7885	Pesticides and other agricultural risk factors for non-Hodgkin lymphoma	The odds ratio for men who ever handled glyphosate was 1.1 (0.7-1.9), low power of the study to assess risk of NHL associated with glyphosate	Agreement	No, because released before 2000	No significantly increased risk of non-Hodgkin lymphoma, limited power of the study
Cocco et al., 2013, ASB2014-7523	Pesticide exposure and lymphoma risk	Odds ratio for glyphosate exposure was 3.1 (0.6-17.4); the study had a very limited power to assess the effects of glyphosate on risk of NHL	Agreement with the reported results and the conclusion on limited power of the study. Only 4 exposed cases and 2 control subjects have been considered in this study.	Yes, page 532	Very limited power of the study (only 4 exposed cases and 2 control subjects)
De Roos et al., 2003, ASB2012-11606	Pesticide exposure and risk of non-Hodgkin lymphoma	See separate assessment in this addendum	See separate assessment in this addendum	Yes, pages 529 and 537	See Table 2.2-2
Eriksson et al., 2008, ASB2012-11614	Pesticide exposure and risk of non-Hodgkin lymphoma	See separate assessment in this addendum	See separate assessment in this addendum	Yes, pages 531 and 540	See Table 2.2-2
Hardell and Eriksson,	Pesticide exposure and risk of non-Hodgkin	The odds ratio for ever-use of glyphosate was 2.3 (0.4-13.4) in a univariate analysis, and 5.8	Agreement with the reported results and the conclusion on limited power	Yes, pages 530 and 534	no conclusion possible because of

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
1999, ASB2012-11838	lymphoma	(0.6-54) in a multivariable analysis. The exposure frequency was low for glyphosate, and the study had limited power to detect an effect.	of the study. Only 4 exposed cases and 3 control subjects have been considered in this study.		limited power of the study (only 4 exposed cases and 3 control subjects)
Hardell et al., 2002, ASB2012-11839	Pesticide exposure and risk of non-Hodgkin lymphoma and hairy cell leukaemia	The study is a pooled analysis of two case-control studies (see Hardell and Eriksson, 1999, TOX1999-686, ASB2012-11838 and Nordström et al., 1998, TOX1999-687 in this addendum). Increased risk was found for glyphosate only in univariate analysis (odds ratio, 3.04 (1.08-8.52)), however, the odds ration decreased in multivariate analysis to 1.85 (0.55-6.20). The exposure frequency for glyphosate was low and the study had limited power.	Agreement with the presented results and the conclusion on limited power of the study. The study is a pooled analysis of two case-control studies (see separate discussion on studies of Hardell and Eriksson, 1999, TOX1999-686, ASB2012-11838 and Nordström et al., 1998, TOX1999-687 in this addendum).	Yes, page 530 and 535	See Table 2.2-2
Kachuri et al., 2013, ASB2014-8030	Pesticide exposure and risk of multiple myeloma	The odds ratio for ever-use of glyphosate was 1.19 (0.76-1.87); no association was found for light users (≤ 2 days per year, odds ratio 0.72 (0.39-1.32), the odds ratio in heavier users (>2 days per year) was 2.04 (0.98-4.23). The study had relatively low response rates.	Agreement	Yes, page 532	No increased risk of multiple myeloma for ever use of glyphosate, higher (not significant) OR if mixing or applying glyphosate >2 days per year, low response rate
Karunanayake et al., 2012, ASB2012-11865	Pesticide exposure and risk of non-Hodgkin lymphoma	Based on 38 cases exposed to glyphosate, the odds ratios were 1.14 (0.74-1.76) adjusted for age and province, and 0.99 (0.62-1.56) when additionally adjusted for medical history variables.	Agreement	Yes, page 531	No increased risk of non-Hodgkin lymphoma
Lee et al., 2004a, ASB2015-	Pesticide exposure and risk of non-Hodgkin Lymphoma among	Subject with a history of asthma had a non-significantly lower risk of NHL than non-asthmatics. The odds ratio associated with	Agreement	No	No significantly increased risk of non-Hodgkin

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
8238	asthmatics	glyphosate use was 1.4 (0.98-21.) among non-asthmatics and 1.2 (0.4-3.3) among asthmatics.			lymphoma for asthmatics and non-asthmatics; non-significantly lower risk of NHL for asthmatics than non-asthmatics
McDuffie et al., 2001, ASB2011-364	Pesticide exposure and risk of non-Hodgkin lymphoma	Odds ratio of 1.26 (0.87-1.80) and 1.20 (0.83-1.74, adjusted for age, province, high-risk exposures) were observed for exposure to glyphosate. In an analysis by frequency of exposure to glyphosate, participants with 2+ days of exposure per year had an odds ratio of 2.12 (1.2-3.73) compared with those with some but ≤ 2 days of exposure. The study was large, but had relatively low participation rates.	See separate assessment in this addendum	Yes, pages 529 and 545	See Table 2.2-2
Nordström et al., 1998, TOX1999-687	Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukaemia	An age-adjusted odds ratio of 3.1 (0.8-12) was observed for exposure of glyphosate. However, the study had limited power, only 4 exposed cases and there was no adjustment for other exposures.	Agreement with reported results and conclusions on limited power, only 4 exposed cases and 5 exposed controls are considered in this study	Yes, page 530	Limited power of the study (only 4 exposed cases and 5 exposed controls)
Orsi et al., 2009, ASB2012-11985	Pesticide exposure and risk of lymphoid neoplasms	The odds ratios associated with any exposure to glyphosate were 1.2 (0.6-2.1) for all lymphoid neoplasms, 1.0 (0.5-2.2) for NHL, 0.6 (0.2-2.1) for lymphoproliferative syndrome, 2.4 (0.8-7.3) for multiple myeloma, and 1.7 (0.6-5.0) for Hodgkin lymphoma.	Agreement with reported results. It should be considered in the discussion on an association between glyphosate and NHL that the OR of NHL in this study (12 exposed cases and 24 exposed controls) was 1.0.	No	See Table 2.2-2
Waddell et al., 2001, ASB2015-8037	Use of organophosphate pesticides and risk of non-Hodgkin lymphoma	IARC compared the numbers of cases and controls in this study with the study of De Roos et al., 2003; however, no information on glyphosate in this study	No information on glyphosate	No, no information on glyphosate	no information on glyphosate

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Zahm et al., 1990, ASB2013-11501	Exposure to 2,4-D and risk of non-Hodgkin Lymphoma	The study was mentioned by IARC because data were used in the study of De Roos et al., 2003	No information on glyphosate	No, no information on glyphosate	no information on glyphosate

Table 2.2-2: Summary of the RMS assessment on the strength of evidence and validity of epidemiological studies mentioned by IARC.

Short evaluation of the crucial studies in the draft of the Renewal Assessment Report (RAR) of the RMS	Main RMS comment after IARC publication on strength of evidence (none, low, medium, high) based on study type, internal and external validity and estimated effect size	Internal validity, such as quality aspects of the study, sample size, measurement biases, statistical uncertainty.	External validity & relevance for the RMS assessment: how close is the measured endpoint to the health endpoint of concern
De Roos et al. (2003, ASB2012-11606) had reported an association between NHL and glyphosate use.	No unequivocal evidence for causation of NHL by glyphosate based on a pooled analysis of three case control studies in the Midwestern United States (NHL diagnosed between 1979-1986) and reported exposures with 47 pesticides. Logistic regression and hierarchical model provide significant effect (OR, 2.1 with 95% confidence interval (CI) 1.1 to 4.0) and non-significant effect (OR, 1.6 with 95% CI 0.9 to 2.8), respectively, the latter with adjustment for multiple exposures and using prior probability of 0.3 for glyphosate as being carcinogenic. Contrary to common standards, the authors consider the result from the hierarchical model as significant. The description of the study design, analysis and results do not allow assessing methodological quality.	The internal validity cannot be assessed fully due to limitations in the reporting of the study. The past exposure status for a wide range of pesticides has been assessed in interviews, which is inherently prone to recall and interviewer bias. The study showed four out of 47 pesticides with lower limits of 95% confidence intervals greater than 1.0, indicative for a significant effect. The 47 pesticides may constitute multiple testing so that 5% of effects may show up by chance alone. The approximation of the relative risk using the OR is justified for NHL being a rare disease.	The relevance of the study for the current risk question is high. It is not known whether exclusion of females from the study population compromises the applicability of the findings to the general, European population.
McDuffie et al. (2001, ASB2011-364) mentioned a non-significant positive association between self-reported glyphosate exposure and NHL in a Canadian study.	OR_{adj} = 1.20 (0.83/1.74): low effect size, not significant; no unequivocal evidence for causation of NHL by glyphosate. Well performed case control study on the male Canadian population from 6 provinces with one	Low/medium Multiplicity of pesticide exposure reported, but not the correlations. Tiered approach starting with pesticide classes, but no adjustment	Low/medium Should be considered for assessment as it is a well performed study exploring the endpoint NHL, which however is a collection of diseases.

Short evaluation of the crucial studies in the draft of the Renewal Assessment Report (RAR) of the RMS	Main RMS comment after IARC publication on strength of evidence (none, low, medium, high) based on study type, internal and external validity and estimated effect size	Internal validity, such as quality aspects of the study, sample size, measurement biases, statistical uncertainty.	External validity & relevance for the RMS assessment: how close is the measured endpoint to the health endpoint of concern
	of four rare tumours (517 cases, 1506 controls). The study has some limitations typical of a case-control study (recall bias, misclassification of pesticide exposure) and without appropriate adjustment for multiple testing (multiple exposures and multiple endpoints).	for multiple testing (many pesticides, four tumours). While in this publication only NHL is considered, the study was planned and evaluated for four tumours.	The problem of multiple exposures is not easily overcome in reality; therefore it should not be over-stressed.
Eriksson et al. (2008, ASB2012-11614) reported a case-control study which included 910 cases of NHL and 1016 controls living in Sweden. The highest risk was calculated for MCPA. Glyphosate exposure was reported by 29 cases and 18 controls, and the corresponding OR was 2.02.	OR = 2.02 (1.10-3.71) medium effect size, significant; a multivariate analysis gave no significant results. Case control study in 4/7 Swedish regions; all new cases during 29 months. 910 cases, 1016 controls from population registry. The study has some limitations typical of a case-control study (recall bias, misclassification of pesticide exposure) and without appropriate adjustment for multiple testing (multiple exposures).	Low/medium OR values and confidence intervals cannot be reproduced. The reported dependency from use intensity sounds logical but might as well be attributable to reporting bias.	Medium Study reported NHL diagnosis and subtypes according to WHO classification
De Roos et al. (2005, ASB2012-11605) make use of the AHS cohort.	OR = 1.1 [0.7, 1.9] for NHL, adjusted for age, demographic and lifestyle factors, and other pesticides.	High/medium In contrast to case-control-studies, a prospective cohort study does not suffer recall-bias. However, the problems of multiple exposures and multiple testing remain.	High/medium This study is the best we can hope for: A prospective cohort study with sensible stratification is optimal for establishing a causal relation. However, the problems of multiple exposures and of the possible effect of frequently used co-formulants remain.
Orsi et al. (2009, ASB2012-11985) did not find an association between NHL and glyphosate handling in a French case control study (OR = 1.0).	OR = 1.0 [0.5, 2.2] for any exposure (12 cases, 24 controls), OR = 1.0 [0.3, 2.7] for professional exposure (5 cases, 24 controls). The study has some limitations typical of a case-control study (recall bias, misclassification of pesticide exposure) and without appropriate adjustment for multiple testing (multiple exposures).	Medium Sensible stratification.	Medium Study reported NHL diagnosis and subtypes according to ICD-O-3 classification

Short evaluation of the crucial studies in the draft of the Renewal Assessment Report (RAR) of the RMS	**Main RMS comment after IARC publication on strength of evidence (none, low, medium, high) based on study type, internal and external validity and estimated effect size**	**Internal validity, such as quality aspects of the study, sample size, measurement biases, statistical uncertainty.**	**External validity & relevance for the RMS assessment: how close is the measured endpoint to the health endpoint of concern**
Hardell et al. (2002, ASB2012-11839) This study pools data from Hardell and Eriksson (1999, ASB2012-11838) with data from Nordström et al. (1998, TOX1999-687). Case-control study which included 515 male cases of NHL/ HCL and 1141 controls living in North and Middle Sweden. NHL and HCL diagnosed between 1987-1992), each case matched with two male controls, for age and country.	Univariate: OR = 3.04 (1.08 - 8.52) –medium effect size, only 8 exposed case and 8 exposed controls Multivariate: OR = 1.85 (0.55 - 6.2) with adjustment for study, study area, vital status, other pesticides Low effect size, Logistic regression model provide no significant effect.	Not reliable as the study combines two studies with different endpoints in order to increase the power. Note that it might have been justified to combine the endpoints in the first place (if it is true that HCL can be considered a subtype of NHL) but combining two weak studies in order to strengthen the result is technically invalid. The results in the multivariate analysis must be interpreted with caution since exposure to different types of pesticides correlate.	Not relevant for the link between glyphosate and NHL as the study reported NHL and HCL diagnosis. Limited power for glyphosate exposure.

HCL, Hairy cell leukaemia; NHL, non-Hodgkin lymphoma; OR, odds ratio

The crucial studies used by IARC in the discussion on a relation between glyphosate exposure and risk of NHL were re-evaluated regarding strength of evidence and validity and there was no unequivocal evidence for a clear and strong association of NHL with glyphosate because of the limitations of these epidemiological studies such as being based on interviews with farmers or family members, the number of cases involved, and no knowledge of the actual amount of glyphosate or the type of glyphosate formula used. Even though the OR for an association between the exposure to glyphosate and NHL was slightly increased in all studies, it was not significant in the McDuffie study (ASB2011-364), significant in the Eriksson study (based on 29 cases) (ASB2012-11614) and not unequivocal in De Roos (2003, ASB2012-11606) (a further study with data from the AHS in 2005 by De Roos (ASB2012-11605) found no clear association between glyphosate and NHL, based on a large number of participating farmers), allowing no solid epidemiological statement on the basis of these three epidemiological studies. The studies need to be put in the context of the other epidemiological and experimental studies undertaken. Probably, further research needs to be carried out to study the usage and the impact of the formulation used in the field situation.

2.3 Case–control studies on other cancer sites

6 case control studies on other cancer sites were reported by IARC. The studies are summarized in Table 2.3-1.

One of these studies (Monge et al., 2007, ASB2012-11909) did not separately assess glyphosate. The other 5 studies reported no increased risk or even a reduced risk of the investigated cancers (adenocarcinoma of stomach and oesophagus, gliomas and soft-tissue sarcoma).

Table 2.3-1: Discussion of studies in section 2.3 'Case-control studies on other cancer sites' and section 2.4 'Meta-analyses' of the IARC monograph

Study (Author/year)	Subject	Evaluation IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Lee et al., 2004b, ASB2012-11883	Pesticide use and risk of adenocarcinomas of stomach and oesophagus	For ever use of glyphosate, the odds ratio was 0.8 (0.4 - 1.4) for cancer of the stomach, and 0.7 (0.3 - 1.4) for oesophageal cancer; the power of the study was limited.	Agreement	Yes, page 531	No increased risk of adenocarcinomas of stomach and oesophagus
Ruder et al., 2004, ASB2015-8078	Pesticide exposure and risk of gliomas	No association was found with any of the pesticides assessed, including glyphosate. Glyphosate use was assessed, but specific results were not presented.	Agreement	No	No increased risk of gliomas
Carreon et al., 2005, ASB2012-11585	Pesticide exposure and risk of gliomas	There was a reduced risk for glyphosate (OR 0.7 (0.4 - 1.3).	Agreement	Yes, page 531	Reduced risk of gliomas
Lee et al., 2005, ASB2012-11882	Pesticide use and risk of gliomas	There was a non-significant excess risk with glyphosate use for the overall group, but there was inconsistency between observations for self-responds and observations for proxy respondents. The study had limited power to detect an effect of glyphosate use and was difficult to interpret.	Agreement	Yes, page 530	Limited power of the study, difficult to interpret
Pahwa et al., 2011, ASB2014-9625	Pesticide exposure and risk of soft-tissue sarcoma	The fully adjusted odds ratio for glyphosate was 0.90 (0.58 - 1.40).	Agreement	Yes, page 532	No increased risk of soft-tissue sarcoma
Monge et al., 2007, ASB2012-11909	Pesticide exposure and risk of childhood leukaemia	Association of childhood cancer with glyphosate were reported only for an "other pesticides" category that also included other chemicals, glyphosate was not specifically assessed.	Agreement	Yes, page 530	No specific assessment of glyphosate

Study (Author/year)	Subject	Evaluation IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Schinasi and Leon, 2014, ASB2014-4819	Meta-analysis, exposure to pesticides and non-Hodgkin lymphoma	The meta-analysis for glyphosate included six studies and yielded a meta-risk ratio of 1.5 (1.1 - 2.0). The working group noted that the most fully adjusted risk estimates from the articles by Hardell et al. (2002, ASB2012-11839) and Eriksson et al. (2008, ASB2012-11614) were not used in this analysis. After considering the adjusted estimates of the two Swedish studies in the meta-analysis, the Working Group estimated a meta-risk-ratio of 1.3 (1.03 - 1.65).	Agreement, see separate assessment in this addendum (section 2.4).	Yes, page 531 and addendum	See separate assessment in this addendum (section 2.4).

OR, odds ratio

2.4 Meta-analyses

Meta-analysis is an accepted investigation tool to provide a statistical summary across a number of studies with the same research question and similar setting. RMS has reviewed the study of Schinasi and Leon (2014, ASB2014-4819) as it is described in the IARC monograph and a meta-risk ratio of 1.3 (95% CI 1.03 - 1.65) I2=0%, P for heterogeneity 0.589) for NHL and glyphosate (glyphosate-based formulations, see discussion in section 2.5), as elicited by the IARC Working Group for glyphosate, could be reproduced by the RMS. The type of selection of the studies by IARC can be followed. This is a matter of definition and weighting the OR/RR from the case-control and cohort studies. The meta-risk ratio - the result of the meta-analysis - appears to show a moderate effect. The result is based on only 6 studies (De Roos et al., 2003, ASB2012-11606; De Roos et al., 2005, ASB2012-11605; Eriksson et al., 2008, ASB2012-11614; Hardell et al., 2002, ASB2012-11839; McDuffie et al., 2001, ASB2011-364; Orsi et al., 2009, ASB2012-11985), which qualified according to the set criteria. Although one of these (De Roos et al., 2005, ASB2012-11605) is a prospective cohort study, it was not ranked higher. And one study (Hardell et al., 2002, ASB2012-11839) was included in the meta-analysis even though its definition of NHL differs from the other studies. Even in the article, it is pointed out that further studies are needed.

The review of epidemiological studies on glyphosate and cancer by Mink et al., (2012, ASB2014-9617) which was sponsored by Monsanto has not been discussed here as it is not mentioned in the IARC monograph. The authors conducted no meta-analysis, but list 7 cohort studies and 11 case-control studies; they found no evidence of consistent positive associations that would be indicative of a causal relationship between any site-specific cancer and exposure to glyphosate. Almost all of these studies were also reviewed by IARC and the RMS.

The conduct of systematic reviews and meta-analysis is considered primary research work and is typically not conducted by public agencies entrusted with assessing market authorisation studies.

2.5 Categorization of evidence from studies in humans

2.5.1 Contribution of co-formulants to the toxicity of glyphosate-based formulations

IARC concluded that the evidence relevant to carcinogenicity of glyphosate from studies in humans is classified into the category "*Limited evidence of carcinogenicity*".

IARC did not consider the differences of toxicity between the active substance glyphosate and of glyphosate-based formulations caused by the higher toxicity of co-formulants. The exposed cases in human studies are always exposed to glyphosate-based formulations and practically never to the active substance only.

All glyphosate-containing plant protection products contain surfactants or - if not present as an integral component - are to be mixed with surfactants as a compulsory additive to produce the ready-to-use dilution. As has already been discussed during the first Annex I inclusion procedure for glyphosate it became apparent that glyphosate-containing products were more toxic than glyphosate alone. This phenomenon was attributed to the presence of particular surfactants predominantly, namely the POE-tallowamines.

Already in the DAR on glyphosate (Germany, DAR, 1998, ASB2010-10302) that was prepared to support the first Annex I listing of the active substance, it was mentioned that surfactants could significantly contribute to the toxicity of glyphosate products.

Furthermore, a toxicological evaluation of tallowamine was prepared in 2010 and was included into the RAR (see pages 871-886 of the RAR (Volume 3 B.6), revised April 2015, ASB2015-1194).

With regard to nearly all toxicological endpoints under investigation, the POE-tallowamine was clearly

more toxic than glyphosate.

The higher toxicity of the surfactant might explain that also Roundup formulations when tested for different endpoints were more toxic than glyphosate ([redacted] 1982, TOX2002-693, and [redacted] 1983, TOX2002-694; Dallegrave et al., 2003, ASB2012-11600, and Dallegrave et al., 2007, ASB2012-2721).

Toxicological end points for which a higher toxicity of POE tallowamine in comparison to glyphosate was evidenced are summarized in Table 2.5-1.

Table 2.5-1: Comparison of toxicity data for glyphosate and the POE-tallowamine surfactant with CAS no. 61791-26-2 (from RAR, revised April 2015, ASB2015-1194).

End point	**Glyphosate**		**POE-tallowamine surfactant**	
Acute oral (rat)	LD_{50} > 5000 mg/kg bw		LD_{50}: 864 mg/kg bw	
Acute dermal (rabbit)	LD_{50} > 2000 mg/kg bw		LD_{50} >907 mg/kg bw	
Skin irritation	Not irritant		Irritant	
Eye irritation	Moderately to severely irritant		Severely irritant	
Skin sensitization	Negative		Sensitising	
DNA damage	Negative		Equivocal (some evidence at high and clearly toxic doses)	
	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)
Short-term toxicity (rat, oral, 90-day)	150	300	20	60
Short-term toxicity (dog, oral, approx. 3 month)	300	1000	21	42
Reproduction toxicity (rat)	700 (parental) 2000 (repro) 700 (offspring)	2000 (parental) >2000 (repro) 2000 (offspring)	38 (parental) 12 (repro) 12 (offspring)	74 (parental) 38 (repro) 38 (offspring)
Developmental studies (rat), maternal toxicity	300	1000	10.8	72
Developmental studies (rat), foetal effects	300	1000	72	216

Additionally to the above cited toxicological evaluation of tallowamine a large number of further, new studies demonstrated a higher toxicity of glyphosate-based formulations in comparison to the lower toxicity of the active substance glyphosate. Some of these studies are reported in the RAR (revised April 2015, ASB2015-1194) in chapter B.6.6.12 (in a comparison of the active substance glyphosate and glyphosate containing formulations concerning developmental and reproductive toxicity and endocrine disruption) and in chapter B.6.8.4 'Further published data released since 2000'.

Even in the new IARC monograph on glyphosate some studies have been reported which clearly demonstrate a higher toxicity of glyphosate-based formulations than of the active substance. (Gasnier et al., 2009, ASB2009-7384; Richard et al., 2005, ASB2009-9024; Benachour et al., 2007, ASB2009-9018, and Walsh et al., 2000, ASB2012-12046).

However, the evidence of a higher toxicity of glyphosate-based formulations caused by co-formulants was not noticed and not considered in the discussion by IARC.

Even though in some of the cited studies the authors clearly reported that a formulation was used, IARC discussed the effects only as glyphosate effects (e.g. IARC concluded in the study of Kreutz et al., 2001, ASB2015-8279, "*A positive association between exposure to glyphosate and immunotoxicity in fish has been reported.*"). However, no active substance glyphosate was used in this study but a formulation

including co-formulants.

2.5.2 Conclusions on the classification of the evidence relevant to carcinogenicity from studies in humans into the IARC-categories

The categories of IARC as explained in the document IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Preamble, Lyon, 2006 explain the evaluation of epidemiological studies into certain categories (IARC, 2006, ASB2015-8291). On page 19 "Evaluation and rationale" it is stated: "*[…] It is recognized that the criteria for these evaluations, described below, cannot encompass all of the factors that may be relevant to an evaluation of carcinogenicity. In considering all of the relevant scientific data, the Working Group may assign the agent to a higher or lower category than a strict interpretation of these criteria would indicate.*"

These categories refer only to the strength of the evidence that an exposure is carcinogenic and not to the extent of its carcinogenic activity (potency). In other words, the categories describe whether there may be a possible carcinogenic effect of the substance, but not the severity of this effect.

IARC notes for categories:

"*1. Evidence suggesting the lack of carcinogenicity: there are several adequate studies covering the full range of levels of exposure that humans are known to encounter, which are mutually consistent in not showing a positive association between exposure to the agent and any studied cancer at any observed level of exposure […]*"

This is clearly not the case since the studies are not mutually consistent in not showing a positive association, instead results are inconsistent: a considerable number show no positive correlation, others may indicate a positive association. IARC states further "*Bias and confounding should be ruled out with reasonable confidence […]*". This is not the case for the epidemiological studies with glyphosate, since in most studies several chemicals are studied (and used) and the substance under consideration has been used in various mixtures with different co-formulants. Furthermore, a problem with estimating the exposure based on several studies using questionnaires and interviews should be considered since these instruments are prone to recall biases. The studies are not showing consistently a positive association. Most studies do not show an association, but some do. However, it is difficult to demonstrate or prove the lack of carcinogenicity using an epidemiological study. Therefore, RMS agrees that glyphosate cannot be classified in category 1.

"*2. Inadequate evidence of carcinogenicity: the available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absences of a causal association between exposure and cancer, or no data on cancer in humans are available*" (as defined by IARC). IARC does not classify glyphosate in this category, since there were limited data available, even though a lot of the studies have low statistical power, when assessing them individually, due to the number of individuals involved. The AHS cohort-study does list a considerable number of participants. Furthermore, the epidemiological studies show serious limitations because of recall bias, mixture of several chemicals, and missing knowledge about the exact products used (formulations) and low sample sizes, etc. The adherence of each primary study to pertinent guidelines for epidemiological studies was not re-assessed by RMS.

Despite limitations of all involved individual primary studies, it would seem inadequate to neglect the body of evidence they can provide in combination. RMS agrees with IARC that glyphosate should not be classified in this category as the description does not fit the available data even though some of them are weak.

In the 3rd category: "*Limited evidence of carcinogenicity: a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered by the working group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence*". This in other words means a trend in some studies is observed, however, no clear causal relationship can be established and no consistent positive association and the result can be an artefact due to chance or confounding. The IARC classifies the epidemiological evidence of glyphosate in this category. However, the authors of the meta-analysis (Schinasi and Leon, 2014, ASB2014-4819)

recommend for all pesticides further studies. The result could also be described as: most studies show no association, but a few studies do and in the most recent meta-analysis a weak trend between glyphosate NHL and a subgroup B cell lymphoma was observed. Therefore, an effect cannot be ruled out. Following the logic of the classification system of IARC, the RMS can accept this interpretation since the categories 1 and 2 do not appear to be correct, neither is the last category 4 with "sufficient evidence of carcinogenicity". It is a matter of expressing the remaining uncertainty in classifying glyphosate, since a lot of studies show no effect of glyphosate but some do with a weak carcinogenic potency as expressed through the odds ratios. It should be noted that the estimated OR of 1.3 by the IARC based on the meta-analysis of Schinasi and Leon, 2014, ASB2014-4819, indicates a rather weak association and that epidemiological associations cannot be interpreted as proof of causality. It is noteworthy that the most powerful study, the AHS, the prospective cohort-study, which in epidemiological terms is best suited to study the relationship, showed no association with cancer incidence overall or with most of the cancer subtypes, only a suggested association with multiple myeloma incidence was found, which needs to be followed up (De Roos et al., 2005, ASB2012-11605). Therefore, the evaluation of the RMS has a slightly different nuance than the evaluation of IARC, as the RMS is more cautious in describing the evidence for a positive relationship, even though the evaluation of the individual studies is similar.

The RMS sees a particular problem with the co-formulants of glyphosate-based formulations. As described in chapter 2.5.1 for the surfactants and thus for the glyphosate-based formulation a higher toxicity may be observed than for the glyphosate on its own. In the epidemiological studies it is not possible to differentiate between glyphosate itself and the other co-formulants, as well as different formulations used.

3 Cancer in Experimental Animals

In its Monograph Volume 112 IARC came to the conclusion, that there is "sufficient evidence" in experimental animals for the carcinogenicity of glyphosate (IARC, 2015, ASB2015-8421). In contrast and based on animal studies evaluated by the RMS Germany, the RMS had come to the conclusion that classification and labelling for carcinogenicity is not considered appropriate (RAR, April 2015, ASB2015-1194).

Potential explanations for the differences in the outcome of the evaluation may be that:

i) *a different database was used by both agencies and/or*

ii) *the data provided by the study reports was evaluated differently, and/or*

iii) *the overall database was interpreted differently, e.g. as the result of different decision criteria.*

Subsequently, all of these potential explanations are discussed.

i) Differences in the data basis

The database used by IARC and/or RMS for evaluation of neoplastic effects of glyphosate in laboratory animals is presented in the Table 3-1 (mice) and Table 3-2 (rats) below.

Overall, IARC evaluated three mouse and seven rat studies. Additionally IARC reported three further mouse studies and three more rat studies, which were however, not evaluated because these studies were not available in sufficient detail to the IARC Working Group.

Overall, RMS evaluated six mouse and ten rat studies. In addition to all studies assessed by IARC, RMS also evaluated the studies mentioned by IARC that were not fully assessed by the IARC Working Group. Hence, the data-basis considered by both agencies is essentially similar with three more mouse and three more rat studies fully evaluated by the RMS.

Table 3-1 and Table 3-2 summarize the studies reported by IARC and/or RMS, providing references and study owners, study type, duration, routes of exposure, dose levels, results (with respect to carcinogenicity) and the respective evaluations by both agencies.

Table 3-1: Animal studies in mice reported by IARC and/or RMS.

Reference, study ID, Lot, purity, owner	Study type duration route dose levels	Results (with respect to carcinogenicity)	Evaluation by IARC	Evaluation by RMS	Comments
[redacted] 1983, TOX9552381, Lots NB 1782608/3 and 1782610/7, 99.7%, Monsanto	Carcinogenicity, 2 year, CD-1, feeding 0, 1000, 5000, 30000 ppm (equal to 157/190; 814/955; 4841/5874 mg/kg bw/d in m/f)	*Males:* Renal tubule adenoma: 0/49, 0/49, 1/50 (2%), 3/50 (6%) [P for trend = 0.016] *Females:* No data provided on the kidney Report from the PWG of the EPA (1986): *Males:* Renal tubule adenoma: 1/49 (2%), 0/49, 0/50, 1/50 (2%) [NS] Renal tubule carcinoma: 0/49, 0/49, 1/50 (2%), 2/50 (4%) [P = 0.037; Cochran–Armitage trend test] Renal tubule adenoma or carcinoma (combined): 1/49 (2%), 0/49, 1/50 (2%), 3/50 (6%) [P = 0.034; Cochran–Armitage trend test]	Positive trend for renal tubule adenoma and carcinoma in male mice	No significant increase in tumour incidence observed in any groups of treated animals	Different statistical approaches reported by RMS and IARC. Due to differences in statistical evaluation RMS did not consider the renal tubule tumours as significant
[redacted] 1993, TOX9552382, Lot 206-JaK-25-1, 98.6%, Cheminova	Carcinogenicity, 2 year, CD-1, feeding 0, 100, 300, 1000 mg/kg bw/d (dietary levels regularly adjusted)	*Males*: Haemangiosarcoma: 0/50, 0/50, 0/50, 4/50 (8%) [P < 0.001, Cochran-Armitage] Histiocytic sarcoma in the lymphoreticular/ haemopoietic tissue: 0/50, 2/50 (4%), 0/50, 2/50 (4%) [NS] *Females:* Haemangiosarcoma: 0/50, 2/50 (4%), 0/50, 1/50 (2%) [NS] Histiocytic sarcoma in the lymphoreticular/ haemopoietic tissue: 0/50, 3/50 (6%), 3/50 (6%), 1/50 (2%) [NS]	Positive trend for haem-angiosarcoma in males	No significant increase in tumour incidence observed in any groups of treated animals	Different statistical approaches reported by RMS and IARC. Due to differences in statistical evaluation RMS did not consider the haemangiosarcomas as significant

Reference, study ID, Lot, purity, owner	Study type duration route dose levels	Results (with respect to carcinogenicity)	Evaluation by IARC	Evaluation by RMS	Comments
[redacted] 2009, ASB2012-11492, Lot H05H016A, 95.7%, Nufarm	Carcinogenicity, 18 month, CD-1 (ICR), feeding 0, 500, 1500, 5000 ppm (equal to 71/98; 234/299; 810/1081 mg/kg bw/d in m/f)	No relevant carcinogenic response reported	Study reported but not evaluated	No significant increase in tumour incidence observed in any groups of treated animals	Study not considered by IARC
[redacted] 2001, ASB2012-11491, Lot 01/06/97, >95.14%, ADAMA	Carcinogenicity, 18 month, Swiss albino, feeding 0, 100, 1000, 10000 ppm (15; 151; 1460 mg/kg bw/d, sexes combined since values were similar)	Higher incidence of malignant lymphoma at top dose level in males and females (significant according to Cochran-Armitage and Peto test)	Study reported but not evaluated	Considering historical control range and consistency, some evidence for carcinogenicity but not sufficient for classification	Study not considered by IARC
[redacted], 1997, ASB2012-11493, T-941209, 97.56% and T-950308, 94.61%, Arysta	Carcinogenicity, 18 month, CD-1 (ICR), feeding 0, 1600, 8000, 40000 ppm (165/153; 838/787; 4348/4116 mg/kg bw/d in m/f)	No relevant carcinogenic response reported	Study reported but not evaluated	No significant increase in tumour incidence observed in any groups of treated animals	Study not considered by IARC
George et al., 2010, ASB2012-11829, glyphosate based formulation (glyphosate, 41%; POEA, ~15%) (referred to as "glyphosate") dissolved in 50% ethanol; DMBA dissolved in 50% ethanol, and TPA dissolved in 50% acetone, Published study	Initiation–promotion study; Skin only 20 M/group Group I: untreated control Group II: glyphosate only: 25 mg/kg bw topically, 3 × /week, for 32 weeks Group III: single topical application of DMBA, 52 µg/mouse, followed 1 week later by TPA, 5 µg/mouse, 3 × /week, for	Skin tumours Group I: 0/20 Group II: 0/20 Group III: 20/20*, 7.8 ± 1.1 *P < 0.05 vs groups VI and VII Group V: 0/20 Group VI: 0/20 Group VII: 0/20 Group VIII: 8/20*, 2.8 ± 0.9 *P < 0.05 vs group VI	Inadequate study for the evaluation of glyphosate carcinogenicity	Inadequate study for the evaluation of glyphosate carcinogenicity	Both evaluations agree

Reference, study ID, Lot, purity, owner	Study type duration route dose levels	Results (with respect to carcinogenicity)	Evaluation by IARC	Evaluation by RMS	Comments
	32 weeks Group IV: single topical application of glyphosate, 25 mg/kg bw, followed 1 week later by TPA, 5 µg/mouse, 3 × /week, for 32 weeks Group V: 3 × /week topical application of glyphosate, 25 mg/kg bw, for 3 weeks, followed 1 week later by TPA, 5 µg/mouse, 3 × /week, for 32 weeks Group VI: single topical application of DMBA, 52 µg/mouse Group VII: topical application of TPA, 5 µg/mouse, 3 × /week, for 32 weeks Group VIII: single topical application of DMBA, 52 µg/mouse, followed 1 week later by topical treatment with glyphosate, 25 mg/kg bw, 3 × /week, for 32 weeks				

Table 3-2: Animal studies in rats reported by IARC and RMS.

Reference, study id, Lot, purity, owner	Study type duration route dose levels	Results	Evaluation by IARC	Evaluation by RMS	Comments
[redacted] 1993, TOX9750499,	Combined chronic toxicity/carcinogenicity; 2 year;	No relevant carcinogenic response reported	No significant increase in tumour incidence observed	No significant increase in tumour	Both evaluations agree

Reference, study id, Lot, purity, owner	Study type duration route dose levels	Results	Evaluation by IARC	Evaluation by RMS	Comments
229-JaK-5-1, 98.9% and 229-JaK-142-6, 98.7%, Cheminova	Sprague-Dawley; feeding		in any groups of treated animals	incidence observed in any groups of treated animals	
[redacted], 1996, TOX9651587, 2 batches used, 96.8/96.0%, ADAMA	Combined chronic toxicity/carcinogenicity; 2 year; Wistar; feeding 0, 100, 1000, 10000 ppm (6.3/8.6, 59.4/88.5, 595.2/886 mg/kg bw/d in m/f)	No relevant carcinogenic response reported	Study reported but not evaluated	No significant increase in tumour incidence observed in any groups of treated animals	No evaluation by IARC
[redacted] 1990, TOX9300244; XLH-264, 96.5%, Monsanto	Combined chronic toxicity/carcinogenicity; 2 year; Sprague-Dawley; feeding 0, 2000, 8000, 20000 ppm (89/113, 362/457, 940/1183 mg/kg bw/d in m/f)	*Males:* Pancreas (islet cell): Adenoma: 1/43 (2%), 8/45 (18%; P = 0.018), 5/49 (10%), 7/48 (15%; P = 0.042) Carcinoma: 1/43 (2%), 0/45 (0%), 0/49 (0%), 0/48 (0%) Adenoma or carcinoma (combined): 2/43 (5%), 8/45 (18%), 5/49 (10%), 7/48 (15%) Liver: Hepatocellular adenoma: 2/44 (5%; P for trend = 0.016), 2/45 (4%), 3/49 (6%), 7/48 (15%) Hepatocellular carcinoma: 3/44 (7%); 2/45 (4%), 1/49 (2%), 2/48 (4%) Hepatocellular adenoma or carcinoma (combined): 5/44 (11%), 4/45 (9%), 4/49 (8%), 9/48 (19%) *Females:* Pancreas (islet cell): Adenoma: 5/60 (8%), 1/60 (2%), 4/60 (7%), 0/59 Carcinoma: 0/60, 0/60, 0/60, 0/59 Adenoma or carcino-ma (combined): 5/60 (8%), 1/60 (2%), 4/60 (7%), 0/59 Thyroid: C-cell	Pancreas: There was no statistically significant positive trend in the incidence of pancreatic tumours, and no apparent progression to carcinoma but a significant increase in adenoma in males in two dose levels Liver: Significant positive trend for hepatocellular adenoma in males, no progression to malignancy Thyroid: Significant positive trend for C-cell adenoma in females	No significant increase in tumour incidence observed in any groups of treated animals	Due to differences in statistical evaluation RMS did neither consider the pancreatic islet cell tumours nor the hepatocellular adenomas nor the thyroid c-cell adenomas for classification

Reference, study id, Lot, purity, owner	Study type duration route dose levels	Results	Evaluation by IARC	Evaluation by RMS	Comments
		adenoma: 2/60 (3%), 2/60 (3%), 6/60 (10%), 6/60 (10%)			
[redacted] 1981, TOX2000-595 and TOX2000-1997, XHJ-64, 98.7%, Monsanto	Combined chronic toxicity/carcinogenicity; 26 months; Sprague-Dawley; feeding 0, 3/3.4, 10.3/11.2, 31.5/34 mg/kg bw/d in m/f (dietary levels adjusted according to values as measured in the 1st week)	*Males:* Pancreas (islet cell): Adenoma: 0/50 (0%), 5/49* (10%), 2/50 (4%), 2/50 (4%) Carcinoma: 0/50 (0%), 0/49 (0%), 0/50 (0%), 1/50 (2%) Adenoma or carcinoma (combined): 0/50 (0%), 5/49 (10%), 2/50 (4%), 3/50 (6%) *Females:* Pancreas (islet cell): Adenoma: 2/50 (4%), 1/50 (2%), 1/50 (2%), 0/50 (0%) Carcinoma: 0/50 (0%), 1/50 (2%), 1/50 (2%), 1/50 (2%) Adenoma or carcinoma (combined): 2/50 (10%), 2/50 (2%), 2/50 (74%), 1/50 (2%)	There was no statistically significant positive trend in the incidence of pancreatic tumours, and no apparent progression to carcinoma, but a significant increase in one of the treated groups of males	No significant dose dependent increase in tumour incidence observed in any groups of treated animals	Both evaluations basically agree; they disagree in the interpretation of the significant increase of pancreatic islet cell adenoma at the lowest dose group in males
[redacted] 2009, ASB2012-11490, H05H016A, 95,7%, Nufarm	Combined chronic toxicity/carcinogenicity; 2 year; Wistar; feeding Combined chronic toxicity/carcinogenicity; 2 year; Wistar; feeding	No relevant carcinogenic response reported	Study reported but not evaluated	No significant dose dependent increase in tumour incidence observed in any groups of treated animals	No evaluation by IARC
[redacted] 2001*, ASB2012-11488, P30, 97.6%, Syngenta	0, 2000, 6000, 20000 ppm (121/145, 361/437, 1214/1498 mg/kg bw/d in m/f)	No relevant carcinogenic response reported	No significant increase in tumour incidence observed in any groups of treated animals	No significant increase in tumour incidence observed in any groups of treated animals	Both evaluations agree
[redacted] 1997, ASB2012-11484, ASB2012-11485, ASB2012-11486, ASB2012-11487,	Combined chronic toxicity/carcinogenicity; 2 year; Sprague-Dawley; feeding 0, 3000, 10000, 30000 ppm (104/115, 354/393, 1127/1247 mg/kg bw/d	No relevant carcinogenic response reported	Study reported but not evaluated	No significant increase in tumour incidence observed in any groups of treated animals	No evaluation by IARC

Reference, study id, Lot, purity, owner	Study type duration route dose levels	Results	Evaluation by IARC	Evaluation by RMS	Comments
T-941209, 97.56% and T-950308, 94.61%, Arysta Life Sciences	in m/f)				
[redacted] 1996*,#, TOX2000-1998, P24, 95.6%, Syngenta	Chronic toxicity; Wistar-derived; 12 months; feeding 0, 2000, 8000, 20000 ppm (141/167, 560/671, 1409/1664 mg/kg bw/d in m/f)	No relevant carcinogenic response reported	No significant increase in tumour incidence observed in any groups of treated animals	No significant increase in tumour incidence observed in any groups of treated animals	Both evaluations agree
Seralini et al., 2012, (re-published 2014) ASB2012-15514, Published study	24-month study (10 males and 10 females per group) Sprague Dawley Drinking water at 0, 5 x 10^{-5} mg/L, 400 mg/L and 2.25 g/L of total glyphosate from a glyphosate based formulation	*Males:* No significant increase in tumour incidence observed in any of the treated groups *Females:* Mammary tumours (mainly fibroadenomas and adenocarcinomas): 5/10 (50%), 9/10 (90%), 10/10 (100%)*, 9/10 (90%) Pituitary lesions (hypertrophy, hyperplasia, and adenoma): 6/10 (60%), 8/10 (80%), 7/10 (70%), 7/10 (70%)	Inadequate study for the evaluation of glyphosate carcinogenicity	Inadequate study for the evaluation of glyphosate carcinogenicity	Both evaluations agree
Chruzielska et al., 2000, ASB2013-9829, Published study	24 month-study Wistar drinking water containing 0, 300, 900 or 2700 mg/L, 55 m/f per group	No relevant carcinogenic response reported	No significant increase in tumour incidence observed in any groups of treated animals	No significant increase in tumour incidence observed in any groups of treated animals	Both evaluations agree

Summary of results by IARC:

Critical results with respect to carcinogenicity identified by IARC included the occurrence of renal tubular adenoma and carcinoma in CD-1 mice in one study ([redacted], 1983, TOX9552381), the occurrence of haemangiosarcoma in male mice in one other study ([redacted], 1993, TOX9552382) and the occurrence of pancreatic islet cell tumours and hepatocellular adenomas in rats ([redacted] 1990, TOX9300244).

IARC summarized: "*[…] there was a positive trend in the incidence of renal tubule carcinoma and of renal tubule adenoma or carcinoma (combined) in males in one feeding study in CD-1 mice. Renal tubule carcinoma is a rare tumour in this strain of mice. No significant increase in tumour incidence was seen in female mice in this study. In the second feeding study, there was a significant positive trend in the incidence of haemangiosarcoma in male CD-1 mice. No significant increase in tumour incidence was seen in female mice in this study. For the five feeding studies in rats, two studies in the Sprague-Dawley strain showed a significant increase in the incidence of pancreatic islet cell adenoma in males - one of these two studies also showed a significant positive trend in the incidences of hepatocellular adenoma in males and of thyroid C-cell adenoma in females. Two studies (one in Sprague-Dawley rats, one in Wistar rats) found no significant increase in tumour incidence at any site. One study in Wistar rats was inadequate for the evaluation because of the short duration of exposure. In the study in Wistar rats given drinking-water containing glyphosate, there was no significant increase in tumour incidence. A glyphosate-based formulation was found to be a skin-tumour promoter in the initiation-promotion study in male Swiss mice. The study of a glyphosate-based formulation in drinking-water in Sprague-Dawley rats was inadequate for the evaluation because of the small number of animals per group, and the limited information provided on tumour histopathology and incidence in individual animals. These studies of a chemical mixture containing glyphosate were considered inadequate to evaluate the carcinogenicity of glyphosate alone.*" (IARC, 2015, ASB2015-8421)

In addition, IARC reported but did not evaluate the studies by [redacted] (1997, ASB2012-11493) in CD-1 mice, [redacted] (2001, ASB2012-11491) in Swiss albino mice and [redacted] (2009, ASB2012-11490) in CD-1 mice.

Summary results by RMS:

As apparent from the Tables above, RMS had not considered any of the tumours listed by IARC as potentially relevant for classification due to a lack of statistical significance and limited consistency between the studies. Critical results in terms of carcinogenicity identified by the RMS included the occurrence of malignant lymphoma in Swiss mice. RMS argued, however, that the murine tumours are not to be considered for classification because of the high background level of these tumours in Swiss mice.

In summary, RMS stated: "*Taking all this information together, a treatment-related effect in the study by [redacted] (2001, ASB2012-11491) in Swiss albino mice cannot be completely excluded. However, the weak increase in malignant lymphoma even over the historical control of the performing laboratory was clearly confined to this single study and strain since it was not reproducible in four other valid long-term studies. Thus, there is only very limited evidence of a carcinogenic potential of glyphosate as a high-dose phenomenon in mice of a susceptible strain. Most likely, perhaps, age-related neoplastic changes might be exacerbated by long-lasting administration of high doses. Swiss albino mice with high background prevalence of malignant lymphoma could be more vulnerable than other strains.*

Since the more frequent occurrence of malignant lymphoma was confined to a very high dose level that was administered over a long period, glyphosate was considered unlikely to pose a carcinogenic risk in humans. Classification and labelling for carcinogenicity is not considered appropriate by the RMS because of the following considerations:

(1) The presumed effect was observed statistically significant in only one of five long-term studies in mice in a strain with a rather high background incidence of malignant lymphoma. Evidence coming from two other studies one more study is even more equivocal because a certain increase there did not gain statistical significance. In a third study, a (non-significant) increase in top

dose incidence was explained and contravened by historical control data. Taking into account the huge amount of information on historical control incidences, there was no evidence of a similar effect in any other study.

(2) Although the increase in lymphoma incidence in the study by [redacted] (2001, ASB2012-11491) was statistically significant in both sexes, it was still within the (small) historical control range of the performing laboratory for females. No evidence of a similar effect in female mice was obtained in any other study.

(3) No evidence of carcinogenicity was obtained in a total of six valid 2-year studies in rats (see above) in which sufficiently high dose levels were employed.

(4) The dose with a significantly higher lymphoma incidence (1460 mg/kg bw/day) is more than 2900 times higher than the proposed ADI and the margin to the expected consumer exposure is even wider." (RAR, April 2015, ASB2015-1194)

ii) Differences in evaluation of individual study reports

Due to the application of different statistical approaches selected for evaluation, IARC and RMS came to diverging conclusions when evaluating cancer incidences in animal studies. IARC included a trend test (generally according to Cochran-Armitage) for statistical evaluation of the data (IARC, 2015, ASB2015-8421). In contrast, initially, the RMS relied on the statistical evaluation provided with the study reports, which was performed and documented as foreseen in the individual study plans (RAR, April 2015, ASB2015-1194). The later were mostly based on pairwise comparison of treatment groups using tests including Fishers exact test, Chi-Square test, or Z-test. As a consequence, IARC reported a positive carcinogenic response in some of these studies, while RMS did not. According to guidance documents for the evaluation of carcinogenicity studies published in support of respective OECD test guidelines (OECD 2012, ENV/JM/MONO(2011)47, ASB2015-8445 and OECD 2002, ENV/JM/MONO(2002)19, ASB2013-3754), both statistical approaches are appropriate.

In order to systematically assess the impact of choice of statistical method, a number of neoplastic endpoints in key-studies were re-evaluated by the RMS for this Addendum using the Fishers exact test and the Cochran-Armitage test, as both are explicitly recommended in the OECD guidance documents cited above. The Cochran-Armitage Test was performed using BMDS version 2.4.0.70. The Fisher-Yates test (Fisher´s exact test) was done using SigmaPlot version 11.2.0.5. The Fisher exact test was replaced by the Chi-square test if N was >50 for all groups.

(a) Renal adenoma and carcinoma in male mice:

The positive trend for renal adenoma and carcinoma in the study by [redacted] (1983, TOX9552381) as reported in the IARC evaluation could be confirmed (Table 3-3). When the trend test was also applied to the incidences of renal tubular tumours in male CD-1 mice as reported by [redacted] (1997, ASB2012-11493), and male Swiss albino mice as reported by [redacted] (2001, ASB2012-11491) further positive results were obtained (Table 3-4). The IARC Working Group did report but not evaluate these studies. In both cases, the pairwise comparison of treatment groups using the Fishers exact test did not show statistically significant differences (Table 3-3 and Table 3-4). No statistical association between renal neoplasia and glyphosate exposure was found in females of these three studies (incidences as follows: [redacted]: 0,0,0,0; [redacted] 0,0,0,0; [redacted] 0,0,0,0). No renal neoplasia were reported in mice of either sex by [redacted] (2009, ASB2012-11492). In the study of [redacted] (1993, TOX9552382), the incidences of renal tubular adenoma + carcinoma were 1+1, 1+1, 0, 0 in groups of males and 0 in all groups of females (control, low, medium, high dose). Incidences of renal tumours in rat studies were not statistically (re-)evaluated.

Table 3-3: **Renal adenoma and carcinoma in male CD-1 mice ([redacted] 1983, TOX9552381), originally reported data and re-evaluation by pathology working group (PWG). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each endpoint a Cochran-Armitage trend test was performed, with p-values reported in a separate row.**

Dose		**report**	**Re-evaluation by PWG**		
(mg/kg bw)	N	adenoma	adenoma	carcinoma	combined
0	49	0	1	0	1
157	49	0 (1.000)	0 (1.000)	0 (1.000)	0 (1.000)
814	50	1 (1.000)	0 (0.495)	1 (1.000)	1 (1.000)
4841	50	3 (0.242)	1 (1.000)	2 (0.495)	3 (0.617)
Trend test (p-value)		0.0080	0.2473	0.0370	0.0339

Table 3-4: **Renal tubular tumours adenoma in two additional studies performed in CD-1 mice ([redacted] 1997, ASB2012-11493) and in Swiss albino mice ([redacted] 2001, ASB2012-11491). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. A Cochran-Armitage trend test was performed, with p-values reported in a separate row.**

Animals	**[redacted] (1997, ASB2012-11493)**		**[redacted] (2001, ASB2012-11491)**	
per group	Dose (mg/kg bw)	adenoma	Dose (mg/kg bw)	adenoma
50	0	0	0	0
50	165	0 (1.000)	15	0 (1.000)
50	838	0 (1.000)	151	1 (1.000)
50	4348	2 (0.495)	1460	2 (0.495)
	Trend test (p-value)	0.0078	Trend test (p-value)	0.0390

b) Haemangiosarcoma in male mice:

The statistically positive trend test for haemangiosarcoma in the study by [redacted] (1993, TOX9552382) as reported by IARC could be confirmed. Direct comparison of the incidences in males of the high dose and the control group using the Fishers exact test resulted in a p-value of 0.059 just above the significance level of 0.05 (**Fehler! Ungültiger Eigenverweis auf Textmarke.**). In addition, there was a positive trend for haemangiosarcoma when the data from [redacted] (1997, ASB2012-11493) was included in the re-evaluation.

Table 3-5: Haemangiosarcoma in male CD-1 mice ([redacted] 1993, TOX9552382; [redacted] 1997, ASB2012-11493). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. A Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose		Haemangiosarcoma	Dose	Haemangiosarcoma
(mg/kg bw)	N	[redacted] (1993, TOX9552382)	(mg/kg bw)	[redacted] (1997, ASB2012-11493)
0	50	0	0	0
100	50	0 (1.000)	165	0 (1.000)
300	50	0 (1.000)	838	0 (1.000)
1000	50	4 (0.059)	4348	2 (0.495)
Trend test (p-value)		0.0004		0.0078

c) Malignant lymphoma in mice:

IARC and RMS reported a significantly increased incidence of malignant lymphoma in males of the high dose group in the study of [redacted] (2001, ASB2012-11491) compared to the concurrent control. Interestingly, when the analysis was performed using the Fischers exact test rather than the Z-test as done by the authors of the study report, a p-value of 0.077 > 0.05 instead of 0.002 < 0.01 was obtained. The trend test (not reported by IARC) also provided a p-value above the significance level of 0.05 (Table 3-6).

However, re-evaluation of the incidences if malignant lymphoma reported by [redacted] (2009, ASB2012-11490) and [redacted] (1997, ASB2012-11493) showed statistically significant increases with dose for male CD-1 mice (Table 3-7 and Table 3-8). Re-analysis of malignant lymphoma data reported by of [redacted] (1993, TOX9552382) confirmed the earlier evaluation, showing no treatment-related increases in incidence (Table 3-9).

Table 3-6: Malignant Lymphoma in Swiss albino mice ([redacted] 2001, ASB2012-11491). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each sex, a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose	male		female	
(mg/kg bw)	N	Malignant Lymphoma	N	Malignant lymphoma
0	50	10	50	18
15	50	15 (0.356)	50	20 (0.837)
151	50	16 (0.254)	50	19 (1.000)
1460	50	19 (0.077)*	50	25 (0.225)*
Trend test (p-value)		0.0655		0.068

* The original study report indicated a statistically significant increase (p<0.05).

Table 3-7: Malignant Lymphoma in CD-1 mice ([redacted] 2009, ASB2012-11490). Chi square test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each sex, a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose	male		female	
(mg/kg bw)	N	Malignant Lymphoma	N	Malignant lymphoma
0	51	0	51	11
71	51	1 (1.000)	51	8 (0.611)
234	51	2 (0.475)	51	10 (1.000)
810	51	5 (0.067)*	51	11 (1.000)
Trend test (p-value)		0.0037		0.3590

* Chi –square test was chosen in accordance to the recommendations of the statistics package used. Using the Fishers exact test, a p-value of 0.056 (two-sided) is calculated. Depending on the tool used for calculation, the two-tailed Z-test produced p-values of 0.0220, 0.0219 and 0.067.

Table 3-8: Malignant Lymphoma in CD-1 mice ([redacted] 1997, ASB2012-11493). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each sex, a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose	male		female	
(mg/kg bw)	N	Malignant Lymphoma	N	Malignant lymphoma
0	50	2	50	6
165	50	2 (1.000)	50	4 (0.741)
838	50	0 (0.495)	50	8 (0.774)
4348	50	6 (0.269)	50	7 (1.000)
Trend test (p-value)		0.0085		0.2971

Table 3-9: Malignant Lymphoma in CD-1 mice ([redacted] 1993, TOX9552382). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each sex, a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose	male		female	
(mg/kg bw/d)	N	Malignant Lymphoma	N	Malignant lymphoma
0	50	4	50	14
100	50	2 (0.678)	50	12 (0.657)
300	50	1 (0.362)	50	9 (0.342)
1000	50	6 (0.741)	50	13 (1.000)
Trend test (p-value)		0.0760		0.4831

d) Pancreatic islet cell adenoma in rats:

IARC noted that based to the tumour incidences reported by [redacted] (1990, TOX9300244), there was a significant increase in pancreatic adenoma in males in two dose levels but no statistically significant positive trend nor a progression to carcinoma. In contrast, RMS did not report any statistically significant effect for pancreatic tumours in this study. When re-evaluating the reported incidences using Cochran-Armitage trend testing and Fishers exact test, absence of a statistically positive trend was confirmed and a significant difference to the incidence in the control group was found for the low dose group only (Table 3-10). The latter result is in agreement with the study summary provided in the revised RAR Volume 3 (April 2015, ASB2015-1194).

Table 3-10: Pancreatic islet cell tumors in SD rats ([redacted], 1990, TOX9300244). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. A Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose	**male**	
(mg/kg bw)	N	adenoma
0	43	1
89	45	8 (0.030)
362	49	5 (0.209)
940	48	7 (0.062)
Trend test (p-value)		0.1687

In addition, IARC reported for the study of [redacted] (1981, TOX2000-595, TOX2000-1997) in SD rats a significant increase in the incidence of pancreatic tumours in one of the treated groups of males in the absence of statistically significant positive trends over all dose groups and no indication for progression to carcinoma. The RMS did not report significant pancreatic tumour findings for this study. Re-evaluation confirmed a significantly increase number of adenomas and combined adenomas + carcinomas for the male low dose group when compared to the concurrent controls. In addition, a significantly positive trend for carcinomas in male animals was found that has not been previously reported. There were no significant findings for pancreatic tumours in the females (Table 3-11 and Table 3-12).

Table 3-11: Pancreatic tumors in male SD rats ([redacted] 1981, TOX2000-595, TOX2000-1997). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each endpoint a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose	**male**			
(mg/kg bw)	N	adenoma	carcinoma	adenoma + carcinoma
0	50	0	0	0
3	49	5 (0.027)	0 (1.000)	5 (0.027)
10.3	50	2 (0.495)	0 (1.000)	2 (0.495)
31.5	50	2 (0.495)	1 (1.000)	3 (0.242)
Trend test (p-value)		0.5284	0.0496	0.3207

Table 3-12: **Pancreatic tumors in female SD rats ([redacted] 1981, TOX2000-595, TOX2000-1997). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each endpoint a Cochran-Armitage trend test was performed, with p-values reported in a separate row.**

Dose	**female**			
(mg/kg bw)	N	adenoma	carcinoma	adenoma + carcinoma
0	50	2	0	2
3.4	50	1 (1.000)	1 (1.000)	2 (1.000)
11.2	50	1 (1.000)	1 (1.000)	2 (1.000)
34	50	0 (0.495)	1 (1.000)	1 (1.000)
Trend test (p-value)		0.9025	0.2969	0.7371

e) Hepatocellular adenoma and carcinoma in rats:

IARC reported a significantly positive trend for hepatocellular adenoma in males in the study of [redacted] (1990, TOX9300244) without indications for progression to malignancy. In contrast, RMS did not report any statistically significant effect for liver tumours in this study. When re-evaluating the reported incidences using Cochran-Armitage trend testing and Fishers exact test, the statistically positive trend was confirmed for adenomas and no positive trend was observed for adenoma and carcinoma combined. In accordance with evaluations by IARC and RMS, a significant difference to the incidence in the control group was not found for the respective treatment groups (Table 3-13).

Table 3-13: **Liver cell tumors in SD rats ([redacted] 1990, TOX9300244). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each endpoint a Cochran-Armitage trend test was performed, with p-values reported in a separate row.**

Dose	**male**	**liver**	
(mg/kg bw)	N	adenoma	adenoma + carcinoma
0	44	2	5
89	45	2 (1.000)	4 (0.739)
362	49	3 (1.000)	4 (0.732)
940	48	7 (0.162)	9 (0.392)
Trend test (p-value)		0.0171	0.0752

f) Thyroid C-cell adenoma in rats:

The IARC Working Group reported a significant positive trend for C-cell adenoma in females of the study of [redacted] (1990, TOX9300244). The RMS did not report any statistically significant effect with respect to thyroid tumours for this study. The statistically significant positive trend could be confirmed using the Cochran-Armitage test (Table 3-14).

Table 3-14: Thyroid C-cell adenoma tumors in female SD rats ([redacted], 1990, TOX9300244). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. A Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose	**female**	**Thyroid**
(mg/kg bw)	N	C-cell adenoma
0	60	2
113	60	2 (1.000)
457	60	6 (0.167)
1183	60	6 (0.167)
Trend test (p-value)		0.0435

iii) Differences in decision criteria

In addition to the statistical significance, the RMS had taken into account consistency of results as a criterion for evaluation. Since no consistent significant increase in any of the tumour types was originally reported in the available studies the apparent effects were not considered sufficient for classification in the RAR (April 2015, ASB2015-1194).

As for the database, a part of the criteria used by both agencies is essentially similar while some deviations exist in terms of classification.

The IARC has used their own published criteria for evaluation of carcinogenic effects (IARC, 2006, ASB2015-8291) while RMS is generally bound to the classification criteria laid down in EU Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging of Substances and Mixtures (in brief referred to as CLP-criteria) (2008, ASB2015-8591).

Criteria IARC:

When considering the level of evidence for a carcinogenic effect, both sets of criteria are similar.

The IARC and CLP criteria state, that:

"*Sufficient evidence of carcinogenicity*: [The Working Group considers that] a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide *sufficient evidence*.

A single study in one species and sex might be considered to provide *sufficient evidence of carcinogenicity* when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.

"Limited evidence of carcinogenicity": The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs." (IARC 2006, ASB2015-8291; Reg (EC) No 1272/2008, Annex 1, 3.6.2, ASB2015-8591).

Conclusion by IARC:

Based on these criteria it is obvious that IARC concludes on "sufficient evidence of carcinogenicity" in experimental animals, because the above criteria for this conclusion are fully met.

Additional Criteria CLP:

The CLP criteria are taking into account the IARC criteria. However, the CLP regulation also states that when evaluating carcinogenic effects, additional criteria have to be taken into account. In Annex I to Reg (EC) 1272/2008 it is summarized:

"*Annex I: 3.6.2.2.4.* Additional considerations (as part of the weight of evidence approach Beyond the determination of the strength of evidence for carcinogenicity, a number of other factors need to be considered that influence the overall likelihood that a substance poses a carcinogenic hazard in humans. The full list of factors that influence this determination would be very lengthy, but some of the more important ones are considered here.

Annex I: 3.6.2.2.5. The factors can be viewed as either increasing or decreasing the level of concern for human carcinogenicity. The relative emphasis accorded to each factor depends upon the amount and coherence of evidence bearing on each. Generally there is a requirement for more complete information to decrease than to increase the level of concern. Additional considerations should be used in evaluating the tumour findings and the other factors in a case-by-case manner.

Annex I: 3.6.2.2.6. Some important factors which may be taken into consideration, when assessing the overall level of concern are:

(a) tumour type and background incidence;

(b) multi-site responses;

(c) progression of lesions to malignancy;

(d) reduced tumour latency;

(e) whether responses are in single or both sexes;

(f) whether responses are in a single species or several species;

(g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity;

(h) routes of exposure;

(i) comparison of absorption, distribution, metabolism and excretion between test animals and humans;

(j) the possibility of a confounding effect of excessive toxicity at test doses;

(k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity." (Reg (EC) No 1272/2008, Annex 1, ASB2015-8591)

Conclusion RMS:

Considering these additional criteria when taking into account the rat studies RMS argued that:

"No evidence of carcinogenicity was obtained in any of these studies." and when considering the majority of mouse studies RMS argues (possibly referring to point (a) and (j)) that: "*Again, there was no evidence of carcinogenicity of glyphosate in any of the studies."*

Accordingly for the malignant lymphoma previously observed in one mouse study only, RMS argues, referring to point (a) of the aforementioned list: *"Taking all this information together, a treatment-related effect in the study by* [redacted] *(2001, ASB2012-11491) in Swiss albino mice cannot be completely excluded. However, the weak increase in malignant lymphoma even over the historical control of the performing laboratory was clearly confined to this single study and strain since it was not reproducible in four other valid long-term studies. Thus, there is only very limited evidence of a carcinogenic potential of glyphosate as a high-dose phenomenon in mice of a susceptible strain. Most likely, perhaps, age-related neoplastic changes might be exacerbated by long-lasting administration of high doses. Swiss albino mice with high background prevalence of malignant lymphoma could be more vulnerable than other strains.*

Since the more frequent occurrence of malignant lymphoma was confined to a very high dose level that was administered over a long period, glyphosate was considered unlikely to pose a carcinogenic risk in humans […]" (RAR , April 2015, ASB2015-1194).

Overall, based on the study results and the CLP criteria RMS concluded that the evidence of carcinogenicity is conclusive but not sufficient for classification.

Summary and conclusion:

The statistical analysis by IARC was confirmed and extended. Based on the data evaluated by the respective agencies and the different criteria used for concluding on a potential carcinogenic effect, it is evident that both agencies have come to reasoned conclusions. The OECD test guideline on the evaluation of carcinogenicity studies states: "*Significance in either kind of test is sufficient to reject the hypothesis that chance accounts for the result.*" (OECD 2002, 2012, ASB2013-3754, ASB2015-8445). Accordingly, renal tumours in male CD-1 mice would be considered as treatment-related based on positive trend tests in two studies ([redacted], 1983, TOX9552381, [redacted] 1997, ASB2012-11493). Malignant lymphoma in males could be considered treatment related in the study by [redacted] (2001, ASB2012-11491) using Swiss albino mice based on the original positive Z-test for the high dose males and the studies of [redacted] (2009, ASB2012-11490) and [redacted] (1997, ASB2012-11493) in CD-1 mice based on positive trend tests for males.

4 Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

4.1.1 Introduction

The introduction in the IARC monograph is in line with the conclusions from the RAR (April 2015, ASB2015-1194). However, in the RAR a broader database was used to assess the microbial metabolism in the gut, suggesting a lower relevance as concluded by IARC.

4.1.2 Absorption

The data presented in the IARC monograph is also nearly completely reported in the RAR (April 2015, ASB2015-1194). The only additional study in the IARC monograph is an *in vitro* model by Vasiluk et al. (2005, ASB2012-12043), describing an increased paracellular permeability due to glyphosate at >10 mg/mL.

4.1.3 Distribution

In general the conclusion for the distribution of glyphosate is comparable between the IARC monograph and the RAR (April 2015, ASB2015-1194), suggesting short half-live times between 10 to 33 h. Also, tissue levels were identified to be highest in kidney.

Two studies presented in the IARC monograph were not reported in the RAR (Yue et al., 2008, ASB2012-12059 and Bernal et al., 2010, ASB2015-7858), however their results do not lead to different conclusions for the distribution of glyphosate.

4.1.4 Metabolism and modulation of metabolic enzymes

Both the IARC monograph and the RAR (April 2015, ASB2015-1194) concluded that glyphosate metabolized to a very small amount into AMPA in mammals. The IARC monograph relied on two studies not included in the RAR (Motojyuku et al., 2008, ASB2015-8160 and Bernal et al., 2010, ASB2015-7858). However in total the RAR provided a broader database for this endpoint. Concerning the modulation of metabolic enzymes all studies used by IARC were also presented in the RAR. No deviating conclusions were drawn in both documents.

4.1.5 Excretion

Except for one study on glyphosate and AMPA levels in urine of a rural population in Colombia (Varona, 2009, ASB2015-8039), which is in line with results from other studies, all references presented by IARC were also cited in the RAR. Also the conclusion that systemically absorbed glyphosate is not metabolized efficiently and is mainly excreted unchanged into the urine is identical. No discrepancies between the RAR (April 2015, ASB2015-1194) and the IARC monograph were identified.

4.2 Mechanisms of carcinogenesis

4.2.1 Genetic and related effects

Glyphosate has been studied for genotoxic potential in a wide variety of assays. The studies which were evaluated by IARC were carried out in exposed humans, in human cells *in vitro*, in other mammals *in vivo* and *in vitro*, and in non-mammalian systems *in vivo* and *in vitro*, respectively, are summarized in Tables 4.1-4.5 of the IARC monograph.

The IARC Working Group has reviewed only reports that have been published or accepted for publication in the openly available scientific literature as well as data from government agency reports that are publicly available.

In contrast, the RMS which undertakes the task of evaluating an active substance according to Regulation (EC) No 1107/2009 (2009, ASB2015-8589) shall review the complete dossier (that contains the full text of the individual test and study reports) and the scientific peer-reviewed open literature on the active substance and its relevant metabolites.

Thus, the RMS has assessed the relevant published data on genotoxicity of glyphosate which has also been reviewed by IARC, and additionally a number of regulatory studies which were not available to IARC, but a great many of them were evaluated in the review article of Kier and Kirkland (2013, ASB2014-9587). The regulatory studies were mostly generated in compliance with internationally agreed test guidelines, which include principles for conducting studies, reporting results, and analysing and interpreting data.

For regulatory purposes, test methods preferred for use are (ECHA, 2015: Guidance on information requirements and chemical safety assessment; Chapter R.7a: Endpoint specific guidance; Version 4.0, ASB2015-8657):

In vitro test methods: OECD 471, OECD 476, OECD 476, OECD 473, OECD 487.

In vivo test methods, somatic cells: OECD 475, OECD 474, OECD 488, OECD 486, OECD 489.

In vivo test methods, germ cells: OECD 483, OECD 478, OECD 488.

To be able to evaluate the mutagenic potential of a substance in a comprehensive way, information is required on its capability to induce gene mutations, structural chromosome aberrations (clastogenicity) and numerical chromosome aberrations (aneugenicity).

Classification of substances for (germ cell) mutagenicity according to CLP criteria:

Hazard classification for germ cell mutagenicity primarily aims to identify substances causing heritable mutations or being suspected of causing heritable mutations. A secondary aim is that the hazard class germ cell mutagenicity offers supporting information with respect to the classification of carcinogenic substances. This is expressed by the broad meaning of the hazard statements 'H340: May cause genetic defects' and 'H341: Suspected of causing genetic defects' which comprises heritable genetic damage as well as somatic cell mutagenicity. Thus, classification as a germ cell mutagen (Category 1A, 1B, and 2) classifies for the hazard heritable genetic damage as well as providing an indication that the substance could be carcinogenic.

Classification as a Category 1A mutagen:

Epidemiological studies have been to date unable to provide evidence to classify a substance as a Category 1A mutagen. Hereditary diseases in humans for the most part have an unknown origin and show a varying distribution in different populations. Due to the random distribution of mutations in the genome it is not expected that one particular substance would induce one specific genetic disorder. Therefore, it is unlikely that such evidence may be obtained by epidemiological studies to enable for classification of a substance as a Category 1A mutagen.

Classification as a Category 1B mutagen:

Classification in Category 1B may be based on positive results of at least one valid *in vivo* mammalian germ cell mutagenicity test. In case there are also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied.

If there are only positive results of at least one valid *in vivo* mammalian somatic mutagenicity test but no respective data on mammalian germ cells are available, additional evidence is required to be able to classify as mutagen in Category 1B. Such additional data must prove that the substance or its metabolite(s) interacts *in vivo* with the genetic material of germ cells. It is also possible to obtain supporting evidence in an *in vivo* genotoxicity test with mammalian germ cells. In addition, genetic damage to germ cells in exposed humans proven to be caused by substance exposure may offer respective information. In case of other supporting evidence or where there are also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied.

Classification as a Category 2 mutagen:

Classification in Category 2 may be based on positive results of a least one *in vivo* valid mammalian somatic cell mutagenicity test, indicating mutagenic effects in somatic cells. A Category 2 mutagen classification may also be based on positive results of a least one *in vivo* valid mammalian somatic cell genotoxicity test, supported by positive *in vitro* mutagenicity results. Genetic damage to somatic cells in exposed humans shown to be caused by substance exposure supported by positive *in vitro* mutagenicity results may also offer respective information warranting classification as a Category 2 mutagen. *In vitro* results can only lead to a Category 2 mutagen classification in a case where there is support by chemical structure activity relationship to known germ cell mutagens. In the case where there are also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied.

Principles for the evaluation of published studies used by the RMS

For the analysis of published studies, the RMS made generally a comparison to the criteria in guidelines used for regulatory purposes. However, these criteria do not represent an absolute judgment standard but can provide a way for evaluating the quality of the protocols used in various published studies. Kier & Kirkland (2013, ASB2014-9587) have summarized a number of relevant issues to be considered: "*Some of the criteria are rarely met in scientific publications and should be given little or no weight in evaluating the studies. For example, data for individual cultures and individual animals are not commonly included in publications in scientific journals. These data are presumably collected but are usually summarized as group means with a measure of variance for the treatment and control groups. This is not considered to be a significant omission in a scientific publication. However, other guideline features are more essential as scientific quality standards and should be considered as having greater weight in evaluating a study. For example, there are consistent recommendations that assays involving visual scoring (e.g. chromosomal aberration, micronucleus and sister chromatid exchange (SCE) endpoints) should use slides that are independently coded so that scoring is performed without any knowledge of the treatment or practice and studies that do not explicitly include a description of coding or ''blind'' scoring in the methodology would appear to have a deficiency either in the methodology, or perhaps a limitation in the description of the methodology used if coding was actually used and either not indicated or was assumed to be indicated by a reference citation. Other examples of guideline features that have clear experimental scientific value are the use of concurrent negative and positive controls and concurrent measurement and reporting of toxicity endpoints in main experiments, especially in in vitro mammalian cell assays.*"

Glyphosate:

Assessment and conclusion of IARC:

According to the conclusion of IARC, there is strong evidence that glyphosate causes genotoxicity. The evidence base includes studies that gave largely positive results in human cells *in vitro* (IARC monograph, Table 4.2), in mammalian model systems *in vivo* (IARC monograph, Table 4.3) and *in vitro*

(IARC monograph, Table 4.4), and studies in other non-mammalian systems *in vivo* (IARC monograph, Table 4.5) and *in vitro* (IARC monograph, Table 4.6). *In vivo* studies in mammals gave generally positive results in the liver, with mixed results for the kidney and bone marrow. The end-points that have been evaluated in these studies comprise biomarkers of DNA adducts and various types of chromosomal damage. Tests in bacterial assays gave consistently negative results (IARC monograph, Table 4.6).

Assessment and conclusion of the RMS:

In vitro studies:

1. Bacterial assays gave consistently negative results.
2. *In vitro* mammalian cell gene mutation tests gave consistently negative results.
3. *In vitro* mammalian chromosome aberration tests and *in vitro* micronucleus tests: several regulatory studies conducted according to internationally agreed test guidelines which gave negative results at concentrations up to 1250 µg/ml (Table 4.2-1). In contrast, induction of chromosomal aberrations in bovine lymphocytes was reported in one non-guideline study without metabolic activation at concentrations of 3-30 µg/mL (Lioi et al., 1998, ASB2013-9836), and induction of micronucleus formation in CHO cells was reported in one non-guideline study with metabolic activation at concentrations of 5-100 µg/mL (Roustan et al., 2014, ASB2014-8086).
4. Further *in vitro* tests (indicator tests): Positive results for induction of sister chromatid exchange (SCE) were reported in cultured human and bovine lymphocytes without metabolic activation in two published non-guideline studies (Table 4.2-2).

   Positive results were also reported for induction of DNA strand breaks in *in vitro* mammalian cell assays in five published non-guideline studies (Table 4.2-2).

   There was no evidence of an increase in unscheduled DNA synthesis (UDS) in rat primary hepatocyte cultures *in vitro* in a published study and a regulatory study (Table 4.2-2).

In vivo studies (in mammals) in somatic cells:

1. Mutagenicity tests: Both the rodent bone marrow micronucleus test and the rodent bone marrow chromosome aberration test were used in a total of 16 studies to examine mutagenic effects of glyphosate.

   In 8 regulatory studies in rats and mice conducted according to internationally agreed test guidelines, glyphosate was administered by oral gavage at dose levels up to 5000 mg/kg bw, which is well above the limit dose of 2000 mg/kg bw according to OECD test guidelines 474 or 475. The tests gave consistently negative results (Table 4.2-3).
   In another 8 studies in rats and mice (4 publications and 4 regulatory studies), glyphosate was administered by intraperitoneal application at dose levels up to 600 mg/kg bw in mice and up to 1000 mg/kg bw in rats. These dose levels may have exceeded the maximum tolerated dose, since the intraperitoneal LD_{50} of glyphosate has been reported to be 134 mg/kg bw in mice (Bababunmi et al., 1978, ASB2015-8535). For rats, the intraperitoneal LD_{50} of glyphosate ranged from 238 mg/kg bw to 1383 mg/kg bw (Bababunmi et al., 1978, ASB2015-8535, [redacted] 1991, TOX9300330). Irrespective of the high dose levels tested, negative results were obtained in 6 studies (one chromosome aberration test in rats, 5 micronucleus tests in mice; Table 4.2-3).
   In one published study in mice (Bolognesi et al., 1997, Z59299), two i.p. doses of 150 mg/kg bw, administered 24 h apart, produced a statistically significant increase in micronuclei when bone marrow was examined 24 h after the second dose. However, the dose tested was in the range of the intraperitoneal LD_{50} of glyphosate reported for mice, and no information on signs of toxicity was provided in the publication.
   In second published study in mice (Mañas et al., 2009a, ASB2012-11892), two i.p. doses of 200 mg/kg bw, administered 24 h apart, produced a statistically significant increase in micronuclei

when bone marrow was examined 24 h after the second dose. However, the result of this study is flawed by a major deviation from internationally agreed test guidelines: "erythrocytes" instead of immature or "polychromatic erythrocytes" (PCE) were scored for micronuclei. In an assay with the reported treatment and sampling times, scoring of all erythrocytes instead of polychromatic erythrocytes would be inappropriate (test guideline OECD 474).

2. Further *in vivo* studies: Evidence for DNA adduct formation and for induction of DNA strand breaks following i.p. administration of glyphosate to mice at a single dose of 300 mg/kg bw has been reported in one publication (Bolognesi et al., 1997, Z59299). Induction of DNA strand breaks was also reported in a published study in mice after oral doses of 40 and 400 mg/kg bw per day over a period of 14 days (Mañas et al., 2013). In contrast, no evidence for DNA adduct formation was reported following intraperitoneal administration of glyphosate isopropylammonium salt to mice at a single dose of 270 mg/kg bw (Peluso et al., 1998, TOX1999-318).
   Since the induction of DNA strand breaks was observed at a dose close to or in excess of the i.p. LD_{50} of glyphosate in mice, the positive result of this assay may be caused by secondary effects of cytotoxicity.

In vivo studies (in mammals) in germ cells:

Glyphosate has been shown to be devoid of mutagenic activity in a dominant lethal assay in mice at oral doses up to 2000 mg/kg bw (EPA, 1980, ASB2015-8547; [redacted] 1980, TOX9552377) and in a dominant lethal assay in rats at oral doses up to 2000 mg/kg bw ([redacted] 1992, TOX9551102).

Overall conclusion:

Glyphosate has been tested in a broad spectrum of mutagenicity and genotoxicity tests *in vitro* and *in vivo*.

In vitro, bacterial assays and mammalian cell gene mutation assays gave consistently negative results. Also, the majority of *in vitro* chromosomal aberration tests and micronucleus tests were negative, in particular, all of the studies performed under GLP conditions resulted in negative findings. *In vitro* tests for induction of indicator endpoints gave positive results for induction of SCE and DNA strand breaks (comet assay) and a negative result for induction of DNA repair (UDS).

In vivo, 14 somatic cell tests for induction of chromosomal aberrations or micronuclei gave negative results, including all the 12 regulatory studies conducted under GLP conditions. Therefore, it is concluded that glyphosate does not induce chromosomal damage *in vivo*, although positive results are reported in two publications. Furthermore, there was no evidence for mutagenic activity in germ cells. Inductions of DNA strand breaks were reported in 2 publications following a high i.p. dose or repeated oral doses.

Taking into account all available data and using a weight of evidence approach, it is concluded that glyphosate does not induce mutations *in vivo* and no hazard classification for mutagenicity is warranted according to the CLP criteria.

AMPA:

AMPA has been tested for mutagenicity and genotoxicity *in vitro* and *in vivo* in an adequate range of assays.

In vitro, two bacterial assays and a mammalian cell gene mutation assay performed under GLP conditions gave negative results, while two micronucleus tests were positive. Two *in vitro* tests for induction of DNA repair (UDS) performed under GLP conditions gave negative results; while a test for induction of DNA strand breaks (comet assay) was positive.

In vivo, two bone marrow micronucleus tests conducted under GLP conditions gave negative results, while a positive result was reported in a published study flawed by methodological limitations. Induction of DNA strand breaks was reported in a publication following repeated oral doses.

Taking into account all available data and using a weight of evidence approach, it is concluded that AMPA does not induce mutations *in vivo* and no hazard classification for mutagenicity is warranted according to the CLP criteria.

Glyphosate-based formulations:

Glyphosate-based formulations have been extensively tested for mutagenicity and genotoxicity *in vitro* and *in vivo* in a wide range of assays. However, since formulation compositions are considered proprietary, the specific composition of the formulations tested was not available for the published studies.

In vitro, bacterial assays gave generally negative results. No regulatory studies of glyphosate-based formulations in *in vitro* mammalian cell chromosomal aberration or micronucleus assays were provided. However, published studies suggested the possibility of activity of glyphosate-based formulations in *in vitro* chromosomal damage assays. No regulatory studies of glyphosate-based formulations in *in vitro* mammalian cell assays for DNA damage were provided. In some published studies, however, positive results for DNA strand breakage and SCE induction were reported.

In vivo mammalian chromosomal aberration or micronucleus assays gave positive results in some published studies for specific glyphosate-based formulations. However, no regulatory studies for these endpoints were provided. Also, no regulatory studies for these endpoints were provided for *in vivo* mammalian assays for DNA damage. However, in some published studies positive results for DNA adducts, DNA strand breakage and SCE induction were reported for specific glyphosate-based formulations. The positive results may be associated with high organ toxicity (liver, kidney) that was primarily due to the non-glyphosate components of the formulation when administered at very high doses via the i.p. route of exposure.

In non-mammalian systems, positive results were reported in *in vivo* studies on chromosomal damage or DNA damage of fish, amphibians and reptiles with different formulations (IARC monograph, Table 4.5). For the representative formulation for the EU renewal procedure 'Roundup Ultra' two studies (Guilherme et al., 2012, ASB2014-7619, Guilherme et al., 2014, ASB2015-8631) reported positive results in comet assays using the European eel as test species.

However, in addition to some technical limitations, there is considerably less experience with these assay systems, and their relevance fur human health assessment is undecided.

Table 4.2-1: Glyphosate; mutagenicity tests in mammalian cells or bacteria *in vitro*

Reference	Evalu-ated by IARC	Test system (endpoint)	Test	Results: Without metabolic activation by authors	Results: With metabolic activation by authors	Concentration range	GLP, Test guideline	RAR 04/2015	Comments BfR
Mañas et al., 2009a, ASB2012-11892	Yes	Human Lymphocytes (Chromosomal damage)	Chromosomal aberrations	–	NT	0.2-6.0 mM (34 - 1015 µg/mL) Purity: 96%	NR, TG 473	p. 401, 436	Only 100 cells scored per treatment. Results not reported separately for replicate cultures.
Fox, 1998, TOX2000-1995	No	Human lymphocytes (Chromosomal damage)	Chromosomal aberration	–	–	-S9/+S9: 100 - 1250 µg/ml Purity: 95.6%	GLP, TG 473	p. 345, 353-357	
Mladinic et al., 2009a, ASB2012-11907	Yes	Human lymphocytes (Chromosomal damage)	Micronucleus formation	–	(+)	-S9/+S9: 0.5 - 580 µg/mL Purity: 98%	Non-GLP, NR	p. 401, 437	$P < 0.01$ (580 µg/mL) Independent coding of slides for scoring not indicated for visually scored slides. Results not reported separately for replicate cultures.
Van de Waart, 1995, TOX9651525	No	Human lymphocytes (Chromosomal damage)	Chromosomal aberration	–	–	-S9: 33 - 333 µg/mL +S9: 237 - 562 µg/mL Purity: 96%	GLP, TG 473	p. 345	
Wright, 1996, ASB2012-11476	No	Chinese hamster lung cells (Chromosomal damage)	Chromosomal aberrations	–	–	-S9/+S9: 312.5 - 1250 µg/mL Purity: 95.3%	GLP, NR	p. 345, 351-353	
Kyomu, 1995, ASB2012-11475	No	Chinese hamster lung cells	Chromosomal aberrations	–	–	-S9: 62.5 - 500 µg/mL +S9:	GLP, TG 473	p. 345-351	

Reference	Evalu-ated by IARC	Test system (endpoint)	Test	Results: Without metabolic activation by authors	Results: With metabolic activation by authors	Concentration range	GLP, Test guideline	RAR 04/2015	Comments BfR
		(Chromosomal damage)				250 - 1000 µg/mL Purity: 95.7%			
Lioi et al., 1998, ASB2013-9836	Yes	Bovine Lymphocytes (Chromosomal damage)	Chromosomal aberrations	+	NT	17 - 170 µM (3 - 30 µg/mL) Purity: ≥ 98%	NR	p. 387	$P < 0.05$ (17 µM) 150 metaphases per concentration were scored for CAs (200 or 300 needed acc. TG 1997 or 2014).
Roustan et al., 2014, ASB2014-8086	Yes	Hamster, Chinese CHO-K1 ovary cell line (Chromosomal damage)	Micronucleus formation	–	+	5 - 100 µg/mL Purity: not given	NR	p. 423-424	$P \leq 0.001$ (10 µg/mL) No continuous treatment (TG 2014).
Clay, 1996, TOX2000-1994	No	Mouse lymphoma cells /L5178Y TK$^{+/-}$ (Mutation)	Mouse lymphoma test	–	–	+/-S9: 296 - 1000 µg/mL Purity: 95.6%	GLP, TG 476	p. 338-341	
Jensen, 1991, TOX9552372	No	Mouse lymphoma cells/L5178Y	Mouse lymphoma test	–	–	-S9: 0.61 - 5.0 mg/mL +S9: 0.52 - 4.2 mg/mL Purity: 98.6%	GLP, TG 476	p. 338	
Li and Long, 1988, TOX9500253 also reported in RAR, TOX9552369, Z35243	Yes	Hamster, Chinese CHO-K_1BH_4 ovary, cell line (Mutation)	*Hprt* mutation	–	–	-S9: 2 - 22.5 mg/mL +S9: 5 - 22.5 or 25 mg/mL Purity: 98.7%	NR	p. 338	Not entirely clear from the original study report which dose level was actually the highest under activation conditions.

Reference	Evalu-ated by IARC	Test system (endpoint)	Test	Results: Without metabolic activation by authors	Results: With metabolic activation by authors	Concentration range	GLP, Test guideline	RAR 04/2015	Comments BfR
Li and Long, 1988, TOX9500253	Yes	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, TA100 (Mutation)	Reverse mutation	–	–	10 - 5000 µg/plate Purity: 98.4%	NR	p. 305	2-aminoanthracen only used as positive control + S9. Only duplicate plating.
Li & Long, 1988, TOX9500253	Yes	Escherichia coli WP2 (Mutation)	Reverse mutation	–	–	10 - 5000 µg/plate Purity: 98.4%	NR	p. 305	2-aminoanthracen only used as positive control + S9. Only duplicate plating.

Results: +, positive; -, negative
NT, not tested; NR, not reported ; S9, 9000 × g supernatant; Hprt, hypoxanthine guanine phosphoribosyl transferase gene;

Table 4.2-2: Glyphosate; genotoxicity tests in mammalian cells or bacteria *in vitro*

Reference	Evaluated by IARC	Test system (endpoint)	Test	Results: Without metabolic activation by authors	Results: With metabolic activation by authors	Concentration range, purity of test substance	GLP, Test guideline	RAR 04/2015	Comments BfR
Mañas et al., 2009a, ASB2012-11892	Yes	Liver Hep-2 (DNA damage)	DNA strand breaks, comet assay	+	NT	3 - 7.5 mM (507.2 - 1268 µ g/mL) Purity: 96%	NR	p. 404, 436	$P < 0.01$ (507.2 µg/mL), dose–response relationship No indication of pH or osmolality control. Results not reported separately for replicate cultures.
Mladinic et al., 2009b, ASB2012-11906	Yes	Human lymphocytes (DNA damage)	DNA strand breaks, standard and hOGG1	+	+	0.5-580 µg/mL Purity: 98%	NR	p. 437	$P < 0.05$ (3.5 µg/mL) With the hOGG1 modified comet assay, + S9, the increase was significant ($P < 0.01$) only at the highest dose tested (580 µg/mL).

Reference	Evaluated by IARC	Test system (endpoint)	Test	Results: Without metabolic activation by authors	Results: With metabolic activation by authors	Concentration range, purity of test substance	GLP, Test guideline	RAR 04/2015	Comments BfR
			modified comet assay						No indication of pH or osmolality control. Results not reported separately for replicate cultures. Authors state that no clear dose-dependent effect was observed.
Alvarez-Moya et al., 2014, ASB2014-6902	Yes	Human lymphocytes (DNA damage)	DNA strand breaks, comet assay	+	NT	0.0007-0.7 mM (0.118- 118 µg/mL) Purity: 96%	NR	p. 404	$P \leq 0.01$ (0.0007 mM) No indication of pH or osmolality control. Results not reported separately for replicate cultures. Inconsistent and not clear dose dependent. Test was conducted with glyphosate isopropylamine .
Monroy et al., 2005, ASB2012-11910	Yes	Fibroblast GM 39 and Fibrosarcoma HT1080 (DNA damage)	DNA strand breaks, comet assay	+	NT	4.0-6.5 nM ($6.76 \cdot 10^{-4}$ – $1.1 \cdot 10^{-3}$ µg/mL, GM39 cells), 4.5-6.5 nM ($7.6 \cdot 10^{-4}$-$1.1 \cdot 10^{-3}$ µg/mL HT1080) cells) Purity: not given	NR	p. 403	Fibroblast: $P < 0.001$ (4 nM) Fibrosarcoma: $P < 0.001$ (4.75 nM) No indication of pH or osmolality control. No concurrent measurement of toxicity reported. Independent coding of slides for scoring not indicated for visually scored slides. Results not reported separately for replicate cultures. Concentrations seem very low.
Lueken et al., 2004, ASB2012-11886	Yes	Fibroblast GM 5757 (DNA damage)	DNA strand breaks, comet assay	(+)	NT	75 mM (12.7 mg/ml) Purity: 98.4%	NR	–	Not regarded as glyphosate was only tested together with H_2O_2.
Koller et al., 2012, ASB2014-7618	Yes	Buccal carcinoma TR146 (DNA damage)	DNA strand breaks, comet assay	+	NT	10-2000 µg/mL Purity: 95%	NR	p. 404	$P \leq 0.05$ (20 µg/mL) No indication of pH or osmolality control. Results not reported separately for replicate cultures. No clear dose-response effect.

Reference	Evaluated by IARC	Test system (endpoint)	Test	Results: Without metabolic activation by authors	Results: With metabolic activation by authors	Concentration range, purity of test substance	GLP, Test guideline	RAR 04/2015	Comments BfR
									Higher activity of formulation than pure a. s.
Bolognesi et al., 1997, Z59299	Yes	Human lymphocytes (Chromosomal damage)	Sister-chromatid exchange	+	NT	0.33 and 6 mg/mL Purity: 99.9%	NR	p. 385, 390, 429	$P < 0.05$ (1 mg/ml) The number of only two subjects to be included in the study appears too low for meaningful evaluation. Furthermore, the data from two experiments were pooled for the two donors and individual values were not given. The study is performed with methodological and reporting deficiencies (no positive controls included in *in vitro* SCE). Test guideline deleted by now.
Li and Long, 1988, TOX9500253	Yes	Rat, Fisher F334 Hepatocytes (DNA damage)	Unscheduled DNA synthesis	–	NT	$1.25 \cdot 10^{-5}$-$1.25 \cdot 10^{-1}$ mg/ml Purity: 98%	NR	–	Only between 5 and 20 cells counted. Test guideline deleted by now.
Rossberger, 1994, TOX9400697	No	UDS assay/ Primary rat hepatocytes/Sprague Dawley	Unscheduled DNA synthesis	–	NT	0.2-111.7 mM (33.8 µg/ml-18.9 mg/ml) Purity: > 98%	GLP, TG 482	p. 342	Instead of autoradiography or LSC procedures, incorporation of radioactivity into DNA was determined on basis of UV absorbance measurement.
Lioi et al., 1998, ASB2013-9836	Yes	Bovine Lymphocytes (Chromosomal damage)	Sister-chromatid exchange	+	NT	17-170 µM (2.9-29 µg/ml Purity: ≥ 98%	NR	p. 387	$P < 0.05$ (17 µM) Data is pooled for the three donors and individual values were not given. Increase of SCE not dose related in highest dose group. Test guideline deleted by now.
Akanuma, 1995, ASB2012-11477	No	B. subtilis H17, M45 (DNA	Rec assay	–	–	7.5-240 µg/disk Purity: 95.7%	GLP, U.S. EPA FIFRA	p. 342-344	Rec assay is not a standard method for this endpoint (DNA damage and repair).

Reference	Evaluated by IARC	Test system (endpoint)	Test	Results: Without metabolic activation by authors	Results: With metabolic activation by authors	Concentration range, purity of test substance	GLP, Test guideline	RAR 04/2015	Comments BfR
		damage/repair)							

Results: +, positive; -, negative; (+) or (-) positive/negative in a study with limited quality
hOGG1, human 8-hydroxyguanosine DNA-glycosylase; NR, not reported; NT, not tested; S9, 9000 × g supernatant; vs, versus

Table 4.2-3: Glyphosate; somatic cell mutagenicity tests in mammals, *in vivo*

Reference	In IARC mono-graph	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
Oral application								
[redacted] 1991, TOX9552374	No	Mouse, Micronucleus test, bone marrow	Glyphosate, 98.6 % oral, 1x 0 or 5000 mg/kg bw, sampled after 24, 48 and 72 h	Negative	GLP, OECD 474 (1983)	*MN/2000 PCE [mean (range)]:* Control: 2.7 (1-4) 24h, 5000 mg/kg: 3.2 (1-5) 48h, 5000 mg/kg: 2.8 (1-6) 72h, 5000 mg/kg: 1.7 (0-4) PosControl: 48.2 (32-58)	5 animals per sex and sampling time. 2000 PCE scored/animal. PCE/NCE: no effect.	p. 358, 364
[redacted] 1993, TOX9551100	No	Mouse, Micronucleus test, bone marrow	Glyphosate, 96.8 % oral, 2x 0, 50, 500 or 5000 mg/kg bw (24 h interval), sampled 24 h after second dose	Negative	GLP, OECD 474 (1984)	*% MNPCE [mean (range)], male/female:* Control: 0.69 (0.1-1.6)/0.51 (0.2-1.0) 50 mg/kg: 0.84 (0.2-1.4)/0.28 (0.0-0.5) 500 mg/kg: 0.73 (0.4-1.6)/0.52 (0.2-1.3) 5000 mg/kg: 0.89 (0.7-1.1)/1.05*(0.4-1.6) PosControl: 2.33* (1.5-3.2)/2.39* (1.4-3.4) *p<0.05	5 animals per sex and dose (Control: 10/sex). 2000 PCE scored/animal. PCE/NCE: no effect (but PosControl). *The MN incidence in females at 5000 mg/kg is within the range of controls considering both sexes.*	p. 357 ff.
[redacted] 1994, TOX940032	No	Mouse, Chromosome aberration test,	Glyphosate, 96.8 % oral, 2 x 0-5000 mg/kg bw	Negative	GLP, OECD 475	*No. of aberrations per 250-250-500 metaphases (male/female/total)* Control: 12/10/22	5 animals per sex. 50 metaphases/animal examined. *Mitotic index (%)*	p. 358

Reference	In IARC mono-graph	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
3		bone marrow	(24 h interval), sampled 24 h after second dose		(1984)	5000 mg/kg: 10/11/21 PosControl: 139*/155*/294* *p<0.05	*(male/female/total)* Control: 13.3/17.4/15.3 5000 mg/kg: 8.9*/9.5*/9.2* PosControl: 14.7/5.5*/10.1*	
[redacted] 1996, TOX2000-1996	No	Mouse, Micronucleus test, bone marrow	Glyphosate, 95.6 % oral, 1x 0 or 5000 mg/kg bw, sampled after 24 and 48 h	Negative	GLP, OECD 474 (1997)	*MN/1000 PCE (mean±SD), male/female:* 24h, Control: 1.6±0.8/1.4±0.7 24h, 5000 mg/kg: 2.1±1.6/2.1±2.5 24h, PosControl: 22.2±6.1*/23.3±4.9* 48h, Control: 1.7 ±1.3/0.7±0.6 48h, 5000 mg/kg: 2.1±1.9/0.8±0.8 *p<0.01	5 animals per sex and sampling time. 2000 PCE scored/animal. PCE/NCE: no effect.	p. 359, 370 ff.
[redacted], 2008, ASB2012-11483	No	Mouse, Micronucleus test, bone marrow	Glyphosate, 99.1 % oral, 1x 0, 500, 1000 or 2000 mg/kg bw, sampled after 24 h 1x 0 or 2000 mg/kg bw, sampled after 48 h	Negative	GLP, OECD 474 (1997)	*MN/2000 PCE [mean (range)]:* 24h, Control: 1.4 (0-3) 24h, 500 mg/kg: 1.6 (1-2) 24h, 1000 mg/kg: 1.6 (1-2) 24h, 2000 mg/kg: 1.4 (0-2) 24h, PosControl: 63.0 (44-92)* 48h, Control: 1.4 (0-3) 48h, 2000 mg/kg: 1.6 (0-3) *p<0.01	5 males per group and sampling time. 2000 PCE scored/animal. PCE/NCE: no effect. Historical control data (293 studies): *% MNPCE [mean±SD, (range)]:* *0.084±0.031 (0.01 – 0.18)*	p. 359, 372 ff.
[redacted] 2012, ASB2014-9277	No	Mouse, Micronucleus test, bone marrow	Glyphosate, 98.9 % oral, 2x 0 or 2000 mg/kg bw (24 h interval), sampled 24 h after second dose	Negative	GLP, OECD 474 (1997)	*% MNPCE [mean (range)]:* Control: 0.033 (0-0.05) 2000 mg/kg: 0.0 (0-0) PosControl: 2.49* (1.1-3.7) *p<0.01	6 males per group. 2000 PCE scored/animal. PCE/NCE: no effect at 2000 mg/kg, increased in PosControl. Historical control data (of 73 studies) *% MNPCE [mean±SD (range)]:* *0.02±0.02 (0.0-0.07)*	p. 359, 374 ff.
[redacted] 2012, ASB2014-	No	Mouse, Micronucleus	Glyphosate, 96.3 % oral,	Negative	GLP, OECD	*MN/2000 PCE [mean±SD, (range)]:* 24h, Control: 3.2±3.6 (0-8)	7 males per group (Control and PosControl: 5 males each).	p. 359. 375 ff.

Reference	In IARC mono-graph	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
9333		test, bone marrow	1x 0 or 2000 mg/kg bw, sampled after 24 and 48 h		474 (1997)	24h, 2000 mg/kg: 2.3±0.5 (2-3) 24h, PosControl: 40.2±18.2* (16-67) 48h, Control: 1.4±1.1 (0-3) 48h, 2000 mg/kg: 1.1±1.3 (0-3) *p<0.01	2000 PCE scored/animal. PCE/NCE: no effect. Historical control data (of 219 studies) *% MNPCE [mean±SD (range of mean group value)]:* 0.108±0.039 (0.01-0.25)	
[redacted] 2009, ASB2012-11479	No	Rat, Micronucleus test, bone marrow	Glyphosate, 98.8 % oral, 1x 0, 500, 1000 or 2000 mg/kg bw, sampled after 24 and 48 h	Negative	GLP, OECD 474 (1997)	*MN/2000 PCE (mean±SD), male/female:* 24h, Control: 1.6±1.1/1.8±0.4 24h, 500 mg/kg: 1.0±1.2/1.2±1.3 24h, 1000 mg/kg: 0.8±0.4/1.6±0.9 24h, 2000 mg/kg: 1.2±0.8/0.8±0.8 24h, PosControl: 30.2±10.5*/24.0±4.9* 48h, Control: 2.0 ±1.9/2.2 ±1.3 48h, 2000 mg/kg: 1.6±0.9/0.8±0.8 *p<0.05	5 animals per sex and dose and sampling time. 2000 PCE scored/animal. PCE/NCE: no effect. Historical control data (24, 48 and 72 h samplings combined): *MN/1000 PCE [mean and (range):* Males: 1.97 (0.4 – 5.7) Females: 1.86 (0.4 – 4.7)	p. 359. 376 ff.
i.p. application								
Li and Long, 1988, TOX9500253 [redacted] 1983, TOX9552369	Yes	Rat, Chromosome aberration test, bone marrow	Glyphosate, 98 % i.p., 1x 0 or 1000 mg/kg bw, sampled after 6, 12 and 24 h	Negative	No GLP, no reference to TG	*% aberrant cells (mean), male/female/total:* 6h, Control: 1.3/2.7/2.0 6h, 1000 mg/kg: 2.3/3.0/2.7 12h, Control: 1.0/1.5/1.2 12h, 1000 mg/kg: 2.0/2.5/2.3 24h, Control: 1.3/2.3/1.8 24h, 1000 mg/kg: 1.0/3.7/2.6 PosControl: 42.2*/23.8*/40.8* * p < 0.05	Consistent with OECD 475 (1984): 6 animals per sex and sampling time. Ca 50 metaphases/animal examined. Slides were coded and scored "blind". Original study reported in RAR as Li, 1983 (TOX9552375).	p. 358, 383
Rank et al., 1993,	Yes	Mouse, Micronucleus	Glyphosate isopropylamine salt,	Negative	No GLP, no	*% MNPCE (mean±SD):* 24h, Control: 0.27±0.11	Consistent with OECD 474 (1983):	p. 385, 388f.

Reference	In IARC mono-graph	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
Z82234		test, bone marrow	purity not stated i.p., 1x 0, 100, 150 or 200 mg/kg bw sampled after 24 and 48 h		reference to TG	24h, 100 mg/kg: 0.20±0.13 24h, 150 mg/kg: 0.2±0.13 24h, 200 mg/kg: 0.25±0.10 24h, PosControl: 2.53±0.59 48h, 150 mg/kg: 0.13±0.09 48h, 200 mg/kg: 0.12±0.09	Mostly 5 animals per sex and dose and sampling time. 1000 PCE scored/animal. Slides were scored randomly. PCE/NCE: no effect.	
Bolognesi et al., 1997, Z59299	Yes	Mouse, Micronucleus test, bone marrow	Glyphosate, 99.9 % i.p., 2x 150 mg/kg bw (24 h interval), sampled 6 or 24 h after second dose	Positive	No GLP, no reference to TG	*MN/1000 PCE (mean±SD):* Control: 0.75±0.46 6h, 2x 150 mg/kg: 1.4±0.9 24h, 2x 150 mg/kg: 2.4±1.5* 24h, PosControl: 80.0±8.5* * p < 0.05	6 males in Control and PosControl group. 3000 PCE scored/animal. PCE/NCE: 0.73±0.06 in Control, 0.6±0.05 at 6h, 0.5±0.2 at 24h. *Deviations from OECD 474 (1997):* *Only 3(4) males examined per sampling time.* *Sampling time of Control not stated.* *Independent coding of slides not stated.*	p. 385, 389
Mañas et al., 2009a, ASB2012-11892	Yes	Mouse, Micronucleus test, bone marrow	Glyphosate, 96 % i.p., 2x 50, 100 or 200 mg/kg bw (24 h interval), sampled 24 h after second dose	Positive	No GLP, OECD 474 (1997)	*MN/1000* ***Erythrocytes*** *(mean±SD):* Control: 3.8 ±0.8 2x 50 mg/kg: 3.7±0.5 2x 100 mg/kg: 4.2±0.5 2x 200 mg/kg: 13.0±3.5* PosControl: 19.2±3.9* * *P* < 0.01	5 animals per dose. PCE/NCE no effect. *Deviations from OECD 474 (1997):* *Sex of animals not reported.* *1000* ***erythrocytes*** *(not* ***PCE****) scored/animal.* *Independent coding of slides not stated.*	p. 402, 410
[redacted] 1999,	No	Mouse, Micronucleus test, bone marrow	Glyphosate, 95 % i.p., 2x 0, 187.5, 375 or 562.5 mg/kg bw (24 h	Negative	GLP, internal SOP	*MN/1000 PCE [mean (range)], male/female:* Control: 0.4 (0-1)/0.8 (0-2) 188 mg/kg: 0.0 (0)/0.6 (0-3)	5 animals per sex and dose. 1000 PCE and 1000 NCE scored per animal. PCE/NCE: no effect (but	p. 358, 367 ff.

Reference	In IARC mono-graph	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
ASB2012-11482			interval), sampled 24 h after second dose			375 mg/kg: 0.6 (0-3)/0.6 (0-2) 563 mg/kg: 0.4 (0-2)/0.6 (0-1) PosControl: 4.8* (4-7)/4.8* (2-12) *p<0.05	PosControl). MN/1000 NCE: no effect (but PosControl). *$LD50_{i.p.}$=750 mg/kg*	
[redacted] 2006, ASB2012-11478	No	Mouse, Micronucleus test, bone marrow	Glyphosate, 95.7 % i.p., 1x 0, 150, 300 or 600 mg/kg bw, sampled after 24 and 48 h	Negative	GLP, OECD 474 (1997)	*% MNPCE [mean±SD, (range)]:* 24h, Control: 0.06±0.06 (0.0-0.15) 24h, 150 mg/kg: 0.07±0.04 (0.0-0.10) 24h, 300 mg/kg: 0.06±0.05 (0.0-0.15) 24h, 600 mg/kg: 0.19±0.07* (0.05-0.25) 24h, PosControl: 3.03±0.49*** (2.20-3.35) 48h, Control: 0.1±0.12 (0.0-0.35) 48h, 600 mg/kg: 0.09±0.11 (0.0-0.30) *p<0.05, ***p<0.001	7 males per group and sampling time. 2000 PCE scored/animal. *Pre-test: Mortality at 800-1000 mg/kg, clinical signs at 150 mg/kg and above.* PCE/NCE: reduced at 600 mg/kg (not in PosControl). Stat. sign. increase in MNPCE at 600 mg/kg (24 h), within historical control. *Control data from 60 groups (24h):* *0.0-0.9 MN/1000 PCE: 40x (67%)* *1.0-1.4 MN/1000 PCE: 14x (23%)* *1.5-2.0 MN/1000 PCE: 3x (5%)* *2.1-2.5 MN/1000 PCE: 3x (5%)*	p. 358, 359 ff.
[redacted] 2008, ASB2012-11481	No	Mouse, Micronucleus test, bone marrow	Glyphosate, 98 % i.p., 2x 0, 15.6, 31.3 or 62.5 mg/kg bw (24 h interval), sampled 24 h after second dose	Negative	GLP, OECD 474 (1997)	*MN/2000 PCE [mean (range)], male/female:* Control: 0.0 (0)/0.0 (0) 15.6 mg/kg: 0.0 (0)/0.0 (0) 31.3 mg/kg: 0.0 (0-1)/0.0 (0) 62.5 mg/kg: 0.6 (0-3)/0.0 (0) PosControl: 23.0* (8-30)/12.2* (7-26) *p<0.01	5 animals per sex and dose. 2000 PCE scored/animal. *Pre-test: Mortality at 500-1000 mg/kg, decreased PCE/NCE at 250 mg/kg and above.* PCE/NCE no effect. Historical control: ca. 3 MN/1000 PCE	p. 358. 364 ff.
[redacted] 2010,	No	Mouse,	Glyphosate, 98 %	Negative	GLP,	*MN/2000 PCE [mean (range)],*	5 animals per sex and dose.	p. 358.

Reference	In IARC mono-graph	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
ASB2014-9284		Micronucleus test, bone marrow	i.p., 2x 0, 125, 250 or 375 mg/kg bw (24 h interval), sampled 24 h after second dose		OECD 474 (1997)	*male/female:* Control: 0.4 (0-2)/0.4 (0-1) 125 mg/kg: 0.2 (0-1)/0.0 (0-1) 250 mg/kg: 0.0 (0)/0.0 (0) 375 mg/kg: 0.2 (0-1)/0.0 (0-1) PosControl: 8.0* (5-11)/6.4* (5-9) *p<0.01	2000 PCE scored/animal. *Clinical signs at 125 mg/kg and above.* PCE/NCE: slight increase at 250 and 375 mg/kg and in PosControl. Historical control: ca. 3 MN/1000 PCE	364 ff.

NCE, normochromatic erythrocytes; MN, micronucleus; MNPCE%, percent of micronucleated polychromatic erythrocytes; PCE, polychromatic erythrocytes; SD, standard deviation

Table 4.2-4: Glyphosate; further tests on DNA adducts and DNA strand breaks in mammals, *in vivo*

Reference	In IARC monograph	Species, test, tissue	Test substance, purity, route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
Bolognesi et al., 1997, Z59299	Yes	Mouse DNA adduct (8-OHdG by LC/UV), liver	Analytical grade glyphosate (purity 99.9 %) i.p.; 1 × 300 mg/kg bw; sampled after 8 and 24 h	- (4 h) + (24 h)	No GLP, no reference to TG	(Estimated from figure in report) Control: approx. 0.6 moles 8-OHdG/10^5 moles dG 4 h: approx. 0.9 moles 8-OHdG/10^5 moles dG 24 h: approx. 3.6 moles 8-OHdG/10^5 moles dG*	3 male animals per group, at least 3 independent repeat experiments	p. 386
Bolognesi et al., 1997, Z59299	Yes	Mouse DNA adduct (8-OHdG by LC/UV), kidney	Analytical grade glyphosate (purity 99.9 %) i.p.; 1 × 300 mg/kg bw; sampled after 8 and 24 h	– (4 & 24 h)	No GLP, no reference to TG	(Estimated from figure in report) Control: approx. 0.6 moles 8-OHdG/10^5 moles dG 4 h: approx. 0.5 moles 8-OHdG/10^5 moles dG 24 h: approx. 0.4 moles 8-OHdG/10^5 moles dG*	3 male animals per group, at least 3 independent repeat experiments	p. 386

Reference	In IARC monograph	Species, test, tissue	Test substance, purity, route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
Peluso et al., 1998, TOX1999-318	Yes	Mouse DNA adduct (^{32}P-DNA post labelling), kidney	Glyphosate isopropylammonium salt i.p.; 1 × 0, 130 or 270 mg/kg bw; sampled after 24 h	–	No GLP, no reference to TG	Not reported	6 animals in control group, 6 in low dose group and 3 in high dose group, sex of animals not clear	p. 386
Peluso et al., 1998, TOX1999-318	Yes	Mouse DNA adduct (^{32}P-DNA post labelling), liver	Glyphosate isopropylammonium salt i.p.; 1 × 0, 130 or 270 mg/kg bw; sampled after 24 h	–	No GLP, no reference to TG	Not reported	6 animals in control group, 6 in low dose group and 3 in high dose group, sex of animals not clear	p. 386
Bolognesi et al., 1997, Z59299	Yes	Mouse DNA strand breaks (alkaline elution assay), liver	Analytical grade glyphosate (purity 99.9 %) i.p.; 1 × 300 mg/kg bw; sampled after 4 and 24 h	+ (4 h) - (24 h)	No GLP, no reference to TG	(Estimated from figure in report) Control: approx. 15 *10^3/mL 4 h: approx. 47 *10^3/mL* 24 h: approx. 20 *10^3/mL	3 male animals per group, at least 4 independent repeat experiments	p. 385
Bolognesi et al., 1997, Z59299	Yes	Mouse DNA strand breaks (alkaline elution assay), kidney	Analytical grade glyphosate (purity 99.9 %) i.p.; 1 × 300 mg/kg bw; sampled after 4 and 24 h	+ (4 h) - (24 h)	No GLP, no reference to TG	(Estimated from figure in report) Control: approx. 17 *10^3/mL 4 h: approx. 55 *10^3/mL* 24 h: approx. 25 *10^3/mL	3 male animals per group, at least 4 independent repeat experiments	p. 385
Manas et al., 2013, ASB2014-6909	No	Mouse comet assay, blood cells	Glyphosate (96%) Drinking water, 14 days, 0, 40 or 400 mg/kg bw per day; sampled after treatment period	+	No GLP, no reference to TG	Tail moment (mean ± SEM): Control: 2.98±1.08 40 mg/kg bw per day: 8.54***±7.82 400 mg/kg bw per day: 9.06***±5.15	6 animals per group sex of animals not clear	p. 404

Reference	In IARC monograph	Species, test, tissue	Test substance, purity, route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
Manas et al., 2013, ASB2014-6909	N	Mouse comet assay, liver cells	Glyphosate (96%) Drinking water, 14 days, 0, 40 or 400 mg/kg bw per day; sampled after treatment period	+	No GLP, no reference to TG	Tail moment (mean ± SEM): Control: 7.14±3.41 40 mg/kg bw per day: 7.92*±3.99 400 mg/kg bw per day: 20.59***±15.47	6 animals per group sex of animals not clear	p. 404

8-OHdG, 8-hydroxy-2' -deoxyguanosine; dG, deoxyguanosine; SEM, standard error of the mean; SCGE, single cell gel electrophoresis

Table 4.2-5: Glyphosate; germ cell mutagenicity tests in mammals, *in vivo*

Reference	In IARC mono-graph	Species, test, tissue	Test substance, purity, application route, dose levels, mating period	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
EPA, 1980, ASB2015-8547 ([redacted] 1980, TOX9552377	Yes	Mouse, Dominant lethal test	Glyphosate, 98.7 % oral, 1x 0, 200, 800 or 2000 mg/kg bw 8 successive one-week mating periods (1 male/2 females)	Negative	GLP, no reference to TG	No increase in post-implantation loss in treated groups. PosControl: stat. significant increase in post-implantation loss.	Only 10 males per group. Post-implantation loss evaluated after mating of non-treated females with glyphosate-treated male mice. Original study reported in RAR as Wrenn et al., (1980, TOX9552377).	p. 378
[redacted] 1992, TOX9551102	No	Rat, Dominant lethal test	Glyphosate, 96.8 % oral, 1x 0, 200, 800 or 2000 mg/kg bw 10 successive one-week mating periods (1 male/1 female)	Negative	GLP, OECD 478 (1984)	No increase in post-implantation loss in treated groups. PosControl: stat. significant increase in post-implantation loss.	30 males per group (Control: 10 males, PosControl: 2 x 5 males). Post-implantation loss evaluated after mating of non-treated females with glyphosate-treated male mice.	p. 378

Table 4.2-6: AMPA; mutagenicity and genotoxicity tests, *in vitro*

Reference	Evaluated by IARC	Test system (endpoint)	Test substance	Results: Without metabolic activation by authors	Results: With metabolic activation by authors	Concentration range	GLP, Test guideline	RAR 04/2015	Comments BfR
Callander, 1988, TOX9500043	No	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, TA100 (reverse mutation)	AMPA, >99%	Negative	Negative	1.6-5000µg/plate	GLP, OECD 471 (1983)	p. 735	No evidence of genotoxicity. The slight increase in revertant numbers in one strain in the first experiment was rather weak and was sufficiently contravened by subsequent trials in which the test material proved clearly negative.
[redacted] 1993, TOX9300378	No	Salmonella typhimurium TA1535, TA1537, TA98, TA100 (reverse mutation)	AMPA, 99.2%	Negative	Negative	310-5000µg/plate	GLP, OECD 471 (1983)	p. 95, 727	
[redacted] 1993; TOX9300380	No	L5178Y mouse lymphoma cells, gene mutation, TK locus	AMPA, 99.2%	Negative	Negative	310-5000µg/mL	GLP, OECD 476 (1983)	p. 727	
Mañas et al., 2009b, ASB2012-11891	Yes	Human lymphocytes, Chromosomal aberrations	Analytical grade AMPA (99%).	Positive	NT	1.8 mM [200 µg/mL] $P < 0.05$	No GLP, no reference to TG		Methodological deficiencies (only 2 dose levels used).
Roustan et al., 2014, ASB2014-	Yes	CHO cells, Micronucleus formation	AMPA, purity not stated	Positive	Positive	-S9: 0.005-0.1 µg/ml +S9: 0.1-5 µg/ml	No GLP, no reference	p. 423	-S9: ≥0.01 µg/mL $P < 0.05$ +S9: ≥0.1 µg/mL $P < 0.01$

Reference	Evaluated by IARC	Test system (endpoint)	Test substance	Results: Without metabolic activation by authors	Results: With metabolic activation by authors	Concentration range	GLP, Test guideline	RAR 04/2015	Comments BfR
8086							to TG		
Bakke, 1991, TOX9552409	No	Primary rat hepatocytes (Fischer F334) (UDS test)	AMPA, 94.38%	Negative	Negative	5-5000µg/mL	GLP, no reference to TG	p. 728, 962 steht nur in der Über-sichts-tabelle	Negative up to 2500 µg/mL, meaningful evaluation of higher concentrations not possible due to cytotoxicity.
Nesslany, 2002, ASB2012-11508	No	Primary rat hepatocytes (Fischer) (UDS test)	AMPA, 99.9%	Negative	Negative	0.625 – 10 mM	GLP, OECD 482 (1986)	p. 728, 743	Negative under the condition of the experiment
Mañas et al., 2009b, ASB2012-11891	Yes	Liver Hep-2, DNA strand breaks, comet assay	Analytical grade AMPA (99%).	Positive	NT	Range 2.5-7.5 µM $P < 0.05$ at 4.5 mM [500 µg/mL]; $P < 0.01$ at up to 7.5 mM Dose–response relationship ($r \geq 0.90$; $P < 0.05$)	No GLP, no reference to TG	p. 422, 434	

Results: +, positive; -, negative
NT, not tested; NR, not reported ; S9, 9000 × g supernatant; Hprt, hypoxanthine guanine phosphoribosyl transferase gene;

Table 4.2-7: AMPA; mutagenicity and genotoxicity tests in mammals, *in vivo*

Reference	In IARC monograph	Species, test, tissue	Test substance, purity, route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
Manas et al.,	Yes	Mouse	Analytical grade	Positive	No GLP,	MNE/1000 analysed cells:	5 animals per group	p. 422, 434

Reference	In IARC monograph	Species, test, tissue	Test substance, purity, route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
2009b, ASB2012-11891		micronucleus test, bone marrow	AMPA (purity 99 %) i.p.; 2 × 100 or 200 mg/kg bw per day; sampled 24 h after second injection		OECD 474 (1997)	Control: 3.8 ±1.8 100 mg/kg bw: 10.0**±1.9 200 mg/kg bw: 10.4**±3.3 PosControl: 19.2**±3.9 PCE/NCE: Control: 0.85±0.17 100 mg/kg bw: 1.14±0.22 200 mg/kg bw: 1.07±0.04 PosControl: 0.80.+-0.20	Sex of animals not reported. *1000* ***erythrocytes*** *(not* ***PCE****) scored/animal. Independent coding of slides not stated.*	
[redacted] 1993, TOX9300379	No	Mouse micronucleus test, bone marrow	AMPA (99.2 %) oral; 1x 5000 mg/kg bw; sampled after 24, 48 and 72 h	Negative	GLP, OECD 474 (1983)	MN/1000 PCE [mean (range)] Control: 0.50 (0-1) 24 h, 5000 mg/kg: 0.20 (0-1) 48 h, 5000 mg/kg: 0.40 (0-1) 72 h, 5000 mg/kg:: 0.60 (0-1) PosControl: 13.1** (10-19)	5 males and 5 females per group. 1000 PCE scored/animal. 1000 NCE scored/animal	728 (mentioned but not reported in detail)
[redacted] 1993, TOX9552413 Study also mentioned by Williams et al., 2000, ASB2012-12053	No	Mouse micronucleus test, bone marrow	AMPA (94.38 %) i.p.; 1x 100, 500, 1000 mg/kg bw; sampled 24, 48 and 72 h	Negative	GLP, OECD 474 (1983)	Mean MN/1000 PCE 24 h, males/females: Control: 0.2±0.4/1.0±1.4 100 mg/kg bw: 0.2±0.4/0.8±0.8 500 mg/kg bw: 0.1±0.3/2.0±2.9 1000 mg/kg bw: 0.8±1.3/0.8±0.8 PosControl: 18.3**±10.9/12.0*±12.3 48 h, males/females: Control: 0.6±1.3/0.4±0.9 100 mg/kg bw: 0.0±0.0/0.2±0.4 500 mg/kg bw: 0.6±0.9/0.2±0.4 1000 mg/kg bw: 0.2±0.4/0.0±0.0 72 h, males/females: Control: 0.2±0.4/0.0±0.0 100 mg/kg bw: 0.0±0.0/1.6*±1.1	5 males and 5 females per group. 1000 PCE scored/animal. *Pre-test: Mortality at 606 mg/kg and above*	728 (mentioned but not reported in detail)

Reference	In IARC monograph	Species, test, tissue	Test substance, purity, route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
						500 mg/kg bw: 0.0±0.0/0.8±0.8 1000 mg/kg bw: 0.0±0.0/0.4±0.9		
Manas et al., 2013, ASB2014-6909	N	Mouse comet assay, blood cells	AMPA (99%) Drinking water, 14 days, 0 or 100 mg/kg bw per day; sampled after treatment period	Positive	No GLP, no reference to TG	Tail moment (mean ± SEM): *Blood cells* Control: 2.98 ± 1.08 100 mg/kg bw per day: 8.45*** ± 6.43 Liver cells Control: 7.14 ± 3.41 100 mg/kg bw per day: 14.99*** ± 9.09	6 animals per group sex of animals not clear	p. 404

MN, micronucleus; MNE, micronucleated erythrocytes; NCE, normochromatic erythrocytes; PCE, polychromatic erythrocytes; SEM, standard error of the mean

4.2.2 Receptor-mediated mechanisms

In section 4.4.2 of the IARC monograph 13 studies are reported. The studies including comments of RMS are summarized in Table 4.2-8.

4 studies compared endocrine disrupting activity of glyphosate and glyphosate-based formulations (Gasnier et al., 2009, ASB2009-7384; Richard et al., 2005, ASB2009-9024; Benachour et al., 2007, ASB2009-9018 and Walsh et al., 2000, ASB2012-12046). The results demonstrate that glyphosate-based formulations have a higher sex hormone disrupting activity than the active substance glyphosate.

Other studies used only a formulation. Based on the results no conclusion on the active substance is possible.

2 studies investigated endocrine disrupting potential of pesticides in general and did not report results on glyphosate.

Based on the study of Thongprakaisang et al. (2013, ASB2013-11991) it was concluded that proliferative effects of glyphosate on T4/D cells would be mediated by oestrogen receptors. However the results of all animal studies and of epidemiological studies demonstrated that glyphosate and glyphosate-based formulations did not cause breast cancer in animals and humans.

Glyphosate was included into the U.S. EPA Endocrine Disruptor Screening Program's (EDSP). Levine et al. (2012, ASB2014-9609) published a short summary of the results. They concluded that, based on the Tier 1 assays that had been performed at different independent laboratories and taking into account the 'higher tier' regulatory safety studies glyphosate might not be considered an endocrine disrupter. Later on, Bailey et al. (2013, ASB2013-3464) summarized results of the male and female pubertal assays in which glyphosate did not exhibit evidence of endocrine disruption.

Table 4.2-8: Discussion of studies in section 4.2.2 Receptor mediated mechanisms of the IARC monograph

Study (Author/year)	Subject	Evaluation by IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Thongprakaisang et al., 2013, ASB2013-11991	Glyphosate effects on human breast cell cancer growth	The findings suggested that the proliferative effects of glyphosate on T4/D cells are mediated by oestrogen receptors.	Agreement with the reported results.	Yes, page 672	It must be emphasised that no increase in mammary tumours was reported in any of the numerous long-term studies in rats or mice and no increased risk of mammary tumours was found in the epidemiological studies.
Gasnier et al., 2009, ASB2009-7384	Toxicity and endocrine disrupting activity of glyphosate in human cell lines	In human hepatocarcinoma HepG2 cells, four glyphosate-based formulations had a marked effect on the activity and transcription of aromatase, while glyphosate alone differed from controls, but not significantly so. Additionally, although four glyphosate-based formulations dramatically reduced the transcription of ERα and ERβ in ERE-transfected HepG2 cells, glyphosate alone had no significant effect. A stronger effect of the formulations was also reported for the effects on androgen-receptor transcription in a breast cell line.	Agreement with the reported results. The study confirms the clearly higher activity of formulations than of the active substance alone.	Yes, page 671, 686	The study confirms the higher activity of formulations (Roundup) than of the active substance alone. This important difference was already highlighted in the first DAR and also in the RAR.
Richard et al., 2005, ASB2009-9024	Effects of glyphosate and Roundup on human placental cells and aromatase	A glyphosate-based formulation caused decreased aromatase activity in human placental cells. Glyphosate alone was without effect.	Agreement with the reported results. The authors Richard et al., 2005 conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in	Yes, page 328, 671, 676 and 682	The study confirms the clearly higher activity of formulations (Roundup) than of

Study (Author/year)	Subject	Evaluation by IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
			mammals.		the active substance alone. This important difference was already highlighted in the first DAR and also in the RAR.
Benachour et al., 2007, ASB2009-9018	Time- and dose-dependent effects of Roundup on human embryonic and placental cells	Glyphosate, at non-overtly toxic concentrations, decreased aromatase activity in fresh human placental microsomes and transformed human embryonic kidney cells transfected with human aromatase cDNA. A glyphosate-based formulation, at non-overtly toxic concentrations, had the same effect. The formulation was more active at equivalent doses than glyphosate alone.	The study confirms the higher activity of formulations (Roundup) than of the active substance alone.	Yes, pages 671, 678 and 683-684	The study confirms the higher activity of formulations (Roundup) than of the active substance alone. This important difference was already highlighted in the first DAR and also in the RAR.
Kojima et al., 2004, ASB2010-14389	Estrogen and androgen activities of pesticides	In human androgen receptor and ERα and ERβ reporter gene assays using the Chinese hamster ovary cell line (CHO-K1), glyphosate had neither agonist nor antagonist activity.	Agreement	No	Several of the 200 tested pesticides were found to have endocrine-disrupting potential; however, no activity of glyphosate was reported.
Kojima et al., 2010, ASB2015-7815	Endocrine disrupting potential of pesticides	In human androgen receptor and ERα and ERβ reporter gene assays using the Chinese hamster ovary cell line (CHO-K1), glyphosate had neither agonist nor antagonist activity.	Agreement	No	Several of the 200 tested pesticides were found to have endocrine-disrupting potential; however, no activity of glyphosate was

Study (Author/year)	Subject	Evaluation by IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
					reported.
Walsh et al., 2000, ASB2012-12046	Inhibition of steroidogenesis by roundup	A glyphosate-based formulation markedly reduced progesterone production in mouse leydig cell tumour cells. The inhibition was dose-dependent. The formulation also disrupted steroidgenic acute regulatory protein expression. Glyphosate alone did not affect steroidogenesis.	Agreement	Yes, pages 327, 328, 332, 677, 678	The study confirms the clearly higher activity of formulations (Roundup) than of the active substance alone. No effects of glyphosate alone have been observed. This important difference was already highlighted in the first DAR and also in the RAR.
Forgacs et al., 2012, ASB2012-11621	Effects of glyphosate and further chemicals on steroidogenesis in a novel murine Leydig cell model	Glyphosate had no effect on testosterone production in a novel murine Leydig cell line. Glyphosate did not modulate the effect of recombinant human chorionic gonadotropin.	Agreement	Yes, page 677	No effects of glyphosate on steroidogenesis.
Omran and Salama, 2013, ASB2014-7614	Endocrine disrupting effects of glyphosate and atrazine in snails.	A glyphosate-based formulation reduced levels of testosterone in gonadal tissue of snails and induced degenerative changes in the ovotestis. CYP450 was increased.	Agreement with the reported results. Only a formulation was tested, therefore, no conclusion on the active substance glyphosate alone is possible.	Yes, page 673	Only a formulation was tested, therefore, no conclusion on the active substance glyphosate alone is possible.
Xie et al., 2005, ASB2012-12056	Estrogenic activities of herbicides and surfactants	Glyphosate did not increase plasma vittelogenin levels in juvenile rainbow trout.	Agreement	Yes, page 332,	No estrogenic activity of glyphosate
Vainio et al., 1983, Z31881	Hypolipidaemia and peroxisome proliferation induced by pesticides	Glyphosate had no effect on formation of peroxisomes or the activity of hepatic carnitine acetyltransferase and catalase, and did not	Agreement	No, study published before 2000	Glyphosate does not have peroxisome proliferator activated

Study (Author/year)	Subject	Evaluation by IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
		cause hypolipidaemia, suggesting that glyphosate does not have peroxisome proliferator activated receptor activity.			receptor activity.
Takeuchi et al., 2008, ASB2013-6443	*In vitro* screening for aryl hydrocarbon receptor agonistic activity in 200 pesticides.	Glyphosate was not an agonist for the aryl hydrocarbon receptor in mouse hepatoma Hepa1c17 cells transfected with a reporter plasmid containing copies of dioxin-responsive element.	Agreement	No	No effect of glyphosate
Paganelli et al., 2010, ASB2010-11410	Teratogenic effects of glyphosate-based herbicides by impairing retinoic acid signalling.	Retinoic acid activity in tadpoles exposed to a glyphosate based formulation was measured. Retinoic activity was increased by the formulation, and a retinoic acid antagonist blocked the effect.	The formulation Roundup classic was used in this study. Therefore, no conclusion on the active substance glyphosate alone is possible.	Yes, page 671, 675, 676, 680	The formulation Roundup classic was used in this study. Therefore, no conclusion on the active substance glyphosate alone is possible.

4.2.3 Oxidative stress, inflammation, and immunosuppression

4.2.3.1 Oxidative stress

Human cells *in vitro*, data on glyphosate:

Gehin et al. (2005, ASB2012-11826) investigated effects of pre-incubation of HaCaT with 100 or 200 µM Vit C, Vit E or both for 0, 24 or 48 h on glyphosate cytotoxicity at doses of up to 25 mM for 24 h. IC_{50} for glyphosate alone, pre-incubated with Vit C, Vit E or both in ranges from 20.9 - 23.9 mM, 20.6 - 23.9 mM, 21.6 - 23.6 mM or 19 - 21.3 mM, respectively. No information is available on the purity of the tested substance.

Elie-Caille et al. (2010, ASB2012-11610) investigated the formation of reactive oxygen species (ROS) after treatment of HaCaT cells at the IC_{50} using 2′,7′-dichlorodihydrofluorescein diacetate. Treatment with 50 mM glyphosate (purity 95%) for 30 min resulted in "*overproduction of H_2O_2*" determined as "*a thicker and more intense fluorescent area*". No quantitative estimate is available.

Kwiatkowska et al. (2014, ASB2014-9603) examined the production of ROS in human erythrocytes (without metabolic activation) using dihydrorhodamine 123. Cells were exposed to glyphosate concentrations of 0.01 - 5.0 mM for 1 h. Positive results are observed from 0.25 mM up to the highest tested concentration that induces cytotoxic effects (increase in percent of haemolysis). No information is available on the purity of the tested substance.

Mladinic et al. (2009, ASB2012-11906) investigated possible effects of *in vitro* exposure of glyphosate on oxidative DNA damage and on oxidative stress parameters (total antioxidant capacity and lipid peroxidation) in human lymphocytes with and without metabolic activation. Cells were exposed to concentrations of 0.5 - 580 µg/mL (up to ca. 3.4 mM). Regarding the induction of cytotoxic effects significantly increased early apoptosis and necrosis at the highest tested concentration of 580 µg/mL were observed. In a modified comet assay oxidative DNA damage was observed without metabolic activation only at a concentration of 3.5 µg/mL whereas an obviously more relevant effect was observed with metabolic activation at the highest tested concentration of 580 µg/mL. Both, determinations of total antioxidant capacity (TAC) as well as the lipid oxidation (determination by level of thiobarbituric reactive substances) indicate an increase of oxidative stress with and without metabolic activation at the highest tested concentrations of 580 µg/mL.

Chaufan et al. (2014, ASB2014-7616, ASB2014-9314) evaluated the effect of glyphosate (purity: 95%) on oxidative stress in HepG2 cells with 2',7'dichlorohydrofluorescin diacetate. Treatment of the cells with 900 mg/mL glyphosate for 24 h does not lead to an increase in ROS. Concentrations up to 1000 mg/mL did not affect the cell viability (MTT test).

Human cells *in vitro*, data on AMPA:

Kwiatkowska et al. (2014, ASB2014-9603) examined the production of ROS in human erythrocytes (without metabolic activation) with dihydrorhodamine 123. Cells were exposed to AMPA concentrations of 0.01 - 5.0 mM for 1 h. Positive results are observed from 0.25 mM up to the highest tested concentration that induces cytotoxic effects (increase in percent of haemolysis). No information is available on the purity of the tested substance.

Chaufan et al. (2014, ASB2014-7616) evaluated the effect of AMPA on oxidative stress in HepG2 cells with 2',7'dichlorohydrofluorescin diacetate. AMPA exposure of the only tested concentration of 900 mg/mL for 24 h does not lead to an increase in ROS. Concentrations up to 1000 mg/mL did not affect the cell viability (MTT test). No information is available on the purity of the tested substance.

Human cells *in vitro*, data on formulations containing glyphosate:

Gehin et al. (2005, ASB2012-11826) investigated effects of pre-incubation of HaCaT with 100 or 200 µM Vit C, Vit E or both for 0, 24 or 48 h on cytotoxicity of a glyphosate-based formulation (containing 21% (p/p) isopropylamine glyphosate salt (170 g/L), 8% (p/p) POEA and 71% (p/p) water and others minor ingredients) at doses of up to 25 mM for 24 h. IC_{50} for Roundup 3 plus® alone, pre-incubated with Vit C, Vit E or both ranged from 17.1 - 18.2 mM, 16.9 - 18.1 mM, 16 - 17.6 mM or 16.7 - 21.8 mM, respectively. The authors inferred a protective effect of vitamin pretreatment indicating that ROS formation might be a mechanism for cytotoxicity of glyphosate-based formulations.

George and Shukla (2013, ASB2014-8034) investigated ROS formation after treatment of HaCaT cells with doses of 0.01, 0.025, 0.05 and 0.1 mM of a glyphosate-based formulation (containing glyphosate 41%, polyethoxethyleneamine (POEA) ≅15%) using 2′,7′-dichlorodihydrofluorescein diacetate. An up to 1.9-fold increase in ROS formation was detected when compared to control and antioxidant N-acetylcysteine (NAC) treated HaCaT cells. The effect was comparable with 10 nM 12-otetradecanoyl-phorbol-13-acetate. The positive control of 100 mM H_2O_2 is questionable as peroxide concentration is expected to decrease in cell cultures after 24 h at 37°C. Pretreatment with NAC statistically significantly decreased ROS formation below vehicle control (apparently not pre-treated with NAC). Some cell proliferation occurred upon treatment with Roundup. However, it was statistically significantly increased only at 0.1 mM glyphosate and after 72 h, but not at lower doses or shorter treatment. The proliferative effect at 0.1 mM after 72 h could be statistically significantly decreased by NAC. Cytotoxicity of the glyphosate formulation occurred from 0.5 mM glyphosate on upwards.

Coalova et al. (2014, ASB2014-7615) examined the impact of a glyphosate-based formulation (glyphosate as isopropylamine salt, 48%) on oxidative stress in HEp-2 cells with 2',7'-dichlorohydrofluorescin diacetate. The exposure of the only tested concentration of 376.4 mg/mL for 24 h leads to an increase in ROS. The tested concentration is equivalent to the determined LC_{50} value for a 24 h-exposure. The exposure of the formulation also increased glutathione and catalase activity whereas glutathione-S-transferase activity and superoxide dismutase activity (SOD) were not affected.

Chaufan et al. (2014, ASB2014-7616) evaluated the effect of a glyphosate-based formulation (74.4% monoammonium salt of N-phosphonomethylglycine) on oxidative stress in HepG2 cells with 2',7'-dichlorohydrofluorescin diacetate. An increase in ROS was observed at the only tested concentration of 40 mg/mL after an exposure of 24 h. The tested concentration is equivalent to the determined LC_{50} value of 41.22 mg/mL for a 24 h-exposure (MTT test).

Non-human mammalian experimental systems, data on glyphosate:

Astiz et al. (2009b, ASB2012-11550) investigated the effect of glyphosate, dimethoate and zineb administered alone or in combination on defence systems of the liver, kidney, brain and plasma antioxidant. Male Wistar rats, weighing 190 ± 20 g, were randomly divided into nine groups (4/group). Animals of one group were injected intraperitoneally (i.p.) with 10 mg glyphosate/kg bw (purity: commercial grade) in polyethylene-glycol 400 (PEG-400) three times a week for five weeks. Two groups served as controls (one group without treatment and one group receiving i.p. injections of PEG-400. Six further test groups were used to examine either zineb or dimethoate or a mixture of glyphosate, dimethoate and zineb (these groups are not further discussed here). At the end of the treatment the animals were killed, blood was collected and plasma was prepared. Homogenates from brains, livers, and kidneys were prepared. Various biomarkers of oxidative stress and cell damage were measured. Lipid peroxidation was assessed as thiobarbituric acid-reactive substances (TBARS); the sum of nitrates and nitrites ([NOx]) was measured as the main end-metabolite products of nitric oxide (NO) and peroxinitrite anion ($ONOO^-$), protein carbonyls as a biomarker of oxidative damage to proteins; enzymatic and non-enzymatic biomarkers of the antioxidant defence system: Ferric Reducing Ability of Plasma assay (FRAP, total antioxidant ability in plasma, Vitamin E (α-Tocopherol) levels in liver and brain), total glutathione (GSH) in plasma and brain; catalase activity (CAT), superoxide dismutase activity (SOD), glutathione peroxidase (GPx) activity, glutathione-S-transferase (GST), glutathione reductase (GR) activity in liver, brain, and kidney; lactate dehydrogenase (LDH) in plasma as a biomarker of cellular damage, and γ-glutamyl transpeptidase (γ–GT) activities as a biomarker of hepato-cellular damage. Results: At the end of treatment with glyphosate no effects were observed on animal

behaviour, body weight or body weight gain. Also no clinical signs of toxicity or observations of tremors or gait abnormalities (open field) were observed during the entire experimental period. The analytical examinations showed the following results: Increase of lipid peroxidation in liver, brain, kidney, plasma (significant, $p < 0.01$); slight increase (not significant) of oxidative damage to proteins seen as protein carbonyls in plasma; increase of [NOx] concentration (significant, $p < 0.01$) in brain and plasma; lower values (significant, $p < 0.01$) of FRAP in plasma, liver kidney and brain; progressive loss (significant, $p < 0.01$, approx. 30%) of α-tocopherol in liver and brain; increase (significant, $p < 0.01$) of GSH (GSH and GSSG, glutathione disulphide, oxidized Glutathione, hydrogen acceptor) in plasma; the following values were determined for the various antioxidant enzyme activities: increase (significant, $p < 0.01$) of SOD in liver and brain, decrease (significant, $p < 0.01$) of CAT in brain, slight increase (not significant) of SOD, CAT, GPx, GR, GST activity in kidney; no effect of LDH in plasma, increase (significant, $p < 0.01$) of γ–GT in plasma. Overall, repeated i.p. injection of glyphosate over a period of 5 weeks resulted in a lower antioxidant status in liver, brain, kidney and plasma, higher oxidized protein and glutathione levels in plasma with a decreased concentration of α-tocopherol in brain and liver. SOD was decreased in liver and brain. Glutathione reductase was inhibited in liver while glutathione peroxidase and transferase were unaffected. Plasma lactate dehydrogenase was not affected, but γ-glutamyl transpeptidase activity was increased. In conclusion the IARC statement can be supported that there are indications of oxidative stress in the blood plasma, liver, brain and kidney of rats upon exposure to glyphosate.

Bolognesi et al. (1997, Z59299) examined the genotoxic activity of glyphosate and its technical formulation 'Roundup'. Glyphosate (purity: 99.9%) was tested in a battery of genotoxicity tests *in vitro* and *in vivo*. These data were documented as part of the summarized data on *in vitro* and *in vivo* genotoxicity testing with glyphosate in section 4.2.1 of IARC Monographs Volume 112 (2015, ASB2015-8421). No information regarding 'increased biomarkers of oxidative stress in liver and kidney' is given.

Non-human mammalian experimental systems, data on AMPA:

No data available.

Non-human mammalian experimental systems, data on formulations containing glyphosate:

Bolognesi et al. (1997, Z59299) examined the genotoxic activity of glyphosate and its technical formulation 'Roundup'. Roundup formulate (30.4% glyphosate as active agent) was tested in a battery of genotoxicity tests *in vitro* and *in vivo*. No information regarding 'increased biomarkers of oxidative stress in liver and kidney' is given. As mentioned above this study was disregarded in the assessment.

Cavusoglu et al. (2011, ASB2012-11588) evaluated the protective effect of Ginkgo biloba L. leaf extract against Roundup® (Roundup Ultra-Max, containing 450 g/L glyphosate as active ingredient) in Swiss albino mice. Male Swiss albino mice (12 - 14 weeks old and weighing 25 - 30 g) were randomly divided into six groups, each consisting of six animals. The control animals received single intraperitoneal (i.p.) injection with dimethyl sulfoxide (0.2 mL). One group received single i.p. injection of 50 mg/kg bw Roundup. Two further groups were given orally G. biloba at doses of, respectively, 50 and 150 mg/kg bw for 8 consecutive days. The fifth group was given orally G. biloba at the dose of 50 mg/kg bw and i.p. injection of 50 mg/kg bw Roundup. The sixth group was given orally G. biloba at the dose of 150 mg/kg of body weight and i.p. injection with 50 mg/kg bw Roundup. For the fifth and sixth group, G. biloba application was started 5 days before exposure to Roundup and was continued alone for 3 consecutive days after single-dose applications of Roundup. Animals were sacrificed at the end of treatment (72 h). Blood, bone marrow, and liver and kidney tissues were investigated. Serum analysis involved the following parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), and creatinine. For the determination of lipid peroxidation and glutathione activity the liver and kidney tissues of each animal were processed for biochemical measurements. Tissue glutathione (GSH) and malondialdehyde (MDA) levels were measured. For evaluation of genotoxic effects the mouse erythrocyte micronucleus (MN) assay, a modified mouse MN test that conventionally scores the MN frequencies in bone marrow polychromatic erythrocytes, was used. For

determination of chromosomal aberrations (CAs) animals were sacrificed 2 h after treatment under ether anesthesia and bone marrow from the femur was aspirated, washed, fixed in Carnoy's fixative, and stained with 5% Grünwald–Giemsa stain. Histopathological examination of the liver and kidneys was performed. Results of Roundup treatment without pre-treatment with the antioxidant: Serum AST, ALT, BUN, and creatinine levels were significantly increased ($p < 0.05$) in mice. The examination of the lipid peroxidation products showed significantly decreased ($p < 0.05$) levels of GSH and significantly increased ($p < 0.05$) levels of MDA in the liver and kidney tissues. The frequency of micronucleated cells was clearly increased (significant, $p < 0.05$) in mature normachromatic erythrocytes, and the mean number of micronucleated cells was significantly higher ($p < 0.05$) compared to controls. Roundup induced an increase in the frequency of CAs and the number of AMNs in bone marrow metaphases. It also significantly decreased the rate of MI. A significant stimulation in the frequency of CA types such as chromatid breaks, acentric fragments, and chromatid gaps in bone marrow cells was noted. Histopathology of the liver revealed severe degenerative and necrotic changes. There were hydropic degeneration, nuclear pyknosis, and loss of some nuclei of hepatocytes in periacinar and midsonal areas. Kupffer cell proliferation and fibrosis were seen in some portal areas. In the kidneys glomerular basement membranes were thickened, accumulation of hyaline droplets and cylinders was detected in some tubular lumina, and some tubular epithelial cells were degenerated.

Results of Roundup treatment with pre-treatment with the antioxidant: The treatment of Roundup together with G. biloba caused a significant reduction in the above described effects of Roundup, especially in indices of hepatotoxicity, nephrotoxicity, lipid peroxidation, and genotoxicity. The strongest effect was observed with G. biloba at 150 mg/kg bw.

Overall, results of serum analysis, evaluation of genotoxic effects and the histopathology indicate that Roundup induced (cyto-)toxicity in liver and kidney, higher frequencies of CAs, MNs, and abnormal metaphases compared with the controls, and oxidative stress in Swiss albino mice. The pre-treatment with G. biloba induced a weakening of oxidative stress by the glyphosate-based formulation. The IARC statement can be supported that there are indications of increases in biomarkers of oxidative stress in liver and kidney of mice upon exposure to the glyphosate-based formulation (Roundup). The supplementation with the antioxidant G. biloba extract can protect against glyphosate toxicity by reduction effects of free radicals.

Jasper et al. (2012, ASB2014-9583) investigated biochemical, hematological and oxidative parameters of glyphosate-Roundup® (= 41% Glyphosate as active ingredient and 16% polyoxyethylene amine (POEA) and apparently other surfactants (not further specified)). Male and female Swiss albino rats (10/sex/dose) received daily oral gavage doses of 50 or 500 mg/kg bw/d Roundup for 15 days (vehicle/control: distilled water). Liver toxicity was assessed by serum enzymes ALT, AST, and γ-GT, renal toxicity assessed by urea and creatinine. Haematology was assessed by RBC, WBC, hemoglobin, hematocrit, MCV, MCH, and MCHC. Oxidative damage assessed by TBARS (thiobarbituric acid reactive substances) and NPSH (non-protein thiols) in liver. There was a significant dose-dependent reduction in body weight gain in both sexes. Significant increases in ALT, AST, and γ-GT at both dose levels, no considerable differences by histology. No significant changes in renal parameters. Hematology: Significant anemic alterations at high dose in both sexes: Reduction of RBC, hematocrit, and hemoglobin, significant increase of MCV. Lipid peroxidation: Males: at both dose levels important increases in lipid peroxidation together with an NPSH reduction in the hepatic tissue. Females: Significant increase in TBARS at both doses, significant decreases in NPSH only at high dose. Results indicate that glyphosate-Roundup® causes anemic effects and increased activities of liver enzymes that indicate liver cell dysfunction (although no abnormal morphology was observed) at subacute exposure and which could be related to the induction of reactive oxygen species.

Cattani et al. (2014, ASB2014-3919) investigated rat hippocampus. The herbicide Roundup Original® (Homologation number 00898793) containing glyphosate 360 g/L (commercial formulation registered in the Brazilian Ministry of Agriculture) was used, no further information on components are given. Wistar rats were exposed to 1% Roundup in drinking water during pregnancy up to lactation day 15 and from their pups, slices of hippocampus were prepared. TBARS assay was used to assess oxidation products, reduced GSH was measured with DTNB (both photometric assays). The experimental procedure is in part unclear: "*After preincubation, hippocampal slices were incubated in the presence or absence of 0.01% Roundup for 30 min*", but values are reported as being from 8 animals from each

treated group. TBARS levels were statistically significantly increased, ($p < 0.05$), GSH levels were statistically significantly decreased ($p < 0.01$). Remarks: It appears that the results might be a combination of *ex vivo* and *in vivo* results. Positive control is lacking, experimental details are missing. Unusual test setting, the reliability of the test system seems to be questionable. Uncertainties on the test method remain as a preparation of tissue slices was reported, but on the other hand, a homogenate was described. It is unknown whether a homogenate from slices was prepared and tested. Conclusion: From the poor description/questions arising from experimental procedure and due to lack of positive control, this study should be disregarded.

George et al. (2010, ASB2012-11829) investigated both, carcinogenicity and the change of expression of proteins by proteomics in skin of mice dermally treated with a glyphosate formulation (Roundup original®). Only the proteomics part is assessed here as it relates to oxidative stress. Method: Four male Swiss albino mice were treated each with a single dose of 50 mg/kg bw of glyphosate in a glyphosate formulation (Roundup original®, glyphosate 41%, POEA = 15%-Monsanto Company, St. Louis, MO, USA, 360 g/L glyphosate) by topical application at the dorsal region (2 cm^2, hair clipped). Untreated controls were included. After 24 h animals were sacrificed and skin tissues from the treatment site were excised and homogenized. Protein spots with a >2 fold change (compared to controls) were considered as differentially expressed, excised and identified via MALDI-TOF/TOF. To confirm the observed changes in protein expression an immunoblot analysis for some of the differently expressed proteins was performed. Results: Changes in expression levels of proteins in skin tissues of treated mice compared to controls, which were confirmed by immunoblot analysis, were observed for the three proteins calcyclin (increased expression, about 2.5 fold change), calgranulin-B (increased expression, about 9.5 fold change) and superoxide dismutase (SOD) (decreased expression, about 5 fold change). SOD is a biomarker of oxidative stress and provides a protective response against ROS. The expression of SOD is supposed to be up-regulated if ROS occur. As a down-regulation of SOD was observed it can be concluded that no direct induction of ROS occurred upon treatment with the glyphosate formulation. Calcyclin and calgranulin-B are not directly linked to ROS or oxidative stress. Calgranulin-B is a protein supposed to be involved in chronic inflammation and calcyclin is a calcium-binding protein often detected up-regulated in expression in proliferating cells. Remarks: Only results of the proteomics experiment confirmed by immunoblot analysis were considered as true changes in protein expression levels as only a small number of animals (4), skin samples and one dose were tested. Moreover, the gels were stained with the semi-quantitative silver staining and the detailed procedure of data analysis was not shown (including the total number of gels performed, the expression data of each protein spot on each gel, the significance value for each observed fold change in expression level of a protein spot compared to controls and the group formation for statistical analysis).

Overall, the conclusions drawn by George et al. (2010, ASB2012-11829) do not support the statement in the IARC report. The study was performed with a glyphosate formulation and not with pure glyphosate as described in the IARC report. No production of free radicals or oxidative stress after dermal exposure to a glyphosate formulation has been observed. An alteration of the expression level of an antioxidant enzyme was found (expression of SOD was down-regulated) but the observed down-regulation of SOD is not indicative of increased ROS formation. Conclusion: The IARC statement that glyphosate increases biomarkers of oxidative stress in skin based on the study of George et al. (2010, ASB2012-11829) cannot be supported.

Non-human mammalian experimental systems, data on mixtures of active substances including glyphosate:

Astiz et al. (2013, ASB2014-7493) treated male Wistar rats with a mixture of Zineb (99% pure, 15 mg/kg/d), glyphosate (99% pure, 10 mg/kg/d) and dimethoate (98% pure, 15 mg/kg/d) i.p., 5 x per week for 5 weeks to investigate the association between oxidative stress and inflammation/steroidogenesis. After treatment period, plasma was sampled and testis homogenates were prepared. For determination of oxidative damage, TBARS and protein carbonyls were determined. Further, the sum of nitrates and nitrites was determined. Statistical analysis was performed. Compared to untreated controls, levels of all biomarkers of oxidative damage were significantly increased in

plasma and testis homogenate. No positive control for oxidative stress was included. As glyphosate was only tested in combination with two other pesticides, no conclusion on glyphosate is possible. The IARC text is in principle correct but a more careful wording on the relevance of the study appears appropriate.

Overall conclusion on Oxidative stress:

In general the documentation of the majority of studies on oxidative stress in section 4.2.3 of IARC Monographs Volume 112 (2015, ASB2015-8421) can be confirmed. It is noted that here is a lack of positive controls for oxidative stress in all *in vitro* and *in vivo* studies described in section 4.2.3 *(ii) Non-human mammalian experimental systems* of the IARC monograph. From the available data on glyphosate, there is some indication of induction of oxidative stress from testing in human cell cultures and in mammalian (*in vivo*) experimental systems. In particular, the IARC statement that there are indications of oxidative stress in the blood plasma, liver, brain and kidney of rats upon exposure to glyphosate can be supported. However, only one of the cited studies (Astiz et al., 2009b, ASB2012-11550) investigated oxidative stress in animals with pure glyphosate. This study was conducted in rats and no other species was tested and increased oxidative stress was observed in combination with cytotoxic/degenerative effects of the targeted organs.

Only *in vitro* data were available on induction of oxidative stress by AMPA. There was no indication for such activity.

A glyphosate-based formulation increased biomarkers of oxidative stress in livers and kidneys of mice treated orally for 1 day or 15 days.

Considering the low level of metabolism and the chemical structure of glyphosate, glyphosate radical formation initiating oxidative stress appears unlikely. However, uncoupling or inhibition of mitochondrial oxidative phosphorylation also represents an established mechanism for ROS generation. Notably, uncoupling of oxidative phosphorylation by glyphosate has been reported in rat liver microsomes (Bababunmi et al., 1979, ASB2015-8535) and a glyphosate formulation (but not glyphosate) (Peixoto, 2005, ASB2012-11994).

Induction of oxidative stress, in general, can provide a mechanistic explanation for any observed cytotoxic/degenerative and indirectly genotoxic effects of substances (Chapter 3.6.2.3.2 Additional considerations for classification of Guidance on the Application of the CLP criteria, ECHA-13-G-10-EN, ECHA 2013, ASB2015-8592). However, from the sole observation of oxidative stress and the existence of a plausible mechanism for induction of oxidative stress through uncoupling of mitochondrial oxidative phosphorylation alone, genotoxic or carcinogenic activity in humans cannot be deduced for glyphosate and glyphosate based formulations.

4.2.3.2 Inflammation and immunomodulation

Six studies were reported by IARC in section 4.2.3 (b). The studies including comments of the RMS are summarized in Table 4.2-9 and are described in detail below.

(i) Humans:

Human cells *in vitro*:

Data on glyphosate:

Nakashima et al. (2002, ASB2012-11919) tested the proliferative activity and the release of cytokines of 1-1000 µM glyphosate on PHA-stimulated human peripheral blood mononuclear cells (PBMC) of unknown origin.

After 24 h incubation, glyphosate had a slight (not significant) inhibitory effect on cell proliferation, INF-y was significantly reduced at 1000 µM glyphosate (-30%) and a minimal reduction of IL-2 was

recorded. No effects on TNF-alpha or IL-1 beta. The authors concluded glyphosate showed only a little damage to the immune system.

Remarks: The study of Nakashima et al. (2002, ASB2012-11919) is limited due to the Japanese language. Only a summary and some figures with labelling in English is available, lack of information on the test method, numerical results and the details on the cell donator. The *in vitro* finding (reduction in INF-Y) is opposite to the *in vivo* response in BAL (increase in INF-y) seen in Kumar et al (2014, ASB2015-8276). The relevance of this study seems to be questionable. The highest test concentration of 1 mM that inhibited cell proliferation may be close to a cytotoxic concentration (no data).

Most of the information was correctly cited by IARC. The reported finding 'modestly inhibited the production of IFN-gamma' can be accepted for IFN-gamma (-30%), but no clear effect was seen for IL-2 up to 1000 µM glyphosate.

(ii) Non-human mammalian experimental systems:

Data on glyphosate:

The study of Kumar et al. (2014, ASB2015-8276) used the 'murine intranasal challenge model' with daily intranasal applications for 7 days or 3x/week for 3 weeks of glyphosate-rich air samples (called as 'Real Env.') suspended in PBS (8.66 µg/mL) or reagent grade glyphosate (of unknown purity) at concentrations 100 ng, 1 µg or 100 µg in 30 µl in wild-type of TLR 4-/- mice. (Cell numbers by flow cytometric analysis on BAL and lung tissue, cytokine levels in BAL, serum, immunohistochemistry in lung tissue).

Increases in numbers of cells, eosinophils, neutrophils per lung or BAL fluid at 1 µg and 100 µg glyphosate, but no dose-response was observed. No effect occurred at 100 ng glyphosate. No increase in mast cell number/lung tissue, but higher serum MCPT-1 indicating increased mast cell degranulation was found.

1 or 100 µg glyphosate induced increased release of cytokines (IL-5, Il-10, IL-13 without dose-response for IL-5 and IL-13) to BAL fluid. Although no dose response was recognized, IFN-Y was increased nasal application of glyphosate at both dose levels. In contrast the increase was not confirmed for the 'Real Env.' exposure. IL-4 was increased for 'Real Env.' but not for glyphosate.

At 1 µg glyphosate, 3-4-fold higher levels of IL-33 and TSLP in BAL and (a qualitative) confirmation by positively immuno-stained (bronchiolar?) lung tissue was reported.

Remarks: The study aimed to identify the potential of glyphosate to induce asthma. To our knowledge there are no validated models to assess the potential for respiratory sensitization.

The validity of the administration route and frequency is limited to assess effects after repeated inhalation. Due to the single intranasal injection of the test fluid there is lack of homogenous concentration and lack of constant exposure conditions over 6 hour per day. This method did not produce a continuously homogeneous test atmosphere at the mucosal surface of the airways. As the test material concentrations will be highest in the nasal cavity, the nasal tissues are the preferred sites for cytokine and morphological examinations. In addition, it remains unclear how many animals/sex/dose were treated and how many samples of BAL and lung tissue per animals were examined.

More weight should be given on the testing of glyphosate. Testing of the glyphosate-rich air samples are considered as less informative as the analytical concentration, composition, homogeneity and stability of the air samples were not examined. In comparison with the sham-(PBS) exposed mice the study identified an increase of biomarkers of airway inflammation as shown by increased numbers of cells and increased numbers of inflammatory cells (eosinophils, neutrophils) and elevated cytokine concentrations in BAL. The positive response could be interpreted as qualitative information indicating a potential for airway inflammation since for the majority of cell parameters and cytokines no dose-response was identified. The absence of a dose-response relationship might have been related to the application mode. Increased levels of IL-33 and TSLP in BAL and abundant staining in lung tissue were interpreted as indicative of (asthma-like) type 2 pathology. These effects as well as increased

concentrations of released cytokines that are related to asthma (IL-5, IL-10, IL-13) and mast cell degranulation were also seen following ovalbumin administration with a similar dosing scheme. The authors interpreted the results as indicating that glyphosate triggers allergic inflammation. As there is no validated model on respiratory sensitization and due to the weaknesses of the study, this conclusion needs confirmation by other studies or human data.

The study results were (almost) correctly reported by IARC. In contrast to the IARC text, no effect was seen at 1 ng glyphosate.

In the study of Chan and Mahler (1992, TOX9551954) Groups of 10 male and 10 female F344N rats and B6C3F1 mice were given glyphosate in feed at dietary concentrations of 0, 3125, 6250, 12500, 25000 or 50000 ppm (corresponding to 0, 205, 410, 811, 1678 or 3393 mg/kg for males rats and 0, 213, 421, 844, 1690 or 2293 mg/kg bw/d for female rats). Ten additional rats/sex were included at each dietary level for evaluation of hematologic and clinical pathology parameters (on days 5, and 21, and at the end of treatment after 13 weeks).

In male rats, reduced body weight (bw) gains were observed in the 25000 and 50000 ppm groups. The final bw in these groups were significantly lower than that of the control group. At necropsy the bw of the 50000 ppm male group was 18% less than that of controls. In female rats of this dose there was only a marginal effect on bw gain with the high dose group 5% lighter than controls at the end of study. In male rats of this dose, small increases in relative organ weight were observed for the liver, kidney, and testicle; a decrease in absolute weight and relative weight was observed for the thymus. The relative weight was 0.80% for high dose males versus 0.92% in control males. No treatment-related effects in females and on food consumption were observed.

Mild increases in haematocrit and RBC were observed in male rats at 13 weeks at ≥12500 ppm and increased haemoglobin in male rats at ≥25000 ppm. In female rats, minimal but significant increases occurred in lymphocyte and platelet counts, WBC, MCH and MCV. Treatment-related alterations in clinical chemistry parameters included increases in alkaline phosphatases in males and females at all-time points, ALAT in males and females at all-time points except 90 days, total bile acid at days 23 and 90 in males and at day 23 in females, total protein in females at all-time points, and sporadic increases in urea nitrogen and albumin.

In the 13-week study in mice, significantly lower final bw, lower relative thymus weights and increased relative weights of liver, heart, testes, lungs and kidneys were seen in high dose male mice, significantly lower final bw and lower absolute thymus and liver weights were observed in high dose female mice. A dose-related cytoplasmatic alteration of the parotid salivary gland in male mice and female mice at all doses (except the low dose) were seen. No data on haematology and clinical chemistry were available.

Remarks: The 13-week studies were conducted in 1988; the used method is not comparable to the current OECD test guideline standard. Increased haematocrit and RBC may indicate a lower water consumption and dehydration status of the animals (no data on water consumption available). Elevated ALAT and total bile acids could be related to hepatobiliary dysfunctions (in the absence of histopathological findings reported). Lower absolute and relative thymus weight alone in high dose males without any corresponding (microscopic) effect on immune organs or immune compartments in other tissues is not sufficient to indicate an immunosuppressive effect of glyphosate. More likely it could be interpreted as a nonspecific (toxic) response together with a lower bw gain that resulted in 18% lower final bw at 50000 ppm. Based on the limited information available it can be concluded that the observations in rats are in agreement with the findings in mice.

To the IARC Documentation:

IARC summed up the main findings as ‘pathological effects of glyphosate on the immune system’ without giving an interpretation of the effects seen. Based on a weight of evidence analysis of the available data from the studies in rats and mice one should conclude that there is no clear indication of an immunosuppressive effect.

Glyphosate-based formulation:

In the study of Blakley (1997, ASB2015-7878) female CD-1 mice received drinking water for 26 days at concentrations from 0, 0.35%, 0.7% or 1.05% Roundup (corresponding to 0, 335, 670 or 1000 mg/kg glyphosate/ kg/day. On day 21 mice were i.p. injected with sheep red blood cells (SRBC) and the production of the T-lymphocyte, macrophage dependent antibody response was evaluated on day 26.

No treatment-related effect on bw gain or water consumption. Roundup did not affect the T-cell mediated antibody production.

Remarks: There is no indication that the humoral immune response is adversely affected in mice that received Roundup for 26 days of treatment.

IARC correctly summed up the study results. The lack of effects on the immune system has not been reflected in their overall conclusion.

Overall conclusion on section (b) inflammation and immunomodulation:

IARC documented the results of one *in vitro* and three *in vivo* studies that examined for glyphosate-related effects on the mammalian immune system in this section.

With regards to the underlying mode of action for the carcinogenic effects IARC concluded that there is 'weak evidence that glyphosate may affect the immune system, both the humoral and cellular response' (section 5.4).

RMS concludes that the evidence from available data do not allow to conclude that glyphosate caused immunosuppression. However it is to note that due to the small number of studies assessed and the fact than all studies show limitations, no robust information is available to conclude on the immunomodulatory action of glyphosate.

Conclusion on glyphosate:

The main study results of the above mentioned studies were correctly summed up by IARC. Some details of the reporting could be improved. In the study of Kumar et al. (2014, ASB2015-8276) no effect was seen at the low dose tested (100 ng glyphosate) in mice. A critical analysis of the limitations of the studies (e.g. on the exposure regimen) is lacking.

The effects of the 13-week study in rats (Chan and Mahler, 1992, TOX9551954) were described by IARC as 'pathological effects of glyphosate on the immune system'. The only finding was a reduced absolute/relative thymus weight in male rats at the highest dose. No other corroborating effect in the immune organs was seen. The lower weight of the thymus is likely to be linked to nonspecific toxic effects such as a lower bw gain and a 18% lower final bw in male rats. No such effect was seen in female rats of this study. No clear pathological (immune suppressive) effect on the immune system can be identified from this study.

The study of Kumar et al. (2014, ASB2015-8276) indicated that glyphosate may induce inflammatory effects in the respiratory tract that by the authors was supposed as being predictive to induce asthma-like effects. Additional and more robust data are needed to confirm this assumption. A potential for inflammatory responses of the respiratory tract is the only immunomodulatory effect identified so far.

Conclusion on glyphosate-containing formulation (Roundup):

The negative results for glyphosate of the Chan and Mahler study (1992, TOX9551954) are in agreement with the negative finding for effects on the immune system of the study of Blakley (1997, ASB2015-7878). Although both studies had limitations (in comparison to current test guideline standards or the test material), the negative outcome was not reflected by IARC. The glyphosate-containing formulation tested in the Blakley study (1997, ASB2015-7878) was negative for T-cell dependent antibody response up to 1000 mg/kg bw/d glyphosate and did not indicate that the humoral and cellular immune responses were affected.

Table 4.2-9: Discussion of studies in chapter 4.2.3 (b) Inflammation and immunomodulation of the IARC monograph

Study (Author/year)	Subject	Evaluation by IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Nakashima et al., 2002, ASB2012-11919	Effects of glyphosate on cytokines production by human peripheral blood mononuclear cells	Glyphosate had a slight inhibitory effect on cell proliferation, and modestly inhibited the production of IFN-gamma and IL_2. The production of TNF-alpha and IL-1 Beta was not affected by glyphosate.	Agreement. The authors conclude that glyphosate might be a pesticide with only a little damage to the immune system. The study of Nakashima et al. (2002) is limited due to the Japanese language. Only a summary and some figures with labelling in English is available, lack of information on the test method, numerical results and the details on the cell donator. The *in vitro* finding (reduction in INF-Y) is opposite to the *in vivo* response in BAL (increase in INF-y) seen in Kumar et al., 2014, ASB2015-8276. The relevance of this study seems to be questionable. The highest test concentration of 1 mM that inhibited cell proliferation may be close to a cytotoxic concentration (no data).	No	The relevance of this study seems to be questionable.
Kumar et al., 2014, ASB2015-8276	Pro-inflammatory effects of glyphosate and farm air samples in mice	Airway exposure to glyphosate significantly increased the total cell count, eosinophils, neutrophils, and IgG1 and IfG2a levels and produced pulmonary inflammation. Glyphosate-rich farm air increased circulating levels of IL-5, IL-10, IL-13 and IL-14 in wildtype and TLR4-/- mice. In wildtype mice glyphosate increased levels of IL-5, IL-10, IL-13 and IFN-Gamma (but not IL-4).	Agreement with reported results. The study aimed to identify the potential of glyphosate to induce asthma. The positive response could be interpreted as qualitative information indicating a potential for airway inflammation since for the majority of cell parameters and cytokines no dose-response was identified. Testing of the glyphosate-rich air samples are considered as less informative as the	No	Agreement with reported results; the positive response could be interpreted as qualitative information indicating a potential for airway inflammation.

Study (Author/year)	Subject	Evaluation by IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
			analytical concentration, composition, homogeneity and stability of the air samples were not examined.		
Chan and Mahler, 1992, TOX9551954	NTP report on toxicity studies of glyphosate in mice	In subchronic studies in rats and mice effects on thymus weight and haematological parameters have been observed.	Further effects on clinical chemistry parameters, body weight and salivary gland have been reported. The 13-week studies were conducted in 1988; the used method is not comparable to the current OECD test guideline standard. The results are not sufficient to indicate an immunosuppressive effect of glyphosate. More likely they could be interpreted as a nonspecific (toxic) response together with a lower bw gain that resulted in 18% lower final bw at 50000 ppm.	Yes, page 259	Supplementary information on subchronic toxicity of glyphosate in rats and mice additionally to the large number of studies reported in the RAR; The results are not sufficient to indicate an immunosuppressive effect of glyphosate. More likely they could be interpreted as a nonspecific (toxic) response.
Blakley, 1997, ASB2015-7878	Effect of Roundup on antibody production in mice	The humoral immune response (antibody production against sheep erythrocytes) was not affected by glyphosate.	Agreement	No, reported before 2000	No effect of glyphosate on humoral immune response.
Kreutz et al., 2011, ASB2015-8279	Effects of glyphosate on haematological and immunological parameters in catfish	"A positive association between exposure to glyphosate and immunotoxicity in fish has been reported."	No agreement with conclusion of IARC. Obviously, no glyphosate but a glyphosate containing formulation was used in this study. Without further information it is a mixture of unknown substances. Therefore, no conclusion on glyphosate is possible.	Yes, page 147	No agreement with conclusion of IARC. Obviously, no glyphosate but a glyphosate containing formulation was used in this study. Without further

Study (Author/year)	Subject	Evaluation by IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
					information it is a mixture of unknown substances. Therefore, no conclusion on glyphosate is possible.
El-Gendy et al., 1998, ASB2015-8422	Effects of glyphosate on the immune response and protein biosynthesis of fish	Effects of a glyphosate-based formulation on immune response in bolti fish are reported.	Some effects are described by IARC as glyphosate effects. However, a formulation was used in this study. Therefore, no conclusion on the active substance glyphosate is possible.	No, reported before 2000	Some effects are described by IARC as glyphosate effects. However, a formulation was used in this study. Therefore, no conclusion on the active substance glyphosate is possible.

4.2.4 Cell proliferation and death

Information on apoptosis and proliferation in neuroprogenitor cells from humans (ReN CX) and mice (mCNS) is available from a HTS assay reported (refer to section 4.3).

4.3 Data relevant to comparisons across agents and end-points

IARC stated that no HTS or other relevant data was available to its working group. This included any data from Tox21 or the ToxCast initiatives.

In the RAR (April 2015, ASB2015-1194) information on androgenic and estrogenic effects from the U.S. EPA Endocrine Disruptor Screening Programme are reported. Based on Tier 1 studies of this programme as well as results published as part of the OECD validation of the steroidogenesis assay, and taking into account higher tier regulatory safety studies, it was concluded that there is no evidence for effects on the androgenic or estrogenic pathways of the endocrine system (refer to section 4.2.2).

In addition, the RAR contained information from a HTS assay for apoptosis and proliferation in neuroprogenitor cells from humans (ReN CX) and mice (mCNS). Glyphosate did not activate proliferation (BrdU assay) or apoptosis (caspase 3, p53 pathways) in concentrations between 0.001 and 100 µM in these tests.

DNA microarray data is available for Japanese medaka treated with 16 mg/L glyphosate or its mixture with 0.5 mg/L surfactant for 48 h (Uchida et al., 2012, ASB2015-8590). None of 138 genes that were induced in the liver by the treatment with the combination was associated with mutagenesis or carcinogenesis. Glyphosate alone did not lead to significant hepatic gene expression changes in this fish.

4.4 Cancer susceptibility data

IARC stated that studies examining relevant susceptibility factors were not identified.

In contrast, the RMS considered Swiss albino mice as a potentially susceptible strain for certain tumours: "*Swiss albino mice with high background prevalence of malignant lymphoma could be more vulnerable than other strains.*" (RAR, April 2015, ASB2015-1194). It was discussed that although it could not be completely excluded that the increase in malignant lymphoma incidence over the historical control of the laboratory reported by Kumar ([redacted] ASB2012-11491) was treatment-related, this (potential) effect was "*confined to this single study and strain*".

In its communication entitled "Does glyphosate cause cancer? Preliminary assessment of the carcinogenic risk of glyphosate with regard to the recent IARC evaluation", it was later noted by the BfR: "*Apart from the statistically significant increase in Swiss mice, a higher number of affected top dose males was also seen in two other studies ([redacted] 1997 [22] and [redacted], 2009 [23]) but was contravened later by historical control data.*" (BfR, 2015, ASB2015-8593). The following comparative table was provided:

Table 4.4-1: Total incidence of malignant lymphoma in long-term studies with glyphosate in different mouse strains (Table reproduced from BfR-communication entitled: "Does glyphosate cause cancer? Preliminary assessment of the carcinogenic risk of glyphosate with regard to the recent IARC evaluation" (BfR, 2015, ASB2015-8593).

Study, Strain		Males				Females			
[redacted] 2009, ASB2012-11492 Crl:CD-1 (ICR) BR	Dose (ppm)	0	500	1500	5000	0	500	1500	5000
	Affected	0/51	1/51	2/51	5/51	11/51	8/51	10/51	11/51
[redacted] 2001, ASB2012-11491 HsdOLA:MF1 (Swiss albino)	Dose (ppm)	0	100	1000	10000	0	100	1000	10000
	Affected	10/50	15/50	16/50	19/50*	18/50	20/50	19/50	25/50*
[redacted] 1997, ASB2012-11493 Crj:CD-1 (ICR)	Dose (ppm)	0	1600	8000	40000	0	1600	8000	40000
	Affected	2/50	2/50	0/50	6/50	6/50	4/50	8/50	7/50
[redacted]., 1993, TOX9552382, CD-1 (not further specified)	Dose (mg/kg bw/d)	0	100	300	1000	0	100	300	1000
	Affected**	4/50	2/50	1/50	6/50	14/50	12/50	9/50	13/50

* increase statistically significant, for females based on percentage and not on total number of affected mice
** based on histological examination of lymph nodes with macroscopic changes

4.5 Other adverse effects

A number of further (adverse) effects observed in humans and laboratory animals were discussed by both IARC and BfR. Respective findings have been taken into account in the chapters above as far as these were considered relevant for the assessment of carcinogenic and/or mutagenic potential.

5 Summary of Data Reported

5.1 Exposure data

Results of four occupational and two para-occupational studies using various glyphosate-containing plant protection products have been cited in the IARC monograph. The studies were carried out between 1988 and 2007 in different countries of North America and Europe. Four of these studies (Centre de Toxicology du Québec, 1988 (ASB2015-7889), Lavy et al., 1992 (TOX9650912), Johnson et al., 2005 (ASB2012-11859) and Curwin et al., 2007 (ASB2012-11597)) have not yet been included in the RAR (April 2015, ASB2015-1194) because a refinement of operator exposure was not necessary.

Within the scope of the risk assessment for the representative formulation in the European procedure for renewal of approval of glyphosate the exposure calculations according to the common models demonstrate safe use of the product.

Nevertheless, all six exposure studies have been roughly evaluated now (see Table A-5.5-2).

In all cases but one, the recorded values in the studies were below or in the same order of magnitude as those predicted in the RAR (April 2015, ASB2015-1194). Thus, it can be stated that there is no glyphosate based health risk anticipated for operators for intended uses applied for in the European Union provided that the plant protection product is used correctly and as intended.

However, in one study (Johnson et al., 2005, ASB2012-11859) the reported glyphosate air concentrations for some operators (vehicle application) were strikingly high, i.e. higher than the air concentrations detected in all other studies by a factor of 1000. But it is assumed that the data in this study were obtained with invalid calibration. For more details see Table A-5.5-2.

In summary, for resources on dietary exposure and for results on biological markers IARC refers to several selected reports from national food- and bio-monitoring programmes as well as to some studies in the public literature. Most of the data on dietary consumer exposure are not included in the RAR (April 2015, ASB2015-1194) due to the GAP-based "safe-use" approach for the assessment of active substances under Regulation (EU) 1107/2009 (2009, ASB2015-8589). All studies on biomarkers were also included in the RAR. No deviating conclusions between RAR and IARC were identified.

5.2 Human carcinogenicity data

Based on the studies on cancer in humans IARC concluded: „*There is limited evidence in humans for the carcinogenicity of glyphosate.*" RMS agrees with IARC that the other IARC categories (Evidence suggesting lack of carcinogenicity, inadequate evidence of carcinogenicity and sufficient evidence of carcinogenicity) are not suitable for the classification of the evidence from studies in humans. The evaluation of the epidemiological studies by the RMS is similar to IARC. However, RMS adopts a more cautious view since no consistent positive association is observed, with the most powerful study showing no effect. The IARC interpretation is more precautionary based on the objectives and scope of the IARC Monographs which represent a first step in carcinogen risk assessment, which involves examination of all relevant information in order to assess the strength of the available evidence that an agent could alter the age-specific incidence of cancer in humans and that the Monographs may also indicate where additional research efforts are needed, specifically when data immediately relevant to an evaluation are not available. Therefore, no recommendation is given with regard to regulation or legislation, which is the responsibility of individual governments or other international organizations.

It was also noted that in the epidemiological studies a differentiation between the effects of glyphosate and the co-formulants is not possible. However, data on glyphosate containing formulations indicate a significantly higher toxicity compared to the pure active substance.

5.3 Animal carcinogenicity data

Based on carcinogenicity studies in experimental animals IARC concluded: „*There is sufficient evidence in animals for the carcinogenicity of glyphosate*" on a positive trend in the incidence of renal neoplasms in male CD-1 mice, a significant positive trend in the incidence of haemangiosarcoma in male CD-1 mice and a significant increase in the incidence of pancreatic islet cell adenoma in two studies in the Sprague-Dawley rats.

A much larger number of animal studies have been performed to evaluate the carcinogenic potential of glyphosate than necessary by the legal requirements. In mice, a total of five long-term carcinogenicity studies using dietary administration of glyphosate were considered. In rats, seven chronic toxicity and carcinogenicity studies using dietary administration of glyphosate and two studies with application via drinking-water were reviewed.

In order to support the interpretation and evaluation of the tumour incidences observed in the CD-1 mice studies Table 5.3-1 was prepared (see below).

Renal tumours

In four studies in CD-1 mice and one study in Swiss albino mice, the incidences of renal tumours in male mice were reconsidered for statistical evaluation. In the first study [redacted] 1983 TOX9552381), the combined incidences for renal adenoma and carcinoma in males were 1, 0, 1 or 3 for the control, low, mid or high dose group, respectively, based on the result of the histopathological re-examination and 0, 0, 1, 3 when based on the original study report. In the second study ([redacted] 1997, ASB2012-11493), the incidences for renal adenoma were 0, 0, 0 or 2 for the control, low, mid or high dose group males, respectively. In Swiss albino mice ([redacted] 2001, ASB2012-11491) reported incidences in males were 0, 0, 1, 2. For these three studies, the statistical analysis with the Cochran-Armitage test for linear trend yielded a significant result, whereas the analysis by pair-wise comparisons (Fisher's exact test) indicated no statistically significant differences between the groups. In the two other studies, as well as the females of all studies, there was no indication for induction of renal adenoma.

For both studies in CD-1 mice, the observed renal tumours were considered spontaneous and unrelated to treatment by the study pathologists. Furthermore, extensive pathological and biometrical re-evaluations of the data from the first study reached the conclusion that the absence of any pre-neoplastic kidney lesion in treated males provided sufficient evidence that the occurrence of these tumours was spontaneous rather than substance-induced [redacted], 1986, TOX9552381). This assessment is supported by the fact that, in both studies, the increased incidences of renal tumours at the high dose groups were not statistically significant when compared with the concurrent controls, and the incidences were within the historical control range for adenomas and carcinomas combined (up to 6%).

The EU CLP regulation provides further important factors which should be taken into consideration for the interpretation and assessment of animal carcinogenicity data. If increased tumour incidences are found only at the highest doses used in a lifetime study, the possibility of a confounding effect of excessive toxicity cannot be excluded. In both studies, the highest dose levels tested (4841 or 4348 mg/kg bw per day) were well in excess of the limit dose for carcinogenicity testing (1000 mg/kg bw per day) as recommended by OECD guidance document 116 (OECD 2012). Also, the OECD test guideline for carcinogenicity studies states that the highest dose level should elicit signs of minimal toxicity, with depression of body weight gain of less than 10%. In both studies, however, the body weight gain in high dose males was decreased by more than 15% compared to controls, and there was a significant increase in central lobular hepatocyte hypertrophy, central lobular hepatocyte necrosis, and chronic interstitial nephritis in high dose males in one study ([redacted] 1983 TOX9552381).

Table 5.3-1: Summary of selected tumour incidences in male CD-1 mice.

	Historical control incidences			**Tumour incidence/number of animals examined**																
Dose (mg/kg bw per day)	*Mean*	*Min*	*Max*	0	0	0	0	71	100	157	165	234	300	810	814	838	1000	4348	4841
Study ID				A	B	C	D	D	B	A	C	D	B	D	A	C	B	C	A
Study duration (months)	*NR*	*18*	*24*	24	24	18	18	18	24	24	18	18	24	18	24	18	24	18	24
Survival	*NR*	*18.3%*	*94%*	20/50	26/50	26/50	39/51	41/51	25/50	16/50	34/50	39/51	29/50	35/51	17/50	27/50	25/50	29/50	26/50
Renal tumours#	*0.43%*	*3.43%*	*6.0%*	1/49	2/50	0/50	0/51	0/51	2/50	0/49	0/50	0/51	0/50	0/51	1/50	0/50	0/50	2/50	3/50
Malignant lymphoma	*4.09%*	*1.45%*	*21.7%*	2/48	4/50	2/50	0/51	1/51	2/50	5/49	2/50	2/51	1/50	5/51	4/50	0/50	6/50	6/50	2/49
Haemangiosarcoma	*1.13%*	*1.67%*	*12.0%*	0/48	0/50	0/50	0/51	0/51	0/50	0/49	0/50	0/51	0/50	0/51	1/50	0/50	4/50	2/50	0/49

Study ID: A = [redacted] (1983, TOX9552381), re-evaluation; B = [redacted] (1993, TOX9552382); C = [redacted] (1997, ASB2012-11493); D = [redacted] (2009, ASB2012-11492).

Renal tumours: combined incidence of adenoma and carcinoma.

HC: Historical control data for Crl:CD-1 (ICR)BR mice ([redacted], 2000). The data was gathered from 51 studies of at least 78 weeks duration which were initiated between January 1987 and December 1996.

Mean: Mean (in percent of total); Min: Minimum (in percent found); Max: Maximum (in percent found).

NR: Not reported.

Haemangiosarcoma

In two studies in CD-1 mice, the incidences of haemangiosarcoma in male mice were reconsidered for statistical evaluation. In the first study ([redacted] (1993, TOX9552382), the combined incidences for haemangiosarcoma were 0, 0, 0 or 4 for the control, low, mid or high dose group. In the second study ([redacted] 1997, ASB2012-11493), the incidences for haemangiosarcoma were 0, 0, 0 or 2 for the control, low, mid or high dose group, respectively. For both studies, the statistical analysis with the Cochran-Armitage test for linear trend yielded a significant result, whereas the analysis by pair-wise comparisons (Fisher's exact test) indicated no statistically significant differences between the groups.

The background incidences for haemangiosarcoma in male CD-1 mice provided by [redacted] (2000; from 51 studies, initiated between 1987 and 1996) were up to 6/50 (12%) if multiple organs were considered, and were up to 5% or 8% in liver and spleen, respectively. Therefore, the conclusion of the study pathologists that the observed incidences for haemangiosarcoma were spontaneous and unrelated to treatment is supported by the RMS.

Pancreatic and other tumours

The statistically significant increase in pancreatic tumours incidences in the male rats of the low dose groups of [redacted] (1981, TOX2000-595, TOX2000-1997) and [redacted] (1990, TOX9300244) are considered incidental. With regard to the positive trend for liver cell adenoma in male rats and thyroid C-cell adenoma in females for the study of [redacted] (1990, TOX9300244), IARC noted lack of evidence for progression.

Malignant lymphoma

IARC did also consider a review article (Greim et al., 2015, ASB2015-2287) containing information on five long-term bioassay feeding studies in mice, in which a statistically significant increase in the incidence of malignant lymphoma was reported, but the IARC Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.

In three studies in CD-1 mice, the incidences of malignant lymphoma in male mice were reconsidered for statistical evaluation. For the control, low, mid or high dose group, the respective incidences in the first study were 0, 2, 2 or 5 ([redacted]., 2009, ASB2012-11492), in the second study the incidences were 2, 2, 0, 6 ([redacted] 1997, ASB2012-11493), and in the third study the incidences were 4, 2, 1, 6 ([redacted]., 1993, TOX9552382). For the first and second study, the statistical analysis with the Cochran-Armitage trend test yielded a significant result, whereas the analysis by pair-wise comparisons (Fisher's exact test) indicated no statistically significant differences between the groups for all three studies.

A study in Swiss albino mice ([redacted] 2001, ASB2012-11491) was also reconsidered for statistical evaluation. The incidences in males were 10, 15, 16 or 19 for the control, low, mid or high dose group, respectively. Neither the Cochran-Armitage trend test nor the pair-wise comparisons using Fisher`s exact test yielded a significant result. However, using the Z-test, the pair-wise comparison between the control and high dose group gave a statistically significant result, as reported in the RAR.

For the assessment of the biological significance of these findings, it is important to consider that malignant lymphomas are among the most common spontaneously occurring neoplasms in the mouse. For the CD-1 mouse strain, incidences of up to 13/60 (21.7%) have been reported in male control groups. Thus, the incidences observed in the above studies, with a maximum of 6/50 (12%), were all within the historical control range. Also in the study with Swiss mice, which have considerably higher background incidences for malignant lymphomas, the observed incidences were within the historical control range. Therefore, the conclusion of the study pathologists that the observed malignant lymphomas were spontaneous and unrelated to treatment is supported by the RMS.

For an overall conclusion, the large volume of animal data for glyphosate should be evaluated using a weight of evidence approach. It should be avoided to base any conclusion only on the statistical

significance of an increased tumour incidence identified in a single study, without consideration of the biological significance of the finding.

In summary, based on the data from five carcinogenicity studies in mice and seven chronic toxicity and carcinogenicity studies in rats, the weight of evidence suggests that no hazard classification for carcinogenicity is warranted for glyphosate according to the CLP criteria.

5.4 Mechanistic and other relevant data

Glyphosate has been tested in a broad spectrum of mutagenicity and genotoxicity tests *in vitro* and *in vivo*. Taking into account all available data and using a weight of evidence approach, it is concluded that glyphosate does not induce mutations *in vivo* and no hazard classification for mutagenicity is warranted according to the CLP criteria.

AMPA has been tested for mutagenicity and genotoxicity *in vitro* and *in vivo* in an adequate range of assays. Taking into account all available data and using a weight of evidence approach, it is concluded that AMPA does not induce mutations *in vivo* and no hazard classification for mutagenicity is warranted according to the CLP criteria.

Glyphosate-based formulations have been extensively tested for mutagenicity and genotoxicity *in vitro* and *in vivo* in a wide range of assays. However, since formulation compositions are considered proprietary, the specific composition of the formulations tested was not available for the published studies. Positive results from *in vitro* chromosomal damage assays and tests for DNA strand breakage and SCE induction were reported in published studies. Also, for specific glyphosate-based formulations, *in vivo* mammalian chromosomal aberration or micronucleus assays as well as tests for DNA adducts, DNA strand breakage and SCE induction gave positive results in some published studies. However, no regulatory studies for these endpoints were provided. Thus, for the different glyphosate-based formulations, no firm conclusions can be drawn with regard to a need for classification according to the CLP criteria.

In general the documentation of the majority of studies on oxidative stress can be confirmed, but it is noted that there is a lack of positive controls for oxidative stress in all *in vitro* and *in vivo* studies described in the IARC monograph. From the available data on glyphosate, there is some indication of induction of oxidative stress from testing in human cell cultures and in mammalian (*in vivo*) experimental systems. In particular, the IARC statement that there are indications of oxidative stress in the blood plasma, liver, brain and kidney of rats upon exposure to glyphosate can be supported. However, only one of the cited studies investigated oxidative stress in animals with pure glyphosate. This study was conducted in rats and no other species was tested and increased oxidative stress was observed in combination with cytotoxic/degenerative effects of the targeted organs.

Considering the low level of metabolism and the chemical structure of glyphosate, glyphosate radical formation initiating oxidative stress appears unlikely. However, uncoupling or inhibition of mitochondrial oxidative phosphorylation also represents an established mechanism for ROS generation. Notably, uncoupling of oxidative phosphorylation by glyphosate has been reported in rat liver microsomes and a glyphosate formulation (but not glyphosate).

Induction of oxidative stress can provide a mechanistic explanation for any observed cytotoxic/ degenerative and indirectly genotoxic effects of substances. However, from the sole observation of oxidative stress and the existence of a plausible mechanism for induction of oxidative stress through uncoupling of mitochondrial oxidative phosphorylation alone, genotoxic or carcinogenic activity in humans cannot be deduced for glyphosate and glyphosate based formulations.

Furthermore, the RMS concludes that the evidence from available data do not allow to conclude that glyphosate caused immunosuppression.

Glyphosate was included into the U.S. EPA Endocrine Disruptor Screening Program's (EDSP). Which concluded that, based on the Tier 1 assays that had been performed at different independent laboratories and taking into account the 'higher tier' regulatory safety studies Glyphosate might not be considered an endocrine disrupter.

5.5 Further conclusions and recommendations

In result of the now available additional data and information on glyphosate formulations it is concluded and recommended:

- The data requirement for the evaluation and authorisation of plant protection products should be general verified and extended, in particular in consideration of possible genotoxic properties and effects caused by the mixture of different active substances or in combination with co-formulants. The described information on the genotoxicity of the different glyphosate formulations clearly shows that a prediction on the genotoxicity based on the single ingredients of a formulation according to the CLP-Regulation (ECHA, 2013, ASB2015-8592) is insufficient. Therefore, in general a specific data requirement for the evaluation and assessment of genotoxic properties of plant protection products is necessary.
- For the representative formulation for the EU renewal procedure 'Roundup Ultra' two studies (Guilherme et al., 2012, ASB2014-7619, Guilherme et al., 2014, ASB2015-8631) reported positive results in comet assays using the European eel as test species. According to Point 7.1.7 of Regulation (EU) No 284/2013 (EU, 2013, ASB2015-8658) the competent Authorities have to discuss case by case the need to perform supplementary studies. The RMS recommends further genotoxicity studies performed in compliance with OECD test guidelines for the representative formulation as confirmatory information for the authorisation of plant protection products.

6 Environmental risk assessment

In addition to the evaluation of the IARC monograph in terms of human health and classification aspects, the significance of the corresponding studies for the environmental risk assessment has also been assessed. Taking into account the results of existing reproduction studies in fish, the genotoxicological effects observed in biomarker studies with the active substance glyphosate listed in the IARC monograph are considered not to be manifest on the population level. However, the genotoxicological effects observed in some studies with POEA-containing glyphosate formulations are considered to be relevant for the authorisation of glyphosate products in the Member States.

7 References relied on

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
1	Acquavella, J.F., Alexander, B.H., Mandel, J.S., Gustin, C., Baker, B., Chapman, P., Bleeke, M.	2004	Glyphosate biomonitoring for farmers and their families: Results from the farm family exposure study Environmental Health Perspectives vol.112, 3 (2004) 321-326 GLP: No Published: Yes BVL-2309536, ASB2012-11528
2	Akanuma, M.	1995	HR-001: DNA Repair Test (Rec-Assay) IET 94-0141 GLP: Yes Published: No BVL-2309325, ASB2012-11477
3	Alavanja, M.C., Samanic, C., Dosemeci, M., Lubin, J., Tarone, R., Lynch, C.F., Knott, C., Thomas, K., Hoppin, J.A., Barker, J., Coble, J., Sandler, D.P., Blair, A.	2003	Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort Am J Epidemiol vol.157, 9 (2003) 800-814 GLP: No Published: Yes BVL-2309554, ASB2012-11535
4	Alavanja, M.C.R.; Sandler, D.P.; McMaster, S.B.; Zahm, S.H.; McDonnell, C.J.; Lynch, C.F.; Pennybacker, M.; Rothman, N.; Dosemeci, M.; Bond, A.E.; Blair, A.	1996	The agricultural health study Environmental Health Perspectives, Volume 104, Number 4, April 1996 362-369 ASB2015-7849
5	Alvarez-Moya, C.; Reynoso Silva, M.; Valdez Ramírez, C.; et al.;	2014	Comparison of the in vivo and in vitro genotoxicity of Glyphosate Isopropylamine salt in three different organisms page 105-110 Genetics and Molecular Biology, 37, 1, 105-110 (2014) GLP: No Published: Yes BVL-2716311, ASB2014-6902
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186	[redacted]	2009	Glyphosate Technical: Dietary carcinogenicity study in the mouse SPL 2060-0011 GLP: Yes Published: No BVL-2309412, ASB2012-11492
187	[redacted]	1980	Dominant lethal mutagenicity assay with technical Glyphosate in mice 401-064 ! IR-79-014 GLP: No (27) Open (9) Yes (1) Published: No (31) Open (6) BVL-1345017, TOX9552377
188	Wright, N.P.	1996	Technical glyphosate: Chromosome aberration test in CHL cells in vitro 434/015 GLP: Yes Published: No BVL-2309319, ASB2012-11476
189	Xie, L.T., Thrippleton, K., Irwin, M.A., Siemering, G.S., Mekebri, A., Crane, D., Berry, K., Schlenk, D.	2005	Evaluation of estrogenic activities of aquatic herbicides and surfactants using an rainbow trout vitellogenin assay Toxicological Sciences vol.87, 2 (2005) 391-398 GLP: No Published: Yes BVL-2310138, ASB2012-12056
190	Yoshioka, N.; Asano, M.; Kuse, A.; Mitsuhashi, T.; Nagasaki, Y.; Ueno, Y.;	2011	Rapid determination of glyphosate, glufosinate, bialaphos, and their major metabolites in serum by liquid chromatography–tandem mass spectrometry using hydrophilic interaction chromatography doi:10.1016/j.chroma.2011.04.021 Journal of Chromatography A, 1218 (2011) 3675–3680 ASB2015-8033
191	Yue, Y., Zhang, Y., Zhou, L., Qin, J., Chen, X.	2008	In vitro study on the binding of herbicide glyphosate to human serum albumin by optical spectroscopy and molecular modeling J Photochem Photobiol vol.B 90, 1 (2008) 26-32 GLP: No Published: Yes BVL-2310144, ASB2012-12059

Annex

Table A-5.5-1: Methods for the analysis of glyphosate

Sample matrix	Assay procedure	Derivati-sation	LOD	LOQ	Validation complete?	Cited outside section 1.3	Reference
Water	HPLC/MS (with online solid-phase extraction)	FMOC	0.08 µg/L	0.20 µg/L	yes	no	Lee et al., 2001, ASB2015-8239
Water	ELISA	no	0.05 µg/L	0.5 µg/L	yes	no	Abraxis, 2005, ASB2015-7847
Water	LC-LC-FD	FMOC	0.02 µg/L	0.10 µg/L	yes	no	Hidalgo et al., 2004, ASB2015-8423
Water	HPLC with post-column reaction and FD	OPA	6.0 µg/L	25 µg/L	yes	no	EPA, 1992, ASB2015-8424
Water	UV visible spectro-photometer (at 435 nm)	no	1.1 µg/L	not determined	no	no	Jan et al., 2009, ASB2015-8285
Soil	LC–MS/MS with triple quadrupole	FMOC	0.02 mg/kg	0.50 mg/kg	yes	no	Botero-Coy et al., 2013, ASB2015-7882
Dust	GC-MS-MID	TFA/ TFEtOH	0.0007 mg/kg	not determined	no	no	Curwin et al., 2005, ASB2012-11595
Air	HPLC/MS with online solid-phase extraction	FMOC	0.01 ng/m^3	not determined	no	yes (1.4.1)	Chang et al., 2011, ASB2015-7895
Fruits and vegetables	HILIC/WAX with ESI-MS/MS	no	0.0012 mg/kg	0.005 mg/kg	yes	no	Chen et al., 2013, ASB2014-8087
Field crops (rice, maize and soybean)	LC–ESI-MS/MS	no	0.007 - 0.12 mg/kg	0.10 mg/kg	yes	no	Botero-Coy et al., 2013b, ASB2015-7883
Plant vegetation	HPLC with single polymeric	FMOC	0.3 mg/kg	1.0 mg/kg	yes	no	Nedelkoska and Low, 2004,

Sample matrix	Assay procedure	Derivati-sation	LOD	LOQ	Validation complete?	Cited outside section 1.3	Reference
	amino column						ASB2015-8134
Serum	LC–MS/MS	no	0.01 µg/mL	0.20 µg/mL	yes	no	Yoshioka et al., 2011, ASB2015-8033
Urine	HPLC with post-column reaction and FD	OPA	1.0 µg/L	not determined	unknown	yes (1.4.1, 4.1.2, 4.1.5)	Acquavella et al., 2004, ASB2012-11528
Urine	ELISA	no	0.9 µg/L	not determined	no	yes (1.4.1, 4.1.2, 4.1.5)	Curwin et al., 2007, ASB2012-11597

LOD Limit of detection
LOQ Limit of quantification
ELISA Enzyme linked immunoassay
FMOC 9-Fluorenylmethyl chloroformate
GC-MS-MID Gas chromatography-mass spectrometry with multiple ion detection
HILIC Hydrophilic interaction liquid chromatography
HPLC/MS High performance liquid chromatography-mass spectrometry
LC-LC-FD Two dimensional liquid chromatography with fluorescence detection
LC–ESI-MS/MS Liquid chromatography with electrospray ionization-tandem mass spectrometry
LC–MS/MS Liquid chromatography-tandem mass spectrometry
OPA *o*-Phthaldialdehyde
TFA Trifluoroacetic acid
TFEtOH Trifluoroethanol
WAX Weak anion exchange (chromatography)

Table A-5.5-2: Occupational and para-occupational exposure to glyphosate

Industry, country, year	Job/process	Results	Comments/additional data by IARC	Comments/additional data by RMS	Reference
Forestry					
Canada, 1986	Signaller Operator Overseer Mixer	Arithmetic mean of air glyphosate concentrations: Morning, 0.63 µg/m^3 Afternoon, 2.25 µg/m^3 Morning, 1.43 µg/m^3 Afternoon, 6.49 µg/m^3 Morning, 0.84 µg/m^3 Afternoon, 2.41 µg/m^3 Morning, 5.15 µg/m^3 Afternoon, 5.48 µg/m^3	Air concentrations of glyphosate were measured at the work sites of one crew (five workers) during ground spraying 268 urine samples were collected from 40 workers; glyphosate concentration was above the LOD (15 µg/L) in 14%	Product used: not given Application rate: not given Only 5 operators in the study: two signaller, one operator, one overseer and one mixer Application equipment: not given Taken together, there is a lack of important information/explanations in this report. From the present point of view, this study would not be acceptable as an OECD guideline-conform operator exposure study. Nevertheless, a rough estimate is presented for the inhalatory exposure of the operators. Highest measured air concentration in the study (mixer): 10.5 µg/m^3 corresponding to an operator exposure of 0.105 mg/person/day[a]. This is well below the estimated inhalation exposure according to the German operator predictive model (high crop hand held scenario, 'worst case'[b]) of 1.26 mg/person/day[c] used for risk assessment in the context of product authorisation. Therefore, no risk is expected to arise from this glyphosate air concentration.	Centre de Toxicologie du Québec, 1988, ASB2015-7889
Finland, year NR	Workers performing silvicultural clearing (n = 5)	Range of air glyphosate concentrations, <1.25 - 15.7 µg/m^3 (mean, NR)	Clearing work was done with brush saws equipped with pressurized herbicide sprayers Air samples were taken from the workers' breathing zone (number of samples, NR) Urine samples were collected during the afternoons of the working week (number, NR) Glyphosate	Product used: Roundup, containing 360 g a.e.[d]/L glyphosate Application rate: not given with regard to treated area Spraying solution: mixture of 8% Roundup, 87% water and 5% of a commercial 'carrier liquid' (40% isopropylamine alcohol) Only five operators in the field study in August 1988 Effective working time 6 hours/day Only two values for glyphosate in air could be determined at the end of the spraying week, i.e. 2.8 µg/m^3 and 15.7 µg/m^3, (all other values <1.25 µg/m^3) Taken together, the number of participants is very low in this study and only two air concentrations could be measured at all. From the present point of view, this study would not be acceptable as an OECD guideline-conform operator exposure study. Nevertheless, a rough estimate is presented for the inhalatory exposure of operators. The air concentration of 15.7 µg/m^3 corresponds to an operator exposure of	Jauhiainen et al., 1991, MET9600092

Industry, country, year	Job/process	Results	Comments/additional data by IARC	Comments/additional data by RMS	Reference
			concentrations in urine were below the LOD (10 µg/L)	0.157 mg/person/day[a]. This is well below the estimated inhalation exposure according to the German operator predictive model (high crop hand held scenario, 'worst case'[b]) of 1.26 mg/person/day[c] used for risk assessment in the context of product authorisation. Therefore, no risk is expected to arise from this glyphosate air concentration.	
USA, year NR	Workers in two tree nurseries (n = 14)	In dermal sampling, 1 of 78 dislodgeable residue samples were positive for glyphosate The body portions receiving the highest exposure were ankles and thighs	Dermal exposure was assessed with gauze patches attached to the clothing and hand rinsing Analysis of daily urine samples repeated over 12 weeks was negative for glyphosate	Product used: Roundup, containing 370 g a.e.[d]/L glyphosate (1.4 kg glyphosate a.e.[d]/gal product) 9 operators, application by trigger spray equipment ('weeders'), 2 operators, application by tractor-drawn spray equipment (applicators) and 2 scouts who did not apply the product themselves Application rate: 0.01 kg product/ha for operators using triggers spray, spraying solution: 1:40 dilution of Roundup or 0.11 kg product/ha for operators using tractor-drawn spray equipment, spraying solution, approximately 1:250 dilution of Roundup Taken together, processing of dermal exposure data is not easily comprehensible in this study, at least some pieces of information/explanations are lacking. From the present point of view, this study would not be acceptable as an OECD guideline-conform operator exposure study. Nevertheless, a rough estimate is presented for the dermal exposure of operators. By far the highest extrapolated total skin exposure in the study amounted to 48,618 µg glyphosate a.e.[d]/day for an applicator/weeder (mixing/loading for all other weeders and application) wearing work clothing and gloves (during both tasks?). This is equivalent to 90.033 mg glyphosate a.e.[d]/kg product/day (0.54 kg product was handled per day) corresponding to 33.312 mg glyphosate a.e.[d]/kg a.e.[d]/day. Taking into account an application rate of 3.6 kg a.e/ha (which is very common for many crops glyphosate is used on) external exposure to 119.9 mg a.e.[d]/person/day would result for operators using the derived data of the study for application on 1 ha. The estimated total external skin exposure according to the German operator predictive model which is used for risk assessment in the context of product authorisation (high crop hand-held scenario for 1 ha, 'worst case', since there are no data for hand-held applications downwards under high crops in this	Lavy et al., 1992, ASB2012-11859

Industry, country, year	Job/process	Results	Comments/additional data by IARC	Comments/additional data by RMS	Reference
				model) makes up 152.8 mg a.e.[d]/person/day (gloves during mixing/loading) or 115.0 mg glyphosate a.e.[d]/person/day (gloves during mixing/loading and application). This is in the same order of magnitude as the above mentioned exposure values derived by the study. Therefore, no risk is expected to arise from this dermal glyphosate concentration.	
Weed control					
United Kingdom, year NR	Municipal weed control workers (n = 18)	Median, 16 mg/m^3 in 85% of 21 personal air samples for workers spraying with mechanized all-terrain vehicle Median, 0.12 mg/m^3 in 33% of 12 personal air samples collected from workers with backpack with lance applications	[The Working Group noted that the reported air concentrations were substantially higher than in other studies, but was unable to confirm whether the data were for glyphosate or total spray fluid.] Dermal exposure was also measured, but reported as total spray fluid, rather than glyphosate	Products used: Roundup Pro Bioactive containing 360 g glyphosate a.e.[d]/L (all-terrain vehicles with front-mounted sprayers) Total Herbicide ready-to-use (NOMIX), Hilite Herbicide ready-to-use (NOMIX) and Roundup Pro Bioactive (Monsanto) diluted with 'Lightning' to give a 40% solution, (controlled drop applicators = lances with spinning discs connected to knapsack container, lance handle with trigger) 6 operators with all-terrain vehicles, monitored in May 1998, 1:14 dilution of product applied, mixing/loading not included into monitoring PPE was worn and described in the report 12 operators with drop applicators, mixing/loading included (where no ready-to-use product), monitored in May - October 1999 Application: 15 cm above the ground PPE was normally worn and described in the report It has to be noted that all glyphosate air concentrations reported for operators using all-terrain vehicles for application in this study are much higher than the concentrations measured for operators using hand-held equipment. Furthermore, it is striking that these concentrations are also ways beyond the values recorded in all other studies summarised in this Table. Median air concentration of glyphosate reported in the study for vehicle application: 15.6 mg glyphosate a.e.[d]/m^3 hand-held applicator: 0.12 mg glyphosate a.e.[d]/m^3 In this context it has been noted that the calibrated range of air sampling was 6.7 - 133 µg/m^3, which is 1000 times lower than the data reported. (Similar to the lower calibrated range also the LOQ is reported in µg/m^3.) For these reasons, either the data are obtained with invalid calibration or there is a typo in the units (mg/m^3 instead of correctly µg/m^3).	Johnson et al., 2005, ASB2012-11859

Industry, country, year	Job/process	Results	Comments/additional data by IARC	Comments/additional data by RMS	Reference
				Due to the inconsistencies concerning determination of air concentrations during application listed above, no comparison of measured and estimated values has been carried out. Once the spray droplets generated during application deposited, operators could only be exposed to released glyphosate-containing vapours. On the basis of the vapour pressure for glyphosate (1.31 x 10^{-5} Pa at 25°C) the saturated vapour concentration can be calculated. Irrespective of the input parameters used, the resulting air concentration will not exceed 1 µg glyphosate/m^3. Dermal exposure was considerably higher for operators using vehicles than for those with hand-held equipment. Median values for potential dermal exposure (without hands) for: vehicle application: 2.0 mL spray solution/h hand-held application: 0.13 mL spray solution/h Median values for hand exposure for: vehicle application: 3.0 mL spray solution/h hand-held application (rather actual exposure in this case): 0.004 mL spray solution/h Median values for foot exposure for: vehicle application: not determined hand-held application (rather actual exposure in this case): 0.001 mL spray solution/h Assuming usual application conditions for many glyphosate-containing products[e] total external dermal exposure would amount to: 180 mg glyphosate a.e.[d]/person (in the case of vehicle application) or 5.0 mg glyphosate a.e.[d]/person (in the case of hand-held application) The above mentioned total external dermal exposure values are in the same order of magnitude compared with the estimated total external dermal exposure according to the German operator predictive model even if the only suitable but less exposing application scenario for operators is taken into account there (field crop tractor-mounted application, 20 ha, application rate of 3.6 kg a.e.[d]/ha[c] assumed, without considering mixing/loading (compare with study design above)): 147 mg glyphosate a.e.[d]/person/day (without personal protective equipment)	

Industry, country, year	Job/process	Results	Comments/additional data by IARC	Comments/additional data by RMS	Reference
				or 37.4 mg glyphosate a.e.[d]/person/day (with protective suit during application) The estimated total external dermal exposure according to the German operator predictive model (hand-held application in high crops ('worst case', since there are no data for hand-held applications under high crops in this model) 1 ha, application rate of 3.6 kg a.e.[d]/ha[c] assumed, without considering mixing/loading ('worst case', since ready-to-use formulations as well as those which had to be diluted were used), with protective gloves and suit during application) makes up 22.2 mg glyphosate a.e.[d]/person/day. This is in the same order of magnitude as the above mentioned exposure values derived by the study.	
Farming					
USA, 2001	Occupational and para-occupational exposure of 24 farm families (24 fathers, 24 mothers and 65 children). Comparison group: 25 non-farm families (23 fathers, 24 mothers and 51 children)	Geometric mean (range) of glyphosate concentrations in urine: Non-farm fathers, 1.4 µg/L (0.13–5.4) Farm fathers, 1.9 µg/L (0.02–18) Non-farm mothers, 1.2 µg/L (0.06–5.0) Farm mothers, 1.5 µg/L (0.10–11) Non-farm children, 2.7 µg/L (0.10–9.4) Farm children, 2.0 µg/L (0.02–18)	Frequency of glyphosate detection ranged from 66% to 88% of samples (observed concentrations below the LOD were not censored). Detection frequency and GM concentration were not significantly different between farm and non-farm families (observed concentrations below the LOD were not censored)	Product applied: not given, at least one out of seven various pesticides (e.g. chlorpyrifos, metolachlor, atrazine) must have been used by the study participants, one of which was glyphosate Application rate: not given Application equipment: not given, but most probably boom sprayers since treated crops were corn and soybean 24 "operators" (farm fathers) monitored including their wives and children, often farm fathers did not apply the plant protection product themselves, but had it applied by contractors in many cases. This had actually no influence on the urinary glyphosate concentrations of the farm fathers according to the study. LOQ for the method for glyphosate analysis is not validated (please see Table A-5.5-1) Taken together, there is a lack of important information in this study report, especially with regard to non-dietary exposure conditions. From the present point of view, this study would not be acceptable as an OECD guideline-conform operator exposure study. Nevertheless, a worst case estimate is presented for the systemic exposure of operators resulting from the use of glyphosate-containing products. The highest measured urinary concentration of glyphosate for farm fathers in this study amounted to 18 µg glyphosate a.e.[d]/L. Assuming a urine volume of	Curwin et al., 2007, ASB2012-11597

Industry, country, year	Job/process	Results	Comments/additional data by IARC	Comments/additional data by RMS	Reference
				2 L per day the systemically available amount of glyphosate would be at least 36 µg (rounded for 40 µg in case that not all of it had been excreted in urine the same day). Hence, a systemic dose of 0.00057 mg a.e.[d]/kg body weight would result for a 70 kg weighing operator. This systemic exposure would account for 0.57% of the proposed AOEL of 0.1 mg/kg bw/day. The above mentioned exposure would be fully covered by the estimated total systemic exposure of 0.0473 mg a.e.[d]/kg bw/day according to the German operator predictive model (field crop tractor-mounted scenario for 20 ha, application rate of 3.6 kg a.e.[d]/ha[c] assumed, without personal protective equipment) used for risk assessment in the context of product authorisation. This exposure would correspond to 47.3% of the AOEL. Therefore, no risk is expected to arise from the detected systemic exposure to glyphosate for operators.	
USA, year NR	Occupational and para-occupational exposures of 48 farmers, their spouses, and 79 children	Geometric mean (range) of glyphosate concentration in urine on day of application: Farmers, 3.2 µg/L (< 1 – 233 µg/(L) Spouses, NR (< 1 to 3 µg/L) Children, NR (< 1 to 29 µg/L)	24-hour composite urine samples for each family member the day before, the day of, and for 3 days after a glyphosate application. Glyphosate was detected in 60% of farmers' samples, 4% of spouses' samples and 12% of children's samples the day of spraying and in 27% of farmers' samples, 2% of spouses' samples and 5% of children's samples 3 days after	Product used: Roundup Ultra in most cases (other products used were not further specified) Application rate: not given Application of either glyphosate-containing products alone or in combination with up to two other pesticides (2,4-D and/or chlorpyrifos), no further details given 48 operators monitored including their wives and children Application by tractors partly with closed cabs equipped with boom sprayers Validation status of the method for glyphosate analysis is unknown (please see Table A-5.5-1) Taken together, there is a lack of information/explanations in this study report, especially with regard to pesticide application conditions. From the present point of view, this study would not be acceptable as an OECD guideline-conform operator exposure study. Nevertheless, a worst case estimate is presented for the systemic exposure of operators (please see also Niemann et al. (2015, ASB2014-11029). By far the highest measured urinary concentration of glyphosate for an operator on the day of application in this study amounted to 233 µg glyphosate a.e.[d]/L. Assuming a urine volume of 2 L per day the systemically available amount of glyphosate would be at least 466 µg (rounded for 500 µg in case that not all of it had been excreted in urine the same day). Hence, a	Acquavella et al., 2004, ASB2012-11528

Industry, country, year	Job/process	Results	Comments/additional data by IARC	Comments/additional data by RMS	Reference
				systemic dose of 0.0071 mg a.e.[d]/kg body weight would result for a 70 kg weighing operator. This systemic exposure would account for 7.1 % of the proposed AOEL of 0.1 mg/kg bw/day. The above mentioned exposure would be fully covered by the estimated total systemic exposure of 0.0473 mg a.e.[d]/kg bw/day according to the German operator predictive model (field crop tractor-mounted scenario for 20 ha, application rate of 3.6 kg a.e.[d]/ha[c] assumed, without personal protective equipment) used for risk assessment in the context of product authorisation. This exposure would correspond to 47.3 % of the AOEL. Even if the use of closed tractor cabins in the study reduced exposure this would be covered by the factor of 6.7 between measured and predicted exposure value. Therefore, no risk is expected to arise from the detected systemic exposure to glyphosate for operators.	

LOD, limit of detection; ND, not detected; NR, not reported

[a] Assuming a breathing volume of 1.25 m^3/h and a working day of 8 h

[b] Although all herbicidal applications are usually directed downwards the 'high crop hand-held scenario' according to the German operator exposure predictive model is taken into account as the 'worst case', since there are no data for hand-held applications under high crops available in this model.

[c] Taking into account an application rate of 3.6 kg acid equivalent/ha which is quite common

[d] a.e., corresponding to acid equivalent

[e] Usual application rate of 3.6 kg a.e./ha in 100 L of water, giving a concentration of 36 mg glyphosate a.e./mL in the spray solution

Renewal Assessment Report

October 2015

Glyphosate

Addendum 1 to RAR
Part Ecotoxicology

Assessment of
IARC Monographs
Volume 112 (2015):
Glyphosate

RMS: Germany

Version history (Addendum 1)

Date	**Reason for revision**
October 2015	First Draft Evaluation IARC Monograph, Ecotoxicology

Preface

The International Agency for Cancer Research (IARC) of the World Health Organization (WHO) evaluated the active substance glyphosate and concluded that glyphosate is “probably carcinogenic to humans (Group 2A)”, based on the studies available to IARC. The full report on glyphosate from the IARC monograph (Volume 112) has been made publicly available on 29 July 2015.

As Rapporteur Member State (RMS) for the European renewal of approval of the active substance glyphosate, Germany was commissioned by EFSA to evaluate the IARC Monographs Volume 112 on glyphosate. Subsequently, an addendum to the original draft Renewal Assessment Report (DRAR from 2015) regarding toxicology and metabolisms of glyphosate was provided by the BfR by 31 August 2015 taking into account new information from the IARC monograph (Volume 112).

While the addendum of BfR addresses the relevance of the information provided by IARC work for the evaluation regarding the classification as potentially carcinogenic to humans, here the newly submitted studies are evaluated regarding their significance for the risk of non-target organisms exposed to glyphosate according to intented uses of plant protection products containing this active substance.. The evaluation presented in this addenndumg ‘ecotoxicology’ refers in particular to the section 4.2.1 (b) (iii) “Genetic and related effects in non-mammalian systems *in vivo*" of the IARC Monograph.

Executive summary

In addition to the evaluation of the IARC monograph regarding the potential carcinogenity of the active substance glyphosate in terms of human health and classification aspects already provided with addendum 1'toxicology' by BfR, herewith we assess the significance of the studies addressing "Genetic and related effects in non-mammalian systems *in vivo*" for the environmental risk assessment of glyphosate.

Taking into account the results of existing reproduction studies in non-mammalian systems, the geno-toxicological effects observed in biomarker studies with the active substance glyphosate listed in the IARC monograph are considered not to be manifest on the population level of non-target organisms. As a consequence, unacceptable effects on the environment due to the genotoxic potential of glyphosate can be excluded. Thus, from the evaluation of the studies regarding genotoxic effects of glyphosate in non-mammalian systems as presented in the IARC monograph, no changes to the environmental risk assessment of the active substance glyphosate do arise compared to our previous assessment outcome.

However, the geno-toxicological effects observed in some studies with glyphosate formulations containing surfactants from the group of the POEA (polyoxyethylene-alkylamine) are considered to be relevant for the authorisation of glyphosate products in the European Member States. RMS highlighted already in the DRAR (December 2013) that Member States should take steps to ensure a safe use of glyphosate products, such as demanding further data on POEA-containing products or substituting POEA (polyoxyethylene-alkylamine) in plant protection products by less critical surfactants.

In addition to the evaluation of the information from the IARC monograph, the RMS reiterates in this addendum the knowledge regarding the effects of glyphosate and other broad spectrum herbicides on the populations of non-target species (especially insects and farmland birds), caused by an alteration of the food web. From the perspective of the RMS, the approval of the active substance glyphosate is associated with the default of assessing, minimizing and compensating this type of risk in the Member States.

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1 Genotoxicity in the Environmental Risk Assessment

The eco-toxicological endpoints used to evaluate the risk of plant protection products‘ intended uses on non target organisms in environmental risk assessment schemes are so-called apical endpoints. These endpoints address adverse ecological effects in terms of the protection goals set out in the relevant legislation which is mainly to ensure the sustainability of populations of non-target organisms. In contrast, endpoints from mechanistic studies addressing effects on biochemical parameters on the chromosomal, enzymatic, or other cellular organization levels are considered only as supporting information in environmental risk assessment. Such studies can provide an indication of potential effects on the organism level. For decision-making, however, the relevance to the population level of the effects observed in such studies has to be assessed by means of apical studies covering the toxicological mechanisms and targets addressed in the mechanistic studies. To this effect, the results obtained in mechanistic genotoxicity studies reported in the IARC monograph have to be linked to regulatory relevant endpoints as well as to the predicted environmental exposure in order to characterize the risk for the protection targets.

Genotoxicity studies characterize toxicological effects of chemicals on the genetic material of organisms. The exposure of an organism to a genotoxic substance can result in an interaction of these chemicals with DNA and can subsequently lead to a structural damage of the genetic material by means of DNA strand breaks. DNA strand breaks can be analysed for structural damage in the neutral and the alkaline comet assay (Tice et al. 2000). Finally, genotoxicity can induce mutations resulting in adverse effects - including cancer in somatic cells and reproductive changes - when manifested in germ cells (Jha, 2008). Therefore, a genotoxic substances might have the potential for adverse effects towards populations of non-target organisms. Generally, genetic changes in parental organisms –e.g. in their gametes - can be passed to subsequent generations and can influence in principle the genetic diversity of populations. This in turn might affect the health, and the survival of the population of non-target organisms (Bickham et al. 2000).

The active substance glyphosate and its metabolite aminomethylphosphonic acid (AMPA) have been studied for genotoxic potential via comet assay in a wide variety of assays in non-mammalian systems *in vivo* and *in vitro*, respectively. To the following, the studies evaluated

by the IARC monograph are summarized and discussed regarding their relevance for the assessment of the risk for non-target organisms exposed to glyphosate according to the indented uses of the plant protection products.

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2 Studies addressing the active substance glyphosate and the metabolite AMPA

In the IARC monograph, the following studies on the active substance glyphosate have been considered i.a.:

1. Akcha, F., Spagnol, C., & Rouxel, J. (2012). Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos. Aquatic Toxicology, 106, 104-113.
2. Alvarez-Moya, C., Reynoso Silva, M., Valdez Ramírez, C., Gómez Gallardo, D., León Sánchez, R., Canales Aguirre, A., & Feria Velasco, A. (2014). Comparison of the in vivo and in vitro genotoxicity of glyphosate isopropylamine salt in three different organisms. Genetics and molecular biology, 37(1), 105-110.
3. Guilherme, S., Santos, M. A., Barroso, C., Gaivão, I., & Pacheco, M. (2012). Differential genotoxicity of Roundup® formulation and its constituents in blood cells of fish (*Anguilla anguilla*): considerations on chemical interactions and DNA damaging mechanisms. Ecotoxicology, 21(5), 1381-1390.
4. Lopes, F. M., Junior, A. S. V., Corcini, C. D., da Silva, A. C., Guazzelli, V. G., Tavares, G., & da Rosa, C. E. (2014). Effect of glyphosate on the sperm quality of zebrafish *Danio rerio*. Aquatic Toxicology, 155, 322-326.
5. Moreno, N. C., Sofia, S. H., & Martinez, C. B. (2014). Genotoxic effects of the herbicide Roundup Transorb® and its active ingredient glyphosate on the fish *Prochilodus lineatus*. Environmental toxicology and pharmacology, 37(1), 448-454.

In addition, the following study on AMPA was considered:

a) Guilherme, S., Santos, M. A., Gaivão, I., & Pacheco, M. (2014). DNA and chromosomal damage induced in fish (*Anguilla anguilla* L.) by aminomethylphosphonic acid (AMPA)—the major environmental breakdown product of glyphosate. Environmental Science and Pollution Research, 21(14), 8730-8739.

Four out of the six studies cited in the IARC monograph (see above) have already been considered by the RMS in the original draft RAR (Volume3 CA-CPB-9Appendix, Studies a), c), e) for glyphosate and study a) for AMPA). For the purpose of this addendum, those studies were re-evaluated and considered together with the new studies. For a summary of the results of the individual studies, refer to table 2.1.

Table 2-1: Discussion of studies by the RMS with the active substance glyphosate in the section 4.2.1 (b) (iii) "non-mammalian systems in vivo" of the IARC Monograph

Study (Author/year)	**Subject**	**Evaluation by IARC**	**Comment RMS on IARC evaluation**	**Report in dRAR April 2015**	**Final conclusion of RMS, considering IARC evaluation**
Moreno et al., 2014.	Genotoxic effects of the herbicide Roundup Transorb and its active ingredient glyphosate on the fish *Prochilodus lineatus*	Time of exposure 6, 24, and 96h. For erythrocytes: *P* = 0.01 after 6 h *P* = 0.014 after 96 h; no significant increase after 24 h For gill cells: *P*=0.02 after 6 h at 2.4 mg/L	**Agreement**	**yes**	Glyphosate and the tested product caused damage to the DNA nucleoids of *P. lineatus* at concentrations higher than those predicted in the environment. Erythrocytes exposed to both concentrations of the formulated product (1 and 5 mg/L) and the active substance glyphosate (0.48 and 2.4 mg/L) showed DNA damage after 96 h. Nevertheless, the study shows slight deficiencies, as it is unclear why the negative control showed increase in damaged nucleotids in gill cells (24.3 after 6h and 45 after 96h). This observation is further seen in the positive control, when DNA damage increases after 96 h only. Further studies might demonstrate the sensitivity of the test system is time dependent. Therefore, further studies would be needed to determine if the changes on DNA strands are reproducible. Furthermore, effects observed in the given experimental setup are not transferable into reproductive success of fish as demonstrated by three regulatory studies, conducted according to internationally agreed test guidelines, where at comparable exposure concentrations not adverse effects on reproductive success could be observed.

Guilherme et al. (2012)	Differential genotoxicity of Roundup(®) formulation and its constituents in blood cells of fish (*Anguilla anguilla*): considerations on chemical interactions and DNA damaging mechanisms.	Time of exposure 1 and 3 days $P < 0.05$	**Agreement**	**yes**	The experiment was conducted using the commercial formulation Roundup® Ultra, distributed by Bayer Crop- Science (Portugal), containing isopropylammonium salt of glyphosate at 485 g/L as the active ingredient (equivalent to 360 g/Lor 30.8 % of glyphosate) and POEA (16%) as surfactant. Fish were exposed to equivalent concentrations of the Roundup product (58, 116 µg/L), glyphosate (17.9, 35.7 µg/L) and POEA (9.3, 18.6 µg/L) for 1 and 3 days. The comet assay was applied to blood cells, either as the standard procedure, or with an extra step involving DNA lesion-specific repair enzymes in an attempt to clarify DNA damaging mechanisms. Mean values of genetic damage indicator displayed significantly higher values in comparison to the control. Both components of the formulated product seem to contribute to the effect on genetic structure of the formulation Roundup® Ultra. Roundup® Ultra displayed levels of damage at both tested concentrations and exposure times. Nevertheless, fish exposed to the highest concentration of glyphosate for 3 days recovered from the damage detected after 1 day exposure. The ability of the test organisms to recover from the observed effects was evaluated by adding repair enzymes to the cells. At the highest tested concentration of glyphosate, a repair of DNA damage was seen. Hence, the observed time-related disappearance of DNA damage could be a result of the intervention of a DNA repair system. Effects observed in the given experimental setup are not directly transferable into reproductive success of fish as demonstrated by three regulatory studies, conducted according to internationally agreed test guidelines, where at comparable exposure concentrations not adverse effects on reproductive success could be observed.

Lopes et al. (2014)	Effect of glyphosate on the sperm quality of zebrafish *Danio rerio*.	After 96 h, DNA integrity was 78.3 ± 3.5%, significantly reduced from control (94.7 ± 0.9%) and 5 mg/L (92.6 ± 1.9%), (*P* < 0.05)	**Agreement**	**no**	The effect of the active substance glyphosate on sperm quality of the fish *Danio rerio* was investigated after 24 and 9 h of exposure at concentrations of 5 mg/L and 10 mg/L. No significant differences in sperm concentration were observed; however, sperm motility and the motility period were reduced after exposure to glyphosate to both exposure periods. The mitochondrial functionality and membrane and DNA integrity were also reduced at the highest concentration for both exposure periods. It should be noted that in this experiment glyphosate was applied at high concentrations, which do not relate to predicted environmental concentrations in the environment (based on modelling assumptions, the tested concentrations is exceeding the predicted concentrations by factor > 50).
Alvarez-Moya et al. (2014).	Comparisonof the in vivo and in vitro genotoxicity of glyphosateisopropyl-amine salt in three different organisms	Time of exposure, 10 days *P* < 0.001 with concentrations > 7µM	**Agreement**	**no**	Based on modelling assumptions, the concentrations expected in surface waters exposed to drift are approx. 0.1 mg a.i./L. No effects on DNA integrity were observed in vitro for *Oreochromis niloticus* erythrocytes at 0.0007 mM, approximately corresponding to concentration expected in the environment. In vivo responses above concentrations >7 µM do not relate to the predicted environmental concentrations and did not show dose–response relationship.

Guilherme et al. (2014)	DNA and chromosomal damage induced in fish (*Anguilla anguilla* L.) by aminomethylphosphonic acid (AMPA)-the major environmental breakdown product of glyphosate	Time of exposure, 1 and 3 days $P < 0.05$ after 1 day of exposure	**Agreement**	**yes**	Mean values of genetic damage indicator measured by comet assay in blood cells of *A. anguilla* exposed to 11.8 and 23.6 µg/L aminophosphoric acid showed significant increases after 1 day. After 3 days, no effects were observed for the tested lower concentration. Possibly, tested fishs had the capacity to use successfully DNA repair mechanisms. No significant alterations were found in erythrocytic nuclear abnormalities (ENA) assays following the first day of exposure; considering the 3-day exposure, a significant increase for the higher concentration of AMPA was observed. Based on modelling assumptions, the concentrations expected in surface waters exposed to drift are 0.041 mg AMPA/L. Effects observed in the given experimental setup are not transferable into reproductive success of fish as demonstrated the regulatory study, conducted according to internationally agreed test guidelines, , where at comparable exposure concentrations not adverse effects on reproductive success could be observed.
Akcha et al. (2012)	Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos.	Time of exposure, 1 h	**Agreement**	**yes**	Both the active ingredient glyphosate, as well as the formulation Roundup® showed no significant effect on the development oysters at the concentrations tested.

In the IARC monograph, the results of the studies listed above were summarized as follows:

"*In fish, glyphosate produced DNA strand breaks in the comet assay in sabalo (Moreno et al., 2014), European eel (Guilherme et al., 2012b), zebrafish (Lopes et al., 2014), and Nile tilapia (Alvarez-Moya et al. 2014) AMPA also induced DNA strand breaks in the comet assay in European eel (Guilherme et al.2014b)). A glyphosate-based formulation produced DNA strand breaks in numerous fish species, such as European eel (Guilherme et al., 2010, 2012b, 2Q14a Marques et al. 2014 , 2015), sabalo (Calvante et al. 2008; Moreno et a, 2014,2014), guppy (De Souza filho et al. 2013), bloch (Nawani et al., 2013), neotropical fish Corydoras paleatus (de Castilhos Ghisi & Cestari, 2013), carp (Gholami-Seyedkolaei et al., 2013), and goldfish (Cavas& Könen, 2007).*

AMPA, the main metabolite of glyphosate, induced erythrocytic nuclear abnormalities (kidney-shaped and lobed nuclei, binucleate or segmented nuclei and micronuclei) in European eel (Guilherme et al., 2014b). Micronucleus formation was induced by different glyphosate- based formulations in various fish (Grisolia, 2002; Cavas& Könen, 2007; De Souza Filho et al., 2013; Vera-Candioti et al., 2013)"

2.1 Discussion and Conclusion

The induction of DNA strand breaks in both somatic and germ cells are of paramount importance. If unrepaired or mis-repaired, such damage might have effects on the immediate fitness as well as on reproductive success of the exposed organisms (Jha, 2008). DNA strand breaks in germs cells might lead to stable effects cells leading to heritable changes in fertility and fecundity. The studies from the peer reviewed literature evaluated by RMS in the draft RAR from 2015 , however related to effects observed in somatic cells. Genetic damage indicators were measured by the standard (alkaline) comet assay in erythrocytes of fish after *in-vivo* exposure of the test organisms.

It could be shown that the active substance glyphosate can cause damage to the DNA molecule in the test organism fish, although at concentrations higher than those predicted in the environment (Moreno et al. 2014, Alvarez-Moya et al. 2014). However, no effects on DNA integrity were observed *in-vitro* to *Oreochromis niloticus* at 0.0007 mM, approximately corresponding to concentration expected in the environment (Alvarez-Moya et al. (2014). In contrast, Guilherme et al. (2012) detected DNA damage at environmentally relevant concentrations of the active substance glyphosate as well as the metabolite AMPA (Guilherme et al. 2014). Both studies evaluated the ability of the test organisms to recover by adding repair enzymes to the cells after exposure to glyphosate or AMPA. Guilherme et al. (2012) exposed fish to environmentally relevant concentrations of a Roundup formulation (58, 116 µg/L), glyphosate (17.9, 35.7 µg/L) and the surfactant system based on POEA (9.3, 18.6 µg/L) for 1 and 3 days. The comet assay was applied to blood cells, either as the standard procedure, or with an extra step involving DNA lesion-specific repair enzymes in order to address DNA damage and possibly reparing mechanisms. Blood cells exposed to both concentrations tested as well as both exposure times displayed significantly higher values levels of the genetic damage indicator compared to control assays. Failure to repair this DNA damage might lead to mutations, reduced fitness and might in the end affect reproductive success. Nevertheless, fish exposed to the highest concentration of the active substance glyphosate for 3 days recovered from the damage that had been detected after 1 day exposure. Results indicated that after 3 days of exposure, DNA damage could no longer be detected, a fact which might point to the ability of an organism to identify DNA damage and induce DNA repair mechanisms (Guilherme et al. 2012).

The reported effects from mechanistic studies (i.e. induced DNA damage) do not allow direct conclusions on potential adverse effects on the population level of non-target organisms - i.e.

on the protection item relevant for decision-making. In order to link those results to the assessment of the risk for non-target organisms exposed to PPP containing the acive substance glyphosate, it has to be assessed whether the observed effects are in principle covered by apical studies with survival, developmental or reproductive endpoints of suitable surrogate species of non-target organisms.

The effect of the active substance glyphosate on sperm quality of the fish *Danio rerio* was investigated at exposure regimens exceeding the predicted environmental concentrations in surface waters (Lopes et al. 2014). High concentrations of glyphosate reduced motility and the motility period of sperm in this study, which could - at environmentally realistic concentration - alter reproductive success. No significant differences in sperm concentration were observed.

In three regulatory studies, conducted according to internationally agreed test guidelines, the active substance glyphosate was assessed for its toxicity on reproduction of fish. In general, chronic NOEC values for fish ≥10 mg/L are indicative of low chronic toxicity. The chronic endpoint from the 255 day “fish full life-cycle” study with the fathead minnow was 25.7 mg a.s./L (DOC: 895-00020). Please refer to Point 1.4 of this report and to Point B 9.2.1. of the original draft RAR of 2015 for further information.

The risk assessment for the metabolite AMPA is based on the results from an "early life stage" study (DOC: 2310943/WL-2010-328), in which fish embryos were exposed for 33 days in a flow through system. The results demonstrate similarly low chronic toxicity to fish as for the parent substance glyphosate, with a NOEC value of 12 mg a.s./L, which was the highest concentration tested. There were no effects on the survival and the growth parameters of *Pimephales promelas* observed at the highest concentration tested.

In conclusion, no significantly increased risks to non-target organisms’ populations compared to the previous risk assessment (DRAR from 2013 and 2015) have to be considered when taking into account the information from the IARC monograph. Even though the observed genotoxicity effects could in principle have negative effects on reproduction in fish, no such effects were observed in the available reproduction studies, falsifying the initial suspicion of potential negative effects arising from the genotoxicity studies. Consequently, no significant changes in population densities and no unacceptable effects on functioning of the ecosystem are expected.

3 Studies with glyphosate-containing formulations

In the IARC monograph, the following studies on different glyphosate-containing formulations marketed mainly under the name “Roundup” have been considered:

1) Akcha, F., Spagnol, C., & Rouxel, J. (2012). Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos. Aquatic Toxicology, 106, 104-113.
2) Cavalcante, D. G. S. M., Martinez, C. B. R., & Sofia, S. H. (2008). Genotoxic effects of Roundup® on the fish *Prochilodus lineatus*. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 655(1), 41-46.
3) Clement, C., Ralph, S., & Petras, M. (1997). Genotoxicity of selected herbicides in *Rana Catesbeiana* tadpoles using the alkaline single cell gel DNA electrophoresis (comt) assay. Environ Mol Mutagen, 29, 277-288.
4) Conners, D. E., & Black, M. C. (2004). Evaluation of lethality and genotoxicity in the freshwater mussel *Utterbackia imbecillis* (Bivalvia: Unionidae) exposed singly and in combination to chemicals used in lawn care. Archives of environmental contamination and toxicology, 46(3), 362-371.
5) De Souza Filho, J., Sousa, C. C. N., Da Silva, C. C., De Sabóia-Morais, S. M. T., & Grisolia, C. K. (2013). Mutagenicity and genotoxicity in gill erythrocyte cells of *Poecilia reticulata* exposed to a glyphosate formulation. Bulletin of environmental contamination and toxicology, 91(5), 583-587.
6) dos Santos, K. C., & Martinez, C. B. (2014). Genotoxic and biochemical effects of atrazine and Roundup®, alone and in combination, on the Asian clam *Corbicula fluminea*. Ecotoxicology and environmental safety, 100, 7-14.
7) Guilherme S, Santos MA, Barroso C, Gaivao I, PachecoM (2012b). Differential genotoxicity of Roundup(®) formulation and its constituents in blood cells of fish (*Anguilla anguilla*): considerations on chemical interactions and DNA damaging mechanisms. Ecotoxicology, 21(5):1381-90.
8) Guilherme, S., Gaivao, I., Santos, M. A., & Pacheco, M. (2010). European eel (*Anguilla anguilla*) genotoxic and pro-oxidant responses following short-term exposure to Roundup®-a glyphosate-based herbicide. Mutagenesis, 25(5), 523-530.
9) Guilherme, S., Santos, M. A., Gaivão, I., & Pacheco, M. (2014). Are DNA-damaging effects induced by herbicide formulations (Roundup® and Garlon®) in fish transient and reversible upon cessation of exposure?. Aquatic Toxicology, 155, 213-221.
10) Marques, A., Guilherme, S., Gaivão, I., Santos, M. A., & Pacheco, M. (2014). Progression of DNA damage induced by a glyphosate-based herbicide in fish

(*Anguilla anguilla*) upon exposure and post-exposure periods—Insights into the mechanisms of genotoxicity and DNA repair. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 166, 126-133.

11) MezaJoya, F. L., RamírezPinilla, M. P., & Fuentes-Lorenzo, J. L. (2013). Toxic, cytotoxic, and genotoxic effects of a glyphosate formulation (Roundup® SL-Cosmoflux® 411F) in the direct?developing frog *Eleutherodactylus johnstonei*. Environmental and molecular mutagenesis, 54(5), 362-373.
12) Mohamed, A. H. (2011). Sublethal toxicity of Roundup to immunological and molecular aspects of *Biomphalaria alexandrina* to *Schistosoma mansoni* infection. Ecotoxicology and environmental safety, 74(4), 754-760.
13) Muangphra, P., Kwankua, W., & Gooneratne, R. (2014). Genotoxic effects of glyphosate or paraquat on earthwom coelomocytes. Environmental toxicology, 29(6), 612-620.
14) Nwani, C. D., Nagpure, N. S., Kumar, R., Kushwaha, B., & Lakra, W. S. (2013). DNA damage and oxidative stress modulatory effects of glyphosate-based herbicide in freshwater fish, Channa punctatus. Environmental toxicology and pharmacology, 36(2), 539-547.
15) Piola, L., Fuchs, J., Oneto, M. L., Basack, S., Kesten, E., & Casabé, N. (2013). Comparative toxicity of two glyphosate-based formulations to *Eisenia andrei* under laboratory conditions. Chemosphere, 91(4), 545-551.
16) Poletta, G. L., Larriera, A., Kleinsorge, E., & Mudry, M. D. (2009). Genotoxicity of the herbicide formulation Roundup®(glyphosate) in broad-snouted caiman (*Caiman latirostris*) evidenced by the Comet assay and the Micronucleus test. Mutation Research/Genetic toxicology and Environmental Mutagenesis, 672(2), 95-102

The objective of the literature survey in the Renewal Assessment of the active substance glyphosate was the evaluation of scientific peer-reviewed open literature published within the last 10 years for the active substance glyphosate. Additionally, studies with glyphosate containing products have been evaluated. Eleven out of the 16 studies cited in the IARC monograph had already been considered by the RMS in the original draft RAR (2013). In this addendum, studies with different glyphosate containing formulations were re-evaluated and considered together with the new studies. For a summary of the results of the individual studies, refer to table 2.2.

Table 3-1:Discussion of studies with the different commercial glyphosate containing formulations in the section 4.2.1 (b) (iii) "non-mammalian systems in vivo" of the IARC Monograph

No.	Author/year	Study	Evaluation by IARC	Comment RMS on IARC evaluation	Study reported in dRAR April 2015	Final conclusion of RMS, considering IARC evaluation
1	Akcha et al. (2012)	Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos.	Time of exposure, 1 h	Agreement	yes	Out of the three bioassays conducted at the laboratory, only one assay revealed glyphosate to have an embryotoxic effect at concentrations of 2.5 µg L ($p < 0.001$) upwards. Taking into account the data from the three bioassays, the main effects ANOVA revealed significant differences between the assays ($p < 0.001$). with assay 2 differing from assays 1 and 3. Therfore no concluding evidence exists for both the active substance glyphosate, as well as the formulation Roundup® Express no have an effect on the development oysters at the concentrations tested.
2	Cavalcante et al., (2008)	Genotoxic effects of Roundup® on the fish *Prochilodus lineatus*.	Single dose tested only, for 6, 24 and 96h. $P < 0.05$ for both erythrocytes and bronchial cells	Agreement	No	No significant differences erythrocytes and bronchial cells compared to control were observed in the piscine micronucleus test. The comet assay increased rates of DNA damage in blood and hepatic cells, when the fish were exposed to 10 mg/L glyphosate formulation. Authors conclude that short-term exposure of the pesticide Roundup® is genotoxic to the bioindicator fish species *C. paleatus*, even at a relatively low concentration. However, this low concentration tested did not result in clastogenic and/or aneugenic effects.

3	Clement et al. 1997	Genotoxicity of select herbicides in *Rana eatesbeiana* tadpoles using the alkaline single-cell gel DNA electrophoresis (comet) assay.	P < 0.05, with 6.75 mg/L; P < 0.001 with 27 mg/L (with 108 mg/L, all died	Agreement	No	Tadpoles exposed to the lowest concentration of Roundup, 1.69 mg/l, exhibited no significant increase in DNA damage relative to the negative controls However, tadpoles exposed to 6.75 and 27 mg/l gave significant increases in DNA damage (P < 0.05 and 0.001, respectively). All tested concentrations were below the recommended application levels.
4	Conners, Black (2004)	Evaluation of lethality and genotoxicity in the freshwater mussel *Utterbaekia imbeeillis* (Bivalvia: Unionidae) exposed singly and in combination to chemicals used in lawn care	2.5 and 5 mg/L for 24 h tested NOEC, 10.04 mg/L	**Agreement**	No	Authors evaluated the lethal and genotoxic effects of chemicals used in lawn care on an early life stage of fresh water mussels (*Utterbackia imbecillis*). For the Parameter mortality LC50 Glyphosate formulation was determined to 18.3 mg/L. The combined toxicity of equitoxic and environmentally realistic mixtures to mussels was additive. No genotoxic significant responses were observed in mussels exposed to glyphosate.
5	De Souza Filho et al. (2013)	Mutagenicity and Genotoxicity in Gill Erythrocyte Cells of *Poecilia reticulata* Exposed to a Glyphosate Formulation	Glyphosate, 64.8%, m/v ((648 g/L) **P < 0.05**	**Agreement**	Yes	The 96h LC_{50} for Roundup®Transorb in the absence and presence of predator stress were 3.76 mg ae/L and 3.39 mg ae/L, respectively. The 10-day LC50 value for Roundup was significantly lower, 2.12 mg ae/L and 1.91 mg ae/L in the absence and presence of predator stress, respectively. Lower concentrations of Roundup (1, 2 and 3 mg ae/L) induced the formation of micronuclei (MN) in the erythrocytes in a concentration-dependent manner. Presence of predator stress seemed to increase the toxicity and genotoxicity of Roundup®; but these effects were not statistically significant.

6	dos Santos Martinez (2014).	Genotoxic and biochemical effects of alrazine and Roundup(®), alone and in combination, on the Asian clam *Corbicula fluminea*	Time of exposure, 96 h; Significant increase when atrazine (2 or 10 mg/L) was added to glyphosate ($P < 0.05$) No increase after exposure to atrazine or glyphosate	**Agreement**	**yes**	The study aimed to evaluate biochemical and genotoxic effects of the herbicides atrazine (ATZ) and Roundups (RD) separately, as well as their mixture, on the fresh water clam after 96 h exposure. Roundup did not increase DNA damage, but induced alterations in biochemical parameter related to oxidative stress (alterations in Superoxide dismutase activity and catalase activity). Exposure to ATZ and RD separately did not increase DNA damage.
7	Guilherme et al. (2010)	European eel (*Anguilla anguilla*) genotoxic and pro-oxidant responses following short-term exposure to Roundup® a glyphosate-based herbicide	$P < 0.05$ (Positive dose-response)	**Agreement**	yes	Micronuclei test (MN) and erythrocytic nuclear abnormalities (ENA) results did not significantly differ between studied treatments and control.Comet assay: significant effects indicating DNA damage in erythrocytes at concentrations of 58 and 116 µg/L. This paper reports physiological studies with a commercial formulation, which could not be taken into account as critical information for the assessment of the active substance glyphosate itself. Nevertheless it shows that commercial formulations of glyphosate might have elicit effects.

8	Guilherme et al. (2012)	Differential genotoxicity of Roundup® formulation and its constituents in blood cells of fish (*Anguilla anguilla*): considerations on chemical interactions and DNA damaging mechanisms.	Time of exposure, 1 and 3 day comet assay improved with the DNA- lesion-specific FPG and EndoIII With FPG $P < 0.05$; with comet assay alone, $P < 0.05$ at 116 µg/L	**Agreement**	yes	The experiment was conducted using the commercial formulation Roundup® Ultra, distributed by Bayer Crop-Science (Portugal), containing isopropylammonium salt of glyphosate at 485 g L-1 as the active ingredient (equivalent to 360 g L-1 or 30.8 % of glyphosate) and POEA (16 %) as surfactant. Fish were exposed to equivalent concentrations of Roundup (58, 116 µg/), glyphosate (17.9, 35.7 µg/L) and POEA (9.3, 18.6 µg/L), during 1 and 3 days. The comet assay was applied to blood cells, either as the standard procedure, or with an extra step involving DNA lesion-specific repair enzymes in an attempt to clarify DNA damaging mechanisms. Mean values of genetic damage indicator displayed significantly higher values in comparison with the control. Both components seem to contribute to the effect on genetic structure of the formulation. Both concentrations and exposure times displayed levels of damage. Nevertheless, fish exposed to the highest concentration of glyphosate for 3 days recovered from the damage detected after 1 day exposure. Moreover, the ability of the test organisms to recover was evaluated by adding repair enzymes to the cells after exposure to glyphosate. Interestingly, then at the highest concentration of glyphosate a repair of DNA damage was seen. Hence, the time-related disappearance of DNA damage could be a result of the intervention of a DNA repair system. The recovery phenomenon was not observe for POEA treatment or Roundup® Ultra treatment. Comparing the respective highest concentrations the surfactant displayed the most elevated levels of DNA damage among the study agents. Effects observed in the given experimental setup are not transferable into reproductive success of fish as demonstrated by three regulatory studies, conducted according to internationally agreed test guidelines.

9	Guilherme et al. (2014a).	Are DNA-damaging effects induced by herbicide formulations (Roundup· and Garlon®) in fish transient and reversible upon cessation of exposure?	Single dose tested only; Time of exposure:3 days; DNA damage, but not oxidative DNA damage, 14 days after exposure; P< 0.05	**Agreement**	yes	The study investigated the ability of fish to recover from the DNA damage induced by short-term exposures to Roundup® and Garlon® (triclopyr-based). Roundup®Ultra, distributed by Bayer CropScience (containing isopropylammonium salt of glyphosate at 485 g/L as the active ingredient (equivalent to 360 g/L or 30.8% w/v of glyphosate) and POEA (16% v/v) as surfactant). Exposure for 3 days, recovery for 1, 7 and 14 days (post-exposure period). Fish were exposed to 116 µg/L Roundup®, which is assumed to display a realistic worse case concentration. A decrease of the non-specific DNA damage induced by Roundup® after 14days in herbicide-free water could be demonstrated, while a complete recovery of DNA stability could not be shown. A recovery was observed when considering non-specific DNA damage on day 14 post-exposure. Effects of commercial formulations of glyphosate on responses like changes on chromosomal damage in the field of environmental risk assessment are currently considered as supporting information, especially since this evaluation aims at assessing the risk of non target organisms exposed to glyphosate. . Nevertheless, it is evident that further studies with the respective formulated product are needed to determine if the changes on biochemical traits are translated to sublethal long-term effects.

	Marques et al. (2014)	Progression of DNA damage induced by a glyphosate-based herbicide in fish (*Anguilla anguilla*) upon exposure and post-exposure periods Insights into the mechanisms of genotoxicity and DNA repair.	Time of exposure, 3 days $P < 0.05$	**Agreement**	yes	The study indicated that concentrations of 8.1 mg/ L and 16.3 mg/L commercial glyphosate containing formulation provoke non-significant induction of nuclear lesions; whereas at concentrations of 24.4 mg/L after 96h significantly higher nuclear lesions induction were observed. Roundup® showed to induce DNA damage (measured both as GDIFPG and GDIEndoIII) in hepatic cells of *A. anguilla* at two concnetrations of a glyphosate containing products . After transfer to herbicide-free water, fish were able to reverse the genetic damage.
	Meza-Joya et al. (2013)	Toxic, cytotoxic,and genotoxic effects of aglyphosate formulation (Roundup®SL-Cosmotlux®411F) in the direct-developing frog *Eleutherodactylus johnstonei*	Exposure to an homogenate mist in a 300 cm2 glass terrarium Time of exposure: 0.5, 1, 2, 4,8 and 24h P < 0.05	**Agreement**	**yes**	The study evaluates the toxic, cytotoxic, and genotoxic effects of a glyphosate formulation (RoundupVR SL–CosmofluxVR 411F) in the direct-developing frog *E. johnstonei* by estimating the median lethal application rate (LC50), median hemolytic application rate (HD50), and extent of DNA damage using the in vitro and in vivo Comet assays. Toxicity results indicated that the application rate [37.4 mg acid equivalent (a.e.)/cm2] equivalent to that used in aerial spraying (3.74 kg a.e./ha) is not lethal in male and female adult frogs, whereas neonates are highly sensitive. LC 50 24h = 3.1 (2.8–3.6) kg a.e./ha LC 50 48h = 1.8 (1.5–2.0) kg a.e./ha LC 50 96 h 1.2 (1.1–1.4) kg a.e./ha In vivo and in vitro exposure of *E. johnstonei* erythrocytes to the glyphosate formulation induced DNA breaks in a dose-dependent manner with statistically significant values (P<0.05) at all doses tested. DNA damage initially increased with the duration of exposure and then decreased, suggesting that DNA repair events were occurring during in vivo and in vitro exposures.

	Mohamed (2011)	Sublethal toxicity of Roundup to immunological and molecular aspects of *Biomphalaria alexandrina* to *Schistosoma mansoni* infection	10 mg/I. Single dose tested only; for 24 h. The percentage of damaged DNA was 21% vs 4% (control)	**Agreement**	No	Investigation on the cellular mechanisms of *Biomphalaria alexandrina* snails' hemocytes against sublethal concentration (10mg/L) of Roundup (48%Glyphosate) during7days. Obtained results indicated that treatment and/or infection led to significant increase (P<0.05) in total hemocytes count.
	Muangphra et al. 2014	Genotoxic effects of glyphosate or paraquat on earthworm coelomocytes	Epidermic exposure 48 h on filter paper; LC50: 251.50 µg/cm2 $P < 0.05$, for total micro-, bi-, and trinuclei frequencies at 0.25 µg/cm2; when analysed separately, micro- and trinuclei frequencies significantly differed from controls only at the LC50	**Agreement**	yes	The genotoxicity of glyphosate containing formulations reflected by chromosomal aberrations, DNA damage, and cytoskeleton damage as measured by pinocytic adherence activity in *P. peguana* earthworm coelomocytes is evaluated. Commercial herbicide formulations with active ingredient 36% (w/v)was used with no further specification. Glyphosate did not cause any significant DNA damage as compared with the controls (Table III) in the Comet assay. The LC50 of glyphosate at 48 h to *P. peguana* was 251.45 µg cm–2, respectively. The product did not cause clastogenic effects, but induced aneugenic effects on coelomcytes including a marked chromosomal loss during anaphase at high concentration (LC50).
14	Nwani et al. (2013)	Induction of micronuclei and nuclear lesions in *Channa punctatus* following exposure to carbosulfan, glyphosate and atrazine	Exposure continued for 35 days; blood and gill cells collected on day 1, 7, 14,21 28 and 35 $P < 0.01$, for blood and gill cells; DNA damage increased with time and concentration	**Agreement**	Yes	The induction of nuclear lesions was concentration and duration dependent with the record of highest frequency at 24.4 mgLL at 96 h.

13	Piola et al. 2013	Comparative toxicity of two glyphosate-based formulations to *Eisenia andrei* under laboratori conditions.	Epidermic exposure during 72 h (on filter paper) $P < 0.001$	**Agreement**	yes	Median lethal concentration showed that formulations can be multiple times more toxic than glyphosate itself, and differences among the toxicity of formulations exist,. Formulations at sublethal concentrations can cause weight loss, and effects on DNA and lysosomal damage. Results highlight the importance of eco-toxicological assessment not only of the active ingredients, but also of the different formulations.
8	Poletta et al. (2009).	Genotoxicity of the herbicide formulation Roundup (glyphosate) in broad-snouted caiman (*Caiman latirostris*) evidenced by the Comet assay and the Micronucleus test	In-ovo exposure; blood sampling at the time of hatching $P < 0.05$ in both Experiments 50-1000 µg/egg in experiment 1; 500 -1750 µg/egg in experiment 2	**Agreement**	No	The study evaluated the genotoxic potential of Roundup® in erythrocytes of broad-snouted caiman (*Caiman latirostris*) after *in ovo* exposure. Comet assay and the Micronucleus test revealed a concentration-dependent effect. No difference was found in the MN frequencies < 500 µg/egg. Under the experimental conditions set, no external malformations were observed in any of the caimans of the different groups. Nevertheless, results demonstrated biochemical alterations, as well as growth delay in caimans when oversprayed with the formulation Roundup®. Concerning developmental parameters, data obtained demonstrated that none of the treatments applied produced severe effect after 12 months

IARC summarized the findings from those studies as follows: "*Glyphosate-based formulations induced DNA strand breaks in other species, including caiman (Poletta et al., 2009), frog (Meza-Joya et al. 2013), tadpoles (Clements et al. 1997), and snail (Mohamed, 2011), but not in oyster (Akcha et al. 2012), clam (dos Santo& Martinez, 2014), and mussel glochidia (Conners & Black, 2004). In earthworms, one glyphosate-based formulation induced DNA strand breaks while two others did not (Piola et al., 2013; Muangphra et al., 2014), highlighting the potential importance of components other than the active ingredient in the formulation.*"

3.1 Discussion and Conclusion

The monograph of IARC discussed in this addendum (IARC Monographs, Glyphosate, Volume 112.2 (2015)) cites numerous genotoxicity studies that use glyphosate-containing formulation in a wide range of assays. Numerous formulations containing glyphosate as the active substance are available, the most prominent marketed under the name Roundup®. However, the specific composition of the formulations - especially the employed surfactant systems - was not available for all the evaluated studies. Given the importance of specific surfactants belonging to the ethoxylated alkylamines -as e.g. POE-Tallowamine- in driving the toxicity of formulated glyphosate products, it is not possible to evaluate the studies regarding their relevance for the reassessment of the active substance glyphosate without any information on the so-called inerts in the tested formulations. It is likely that several studies using the formulation Roundup® might test a product with POEA (polyoxyethylene-alkylamine) (especially Roundup® Original). The results of these tests have limited relevance for effects of glyphosate containing products that do not contain POEA and for the active substance glyphosate itself. It should be considered that the lead formulation for the assessment of the active substance glyphosate in the European Union does also not contain POEA as surfactant. Therefore, the studies carried out with POEA-containing glyphosate formulations are considered as additional information for the assessment of the re-authorization of the active substance glyphosate.

Likewise, RMS considered it adequate to provide general background information to other EU Member States to facilitate the assessment of the risk arising from glyphosate-based formulations other than the lead formulation. Please refer for detailed information to the draft RAR Chapter B.9.11 “Surface active substances in glyphosate-based formulations”.

The monograph of IARC discussed in this addendum (IARC Monographs, Glyphosate, Volume 112.2 (2015)) cites peer-reviewed open literature reporting on tests that might likely have been performed with several different formulations marketed under the name Roundup®. The results of these tests demonstrates that such formulations can cause chromosomal damage in several non-mammalian systems. Positive results were reported in in-vivo studies in fish (Guilherme et al. 2010, 2012b, 2014; Marques et al. 2014; Calvante et al. 2008; De Souza Filho et al. 2013; Nwani et al. 2013, De Castilhos Ghisi et al. 2012), amphibians (Clements et al., 1997, Meza-Joya et al. 2013, Yadav et al. 2013) reptiles (Poletta et al. 2011) and snails (Mohamed, 2011). Moreover, fruit flies, worm and plant systems

showed increased susceptibility to chromosomal damage as a result of exposure to glyphosate containing products (Kale et al. 1993, Piola et al. 2013, Muanghra et al. 2014 and Rank et al. 1993, Dimitrov et al., 2006). However, no severe effects were observed in oyster (Akcha et al. 2012), clam (dos Santo& Martinez, 2014), and mussel glochidia (Conners & Black, 2004).

The commercial formulations under the name Roundup® showed a concentration-dependent effect on the genetic integrity of fish species by means of the comet assay. Increased frequency rates of DNA damage in blood cells of fish after short-term exposure of Roundup® has been documented at concentrations ≥24.4 mg/L (Nwani et al. 2013, Marques, et al. 2014, Calvante et al. 2008, Guilherme et al., 2012). Moreover, it was shown that environmentally relevant concentrations of Roundup® formulations can induce positive results in comet assays using the European eel as a test species (Guilherme et al. 2012 and 2014).
Guilherme et al. (2012b and 2014) detected DNA damage at environmentally relevant concentrations using the commercial formulation Roundup® Ultra, containing isopropylammonium salt of glyphosate as the active ingredient and POEA as surfactant and included the test substances glyphosate and POEA separately. Fish were exposed to environmentally relevant concentrations of Roundup® Ultra (58, 116 µg/L), glyphosate (17.9, 35.7 µg/L) and POEA (9.3, 18.6 µg/L) for 1 and 3 days. The comet assay was perfomed with blood cells, either as the standard procedure, or with an extra step involving DNA lesion-specific repair enzymes as to clarify DNA damaging as well as their possible repair mechanisms. Both concentrations Roundup® Ultra and exposure times displayed significantly higher values levels of the genetic damage indicator. In contrast to the active substance glyphosate, a recovery phenomenon was not observed for blood cells of fish exposed either to POEA treatment or Roundup® Ultra treatment. In a second study, Guilherme et al. (2014) exposed fish to 116 µg/L Roundup® Ultra – a concentration which is assumed to cover realistic worse case exposure. Genetic damage indicators revealed a decrease of the non-specific DNA damage, but not a complete recovery of DNA stability. In a study by (Marques et al. 2014) a Roundup® formulation induced DNA damage which was hovewer shown to be reversible when fish were allowed to recover in herbicide-free water.

Furthermore, among the peer reviewed publications, micronucleus test were performed (Guilherme et al. 2010, Calvante et al. 2008). Since these test are capable of quantifyming chromosome and/or genome mutations by determining frequencies of micronucleus abnormalities, a combination of the comet assay and the micronucleus test might be

recommended for genotoxicity testing, because only a small amount of induced DNA damage might lead to fixed mutations depending on e.g. cellular status, repair capacity, genetic background of the cells.. In the study of Calvante et al. (2008) the frequencies of micronucleus and other erythrocyte nuclear abnormalities (ENAs) were not significantly different between Roundup®-exposed fish and their respective negative controls. By contrast, De Souza Filho et al. (2013) showed for a different formulation and test species that induced formation of micronuclei in erythrocytes are possible. Both results demonstrate the demand to generate data for glyphosate containing products with surfactants individually for each product in the framework of product authorization.

Considering the wide use of products formulated with glyphosate and surfactants that might increase the toxicity of the active substance, it it important to understand the mechanisms behind the observed damages in the genetic material of non-target organisms. Comparing all eco-toxicological data available on the effects of the active substance glyphosate with studies using glyphosate-based formulation with POEA, formulated products might cause damage at environmental relevant concentrations and elicidet effects that were not directly reversible (as in the case of the active substance glyphosate alone, see Guillerme et al. 2012b)..

In order to finally conclude on the implication of the reported results on the protection targets, e.g. individuals, populations, communities of organisms exposed to glyphosate containing PPP in environmental relevant concentrations, results related to genetic damage in in-vitro systems with artificial exposure conditions currently need to be related to ecological effects such as changes in population growth or reproductive output. At present, the long term endpoints derived in reproduction studies are considered to supersede results from biomarker studies.

By examining the interactions between the response of non target organisms and chemical exposure, criteria may be established in order to identify early onset biomarkers indicative of toxic effects elicided by chemicals. Nevertheless, in the submitted and evaluated apical studies, population-related effects at low concentrations were attributable to the effect of POE-alkyl amines.

Finally RMS highlights that -besides regulatory defined frameworks and agreed protection targets in assessment of the risk deriving from intended uses of PPP in the EU-, observed effects leading to DNA damage have the potential to induce physiological alterations, e.g. energy metabolism shifts, changes on fitness and reproductive success, and in the long run

might lead to population relevant effects - even if the submitted apical studies did not detect effects in the tested non-target organisms. As relevant effects were reported in two studies with formulation containing POEA surfactant (e.g. Roundup®Ultra Guilherme et al., 2012, ASB2014-7619, Guilherme et al., 2014, ASB2015-8631) European Member States are stronlgy encouraged to consider the substitution of POEA in plant protection products with other less toxic surfactant systems. According to Point 7.1.7 of Regulation (EU) No 284/2013, the competent Authorities have to discuss case by case the need to perform supplementary studies with formulated products in addition to studies with the active substance alone. The RMS recommends to discuss at EU level and to agree on a strategy to address further the genotoxic potential of active substances and formulated PPP products to non-target organisms. In the framework of the renewal of approval of the active substance glyphosate, apical studies on reproduction of non-target organisms with the representative formulation as confirmatory information should be performed.

4 Data relevant to compare information from peer reviewed literature to regulatory demands

The aim of an environmental risk assessment is to prevent inacceptable effects on ecosystems. For this purpose exposure concentrations and eco-toxicological data are assessed. Ecological risks are estimated, by the relation of potential exposure and the possible effects (risk = toxicity to exposure ratio). In order to ensure a uniform approach in the EU, data requirements and the procedure are defined in various guidance documents, inter alia, the Guidance Document on Aquatic Ecotoxicology (EFSA Journal 2013;11(7):3290). The predicted environmental concentrations and further details of calculation for glyphosate acid in surface water (PEC_{sw}), arising as a consequence of drift, drainage and run-off, are calculated and are provided in RAR Volume 3 CA- B8, 2015. The PEC_{sw} values for glyphosate acid and the major metabolites were calculated using the FOCUS (2000) surface water models. Additionally, PEC_{SW} values were estimated for the metabolites AMPA and HMPA. As worst case covering all intended uses, PEC_{sw} were derived for pre-emergence application of glyphosate to various field crops and for post-weed emergence use of glyphosate to the soil and trunks trees representing the intended use in perennial crops.

For glyphosate, maximum PEC_{SW} at FOCUS Step 1 were 104 µg/L, both for the pre-emergence use in field crops and post-weed emergence use in perennial crops. Maximum PEC_{SW} of glyphosate at Step 2 for the intended pre-emergence use in field crops ranged from 18 to 23µg/L. For the intended post-weed emergence use in perennial crops, maximum PECSW of glyphosate at Step 2 were 39 µg/L. In general only a few studies have investigated toxic effects at environmentally relevant concentrations. A few positive results were obtained at concentrations greater than the predicted environmental predicted exposure for the active substance glyphosate (Moreno et al., 2014; Lopes et al., 2014; Alvarez-Moya et al., 2014). In the only peer reviewed study investigating the potential DNA damaging ability of glyphosate and using environmentally realistic concentrations in the experimental setup (Guilherme et al. (2012b) fish exposed to 36µg/L glyphosate for 3 days recovered from the damage detected after 1 day exposure.

The main eco-toxicological endpoints used in environmental risk assessment aims at addressing the likelihood of adverse ecological effects according to the protection goals set out in the relevant legislation. The relevant aspects that need to be protected according to environmental protection goals is set by risk managers in the EU, including suitable

protection units, e.g. individuals, populations, communities (e.g. Guidance Document on Aquatic Ecotoxicology (EFSA Journal 2013;11(7):3290). RMS wants to point out that the definition of protection goals regarding non-target organisms itself already implies that all types of adverse effects of a PPP on a defined subject of protection has to be considered. Genetic resources were identified as important ecosystem services in agricultural landscapes which are potentially affected by pesticides (EFSA Journal 2010; 8(10):1821)

The RMS believes that it is necessary to evaluate the effects on the diversity and abundance of non-target organisms at higher trophic level in the light of the current scientific knowledge in order provide the base for risk management to ensure that the protection goals with respect to non-target organisms and therefore biodiversity in general can be achieved.

Endpoints or ecological effects that are measured in ecotoxicity tests currently include mortality, reduction in growth, reproductive impairment, changes in numbers of species, bioaccumulation of residues in non-target organisms, with the aim of protecting the long-term conservation of populations of non-target organisms in space and time.

Thereby, other toxicity endpoints may be used if lines of evidence determine that they can be linked to assessment endpoints in a reasonable manner. The determination of qualitative or biochemical parameters on, chromosomal, enzymatic, or other cellular organization levels are considered as supporting information, because this information might to provide valuable knowledge on genetic disrupting mechanisms at an early stage before a manifestation at the apical level can be observed. Currently, it represents a useful addition for interpreting observed apical effects in higher reproduction or long term studies. Generally applying information on molecular or cellular levels would enable a shift in the practice of risk assessment away from the use of apical endpoints, towards the use of biological information generated with in-vitro methods provided that effects would lead to apical responses. Data on mutagenicity and carcinogenicity is not routinely considered as representative of an adverse ecological effect, but instead is predominantly used in human cancer risk assessment. However, the above paradigm might unjustifiable and the genetic ecotoxicology might be moved into quantitative risk assessment comparably with mutagenicity and carcinogenicity data in a human risk context.

Finally, the identification of a genetic disrupting substance is considered to be reflected in a population-related effect that is reported by reproductive endpoints. Currently the population-related effects are detected with Fish Short-Term Reproduction-Assay (OECD 229), Fish

Screening Test (OECD 230), Fish Sexual Development Test (OECD 234) and Fish Full-Life-Cycle Test (US EPA OPPT 850.1500). Biochemical studies are able to provide valuable supporting information on genetic disrupting mechanisms already at an early stage before it manifests in the organism at the apical level.

In the present European renewal assessment for the active substance glyphosate, three regulatory long-term studies were conducted according to internationally agreed test guidelines. The active substance glyphosate was assessed for its toxicity towards the reproduction capacity of fish. Additionally the metabolite AMPA was assessed in a chronic fish study. A summary of data for chronic toxicity of fish is shown in RAR Volume 3 CA- B9 Part Ecotoxicology and in the following table:

Table 4-1: Chronic toxicity studies with glyphosate acid and metabolites AMPA

Species	**Substance**	**Test design**	**NOEC**	**Reference**
Pimephales promelas	Glyphosate acid	255d	25.7 mg a.s./L	Review Report for the active substance Glyphosate (SANCO/6511/VI/99-final), Anonym, 95-00020
Brachydanio rerio	Glyphosate acid	168 h	1 mg a.s./L	Doc ID: 2310938 /RF-D62.16/99
Oncorhynchus mykiss	Glyphosate acid	85-day (60 days post-hatch	9.63 mg a.s./L	Doc ID 2310941 /1005.029.321
Pimephales promelas	AMPA	33-day (7days post-hatch	12 mg a.s./L	Doc ID 2310943 /WL-2010-328

The active substance glyphosate acid has low chronic toxicity to aquatic vertebrates. The chronic endpoint for the 255 day fish full life-cycle study with the fathead minnow was 25.7 mg a.s./L ((DOC: 895-00020).). In general, chronic NOEC values for fish ≥10 mg/L are indicative of low chronic toxicity. In this study spawning, eggs per female and eggs per spawn did not differ significantly between controls and fish exposed to concentrations of glyphosate as high as 25.7 mg/L. Percentage of live fry hatching in concentrations of glyphosate as high as 25.7 mg/L was not different from values observed in controls.

For the performance of a quantitative risk assessment for non-target organisms, a short-term study (DOC: 2310938 /RF-D62.16/99) was considered, which provides a NOEC=1mg/L in combination with an assessment factor of 10. In this study, fertilized spawn (*Danio rerio*) was exposed in a continuous flow system for 7 days to glyphosate. Exposure started in an early

life stage and is considered to potentially affect a higly sensitive stage of development. In this study, an increase in the mortality rate was observed at a concentration of 3.2 mg glyphosate/L (statistically significant at a concentration of 5.6 mg/L).

A fish early life-stage study with the active substance glyphosate demonstrates similarly low chronic toxicity to fish with a NOEC value of 9.63 mg a.s./L, which was the highest concentration tested (DOC: 2310941/1005.029.321). In this study, fertilized spawn (*Oncorhynchus mykiss*) were exposed in a continuous flow system for 85 days. The study was rated as valid and there were no significant effects on the embryo vitality, the survival rate and growth parameters observed.

The risk assessment for the metabolite AMPA is based on an early life stage study (DOC: 2310943/WL-2010-328), in which fish embryos were exposed for 33 days in a flow through system. The results demonstrate similarly low chronic toxicity to *Pimephales promelas*, with a NOEC value of 12 mg a.s./L, which was the highest concentration tested. There were no effects on the survival and the growth parameters observed at the highest concentration tested.

The calculated toxicity-exposure-ratio (TER) values taking into account the lowest endpoints derived from regulatory studies as well as endpoints derived from peer-reviewed literature are provided RAR Volume 3 CA- B9 Part Ecotoxicology Chapter B 9.2. The TER values for glyphosate acid exposure to aquatic organisms all exceed the Annex VI acceptability trigger of 10, indicating that glyphosate acid does not pose an unacceptable risk to aquatic organisms following application according to the proposed uses of the glyphosate containing lead formulation.

5 Contribution of co-formulants to the toxicity of glyphosate-based formulations

During the assessment of the peer-reviewed literature in the framework of the renewal of approval of the active substance glyphosate, a major difficulty is a clear differentiation between studies assessing the active substance glyphosate itself and such employing formulatied products with glyphosate as active substance and several co-formulants, often company confidential and therefore unknown to the public. Often, the formulation name "Roundup" is described as "glyphosate" subsuming even several different formulations. At a regulatory level for the renewal of an active substance assessment, information is evaluated on the active substance itself, glyphosate, and a representative lead formulation. The lead formulation for the assessment of glyphosate as active substance for plant protection products in the EU does not contain POEA (polyoxyethylene-alkylamine) as surfactant. Since several glyphosate-based products are formulated with POEA, RMS considered it adequate to provide general background information to other EU Member States to facilitate the environmental risk assessment arising from glyphosate-based plant protection products other than the lead formulation. For detailed information please refer to RAR Volume 3 CA- B9 Part Ecotoxicology Chapter B 9.11.

The DNA damaging potential of POEA is reported in the peer reviewed literature for several non-mammalian systems (Tsui and Chu 2003; Avigliano et al. 2014). Howe et al. (2004) tested the toxic potential of POEA to different species of amphibians. Guilherme et al. (2012b and 2014) detected DNA damage at environmentally relevant concentrations of the active substance glyphosate as well as for POEA in the test species fish. The experiment was conducted using the commercial formulation Roundup®Ultra (containing isopropyl-ammonium salt of glyphosate as the active substance and POEA as surfactant) and included the test substances glyphosate and POEA separately (see evaluation of the study in this addendum, chapter 3). In contrast to the active substance glyphosate a recovery phenomenon was not observed for the formulation Roundup® Ultra nor for the surfactant POEA itself. When analysing the results, the data demonstrate the highest levels of DNA damage in the treatments with the highest concentrations the surfactant. This results support the data presented by Tsui and Chu (2003) and Avigliano et al. (2014), indicating that the particular formulant POEA is far more toxic to aquatic organisms than the active substance glyphosate.

In conclusion, RMS highlights that POEA containing formulations can cause geno-

toxicological effects and concludes that existing information does not allow to exclude unacceptable effects on the population level following the intended uses of plant protection products containinig glyphosate and alkylamine ethoxylated co-formulants. Higher tier apical studies with such formulated products not always available. Therefore, EU Member States should pay particular attention when assessing and managing the risk for POEA-containing glyphosate products, e.g by requesting the generation of further data. Member Stated might also consider to substitute POEA in plant protection products by less critical surfactants.

6 Final Discussion

Studies addressing the active substance glyphosate and the metabolite AMPA

For a reasoned opinion on the reneval of approval of the active substance glyphosate, the results of biomarker studies can be used as supporting information, but do not supersede the results of apical tests at the level of organisms. Usually is not possible to predict whether results from biomarker test will be manifest in the populations of non-target organisms to be protected. Currently, in the assessment of the risk of non-target organisms exposed to the intended use of a plant protection product only studies with apical endpoints are employed, , as these do reflect closer the potential impact at population level, e.g. survival of individuals, their reproductive performance and the vitality of their offspring. An initial indication of a potential adverse effect on organisms which results from biomarker studies is currently superseded by studies related to the reproductive performance of such organisms. In available reproduction studies with the test species fish, no evidence of impairment as a result of possible damage to the genetic material of the non-target organisms as well as other harmful effects have been observed at environmental relevant concentrations of glyphosate. From the evaluation of the studies as reported in the IARC monograph no changes for the environmental risk assessment of the active substance glyphosate do arise as compared to the assessment of the RMS in the draft RAR from July 2015.

Studies with glyphosate-containing formulation

As the lead formulation for the assessment of the active substance glyphosate does not contain alkylamine ethoxylated surfactants (POEA), the studies carried out with POEA-containing glyphosate formulations are not considered relevant for the reneval of approval of the active substance glyphosate. However, RMS considered it adequate to provide general background information to other EU Member States to facilitate the assessment of the risk arising from glyphosate-based products other than the lead formulation. Please refer also to RAR Chapter B.9.11 “Surface active substances in glyphosate-based formulations” for detailed information. All (eco) toxicological data available give strong evidence that the toxicity of glyphosate-based formulation with POEA arises from the effects elicited by this type of surfactants. Similar to the risk assessment of the lead formulation, in the framework of the authorization of plant protection products formulated with POEA, fish reproduction studies were submitted. In these regulatory studies, population-related effects at low concentrations were attributable

to the effect of POEA, since the derived endpoints were significantly lower than the effects observed in studies with the active substance glyphosate alone. Moreover, effects of glyphosate products formulated with POEA were reported in two geno-toxicological studies as assessed in this addendum(Guilherme et al., 2012, ASB2014-7619, Guilherme et al., 2014, ASB2015-8631). Therefore, RMS highlighted that EU Member States might demand the generation of further data at the authorization of glyphosate-based products with surfactants. Moreover, on the basis of this data EU Member States are encouraged to consider the substitution of POEA in plant protection products.

7 Conclusions and recommendations

1) RMS highlighted that EU Member States might demand the generation of further data in the framework of the authorization of glyphosate-based products formulated with specific surfactants. Member States are encouraged to consider the substitution of alkylamine ethoxylates (POEA) in plant protection products with less toxic surfactants.

2) Data on genotoxicity measured in bioassays is not routinely considered as representative of an adverse ecological effect, but is predominantly used in human cancer risk assessment. Generally, understanding available information on molecular or cellular levels might in future help reducing the use of apical endpoints, towards biological information generated with in-vitro methods - provided that effects seen in in-vitro test do lead to apical responses.

3) RMS would support further activities in order to assess the outcome of genotoxic studies in environmental risk assessment. Guidance is needed in order to identify sensitive and validated biomarker models and to qualitatively and quantitatively relate respective information to regulatory endpoints.

4) The assessment of the possible risks of the use of plant protection products containing glyphosate by the RMS has revealed that no unacceptable impact on natural environment and groundwater might be expected only if the application of such products is accompanied by appropriate risk mitigation measures. As a consequence, RMS has requested in its draft RAR for the EU Member States that the approval of the

active substance glyphosate for the use in plant protection products in the EU is associated with default measures in order to minimizie and compensate the identified high risks for the populations of non-target organisms – especially insects and farmland bird species – arising from a disruption of the food web (also referred to as effects on biodiversity).

5) Irrespective of the environmental risks (especially food-web disruption) associated with the application of the active substance glyphosate, and from an eco-toxicological perspective, the mere substitution of the active substance glyphosate by other herbicidal chemicals is not considered to be appropriate by RMS, since alternative substances might not be more favourable in terms of environmental properties compared to the active substance glyphosate.

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